

# **Cytoskeletal genes regulating brain size** Jacquelyn Bond<sup>1</sup> and C Geoffrey Woods<sup>2</sup>

One of the most notable trends in human evolution is the dramatic increase in brain size that has occurred in the great ape clade, culminating in humans. Of particular interest is the vast expanse of the cerebral cortex, which is believed to have resulted in our ability to perform higher cognitive functions. Recent investigations of congenital microcephaly in humans have resulted in the identification of several genes that nonredundantly and specifically influence mammalian brain size. These genes appear to affect neural progenitor cell number through microtubular organisation at the centrosome.

#### Addresses

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# Introduction

The cerebral cortex is a thin layered sheet of neurons at the surface of the brain. During the evolution of *Homo sapiens* the surface area of the cortex has increased by three orders of magnitude relative to body size. To contain this efficiently within the skull the human brain has developed a convoluted (gyrencephalic) appearance. The disproportionate increase in the size of the human cerebral cortex is thought to underlie the growth in intellectual capacity shown by humans and their ability to execute complex cognitive functions, such as language [1].

The formation of the cerebral cortex is a complex and closely regulated sequence of neuronal proliferation, migration and differentiation. Neurons are derived from neural progenitor cells residing in the neuroepithelium of the ventricular zone (VZ), the region surrounding the ventricles of the developing brain [2]. Before neurogenesis the neural progenitor cell pool is expanded by multiple rounds of symmetric (proliferative) division, occurring when the mitotic spindle is horizontal to the neuroepithelial ventricular surface, creating two daughter progenitor cells. The majority of neurons are produced by asymmetric (neuronogenic) division, where the mitotic spindle rotates until it lies vertical to the neuroepithelium. Division in this plane yields two daughter cells with different developmental fates, one neural progenitor cell and one neuron [3,4]. A minority of neurons are produced by terminal symmetrical division of intermediate cells producing two neurons. Post-mitotic neurons follow specific migration pathways in their journey to the cortex along radial glia [5]. Once at the cortex they must move past earlier neurons to form the outermost neuronal layer, until, finally, a six-layer laminar neocortex has been formed.

The centrosome plays a major role in regulating cell division, functioning as a microtubule organising centre (MTOC). One of the most dramatic intracellular changes occurring in mitosis is the loss of the interphase astral microtubule array radiating from the centrosome to the cell cortex and the assembly of a bipolar mitotic spindle. During this process the centrosome functions to nucleate microtubule polymerisation from free tubulin subunits and to organise the microtubule arrays (reviewed in [6]).

When studying the regulation of cerebral cortical size it has often proved valuable to investigate Mendelian disorders that directly affect the size of the brain. Prime candidate genes are those involved in disorders of neurogenesis, such as primary microcephaly (where small brain size is present at birth), in which gene mutations have demonstrated their ability to alter brain size. This review profiles four genes — Microcephalin (MCPH1) [7], ASPM (abnormal spindle-like microcephaly-associated [Drosophila]) [8], CDK5RAP2 (cyclin-dependent kinase 5 regulatory-associated protein 2) and CENPJ (centromere associated protein J) [9<sup>•</sup>] (see Box 1 for electronic database information) - which have recently been identified in which mutations cause a pathological reduction in human brain size, most significantly a decrease in cerebral cortical size. We discuss protein structure and expression profiles and, using species comparisons, speculate upon the neurogenic functions of the MCPH proteins. Intracellular distribution patterns and expression in the neuroepithelial layer of the developing cortex of these cytoskeleton-related proteins puts them in the right place at the right time to sustain control over neurogenic mitosis. Their proposed roles are in assembling, maintaining and orienting the mitotic spindle in neuronal precursor cells. The identification and analysis of these genes has suggested the existence of a common centrosomal mechanism controlling mammalian brain size.



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A comparison of magnetic resonance images from an individual with an *MCPH5* mutation and an unaffected individual of a similar age. **(a)** Sagittal T1-weighted image from an unaffected 11-year-old female control. **(b)** Sagittal T1-weighted image from a 13-year-old female with an *MCPH5* mutation showing striking reduction in the size of the cerebral cortex, prominent sloping of the forehead and preserved midline structures. Scale bar = 2 cm. Figure reproduced from an original figure (Figure 1 from [8]) with consent from Nature Publishing Group and CA Walsh.

# MCPH, a disorder of neurogenic mitosis affecting foetal brain growth

Autosomal recessive primary microcephaly (MCPH [MIM 251200]) is a rare congenital disorder of reduced growth of the cerebral cortex and supporting structures (Figure 1). The disorder is defined by a reduced head circumference (-4 to -11 standard deviations adjusted for age and sex) (reviewed in [10]) and is associated with mental retardation but no other neurological or growth disorders [11]. Although the MCPH brain is small it exhibits the normal six-layer neuronal architecture, suggesting MCPH results from a decrease in neural progenitor cell number [8,12] (Box 1).

The genetic complexity of cortical neurogenesis is reflected in the genetic heterogeneity of MCPH, which is associated with at least six recessive loci causing Box 1 Electronic database information.

MCPH [MIM 251200] ASPM [MIM 650481] CDK5RAP2 [MIM 608201] CENPJ [609279] Microcephalin [607117] From Online Mendelian Inheritance in Man (OMIM), http://www. ncbi.nlm.nih.gov/omim/

indistinguishable disorders (reviewed in [13<sup>•</sup>]). Autozygosity mapping of large consanguineous families and positional cloning strategies have recently led to the identification of genes at four MCPH loci; Microcephalin (MCPH1, [7]), ASPM (MCPH5, [8]) and CDK5RAP2 and *CENPJ* at the *MCPH3* and *MPCH6* loci, respectively [9<sup>•</sup>] (Table 1). All MCPH-associated mutations are nonsense mutations predicted to result in premature protein truncation with the exception of a single missense mutation in CENPJ, which may alter the binding affinity of the Tcp10 domain for the non-erythrocyte 4.1R protein 135 splice variant [9<sup>•</sup>]. Each of the four genes is expressed in the ventricular zone of the embryonic brain [7,8,9<sup>•</sup>] during the period of neurogenesis, suggesting that the MCPH phenotype originates in the neural progenitor cells.

# **MCPH** and mitosis

# ASPM, a microtubule-associated protein

ASPM [MIM 605481] is the human homologue of the *Drosophila* Asp protein. Homologues to ASPM have been identified in many species; however, ASPM does not appear to be related to any human gene family. Species homology and bioinformatic analyses suggest that it is a 3477-aa microtubule-associated protein containing an N-terminal microtubule binding domain [14], two calponin homology domains [15] commonly involved in actin binding [16], up to 81 IQ (isoleucine glutamine) repeat motifs, which potentially bind calmodulin [8,15], and a C-terminal region of unknown function [17]. Together

Gene	Intracellular mitotic localisation	Domains	Predicted role of protein	References
ASPM	Surface of spindle poles	MT association Calponin homology domain IQ motifs	MT and central spindle organisation during mitosis	[8,13°,14,15,17,19,33°°]
CDK5AP2	Spindle poles	N-terminal α-tubulin association	Enhance centrosomal MT production Negative control of CDK5	[9 <b>*</b> ,37–42]
CENJP	Spindle poles	Tcp 10 domain	Regulation of MT nucleation MT depolymerisation	[9°,44°,45,46°,47°°,48–50]
MCPH1	Unpublished	BRCT domains	DNA damage response Cell cycle control Telomerase regulation	[7,52–57]

this suggests that ASPM can bind to the cellular cytoskeleton and through calmodulin binding may assemble as a semi-rigid rod.

Homozygous nonsense mutations in the human *ASPM* gene are the most common cause of MCPH in the Northern Pakistani population, with little variation occurring in the severity of the phenotype [8,17,18]. To date at least 26 mutations in *ASPM* have been identified, spread throughout the gene [8,17,19–21], that are predicted to result in premature truncation. A C-terminal 150-aa region is common to all deletions; this region is of unknown function [17]. Evolutionary studies of *ASPM* 

#### Figure 2

have determined the gene has undergone positively selected changes; this suggests that ASPM may be a significant genetic component involved in the evolutionary expansion of the human brain  $[22-24,25^{\circ}]$ .

A role for ASPM in human neurogenesis is supported by its preferential expression in the neuroepithelium of the lateral ventricles during the neurogenic cycle (J Bond, CG Woods and S Lindsay, unpublished). The role of ASPM and Asp during cellular division is under investigation in both human and fly. During mitosis both are localised at the face of the spindle poles (Figure 2a) [14,15,26]. This localisation is dynein–dynactin dependant [27<sup>••</sup>]. While



Spindle pole localisation of ASPM, CDK5RAP2 and CENPJ during metaphase. (a) Confocal microscopy analysis of fixed HeLa cells showing centrosomal localisation of human ASPM (green), rat monoclonal  $\alpha$ -tubulin antibody (red), and DNA (blue) during metaphase. (b) Confocal microscopy analysis of fixed HeLa cells showing centrosomal localisation of human CDK5RAP2 (green), rat monoclonal  $\alpha$ -tubulin antibody (red) and DNA (blue) during metaphase [9\*]. (c) Confocal microscopy analysis of fixed HeLa cell confirming centrosomal localisation of human CENPJ (green),  $\alpha$ -tubulin antibody (red) and DNA (blue) during metaphase [9\*]. (c) Confocal microscopy analysis of fixed HeLa cell confirming centrosomal localisation of human CENPJ (green),  $\alpha$ -tubulin antibody (red) and DNA (blue) during metaphase [9\*]. CENPJ and CDK5RAP2 localisation are reproduced from an original figure (Figure 2 from [9\*]) with consent from Nature Publishing Group.

mutation in ASPM in humans results in a mitotic phenotype specific to the brain, Drosophila asp recessive mutants are larval-lethal or infertile. In the mutant larval brain, dividing neuron progenitors are unable to complete asymmetric cell division and arrest in metaphase [28]. Abnormal spindles of Asp mutants have disorganised broad poles exhibiting abnormal y-tubulin distribution [29]. Asp is activated by Polo Kinase [30]. Roles for Asp have been determined in meiosis and mitosis [30,31], in the organisation and binding together of microtubules at the spindle poles [27<sup>••</sup>,29], and in the formation of the central mitotic spindle, whose perturbation leads to disruption of the contractile ring machinery and failure of cytokinesis [32,33<sup>••</sup>]. ASPM mitotic spindle function may be regulated by BRCA1 [26]. Extrapolating from Asp function, the mandatory neurogenic functions of ASPM are microtubule organisation at the spindle pole during mitosis and organisation of the central spindle during cytokinesis.

ASPM expression has been reported in several human embryonic and adult tissues and is upregulated in human cancer [15]. Several theories exist to explain why nonsense mutations in ASPM appear to affect neuronal cell division but do not affect other cells shown to express the protein. The three most probable explanations are as follows. First, ASPM may have evolved a specialised and non-redundant mitotic function in neural progenitor cells. Second, the tissue expression pattern suggests ASPM has a specific role in mitosis, but that a functional equivalent to ASPM is expressed in non-neural cells. Structural and functional parallels can be drawn between ASPM and the mammalian-specific, apparently ubiquitously expressed spindle pole protein nuclear mitotic apparatus (NuMA) [34-36]. Both have the same potential linear structure, bind microtubules and are present at the spindle poles during mitosis. Drosophila does not have a NuMA equivalent and mutation in *asp* is larval-lethal [29]. Third, isoforms of ASPM have been identified that may contain sufficient domains to be functional in other tissues [15]. The latter explanation seems unlikely as the most N-terminal MCPH ASPM mutation (349C>T in exon 2) [17], identified in an MCPH patient, results in truncation after only 116aa, before any of the alternative splice sites occur.

#### CDK5RAP2, Cnn and Myomegalin

CDK5RAP2 [MIM 608201] was initially identified in a rat brain yeast-2-hybrid search for rat cerebral cortex proteins interacting with cyclin-dependent kinase 5 regulatory protein 1 (CDK5R1) [37,38]. CDK5R1 activates cyclindependent kinase 5 (CDK5). CDK5 has been shown to be structurally and functionally related to the cyclin-dependant kinase family, but is not involved in control of the cell cycle. CDK5 is a promiscuous protein kinase only functioning in the brain and with numerous neuronspecific roles in processes including neurogenesis, neural migration and neurodegeneration (reviewed in [39]). CDK5RAP2 is an inhibitor of CDK5R1 and hence also an inhibitor of CDK5 [38].

CDK5RAP2 has been shown to be located at the centrosome throughout the cell cycle (Figure 2b), and therefore would be hypothesised to only inhibit a CDK5 centrosomal function [9°]. Furthermore, studies of embryonic and foetal human and mouse brain show that the gene is expressed predominantly in the neuroepithelium lining the cavities of the developing brain ([9°]; Cox, personal communication). This suggests that one potential role of CDK5RAP2 is the inhibition of centrosomal CDK5 during neurogenesis.

However CDK5RAP2 function in mammals has not been elucidated. Bioinformatics gives little assistance in predicting functional protein domains but does reveal a potential orthologue in Drosophila, Centrosomin (Cnn) ([9<sup>•</sup>], reviewed in [13<sup>•</sup>]), and a homologue in mammals, Myomegalin. The Drosophila cnn mutant phenotype has been extensively studied [40]. A reduction in cell number in the Drosophila central and peripheral nervous system is found in null *cnn* mutants as well as developmental effects on gut [41]. Mutant embryos exhibit a high occurrence of apparently linked spindles that share poles, which contain decreased amounts of centrosomal proteins such as ytubulin, and late stage embryos of these mutants often do not have astral microtubules attached to their spindle poles [42]. The N-terminus of CDK5RAP2 interacts with the  $\gamma$ -tubulin ring complex in the centrosome, where it can initiate microtubule nucleation [41]. y-tubulin ring complexes are located within the centrosome and are the predominant site of microtubule nucleation within the cell. In particular, they produce the microtubules that form the mitotic spindle and astral microtubules that anchor the centrosome to specific regions of the cell cortex.

The widespread effects of the Cnn mutant in flies contrast with the restricted phenotype of CDK5RAP2 in humans. This may be explained by Myomegalin (PDE4-DIP), a second orthologue of Cnn in mammals. Myomegalin is also a centrosomal protein but has an expression pattern that is predominantly complementary to CDK5RAP2 [43]. Therefore, assuming a functional homology with Cnn, CDK5RAP2 may have two nonredundant roles in neurogenesis: to enhance the production of centrosomal microtubules and to exert a negative control over CDK5.

#### CENPJ, the $\gamma$ tubulin ring and the centrosome

The official designation 'centromeric protein J' is somewhat puzzling as two independent studies have shown that this protein is located at the centrosome throughout mitosis (Figure 2c) [9°,44°]. CENPJ [MIM 609279] was initially named centrosomal protein 4.1-associated protein (CPAP) because of its cell localisation and interaction with the 4.1R protein non-erythrocyte 135 splice variant (4.1R-135) [45]. 4.1R-135 interacts with NuMA in the interphase nucleus and forms a complex with components of the mitotic apparatus [46°,47°°,48]. The N-terminus of 4.1R-135 interacts with the C-terminal Tcp10 domain of CENPJ, and CENPJ is associated with the  $\gamma$ -tubulin ring complex [44°]. It has therefore been hypothesised that 4.1R-135 protein serves as an adaptor that anchors the CENPJ/ $\gamma$ -tubulin complex to the centrosome [45]. *In vitro* evidence shows that CENPJ can inhibit microtubule nucleation from the  $\gamma$ -tubulin ring complex and is also able to depolymerise Taxol-stabilised microtubules [44].

While a single CENPJ gene exists in other species, including *Drosophila*, to date the mutant phenotype has only been described in man. Other functions of CENPJ are as a coactivator of NF- $\kappa$ B-mediated transcription and to augment Stat5-mediated transcription, but these seem less likely to influence neurogenesis [49,50].

#### Microcephalin, cell cycle and DNA repair

The 835-aa Microcephalin (MCPH1, [MIM 607117]) protein contains three BRCA1 C-terminal (BRCT) domains, components common to DNA repair and cell cycle proteins [51]. BRCT domains are believed to be modulated by phosphorylation during the cell cycle checkpoints or through DNA damage [52,53]. Three roles have recently been identified for Microcephalin: small-interfering-RNA (siRNA)-mediated depletion of *MCPH1* identified a role in the cell cycle in regulating chromosome condensation [54]; a role in DNA damage response through the regulation of BRCA1 and checkpoint kinase 1 was shown [52]; and MCPH1 (as BRIT1) was identified as a negative regulator of the catalytic subunit of telomerase [55].

MCPH1 orthologues have been identified in many species where domain structure is conserved. Evolutionary studies of Microcephalin show Darwinian positive selection throughout the simian lineage, influenced by Darwinian positive selection [56,57]. The intracellular distribution of MCPH1 and its role in neurogenesis are as yet unknown; however, mutation of *MCPH1* may result in MCPH through cell cycle mis-regulation in neural progenitors [7,54].

# Conclusions

Control of neural progenitor division is essential to produce the requisite number of neurons during neurogenesis. The architecturally normal but small brain observed in MCPH results from a reduction in the number of neural cells. The neurogenic function of genes involved in primary microcephaly is as yet undetermined but their localisation in the cell is centrosomal. In all probability the role of the MCPH proteins is associated with control of progenitor cell expansions or with the decision of neural progenitor cells to switch inappropriately from symmetric to asymmetric cell division, as observed in the murine Nde1 (nuclear distribution gene E homologue 1)-null microcephalic brain [58<sup>••</sup>].

The genes at the *MCPH2* and *MCPH4* loci have yet to be identified and multiple MCPH families remain unlinked to the known loci [18,20], indicating the existence of at least three further genes with neurogenic regulatory functions. Identification of *MCPH* genes and elucidation of their functions will provide further insights into the centrosomal mechanisms regulating cortical neurogenesis and will improve our understanding of the mitotic pathway leading to the evolutionary expansion of hominid brain size.

# Update

A second case of microcephaly occurring as a consequence of a missense mutation was recently published. A novel *MCPH1* homozygous 80C>G alteration was identified, resulting in the substitution of a highly conserved threonine residue by an arginine (Thr27Arg) within the N-terminal *BRCT* domain [59]. In contrast to the MCPH missense mutation previously identified in *CENPJ* [9<sup>•</sup>], the *MCPH1* missense mutation produces a specifically mild MCPH phenotype (head circumference of -3SD, mild mental retardation and a slight enhancement of chromosome condensation).

A second publication indicates that MCPH1 (as BRIT1) controls multiple DNA damage checkpoint regulators required for both the intra-S and G2/M checkpoints [60].

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