

# Adaptive evolution of *ASPM*, a major determinant of cerebral cortical size in humans

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A prominent trend in the evolution of humans is the progressive enlargement of the cerebral cortex. The *ASPM* (*Abnormal spindle-like microcephaly associated*) gene has the potential to play a role in this evolutionary process, because mutations in this gene cause severe reductions in the cerebral cortical size of affected humans. Here, we show that the evolution of *ASPM* is significantly accelerated in great apes, especially along the ape lineages leading to humans. Additionally, the lineage from the last human/chimpanzee ancestor to humans shows an excess of non-synonymous over synonymous substitutions, which is a signature of positive Darwinian selection. A comparison of polymorphism and divergence using the McDonald–Kreitman test confirms that *ASPM* has indeed experienced intense positive selection during recent human evolution. This test also reveals that, on average, *ASPM* fixed one advantageous amino acid change in every 300 000–400 000 years since the human lineage diverged from chimpanzees some 5–6 million years ago. We therefore conclude that *ASPM* underwent strong adaptive evolution in the descent of *Homo sapiens*, which is consistent with its putative role in the evolutionary enlargement of the human brain.

## INTRODUCTION

In the evolutionary lineage leading to *Homo sapiens*, one of the most notable trends is the dramatic enlargement of the brain (1). This is especially so for the cerebral cortex, the site of higher cognitive functions (2). Efforts to study the evolution of the human brain have traditionally focused on anatomy, physiology and behavior. By contrast, the genetic basis of brain evolution remains poorly explored. It has been suggested that genes involved in the development of the brain are likely to also play a role in the evolution of the brain. However, this proposition is yet to be validated by empirical data.

Microcephaly (small head) is a congenital condition characterized by severe underdevelopment of the cerebral cortex and its supporting structures (3,4). Primary (or true) microcephaly is a subclass of microcephaly, whereby patients exhibit significant reductions in cerebral cortical size without displaying other gross abnormalities. Thus far, five recessive loci have been linked to clinically indistinguishable forms of primary microcephaly (5–10). The gene underlying one of these loci, *ASPM*, was recently identified by genetic linkage studies (11,12). Consistent with the function of human *ASPM* in controlling

brain size, the mouse *Aspm* gene is expressed prominently at the site of cerebral cortical neurogenesis (11), and the *Drosophila* homolog, *asp*, encodes a microtubule-binding protein required for proper mitotic spindle organization during neuroblast proliferation (13–15). Because genes implicated in primary microcephaly, including *ASPM*, are specifically involved in determining cerebral cortical size, it is tempting to speculate that these genes may also play a role in the evolutionary enlargement of the human cerebral cortex. If this is the case, then these genes may show signatures of adaptive evolution in the lineage leading to humans. In this study, we present evidence that the molecular evolution of *ASPM* indeed exhibits strong signatures of adaptive evolution in the descent of humans. This finding is consistent with a possible role of *ASPM* in the evolutionary enlargement of the human cerebral cortex.

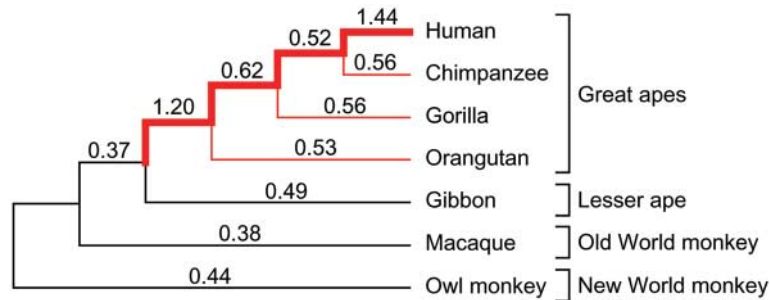
## RESULTS

### Molecular evolution of *ASPM* in primates

We sequenced the 10.4 kb coding sequence of *ASPM* in six primate species representing key positions of the primate

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**Figure 1.** Phylogeny of *ASPM* in primates. The great ape lineages are highlighted in red, and the ape lineages leading to humans in bold. The  $K_a/K_s$  ratios of individual segments of the phylogenetic tree are indicated.

phylogeny. A phylogenetic tree was constructed using these sequences along with the publicly available human sequence (Fig. 1; an alignment of *ASPM* in these species is provided in the Supplementary Material, Fig. S1). The ratio of non-synonymous substitution rate ( $K_a$ ) to synonymous substitution rate ( $K_s$ ), which measures the pace of protein evolution scaled to mutation rate, was determined for each segment of the phylogenetic tree (these ratios are indicated in Fig. 1). This revealed several noteworthy features. First, there is a general trend whereby phylogenetic lineages closer to humans tend to have higher  $K_a/K_s$  ratios. For example, in the great ape lineages (highlighted in red in Fig. 1), the combined  $K_a/K_s$  ratio is significantly higher than that of the more basal primates ( $P = 0.02$ ; see Materials and Methods). Second, this elevation in  $K_a/K_s$  is most pronounced in the ape lineages leading to humans (bold in Fig. 1), in which  $K_a/K_s$  ratios are among the highest of the phylogenetic tree and are statistically different from the other lineages ( $P = 0.02$ ). Third, the terminal human branch (i.e. the lineage from the last human/chimpanzee ancestor to humans) has a  $K_a/K_s$  value much greater than 1. This value, the highest in the tree, both indicates positive Darwinian selection (16) (see below for further evidence of positive selection), and renders the terminal human lineage statistically distinct from all other primate lineages ( $P = 0.03$ ). Finally, the lineage leading to the common ancestor of great apes also has a  $K_a/K_s$  value greater than 1, suggesting positive selection during this period of ape evolution (see Discussion). These findings indicate that, within primates, the evolution of *ASPM* protein sequence is accelerated most prominently along the ape lineages leading to humans, particularly in the terminal human branch after human/chimpanzee divergence.

### Molecular evolution of *ASPM* in diverse mammals

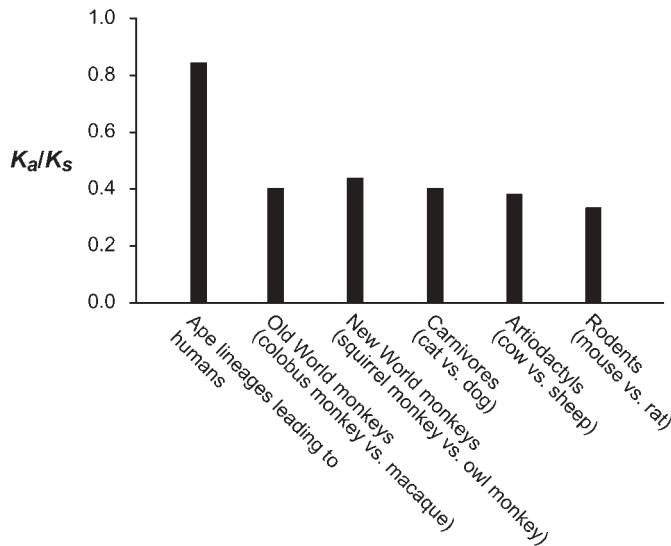
We next sequenced *ASPM* in colobus monkey (an Old World monkey), squirrel monkey (a New World monkey), cow, sheep, cat and dog. We also obtained mouse and rat *Aspm* sequences from the public database. With these sequences, we were able to estimate the rates of *ASPM* protein evolution within Old World monkeys (based on colobus monkey and macaque sequence comparison), New World monkeys (squirrel monkey and owl monkey comparison), carnivores (cat and dog comparison), artiodactyls (cow and sheep comparison) and rodents (mouse and rat comparison). As shown in Figure 2, the evolutionary rates of *ASPM* in Old World monkeys, New

World monkeys, carnivores, artiodactyls and rodents are relatively similar to one another. In contrast, the evolutionary rate of the ape lineages leading to humans as highlighted in Figure 1 is significantly higher ( $P < 0.001$ ). These observations indicate that the elevated rate of *ASPM* protein evolution is a distinct feature of the ape lineages leading to humans, and is not found in either non-hominoid primates or other non-primate mammalian orders examined. We therefore conclude that the evolution of *ASPM* is significantly and uniquely accelerated in the descent of *H. sapiens* relative to other mammalian lineages.

It has been noted that the *ASPM* proteins in worms, flies, mice and humans contain progressively greater numbers of the putative calmodulin-binding IQ domains (11,14). This observation is intriguing, given that the nervous system is also progressively larger and more complex across these species. We found that *ASPM* contains the same number of IQ domains within the primate order, and among primates, carnivores, artiodactyls and several other mammalian orders (data not shown). Given the closer evolutionary relatedness of primates and rodents to each other than either is to carnivores (17), the identical number of IQ domains found in primates and carnivores is probably the ancestral state, whereas the smaller number of IQ domains in rodents probably reflects a rodent-specific deletion of ~900 nucleotides. Thus, the number of IQ domains does not appear to bear any relationship to brain size in mammals, and it certainly has no relevance to the enlargement of the brain during hominoid evolution.

### Signature of positive selection in $K_a/K_s$ profiles

The accelerated evolution of *ASPM* in the descent of humans could reflect the influence of either positive Darwinian selection or relaxed functional constraint. We believe that relaxed constraint is highly unlikely because *ASPM* has been shown to bear a crucial function in the development of the human brain (11). This consideration notwithstanding, we sought to rigorously test for the presence of positive selection. In one analysis, we obtained a sliding-window profile of  $K_a/K_s$  values along the *ASPM* coding sequence in the ape lineages leading to humans as highlighted in Figure 1. This revealed the presence of extreme peaks and valleys along the *ASPM* gene (Fig. 3). Many peaks have  $K_a/K_s$  ratios much greater than the value of 1 expected under neutrality. For three of these peaks, the excess of non-synonymous changes over neutral expectation is



**Figure 2.** The  $K_a/K_s$  ratios of *ASPM* in various mammalian taxa. Species used to derive the ratios are indicated below the bars. The ape lineages leading to humans are defined as the lineage from the last common ancestor of apes to modern humans (i.e. the bolded lineages in Fig. 1).

statistically significant ( $P \leq 0.025$ ; Fig. 3). In addition to these individual peaks, the overall topology of the  $K_a/K_s$  profile departs significantly from the neutral expectation ( $P = 0.02$ ; see Materials and Methods for details). It is worth noting that, among the functional domains of *ASPM*, the  $K_a/K_s$  peaks are most concentrated in the putative microtubule-binding domain and the putative calmodulin-binding IQ domains, whereas the calponin-homology domain and the C-terminal region are much more conserved (Fig. 3). These results argue strongly that positive selection has driven amino acid changes in *ASPM* along the ape lineages leading to humans, particularly within the putative microtubule-binding domain and the IQ domains. In contrast, the  $K_a/K_s$  profiles of Old World monkeys, New World monkeys, carnivores, artiodactyls and rodents do not contain any peaks significantly greater than 1 (Fig. 3).

#### Signature of positive selection by the McDonald–Kreitman test

In a second analysis, we applied the McDonald–Kreitman test (18), a stringent test of positive selection (19,20), to the *ASPM* coding sequence. This test is predicated on the null expectation that the ratio of non-synonymous to synonymous substitutions between species should be equivalent to the ratio of non-synonymous to synonymous polymorphisms within a species if a gene is evolving neutrally or under constant purifying selection. A significant departure from this expectation, whereby the ratio between species is much greater than that within species, is a strong signature of positive Darwinian selection (19). To perform the McDonald–Kreitman test, we obtained the polymorphism distribution in the entire *ASPM* coding sequence from 40 individuals representing world-wide human populations (80 haploid copies; about 835 kb of total

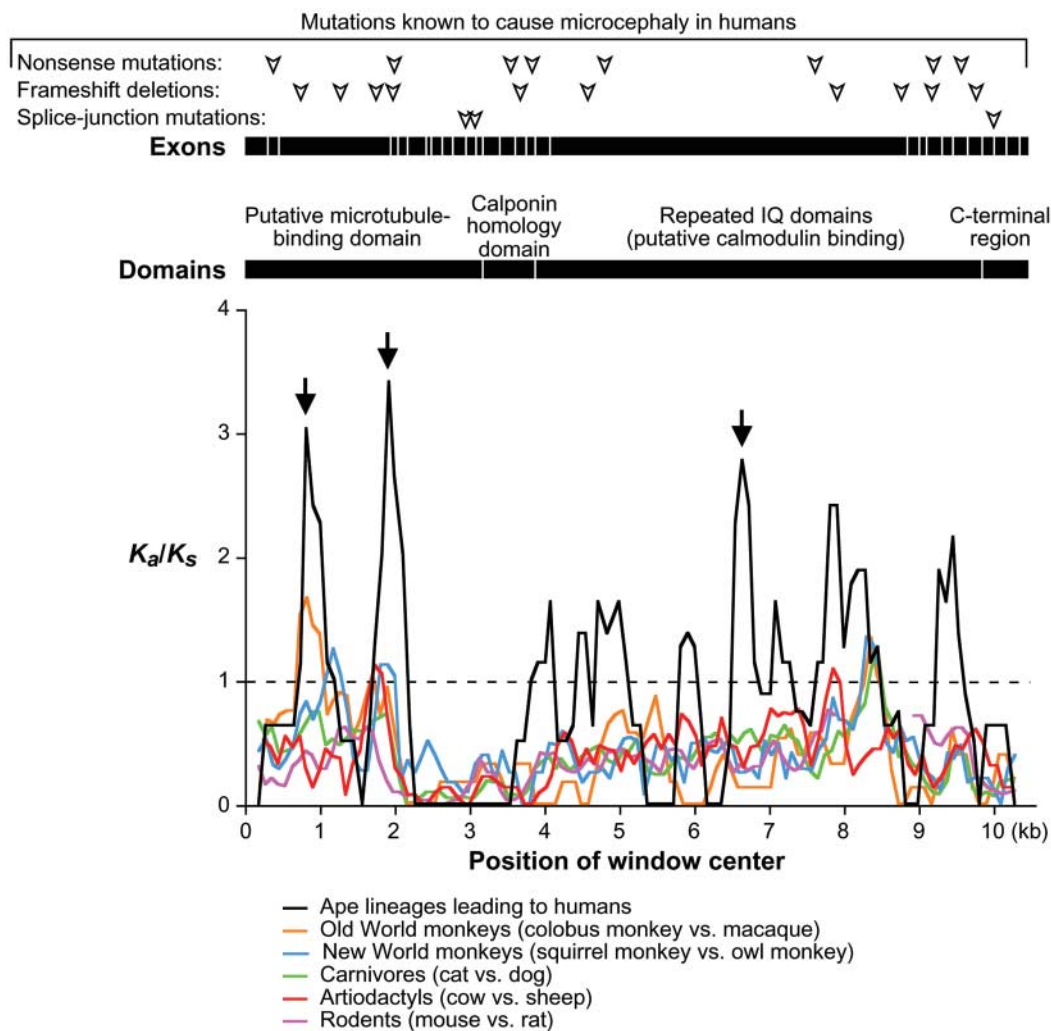
sequence). As shown in Table 1, there is a significant excess of non-synonymous substitutions in the terminal human branch (i.e. from the last human/chimpanzee ancestor to humans) as compared with non-synonymous polymorphisms within humans ( $P = 0.02$ ). While changes in the historical population size could also contribute to genome-wide departures from the McDonald–Kreitman expectation, the extent of the departure seen in *ASPM* is so great that it cannot be reasonably attributed to population size changes. Additionally, there is no evidence that population size changes during human evolution had caused a genome-wide departure from the McDonald–Kreitman expectation. Thus, results of the McDonald–Kreitman test on *ASPM* provides strong evidence that positive Darwinian selection has operated on this gene in the descent of humans. To the extent that the ratio of non-synonymous to synonymous polymorphisms within humans reflects the strength of constant purifying selection, the excess non-synonymous substitutions between species should be the result of adaptive evolution. Accordingly, roughly 15 of the 19 non-synonymous substitutions that occurred between the last human/chimpanzee ancestor and humans may have been adaptive and were driven to fixation by positive selection. This translates to about one adaptive amino acid change in *ASPM* for every 300 000–400 000 years since the human lineage diverged from chimpanzees.

#### DISCUSSION

Homozygous loss-of-function mutations in the *ASPM* gene cause severe underdevelopment of the cerebral cortex and, consequently, microcephaly (11). Besides underdevelopment of the cortex, however, there does not seem to be any other overt developmental defects in the affected patients. Such specificity in phenotype is striking, given that most genes known to regulate corticogenesis are typically also involved in the development of several other tissue systems. In light of the apparent functional specificity of *ASPM* in corticogenesis, it is tempting to speculate that this gene might have also played a role in the enlargement of the cerebral cortex during human evolution. If this is indeed the case, then *ASPM* might exhibit signatures of adaptive evolution in the lineage leading to humans. In this study, we uncovered several such signatures, including a significant acceleration of protein evolution in the ape lineages leading to humans, an excess of non-synonymous substitutions over the neutral expectation, and a much higher non-synonymous to synonymous substitution ratio between species than within species as revealed by the McDonald–Kreitman test. Collectively, these multiple lines of evidence argue strongly that *ASPM* has experienced strong adaptive evolution in the descent of *H. sapiens*, a conclusion consistent with its possible role in the evolutionary enlargement of the human cerebral cortex.

Our study places *ASPM* among a number of human genes linked to adaptive evolution (reviewed in 21). While *ASPM* does not have the highest  $K_a/K_s$  ratio among these genes, it is unique in that it is the only gene involved predominantly—if not exclusively—in determining cerebral cortical size.

Another point worthy of note is that the acceleration of *ASPM* evolution (i.e. high  $K_a/K_s$  ratio) is not confined to the most recent history of *H. sapiens*, but is noticeable along the



**Figure 3.** Sliding-window analysis of  $K_a/K_s$  along the *ASPM* coding region. A gap is introduced into the rodent profile to account for the large rodent-specific deletion in this region. Peaks above the dotted line have  $K_a/K_s$  values greater than 1, and therefore an excess of non-synonymous substitutions over the neutral expectation. The ape lineages leading to humans are defined as the lineage from the last common ancestor of apes to modern humans. Exon structure of *ASPM* and domain structure of the encoded protein are depicted on top of the  $K_a/K_s$  profile. Solid arrows indicate peaks in the  $K_a/K_s$  profiles that show a statistically significant excess of non-synonymous substitutions over neutral expectation ( $P \leq 0.025$ ). Open arrow heads indicate mutations in *ASPM* that cause microcephaly as previously reported (11,12). All the splice-junction mutations are point mutations thought to abolish or impair splicing at the affected junctions. Among these, the second one changes the last base of the exon, while the other two change the splice donor sequence at the beginning of the intron (12).

entire lineage from the last ape ancestor to modern humans (Fig. 1). This observation is consistent with a gradualistic view of human evolution, which contends that many aspects of the human phenotype did not arise abruptly in the most recent history of *Homo*, but are instead the consequence of a lengthy and relatively continuous evolutionary process. This view is consistent with the fact that many (though perhaps not all) biological traits seen most prominently in humans are also found in non-human primates, albeit to lesser degrees. Indeed, with regards to brain size and morphology, the successive emergence of more derived primate taxa along the lineage leading to humans is marked by the progressive expansion and folding of the cerebral cortex (22). Hence, the increase in cerebral cortical size had apparently started long ago in early primates, and affected their descendant humans as well as non-human primates, although this expansion is perhaps most

dramatic in the terminal branch of the human lineage. From our data, it appears that positive selection began operating on *ASPM* at least as early as the last ape ancestor some 18 million years ago (23), and lasted throughout the lineage leading to modern humans. It is perhaps no coincidence that the terminal human branch (i.e. the branch post human/chimpanzee divergence) and the lineage leading to great apes (i.e. from the last ape ancestor to the common ancestor of great apes) have the first and second highest  $K_a/K_s$  ratios, respectively (Fig. 1). These are the periods when hominoid brains underwent dramatic expansion in size, as well as growth in structural and functional complexity (22).

How might the molecular evolution of *ASPM* contribute to the progressive enlargement of the cerebral cortex? There is currently no direct data on the biochemical function of *ASPM* in mammals. However, the *Drosophila* homolog, *asp*, has been



**Table 1.** The numbers of non-synonymous (*N*) and synonymous (*S*) nucleotide changes in *ASPM*

	<i>N</i>	<i>S</i>	<i>P</i> -value
Polymorphism within human populations ( <i>n</i> = 80)	6	10	
Divergence between human and last human/chimpanzee ancestor	19	7	0.025

*n* denotes the number of haploid genomes sampled; *P*-value is from Fisher's exact test.

studied extensively, and shown to have a critical function in organizing the mitotic and meiotic spindle structures (13–15). During the development of the brain, the proliferating progenitor cells of the neuroepithelium can undergo two modes of cell divisions: symmetric divisions with mitotic spindles in the plane of the neuroepithelium to generate two identical progenitor cells, and asymmetric divisions with mitotic spindles perpendicular to the plane of the neuroepithelium to generate one progenitor and one neuron (24). The regulation of mitotic spindle orientations (and hence the proportions of symmetric versus asymmetric cell divisions) in the proliferating neuroepithelium is thought to have a profound impact on the final size of the cerebral cortex, with higher proportions of symmetric divisions during early neuroepithelium development corresponding to larger cortex (25). Based on mouse data, *ASPM* is expressed specifically in the developing neuroepithelium (11). If the mammalian *ASPM* protein, like its *Drosophila* homology, is also involved in the organization of mitotic spindle structures, then it is conceivable that alterations to the amino acid sequence (and hence the biochemical properties) of *ASPM* during evolution could affect the regulation of mitotic spindle orientation in proliferating cells of the neuroepithelium. This in turn could alter the size of the cerebral cortex. These possibilities are potentially testable in future studies.

## MATERIALS AND METHODS

### Sequence acquisition and analysis

The coding sequences of *ASPM* were experimentally obtained from the following species: common chimpanzee (*Pan troglodytes*; accession: AY488910), lowland gorilla (*Gorilla gorilla*; accession: AY488938), orangutan (*Pongo pygmaeus*; accession: AY488966), white-handed gibbon (*Hylobates lar*; accession: AY488994), crab-eating macaque (*Macaca fascicularis*; accession: AY485418), black-and-white colobus monkey (*Colobus guereza*; accession: AY489022), owl monkey (*Aotus* spp.; accession: AY485422), Bolivian squirrel monkey (*Saimiri boliviensis*; accession: AY485419), domestic cat (*Felis catus*; accession: AY485420), domestic dog (*Canis familiaris*; accession: AY485421), cow (*Bos taurus*; accession: AY485424), and sheep (*Ovis aries*; accession: AY485423). For chimpanzee, gorilla, orangutan, gibbon and colobus monkey, genomic DNA was obtained from cell lines. For the rest of the species, total RNA was extracted from brain specimens, and primed by random hexamers or poly-T oligomers to synthesize first-strand cDNA. Standard PCR conditions were used to amplify *ASPM*

coding region from either genomic DNA (100–500 ng per 30 µl reaction) or cDNA (0.5–5 ng per reaction). PCR primers were initially based on human *ASPM* sequence until species-specific sequences were obtained. All sequencing was performed using standard dye-terminator chemistry on PCR products. *ASPM* sequences of human (*H. sapiens*; accession: NM\_018136), mouse (*Mus musculus*; accession: NM\_009791) and rat (*Rattus norvegicus*; obtained by piecing together genomic segments corresponding to exons retrieved from GenBank; accession: XM\_213891) were retrieved from NCBI databases. Nucleotide sequences were aligned in-frame using the Megalign program in the DNASTAR software package (DNASTAR, Madison, WI, USA). In multiple-species alignments, the ancestral sequence was deduced by parsimony. The Diverge function from the Wisconsin Package version 10.2 (Accelrys Inc., San Diego, CA, USA) was used to analyze evolutionary divergence (i.e. the numbers of non-synonymous and synonymous substitutions, and  $K_a$  and  $K_s$  rates) according to the Li (26) method. For some species, we also estimated the rates of sequence divergence within the *ASPM* intronic sequences and found them to be comparable to  $K_s$  rates (data not shown), which was in line with expectation. To calculate the combined  $K_a/K_s$  ratio across multiple lineages, non-synonymous substitutions in all these lineages were added to calculate the combined  $K_a$ , and a similar procedure was used to calculate the combined  $K_s$ . The ratio of these two values was the combined  $K_a/K_s$ . For sliding-window analysis of the  $K_a/K_s$  ratio,  $K_a$  was calculated for window size of 100 codons and sliding increment of 30 codons, and  $K_s$  of the entire gene was used as denominator to avoid problems associated with stochastic variation that can lead to division by zero.

### Analysis of human polymorphism

Contiguous double-stranded sequences were obtained from 40 human individuals for the 10 434 bp coding regions of *ASPM*. These individuals were chosen from the Coriell Human Variation Panels to represent a diverse selection of world-wide populations. They include six North Africans (Coriell number: 17378, 17379, 17380, 17381, 17382, 17383), five South Africans (17341, 17342, 17343, 17344, 17349), six Chinese (16654, 16688, 16689, 17014, 17015, 17016), six Russians (13820, 13838, 13852, 13876, 13877, 13911), three Basques (15883, 15884, 15885), three Iberians (17091, 17092, 17093), three Pacific Islanders (17385, 17386, 17387), three Andeans (17301, 17302, 17303), three Southeast Asians (17081, 17082, 17083), and two Middle Easterners (17331, 17332). PCR primers were designed to amplify various exonic regions of *ASPM*, and PCR products were sequenced on both strands using standard dye-terminator chemistry. Sequence chromatograms were aligned by the Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA). Polymorphisms were detected by direct visual inspection of sequence chromatograms as displayed by Sequencher.

### Statistical analysis

To assess the statistical significance that the  $K_a/K_s$  ratio of one lineage is distinct from that of another lineage, the numbers of non-synonymous and synonymous substitutions were calculated for both lineages using the Li (26) method.

The resulting four values were placed in a  $2 \times 2$  contingency table, and assessed for statistical significance by the two-tailed Fisher's exact test. To perform the McDonald–Kreitman test, the numbers of non-synonymous and synonymous substitutions were obtained for both interspecies divergence and within-species polymorphism. For polymorphism, we excluded segregating sites with very rare alleles ( $\leq 0.25\%$ ) as was done customarily (27,28), because these rare alleles had a high probability of being deleterious and destined for extinction. The resulting four values were placed in a  $2 \times 2$  table, and assessed for statistical significance by the Fisher's exact test. A published method (29) was adapted to test whether the sliding-window profile of  $K_a/K_s$  is the product of positive selection. First, the occurrence of point mutations in the coding sequence of *ASPM* was simulated by assuming complete neutrality of all changes. When simulated synonymous changes reached the observed number, a sliding-window profile of  $K_a/K_s$  was generated from the simulated sequence. In windows where  $K_a/K_s$  ratios exceed 1, the portions of  $K_a$  that exceed neutral expectation were summed. The fraction of simulations in which this sum was equal to or greater than the sum observed for the real sequence was taken as the statistical significance that the observed  $K_a/K_s$  profile departs from the neutral expectation. For *ASPM*, a total of 10 000 simulations were performed.

## SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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## REFERENCES

1. Brodmann, K. (1912) Ergebnisse über die vergleichende histologische Lokalisation der Grosshirne mit besonderer Berücksichtigung des Stirnhirns. *Anat. Anz. Suppl.*, **41**, 157–216.
2. Finlay, B.L. and Darlington, R.B. (1995) Linked regularities in the development and evolution of mammalian brains. *Science*, **268**, 1578–1584.
3. Mochida, G.H. and Walsh, C.A. (2001) Molecular genetics of human microcephaly. *Curr. Opin. Neurol.*, **14**, 151–156.
4. Dobyns, W.B. (2002) Primary microcephaly: new approaches for an old disorder. *Am. J. Hum. Genet.*, **112**, 315–317.
5. Jackson, A.P., McHale, D.P., Campbell, D.A., Jafri, H., Rashid, Y., Mannan, J., Karbani, G., Corry, P., Levene, M.I., Mueller, R.F. *et al.* (1998) Primary autosomal recessive microcephaly (MCPH1) maps to chromosome 8p22-pter. *Am. J. Hum. Genet.*, **63**, 541–546.
6. Roberts, E., Jackson, A.P., Carradice, A.C., Deeble, V.J., Mannan, J., Rashid, Y., Jafri, H., McHale, D.P., Markham, A.F., Lench, N.J. *et al.* (1999) The second locus for autosomal recessive primary microcephaly (MCPH2) maps to chromosome 19q13.1–13.2. *Eur. J. Hum. Genet.*, **7**, 815–820.
7. Moynihan, L., Jackson, A.P., Roberts, E., Karbani, G., Lewis, I., Corry, P., Turner, G., Mueller, R.F., Lench, N.J. and Woods, C.G. (2000) A third novel locus for primary autosomal recessive microcephaly maps to chromosome 9q34. *Am. J. Hum. Genet.*, **66**, 724–727.
8. Jamieson, C.R., Govaerts, C. and Abramowicz, M.J. (1999) Primary autosomal recessive microcephaly: homozygosity mapping of MCPH4 to chromosome 15. *Am. J. Hum. Genet.*, **65**, 1465–1469.
9. Jamieson, C.R., Fryns, J.P., Jacobs, J., Matthijs, G. and Abramowicz, M.J. (2000) Primary autosomal recessive microcephaly: MCPH5 maps to 1q25–q32. *Am. J. Hum. Genet.*, **67**, 1575–1577.
10. Pattison, L., Crow, Y.J., Deeble, V.J., Jackson, A.P., Jafri, H., Rashid, Y., Roberts, E. and Woods, C.G. (2000) A fifth locus for primary autosomal recessive microcephaly maps to chromosome 1q31. *Am. J. Hum. Genet.*, **67**, 1578–1580.
11. Bond, J., Roberts, E., Mochida, G.H., Hampshire, D.J., Scott, S., Askham, J.M., Springell, K., Mahadevan, M., Crow, Y.J., Markham, A.F. *et al.* (2002) *ASPM* is a major determinant of cerebral cortical size. *Nat. Genet.*, **32**, 316–320.
12. Bond, J., Scott, S., Hampshire, D.J., Springell, K., Corry, P., Abramowicz, M.J., Mochida, G.H., Hennekam, R.C., Maher, E.R., Fryns, J.P. *et al.* (2003) Protein-truncating mutations in *ASPM* cause variable reduction in brain size. *Am. J. Hum. Genet.*, **73**, 1170–1177.
13. Ripoll, P., Pimpinelli, S., Valdivia, M.M. and Avila, J. (1985) A cell division mutant of *Drosophila* with a functionally abnormal spindle. *Cell*, **41**, 907–912.
14. Saunders, R.D., Avides, M.C., Howard, T., Gonzalez, C. and Glover, D.M. (1997) 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.
15. do Carmo Avides, M., Tavares, A. and Glover, D.M. (2001) Polo kinase and *Asp* are needed to promote the mitotic organizing activity of centrosomes. *Nat. Cell. Biol.*, **3**, 421–424.
16. Li, W.H. (1997) *Molecular Evolution*. Sinauer Associates, Sunderland, MA.
17. Murphy, W.J., Eizirik, E., Johnson, W.E., Zhang, Y.P., Ryder, O.A. and O'Brien, S.J. (2001) Molecular phylogenetics and the origins of placental mammals. *Nature*, **409**, 614–618.
18. McDonald, J.H. and Kreitman, M. (1991) Adaptive protein evolution at the *Adh* locus in *Drosophila*. *Nature*, **351**, 652–654.
19. Kreitman, M. (2000) Methods to detect selection in populations with applications to the human. *A. Rev. Genomics Hum. Genet.*, **1**, 539–559.
20. Wyckoff, G.J., Wang, W. and Wu, C.I. (2000) Rapid evolution of male reproductive genes in the descent of man. *Nature*, **403**, 304–309.
21. Wolfe, K.H. and Li, W.H. (2003) Molecular evolution meets the genomics revolution. *Nat. Genet.*, **33** (suppl.), 255–265.
22. Noback, C.R. and Montagna, W. (1970) *The Primate Brain*. Meredith Corporation, New York.
23. Goodman, M., Porter, C.A., Czelusniak, J., Page, S.L., Schneider, H., Shoshani, J., Gunnell, G. and Groves, C.P. (1998) Toward a phylogenetic classification of Primates based on DNA evidence complemented by fossil evidence. *Mol. Phylogenet. Evol.*, **9**, 585–598.
24. Caviness, V.S. Jr. Takahashi, T. and Nowakowski, R.S. (1995) Numbers, time and neocortical neuronogenesis: a general developmental and evolutionary model. *Trends Neurosci.*, **18**, 379–383.
25. Rakic, P. (1995) A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci.*, **18**, 383–388.
26. Li, W.H. (1993) Unbiased estimation of the rates of synonymous and nonsynonymous substitution. *J. Mol. Evol.*, **36**, 96–99.
27. Fay, J.C., Wyckoff, G.J. and Wu, C.I. (2001) Positive and negative selection on the human genome. *Genetics*, **158**, 1227–1234.
28. Fay, J.C., Wyckoff, G.J. and Wu, C.I. (2002) Testing the neutral theory of molecular evolution with genomic data from *Drosophila*. *Nature*, **415**, 1024–1026.
29. Fares, M.A., Elena, S.F., Ortiz, J., Moya, A. and Barrio, E. (2002) A sliding window-based method to detect selective constraints in protein-coding genes and its application to RNA viruses. *J. Mol. Evol.*, **55**, 509–521.