

# The Genetics of Primary Microcephaly

## Clinical definition

Autosomal recessive primary microcephaly (MCPH) is the term used to describe a genetically determined form of microcephaly previously referred to as *microcephaly vera* or *true microcephaly*.<sup>1,2</sup> It is clinically diagnosed using the following guidelines:

1. Microcephaly ( $> -3SD$ ) is present at birth.
2. Degree of microcephaly does not progress throughout lifetime.
3. Mild to severe mental retardation without other neurological findings (fits are rare).
4. Height/weight/appearance are all normal (except with mutation in *MCPH1*, where some reduction in height may be observed).

MCPH affects neurogenesis *in utero*. The brains of affected individuals are characterised by a significant reduction in the size of the cerebral cortex (presumably the cause of the observed mental retardation). There is also a smaller general reduction in the rest of the central nervous system (CNS), although the architecture is preserved.<sup>3,4</sup>

## Inheritance of MCPH

This disorder is rare in most populations, with incidences ranging from 1/30,000 to 1/2,000,000, but is more frequent in populations practicing consanguineous marriage.

Despite MCPH cases presenting with almost indistinguishable features, this disorder is genetically heterogeneous. Six autosomal recessive loci have been identified so far and named *MCPH1-6* (reviewed in reference 1). Each locus was mapped from a single large consanguineous family by autozygosity mapping using microsatellite markers or SNPs spaced throughout the human genome. Studies conducted to date suggest that *MCPH5* is the common locus in all populations.<sup>5,6</sup>

For these loci, four of the genes which are mutated in MCPH have been identified, *MCPH1* (*Microcephalin*), *MCPH3* (*CDK5RAP2*), *MCPH5* (*ASPM*) and *MCPH6* (*CENPJ*). The current knowledge for each form of MCPH is summarised in Table 1.

## The MCPH genes

A brief summary of current knowledge for each is given below, in chronological order of discovery.

### *MCPH1*

*MCPH1* encodes an 835aa protein which was named Microcephalin.<sup>2</sup> *MCPH1* mutations are a rare cause of primary microcephaly, and affected individuals display a broader phenotype than reported for other forms of MCPH.<sup>2</sup> It has been shown that *MCPH1* primary microcephaly is allelic to premature chromatin condensation syndrome (PCC),<sup>7</sup> which led to the identification of Microcephalin as a negative regulator of Condensin II, a protein complex involved in chromosome packaging.<sup>8</sup> Clinically, patients with mutations in *MCPH1* display an increased number of prophase-like cells on standard cytogenetic analysis – a clinically useful discrimination unique to *MCPH1* microcephaly.

Microcephalin contains three BRCA1 C-terminal (BRCT) domains, also found in DNA repair and cell cycle checkpoint proteins.<sup>2</sup> These domains seem to bind phosphoproteins to control DNA damage-induced cell cycle checkpoints.

Three functions have so far been reported for Microcephalin: small-interfering-RNA (siRNA)-mediated depletion of *MCPH1* identified a role in regulating chromosome condensation during the cell cycle (hence PCC); a role in DNA damage response through the regulation of BRCA1 and Chk1; and *MCPH1* (as BRIT1) was identified as a negative regulator of the catalytic subunit of telomerase.<sup>1,7,8</sup>

### *MCPH5*

*MCPH5* mutation is the most common cause (~50% of cases) of the MCPH phenotype.<sup>5</sup> It is a large gene and encodes the human orthologue of the *Drosophila* gene *abnormal spindle* (*asp*), called “abnormal spindle mutated in microcephaly” (*ASPM*). The reported mutations are spread throughout the *ASPM* gene and result in truncated *ASPM* protein products ranging in size from 116 – 3357aas.<sup>1,6</sup>

*ASPM* is predicted to contain an N-terminal microtubule binding domain, two calponin homology domains (common to actin binding proteins), 81 isoleucine-glutamine (IQ) repeat motifs (predicted to change conformation when bound to calmodulin), and a C-terminal region of unknown function.<sup>4</sup>

Structural projections and comparison with myosin suggest that when *ASPM* is present at the centrosome, it assumes a semi-rigid-rod-conformation, with microtubules bound by the N-terminus and centrosomal components interacting at the C-terminus.

*ASPM* is found near the centrosome and is thought to play an essential role during neurogenic mitosis. Studies have shown that *Drosophila asp* recessive mutants are larval lethal or infertile with dividing neuron progenitors unable to conclude asymmetric cell division.<sup>9</sup> The *asp* protein is required for microtubule organisation of the mitotic spindle poles and the central spindle in mitosis and meiosis.<sup>9,10</sup> In contrast, *ASPM* mutations in humans produce a mitotic defect restricted to the brain. This may be due to a functional overlap between *ASPM* and NuMA (Nuclear mitotic apparatus protein 1), another protein shown to regulate spindle dynamics.

### *MCPH3*

*MCPH3* encodes Cyclin dependent kinase 5 regulatory associated protein 2 (*CDK5RAP2*).<sup>11</sup> Little is yet known about the function of *CDK5RAP2*, however it was originally identified as a negative regulator of cyclin dependent kinase 5 (*CDK5*) through its inhibition of *CDK5* regulatory protein 1 (*CDK5R1*). *CDK5* is divergent from the rest of the *CDK* family, other members of which are ubiquitously expressed and regulate mitotic checkpoints. In contrast *CDK5* expression is restricted to the brain, where it regulates the creation,



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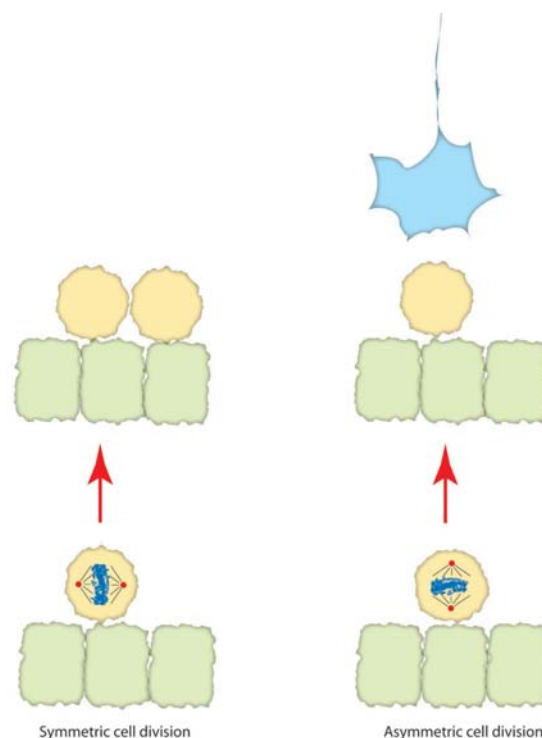


Figure 1: Cell divisions during neurogenesis. Neural progenitor cells (yellow) lie above the neuroepithelium (green cells) in the developing nervous system. During cell division, the centrosomes (red circles) produce a mitotic spindle (black lines) of microtubules attached to the condensed chromosomes (indicated in blue). The orientation of the spindle to the neuroepithelium is dependant on the positioning of the centrosomes. Alignment parallel to the neuroepithelium results in symmetric cell division, increasing the progenitor pool (left hand diagram). In contrast, alignment of the spindle perpendicular to the neuroepithelium results in asymmetric cell division which maintains the progenitor pool cell numbers whilst producing neuronal committed precursors (right hand diagram).

Table 1: The MCPH loci

MCPH	Locus	Gene	Known/predicted domains	Known/predicted functions	Localisation at mitosis
1	822-pter	Microcephalin	• 3 BRCT domains	• Regulation of chromosome condensation during cell division • DNA damage repair through BRCA1 regulation • Inhibitor of Topoisomerase catalytic subunit	Centrosome
2	19q13 1-13.2				
3	9q34	CDK5RAP2	• N-terminal $\alpha$ -tubulin association	• Negative regulation of CDK5 • Promotes production of microtubules at centrosomes	Centrosome
4	15q5-q21				
5	1q31	ASPM	• Microtubule association domain • Calponin homology • IQ motifs	• Direct interaction with cytoskeleton • Binds microtubules at N-terminus • May bind centrosomal components at C-terminus	Centrosome
6	13q12.2	CENPJ	• Tcp10 domain • Microtubule depolymerising domain • 4.1R-135 binding domain	• Depolymerises microtubules • Depletion causes multiple spindles • Interacts with 4.1R-135	Centrosome

migration and degeneration of neurons.<sup>12</sup> The *Drosophila* orthologue *centrosomin* (*cnm*) has been studied and *cnm* mutants display reduced cell numbers in both the central and peripheral nervous system.<sup>13</sup> CDK5RAP2 is located at the centrosome throughout the cell cycle and its N-terminus interacts with the  $\gamma$ -tubulin ring complex, which initiates microtubule nucleation,<sup>11</sup> required for spindle formation. The restriction of CDK5RAP2 mutations to MCPH and not a more widespread growth disorder is probably due to the complementary tissue expression pattern of a mammal specific homologue called Myomegalin.

#### MCPH6

The *MCPH6* gene encodes centromere-associated protein J (*CENPJ*, also known as *CPAP*, centrosomal protein 4.1-associated protein).<sup>11,14</sup> Despite its name, *CENPJ* is a centrosomal protein, and this localisation depends on non-erythroid protein 4.1 splice isoform 135 (4.1R-135).<sup>14</sup> Intriguingly, this protein is also responsible for recruiting NuMA to the centrosome. It has been demonstrated that *CENPJ* associates with the  $\gamma$ -tubulin ring complex, and *in vitro* evidence suggests that *CENPJ* may modulate microtubule nucleation and depolymerise microtubules.<sup>15</sup> This may suggest that an inverse relationship exists between *CENPJ* and CDK5RAP2 in regulating microtubule dynamics. RNAi depletion of *CENPJ* in HeLa cells resulted in a mitotic arrest with >40% of cells containing multipolar spindles, a finding similar to *asp* mutant neuroblasts in *Drosophila*.<sup>9,16</sup>

Both *Drosophila* and *C. elegans* contain a single orthologue to *CENPJ*. In worms, the orthologue SAS-4 is one of only five proteins essential for centriole duplication during mitosis in *C. elegans*.<sup>17</sup>

#### Are MCPH proteins key regulators of brain development?

All four MCPH genes identified are expressed in the ventricular zone (site of prenatal neuron production) during neurogenesis.<sup>1</sup> Furthermore, all of the genes encode proteins which are implicated in regulating mitosis, and the localisation data for these proteins suggests a key role for the centrosome or spindle pole body in the aetiology of this disorder.

This makes sense, as neurogenesis has been shown to rely on balancing symmetric versus asymmetric cell divisions in neural precursors. Symmetric divisions increase the progenitor pool, whilst asymmetric divisions result in the production of one progenitor and one neuron (Figure 1). This outcome is determined by the orientation of the mitotic spindle relative to the neuroepithelium, which in turn is determined by the positioning of the centrosomes upon mitotic commitment.<sup>18</sup> It is intriguing to speculate therefore that the reduced number of neurons leading to MCPH may be a result of a failure to regulate spindle assembly/orientation during the critical period of neurogenesis. If this is so, then the study of these proteins may provide valuable insight into the production of neurons by neural stem cells.

#### Conclusions

Studies to identify the proteins disrupted in the recessive disorder primary microcephaly have identified four centrosomal proteins that seem to be crucial for mitosis during neurogenesis. Currently it is unclear which specific function of each protein is critical to neuron production, although studies of orthologous proteins in model organisms have provided some clues. However, the MCPH proteins have all undergone Darwinian positive selection in the primate/human lineages, which may also have altered their functions.<sup>1,19,20</sup>

As the field evolves, studies of the effect of MCPH protein disruption in affected individuals may provide us with a clearer model of the factors regulating human neurogenesis. Furthermore, combining the study of MCPH protein function with the nascent field of neural stem cell based therapies may ultimately enable controlled production of specific neural lineages – a potential benefit to those suffering from a number of neurodegenerative disorders.

In the immediate future however, the identification of the causative genes for MCPH provides a number of benefits for affected populations. Prenatal diagnosis allows detection of the recurrence of the disorder in affected families, postnatal diagnosis allows us to distinguish the disorder from other possibilities, and most importantly, carrier testing can be offered to consanguineous families where the disorder is known to occur.

#### References

- Woods CG, Bond J, Enard W. Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings. *Am J Hum Genet* 2005;76(5):717-28.
- Jackson AP, et al. Identification of microcephalin, a protein implicated in determining the size of the human brain. *Am J Hum Genet* 2002;71(1):136-42.
- Barkovich AJ, Kuzniecky RI, Dobyns WB. Radiologic classification of malformations of cortical development. *Curr Opin Neurol* 2001;14(2):145-9.
- Bond J, et al. ASPM is a major determinant of cerebral cortical size. *Nat Genet* 2002;32(2):316-20.
- Roberts E, et al. Autosomal recessive primary microcephaly: an analysis of locus heterogeneity and phenotypic variation. *J Med Genet* 2002;39(10):718-21.
- Bond J, et al. Protein-truncating mutations in ASPM cause variable reduction in brain size. *Am J Hum Genet* 2003;73(5):1170-7.
- Trimborn M, et al. Mutations in microcephalin cause aberrant regulation of chromosome condensation. *Am J Hum Genet* 2004;75(2):261-6.
- Trimborn M, et al. Misregulated chromosome condensation in MCPH1 primary microcephaly is mediated by condensin II. *Cell Cycle* 2006;5(3):322-6.
- Gonzalez C, et al. Mutations at the *asp* locus of *Drosophila* lead to multiple free centrosomes in syncytial embryos, but restrict centrosome duplication in larval neuroblasts. *J Cell Sci* 1990;96(Pt 4):605-16.
- do Carmo Avides, M, Tavares A, and Glover DM. Polo kinase and Asp are needed to promote the mitotic organizing activity of centrosomes. *Nat Cell Biol* 2001;3(4):421-4.
- Bond J, et al. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. *Nat Genet* 2005;37(4):353-5.
- Cruz JC, Tsai L-H. A Jekyll and Hyde kinase: roles for Cdk5 in brain development and disease. *Current Opinion in Neurobiology*, 2004;14(3):390-4.
- Li K, Kaufman TC. The homeotic target gene centrosomin encodes an essential centrosomal component. *Cell* 1996;85(4):585-96.
- Hung LY, Tang CJ, Tang TK. Protein 4.1 R-135 interacts with a novel centrosomal protein (CPAP) which is associated with the gamma-tubulin complex. *Mol Cell Biol* 2000;20(20):7813-25.
- Hung LY, et al. Identification of a novel microtubule-destabilizing motif in CPAP that binds to tubulin heterodimers and inhibits microtubule assembly. *Mol Biol Cell* 2004;15(6):2697-706.
- Cho JH, et al. Depletion of CPAP by RNAi disrupts centrosome integrity and induces multipolar spindles. *Biochem Biophys Res Commun* 2006;339(3):742-7.
- Leidel S, Gonczy P. Centrosome duplication and nematodes: recent insights from an old relationship. *Dev Cell* 2005;9(3):317-25.
- Haydar TF, Ang Jr E, Rakic P. Mitotic spindle rotation and mode of cell division in the developing telencephalon. *Proc Natl Acad Sci USA* 2003;100(5):2890-5.
- Evans PD, et al. Adaptive evolution of ASPM, a major determinant of cerebral cortical size in humans. *Hum Mol Genet* 2004;13(5):489-94.
- Evans PD, Vallender EJ, Lahn BT. Molecular evolution of the brain size regulator genes CDK5RAP2 and CENPJ. *Gene In Press*, Corrected Proof.