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The Genetics of Primary Microcephaly

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Abstract

Primary microcephaly (MCPH, for "microcephaly primary hereditary") is a disorder of brain development that results in a head circumference more than 3 standard deviations below the mean for age and gender. It has a wide variety of causes, including toxic exposures, in utero infections, and metabolic conditions. While the genetic microcephaly syndromes are relatively rare, studying these syndromes can reveal molecular mechanisms that are critical in the regulation of neural progenitor cells, brain size, and human brain evolution. Many of the causative genes for MCPH encode centrosomal proteins involved in centriole biogenesis. However, other MCPH genes fall under different mechanistic categories, notably DNA replication and repair. Recent gene discoveries and functional studies have implicated novel cellular processes, such as cytokinesis, centromere and kinetochore function, transmembrane or intracellular transport, Wnt signaling, and autophagy, as well as the apical polarity complex. Thus, MCPH genes implicate a wide variety of molecular and cellular mechanisms in the regulation of cerebral cortical size during development.

INTRODUCTION

The human brain is dramatically larger than the brains of other mammals, including primates. The cerebral cortex, which is responsible for several human-specific cognitive abilities, such as language, may have been particularly affected by evolutionary expansion. Microcephaly ("small head") is defined by a head circumference that is more than 3 standard deviations (SD) below the mean for the age and gender of the individual (56). It can result from exposure to in utero infections, toxin or teratogen exposure (e.g., fetal alcohol syndrome), metabolic conditions (e.g., maternal phenylketonuria), and genetic syndromes. When it presents at birth, it is generally a neurodevelopmental defect and is termed primary microcephaly (MCPH, for "microcephaly primary hereditary"); microcephaly that develops after birth is often degenerative and progressive in nature and is termed secondary microcephaly (39, 178). Individuals with MCPH usually have intellectual disability and language delay, with varying degrees of motor delay (56). Although microcephaly is a relatively rare condition, understanding its etiology has cast important light on core questions in the field of neocortical development.

Several genes have been identified as causes of MCPH, including *MCPH1*, *WDR62*, *CDK5RAP2*, *CEP152*, *ASPM*, *CENPJ*, *CEP63*, and *STIL* (18, 21, 22, 62, 80, 99, 126, 160, 186). **Table 1** provides a more complete list of loci (numbered MCPH1–MCPH18 at last count, and growing) and their associated genes. These genes are generally expressed in the primary germinal zone in the cerebral cortex, called the ventricular zone (VZ), during cortical neurogenesis, which is consistent with a role in proliferation of neural progenitor cells (NPCs) (23). Intriguingly, the centrosome has been implicated in the pathogenesis of several MCPH syndromes (114). Since these genes encode proteins that are ubiquitously expressed in the centrosomes of most mitotic cells of the body (128), it is not obvious why mutations in them should preferentially affect the brain in many cases. Here, we focus on the subset of MCPH genes that encode centrosomal proteins and briefly discuss MCPH genes that fall under other categories.

FITTING A SUBSET OF PRIMARY MICROCEPHALY GENES INTO A CENTRIOLE BIOGENESIS PATHWAY

More than half of microcephaly genes encode proteins that localize to the centrosome and play important roles in centriole biogenesis or duplication (**Figure 1**, **Table 1**). Centrosomes consist of a mature mother centriole and a less mature daughter centriole that duplicate at G_1/S phase and nucleate microtubules during mitosis (24). Mutations in *CENPJ/CPAP/SAS-4*, *CEP152*, and *CEP63* all cause autosomal recessive MCPH in humans (22, 62, 160). CENPJ/CPAP/SAS-4 plays a critical role in centriole biogenesis and lengthening (95, 154, 168), and studies in *Sas-4* mutant mice suggest that neurogenesis defects in microcephaly may be due to loss of centrioles and a secondary loss of cilia (14, 79). Consistent with the requirement of CENPJ/CPAP/SAS-4 for centriole and centrosome duplication (95, 168), the authors observed a progressive depletion of centrosomes, as well as of primary cilia that grow from the few functional centrioles remaining in the *Sas-4* conditional mutant cortex. *Sas-4* mutant mice show p53-mediated apoptosis (14, 79). CEP63 and CEP152 proteins colocalize in a ring-shaped pattern surrounding the proximal end of the maternal centriole in human and chicken cells and interact with each other physically (160). CEP152 and its fly ortholog, Asterless, are required for centriole duplication (19, 41, 70). Likewise, CEP63 and its mouse ortholog are essential for efficient centriole duplication in human and mouse cells (25, 160).

Recent work has characterized the functions of *ASPM* and *WDR62*, the two most common genetic causes of MCPH, in centriole biogenesis and neocortical development (81). Mutations in *ASPM* (located on chromosome 1q31) cause microcephaly with relatively well-preserved gyral

Table 1 Genes associated with primary microcephaly that encode centrosomal proteins functioning in centriole biogenesis

Locus	Gene	Chromosomal location	Subcellular location	Pathway	Reference(s)
MCPH1	*MCPH1*	8p23.1	Nucleus	DNA damage response and regulation of chromosome condensation	80, 170
MCPH2	*WDR62*	19q13.12	Centrosome (interphase) and spindle poles (mitosis)	Centriole biogenesis	18, 81, 126, 186
MCPH3	*CDK5RAP2*	9q33.2	Centrosome	Centriole biogenesis	22, 94
MCPH4	*CASC5*[a]	15q15.1	Kinetochore	Microtubule attachment to centromere and spindle-assembly checkpoint activation in mitosis	55
MCPH5	*ASPM*	1q31.3	Centrosome (interphase)	Centriole biogenesis	21, 81
MCPH6	*CENPJ* (also known as *CPAP* or *SAS-4*)	13q12.12–12.13	Centrosome (interphase)	Centriole biogenesis	22, 81
MCPH7	*STIL*	1p33	Centrosome	Procentriole formation and centriole biogenesis	99, 169
MCPH8	*CEP135*	4q12	Centrosome	Centriole assembly	75, 106
MCPH9	*CEP152*	15q21.1	Centrosome	Centriole biogenesis	62, 94
MCPH10	*ZNF335*	20q13.12	Nucleus	Transcriptional regulation of brain-specific genes controlling cell fate via REST/NRSF	184
MCPH11	*PHC1*	12p13.31	Nucleus	Negative regulation of GMNN (which itself regulates the cell cycle and inhibits DNA replication)	10
MCPH12	*CDK6*	7q21.2	Centrosome (mitosis)	Unknown	76
MCPH13	*CENPE*	4q24	Kinetochore/ centromere	Unknown	115
MCPH14	*SASS6*	1p21.2	Centrosome	Centriole assembly with CEP135 and CENPJ/CPAP/SAS-4	86, 106
MCPH15	*MFSD2A*	1p34.2	Plasma membrane	Omega-3 fatty acid transport across blood–brain barrier	2, 61
MCPH16	*ANKLE2*	12q24.33	Not well characterized	Fly model shows decreased proliferation and increased apoptosis	182
MCPH17	*CIT*	12q24.23	Midbody	Cytokinesis	67, 104
MCPH18	*WDFY3* (also known as *ALFY*)	4q21.23	Nucleus and cytoplasm	Autophagy and regulation of Wnt signaling	83

The information in this table is taken from the Online Mendelian Inheritance in Man catalog and Reference 43.

[a]Until the recent discovery of *CASC5*, *CEP152* was the gene assigned to the MCPH4 locus.

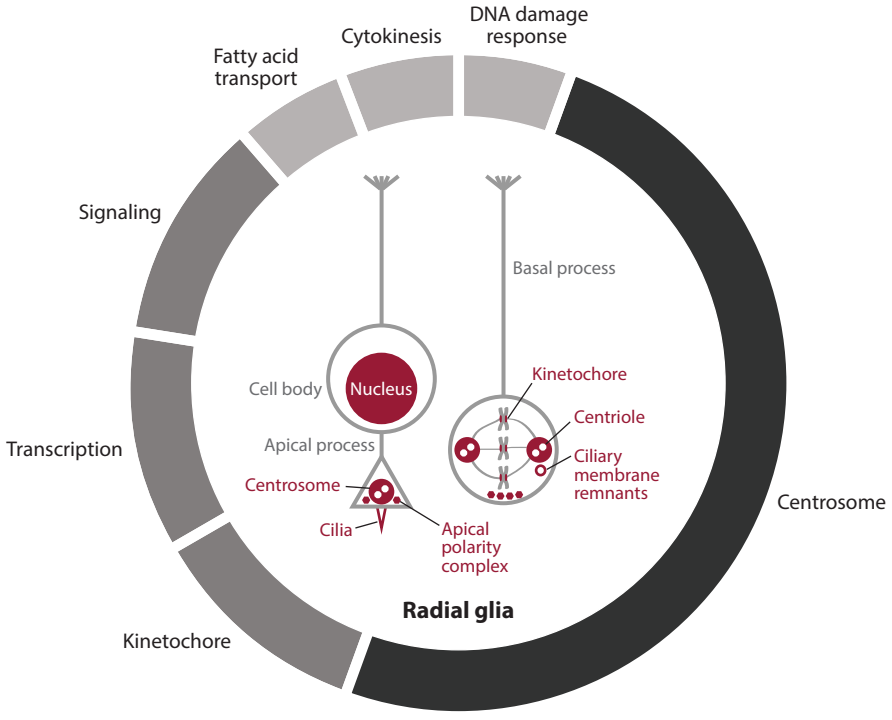

Figure 1

Causative genes of primary microcephaly (MCPH) control the cell fate of radial glial cells, the primary neural progenitor cell type in the developing cerebral cortex. Eighteen MCPH genes have been identified, of which nine encode centrosomal proteins. The other genes are involved in diverse aspects of radial glial cells. The subcellular organelles implicated in MCPH are shown in red.

pattern and cortical architecture (21), whereas mutations in *WDR62* (located on chromosome 19q13) cause microcephaly with additional developmental defects, including abnormal formation of the gyri (18, 126, 186). Using mouse and cell models, Jayaraman et al. (81) discovered that *Wdr62* and *Aspm* not only interact genetically but also encode proteins that physically interact and share a common, essential function in centriole duplication. A lack of both *Wdr62* and *Aspm* is embryonically lethal, while heterozygous deletion of either gene greatly enhances the phenotype of mutations in the other gene. Total loss of either *Wdr62* or *Aspm* or partial loss of both genes impairs centriole duplication, with the severity of the cellular defect proportional to the severity of the microcephaly, and leads to a reduction in centrosomes and cilia in the embryonic mouse brain. Even the transheterozygote ($Wdr62^{+/-};Aspm^{+/-}$) has a mild cellular and brain phenotype, consistent with nonallelic noncomplementation between the two genes, which often implies a physical interaction between the gene products. In human cells, WDR62 and ASPM proteins localize to the proximal end of the mother centriole during interphase and form a physical complex. Finally, Jayaraman et al. (81) also found that the *Wdr62* mutant mouse shows a decrease in centrosomes and cilia and other associated defects during neurogenesis. Together, these results implicate microcephaly genes like *WDR62* and *ASPM* in centriole biogenesis as well as regulation of brain size.

The study by Jayaraman et al. (81) not only revealed a novel cellular function for *WDR62* and *ASPM* but also placed both genes in a pathway involving other microcephaly genes, whose gene products are all required sequentially in centrosome biogenesis. In fact, WDR62 and ASPM

are part of a larger protein complex that includes CEP63. Knockdown of *CEP63* in human cells abolishes the WDR62–ASPM interaction, suggesting that CEP63 is required to mediate this interaction. Immunocytochemistry in *Wdr62* mutant mouse embryonic fibroblasts and in *WDR62*-depleted human cells showed that WDR62 is required for adequate centrosomal localization of CEP63 (but not CEP152) to the centrosome, placing WDR62 between CEP152 and CEP63 in order of recruitment to the centrosome. Similar RNA interference (RNAi) knockdown experiments demonstrated that both WDR62 and CEP63 localize to the centrosome before ASPM does, which in turn helps localize CENPJ/CPAP/SAS-4 to the centrosome, thereby placing ASPM between CEP63 and CENPJ/CPAP/SAS-4 in order of recruitment to the centrosome (81). Given the crucial role of CENPJ/CPAP/SAS-4 in centriole biogenesis, one major role of all these microcephaly-associated proteins is to ultimately bring CENPJ/CPAP/SAS-4 to the centrosome. These and other findings (94) together support a model in which MCPH-associated proteins recruit each other sequentially to the centrosome, thereby enabling centriole duplication to occur (57).

Molecules regulating microcephaly-associated centrosomal proteins, such as Plk, are also implicated in microcephaly (111). Members of the Plk family are critical to centrosome biogenesis because loss of *Plk* in yeasts, flies, frogs, and humans results in formation of monopolar spindles (100, 136, 145, 164). Some members of the Plk family interact with WDR62 and ASPM. *Drosophila* Plk interacts with and phosphorylates Asp, the fly homolog of ASPM (38). In mammals, Plk3 localizes to the spindle poles at metaphase, much like WDR62 (153), and Plk1 interacts with the centrosomal protein Cep170 (60). CEP170 was identified in a large-scale proteomic study as a binding partner for WDR62 (77). Overexpressed WDR62 colocalizes with endogenous CEP170 in a ringlike, pericentrosomal pattern during mitosis (186). Recent work suggests that WDR62 is phosphorylated by PLK1 in human cells (117) and that the fly homolog of *WDR62*, in turn, is essential for maintenance of Plk localization and activity at the apical centrosome (149). Thus, mutations in *PLK4* in humans cause a syndrome of microcephaly, dwarfism, and retinopathy (111).

OTHER BROAD PATHWAYS INTO WHICH MICROCEPHALY GENES FALL

Besides centriole biogenesis, there are other pathways into which some MCPH genes can be organized. One such category has to do with DNA replication, DNA repair, cell cycle progression, and maintenance of genome stability. In fact, the first microcephaly locus (MCPH1) was linked to the gene encoding microcephalin (*MCPH1*), which implicated the DNA damage response pathway (3, 80, 105, 147, 170, 181). *PNKP* is another example of a gene that encodes a protein required for DNA repair and that causes microcephaly with seizures when mutated (157). This broad category also includes *ATR*, *NBS1*, and *PHC1* (**Table 2**).

A group of disorders collectively referred to as microcephalic primordial dwarfism (MPD) is characterized by microcephaly accompanied by intrauterine growth restriction and postnatal growth delay. While a full description of these syndromes is beyond the scope of this review, they are worth mentioning because of the phenotypic and mechanistic overlap with MCPH and the insights they can provide into the underlying cell biology. Syndromes that fall under MPD include microcephalic osteodysplastic primordial dwarfism (MOPD) types 1–3, Seckel syndrome, and Meier–Gorlin syndrome, based on the clinical phenotype and the cellular pathways involved (89). MOPD type 2 is caused by mutations in pericentrin (*PCNT*), which encodes an important pericentrosomal protein that nucleates spindle microtubules (40), whereas Meier–Gorlin syndrome is caused by mutations in key components of DNA replication complexes, including ORC1, ORC4, ORC6, CDT1, GMNN, and CDC45 (16, 17, 28, 46, 63).

Table 2 Genes linked to microcephaly that encode proteins involved in DNA repair

Disease	Gene	DNA repair function	Neurological phenotype	Other phenotype(s)	Reference(s)
Nijmegen breakage syndrome	*NBS1*	DSB repair	Microcephaly	Immunodeficiency and cancer	29, 112, 173
Seckel syndrome	*ATR*	Damage sensor	Microcephaly	Severe growth retardation and cancer	135
Cernunnos deficiency	*XLF*	DSB repair (NHEJ)	Microcephaly	Immunodeficiency	27
Ligase IV deficiency	*LIG4*	DSB repair (NHEJ)	Microcephaly	Immunodeficiency and cancer	13, 50, 134
XRCC4 deficiency	*XRCC4*	DSB repair (NHEJ)	Neuronal death	Immunodeficiency and cancer	52, 155
Ku70 and Ku80 deficiency	*Ku70/80*	DSB repair (NHEJ)	Neuronal death	Immunodeficiency and cancer	59
DNA-dependent kinase deficiency	*DNA-PK*	DSB repair (NHEJ)	Neuronal death	Immunodeficiency	174
XRCC2 deficiency	*XRCC2*	DSB repair (homologous recombination)	Neuronal death	Embryonic lethal	36, 137
Microcephaly vera 1	*MCPH1*	DNA repair or cell cycle control	Microcephaly	Unknown	3, 80, 105, 147, 170, 181
Microcephaly with seizures	*PNKP*	SSB (BER) and DSB repair	Microcephaly and seizures	No cancer or immune defects	157

Abbreviations: BER, base excision repair; DSB, double-strand break; NHEJ, nonhomologous end joining; SSB, single-strand break.

Of the MOPDs, Seckel syndrome is perhaps the most pertinent to MCPH, as the phenotypic spectrum and underlying cellular pathways are increasingly blurring the line separating them. Similarly to MCPH, Seckel syndrome is caused by mutations in genes encoding proteins involved in centriole biogenesis, such as *CENPJ*, *CEP63*, and *CEP152*, and in the DNA damage response or maintenance of genome stability, including *ATR*, *TRAIP*, and *RBBP8* (see **Table 3**). Microcephaly with head circumference 2 SD or more below the mean is a common feature of both MCPH and Seckel syndrome. Height used to be the distinguishing feature between Seckel syndrome and

Table 3 Seckel syndrome phenotypes caused by mutations in genes encoding proteins involved in centriole biogenesis and the DNA damage response

Phenotype	Chromosomal location	Gene/locus	Pathway	Reference(s)
SCKL1	3q23	*ATR*	DNA damage response	135
SCKL2	18q11.2	*RBBP8*	DNA damage response	146
SCKL3	Not identified	Not identified	Not identified	—
SCKL4	13q12.12–12.13	*CENPJ*	Centriole biogenesis	1
SCKL5	15q21.1	*CEP152*	Centriole biogenesis	84
SCKL6	3q22.2	*CEP63*	Centriole biogenesis	160
SCKL7	14q22.1	*NIN*	Centriole subdistal appendage protein required to anchor microtubules	34, 92
SCKL8	10q21.3	*DNA2*	DNA damage response	155
SCKL9	3p21.31	*TRAIP*	DNA damage response	68
SCKL10	8q24.13	*NSMCE2*	DNA damage response	142

MCPH, with stature 1–2 SD below the mean for MCPH and 4–12 SD below the mean for Seckel syndrome (175). However, mutations in some genes (notably *CENPJ/CPAP/SAS-4*, *CEP152*, *CDK5RAP2*, and *CEP63*) have been linked to classic Seckel syndrome and the MCPH phenotype, as well as to individuals with intermediate stature (2–4 SD below the mean) (1, 22, 62, 84, 160, 185). In fact, *CENPJ/CPAP/SAS-4* and *CEP152* are classified as both MCPH and Seckel syndrome genes (see **Tables 1** and **3**). As a result, it is plausible that both MCPH and Seckel syndrome exist on a phenotypic spectrum with varying degrees of short stature relative to the microcephaly (175).

Two studies recently identified *DONSON* gene mutations as a novel cause of MPD and implicated the DNA damage response (42, 151). One study used RNA sequencing in a single large First Nations community with a neonatal lethal syndrome of profound microcephaly, intrauterine growth restriction, skeletal (especially limb) dysplasia, and craniofacial dysmorphisms to identify an intronic variant causing aberrant splicing of *DONSON* (42). The authors also demonstrated that *DONSON* is coexpressed with key DNA replisome components and that small interfering RNA (siRNA) knockdown of *DONSON* results in upregulation of p21 and downregulation of cyclin D2 and E2, consistent with cell cycle arrest at the G_1/S checkpoint. The other study used whole-exome sequencing to discover biallelic *DONSON* mutations in 29 individuals from multiple families in Asia, Africa, Europe, and the Middle East with microcephalic dwarfism (151). The authors characterized DONSON as a component of the DNA replication fork that maintains genome stability by stabilizing stalled or damaged replication forks and activating cell cycle checkpoints. This function of DONSON is most likely mediated by ATR, which was itself implicated in both Seckel syndrome and the DNA damage response (135, 151).

A few novel microcephaly genes have recently been discovered that encode proteins functioning in cytokinesis, implicating an entirely new cellular pathway in the pathogenesis of microcephaly. The first example was *CIT*, which encodes citron kinase, a component of the midbody important in cytokinesis (67, 104). Mutations in *KIF14* were identified as another cause of microcephaly and short stature; this gene also encodes a cytokinesis-associated protein (118). Interestingly, ASPM colocalizes with citron kinase at the midbody during cytokinesis (138), and mass spectrometry confirmed CIT as a binding partner of ASPM (81). These findings suggest a possible novel role for microcephaly proteins in cytokinesis.

The centromere/kinetochore pathway was recently implicated in two novel microcephaly and MPD syndromes. Mutations in *CASC5*, which encodes a kinetochore protein required for microtubule attachment to the centromere and for spindle-assembly checkpoint activation in mitosis, were linked to MCPH in humans (55). *CENPE* likewise encodes a protein that localizes to the kinetochore/centromere; mutations in *CENPE* were identified as a novel cause of MPD (115).

One of the most recently identified MCPH genes, *WDFY3/ALFY*, highlighted the role of Wnt signaling in the developing brain and implicated autophagy for the first time in the pathogenesis of microcephaly. A dominantly inherited mutation in *ALFY*, which encodes an autophagy scaffold protein, causes MCPH in humans and in transgenic flies engineered to carry the human mutation. *ALFY* also negatively regulates the canonical Wnt pathway by autophagy-mediated clearance of aggregates of DVL3, a downstream target of Wnt (83). *ASPM* may also affect NPC proliferation at least partly through the Wnt signaling pathway. *ASPM* was identified as a positive regulator of the Wnt signaling pathway in a genome-wide siRNA screen (109). In utero electroporation of short hairpin RNAs (shRNAs) to *Aspm* in mice caused defects in neurogenesis and reduced Wnt-mediated transcriptional activity in the developing neocortex that were successfully rescued by coexpression of stabilized β-catenin (26). Transgenic mice in which β-catenin (a downstream signaling target of Wnt) was constitutively expressed in NPCs developed large brains with increased surface area and folding of the lateral ventricles analogous to gyri, consistent with an expansion of the progenitor pool (33). These findings suggest that the Wnt pathway may mediate proliferation

defects caused by *ASPM* loss of function, *ALFY* gain of function, and possibly mutations in other microcephaly genes yet to be identified.

Another broad category of causative genes for microcephaly encode proteins involved in transmembrane or intracellular transport. For instance, mutations in *COH1*, which encodes a transmembrane protein playing a role in vesicle-mediated intracellular trafficking, cause Cohen syndrome, an autosomal recessive disorder of microcephaly, dysmorphic facies, retinal dystrophy, and intermittent neutropenia (96, 121). Similarly, an autosomal recessive syndrome of microcephaly and periventricular heterotopia is caused by mutations in *ARFGEF2*, which encodes a protein essential for vesicle trafficking of proteins (including β-catenin) from the Golgi apparatus to the cell membrane (156). *TRAPPC9* mutations cause autosomal recessive intellectual disability and variable postnatal microcephaly, and the protein has been implicated in intracellular protein trafficking in postmitotic neurons (120). *CHMP1A* mutations cause pontocerebellar hypoplasia (small cerebellum) as well as microcephaly. *CHMP1A* encodes charged multivesicular body protein 1A, also known as chromatin-modifying protein 1A, which is a component of the ESCRT-III complex and also regulates chromatin structure and function via BMI-INK4A (119). These dual functions of CHMP1A serve as a link between intracellular transport and chromatin modifications that regulate neural stem cell proliferation.

Finally, a new category of microcephaly genes has come to light in the last few years that encode proteins involved in amino acid or protein synthesis. The first of these was *QARS*, which encodes glutaminyl-tRNA synthetase and causes progressive microcephaly with severe seizures and atrophy of the cerebral cortex and cerebellum when mutated (188). Similarly, loss-of-function mutations in *AARS*, which encodes alanyl-tRNA synthetase, cause progressive microcephaly, intractable seizures, hypomyelination, and spasticity (125). Finally, mutations in *PYCR2*, encoding pyrroline-5-carboxylate reductase 2 (an enzyme in the proline biosynthesis pathway), cause postnatal microcephaly and hypomyelination, likely resulting from increased apoptosis (124). While these postnatal or progressive microcephaly syndromes are best classified as secondary microcephaly and likely reflect neuronal atrophy rather than decreased proliferation or cell fate changes (unlike many MCPH syndromes), they illustrate the importance of protein and amino acid synthesis pathways in ensuring adequate neuronal survival in the developing central nervous system.

PROGENITOR DIVERSITY IN NORMAL DEVELOPMENT OF THE MAMMALIAN NEOCORTEX

To understand microcephaly, it is important to understand the normal development of the mammalian neocortex and the diverse types of NPCs involved (**Figure 2**). At the earliest stages in the development of the mammalian neocortex, the wall of the embryonic telencephalon is organized as a pseudostratified epithelium consisting of undifferentiated neuroepithelial cells with apicobasal polarity, the ventricular surface being the apical side and the pial surface being the basal side (139, 179). These neuroepithelial cells move their nuclei along the apicobasal axis according to which phase of the cell cycle they are in and undergo multiple symmetric rounds of division to expand the progenitor pool (139). Once neurogenesis begins, the embryonic neocortex contains two major sites of neurogenesis: the VZ lining the ventricles, and the adjacent subventricular zone (SVZ) (132). Radial glial cells inhabit the VZ and undergo multiple rounds of asymmetric division to self-renew and generate neurons (130, 131). Like neuroepithelial cells, these apical ventricular radial glial (VRG) cells are also bipolar in morphology, contacting both ventricular and pial surfaces via apical and basal processes, respectively (139). VRG cells can also give rise to basal intermediate progenitor (IP) cells, which delaminate from the apical surface, assume multipolar morphology, and translocate to the SVZ. Within the SVZ, these IP cells undergo a limited number of

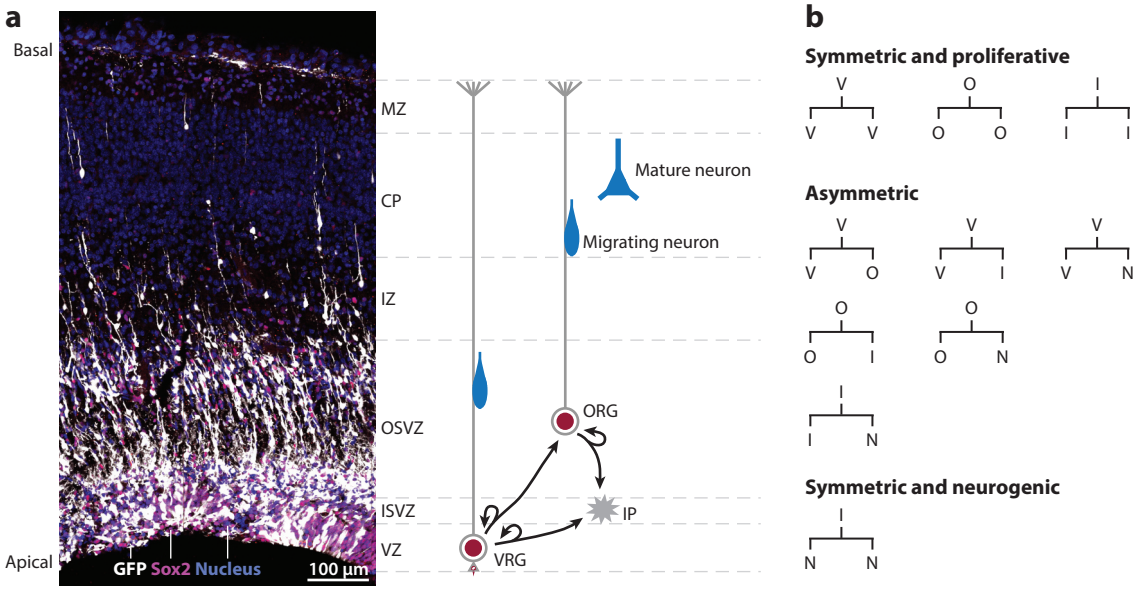

Figure 2

The developing cerebral cortex contains multiple neural progenitor cell types, including apical ventricular radial glial (VRG) cells, basal outer radial glial (ORG) cells, and basal intermediate progenitor (IP) cells. Transcriptionally, VRG and ORG cells express similar sets of genes, whereas IP cells express a distinct set. Morphologically, VRG cells have both apical and basal processes, ORG cells have only a basal process, and IP cells lack long processes and are multipolar. (*a*) The developing ferret cerebral cortex has all three major progenitor types. Here, the ferret cortex was in utero electroporated with plasmids expressing GFP at embryonic day 34 and analyzed at embryonic day 39. VRG cells reside in the ventricular zone (VZ), IP cells in the inner subventricular zone (ISVZ), and ORG cells in the outer subventricular zone (OSVZ) and intermediate zone (IZ). Newborn neurons migrate to the cortical plate (CP) along the basal processes of VRG cells and ORG cells. Additional abbreviation: MZ, marginal zone. (*b*) All progenitor cell types undergo both symmetric proliferative divisions and asymmetric divisions, and sometimes also undergo symmetric neurogenic divisions, which are terminal. Abbreviations: I, intermediate progenitor cell; O, outer radial glial cell; N, neuron; V, ventricular radial glial cell. Image in panel *a* courtesy of Richard S. Smith.

symmetric transit-amplifying or neurogenic divisions (44). Neurons generated by VRG and IP cells migrate radially outward along the basal processes of the VRG toward the cortical plate, where they settle in an inside-out fashion (132).

One way in which microcephaly appears to occur is a premature change in cell fate from apical VRG to basal IP cells. This would be predicted to cause a premature increase in basal IP cells at the expense of apical VRG cells and eventually exhaust the progenitor pool, resulting in the generation of fewer neurons and a smaller brain. An example of this phenomenon can be seen in mice that carry mutations in *Wdr62* and *Aspm*. Notably, immunohistochemical analysis of *Wdr62*$^{-/-}$ and *Wdr62*$^{+/-}$;*Aspm*$^{-/-}$ mice shows an expansion of Tbr2^{+} IP cells in the SVZ and beyond at the expense of Sox2^{+}, Pax6^{+} VRG cells (81). These findings are consistent with a cell fate change from apical VRG to basal IP cells, which in turn delaminate (likely from losing the apical processes tethering them to the ventricular surface) and leave the VZ and SVZ.

The mature neocortex is organized into six layers, with earlier-born neurons inhabiting the deep layers and later-born neurons inhabiting the superficial layers (7, 158). For example, the early-born corticothalamic projection neurons, which are Tbr1^{+}, settle in layer VI and send axonal projections to the thalamus (72), followed by Ctip2^{+} corticospinal motor neurons that settle in layer V and send projections to the spinal cord (8, 103). Later-born callosal projection neurons

that stain for Cux1 and Brn1 migrate past the deep layers to settle in superficial layers II–IV and send projections across the corpus callosum to the contralateral side of the cortex (113, 127). Most projection neurons, which are glutamatergic and excitatory in nature, originate in the germinal zones of the dorsal telencephalon or pallium (31, 58, 69). Most cortical interneurons, which are inhibitory and GABAergic in nature, originate in the ventral telencephalon or subpallium, including the medial and lateral ganglionic eminences, and then migrate long distances toward their final destination in the neocortex (6, 35, 101, 167).

Several mouse models of microcephaly show a preferential reduction in the superficial layers of the cortex, paralleling the expansion of the superficial layers in the course of mammalian and primate evolution. For example, *Wdr62*$^{-/-}$ brain shows a reduction in the ratio of Cux1^{+} (superficial layer) to Ctip2^{+} (deep layer) neurons compared with controls, and *Aspm*$^{-/-}$ brain shows a preferential decrease in the thickness of the superficial cortical layers. *Wdr62*$^{+/-}$;*Aspm*$^{-/-}$ mice show an even greater reduction in superficial cortical thickness compared with *Aspm*$^{-/-}$ mice, suggesting that the two genes are not redundant (81). It is likely no coincidence that the superficial cortical layers, which appear later in development, are also the layers that developed later in the evolution of the human brain (110). Not surprisingly, some of the genes linked to microcephaly have also been implicated in the evolution of the human brain. For example, studies have demonstrated evidence of positive selection for *ASPM* and *CDK5RAP2*, with a strong correlation between evolutionary changes in *ASPM* and *CDK5RAP2* and brain size in primates and other placental mammals (11, 122). It is plausible that evolution has acted on the amino acid sequence of several microcephaly-associated proteins to manipulate the size of the brain.

Recently, a new, expanded proliferative region located in the outer region of the SVZ called the outer subventricular zone (OSVZ) has been identified in primates and in placental mammals with large brains. A new category of cortical progenitors called outer radial glial (ORG) cells or basal radial glial cells inhabit the OSVZ. They have a similar gene expression pattern to VRG cells (i.e., Pax6^{+}/Sox2^{+}/Tbr2^{-}) but are distinguished from apical VRG cells by their patterns of movement and unipolar morphology, with only a basal process (15, 47, 66, 150, 161). These basal ORG cells may be a source of additional neurons as well as providing support to radially migrating neurons via their basal processes (108). Much work is still being done to elucidate how these ORG cells fit into the neuronal lineage and how apical VRG cells transition to basal ORG and IP cells and eventually to neurons.

There is an active debate in the field of neocortical development concerning the roles played by ORG cells in the evolutionary expansion of the neocortex in carnivores and primates. While ORG cells are plentiful in carnivores and primates, which have a well-developed OSVZ, these cells are almost absent in mice. As gyri or cortical folds help maximize the surface area that can be accommodated in a given volume, and the evolutionary expansion of the neocortex is closely associated with the formation of gyri (166), studies in multiple mammalian species have attempted to characterize the roles of ORG cells in the processes of cortical gyrification and expansion. In fact, ORG cells have been found in both the Amazonian agouti, a large, gyrencephalic rodent, and the marmoset, a small, lissencephalic (lacking gyri) primate, suggesting that the existence of ORG cells alone is not sufficient for the formation of gyri, although it may be important in regulating gyral patterning (54, 85). In light of the cell fate change from apical VRG cells to basal IP cells in mice carrying mutations in *Wdr62* and *Aspm* (81), it may also be helpful to study other genetic animal models of microcephaly (such as ferrets) to determine whether ORG cells are increased or otherwise affected in microcephaly.

Another unresolved debate on NPCs has to do with how these progenitors can give rise to postmitotic neurons with divergent morphologies, molecular signatures, electrophysiological characteristics, and synaptic connections. A large body of evidence, both in the neocortex and in other

parts of the nervous system, such as the retina and spinal cord, suggests that a common ancestral progenitor type undergoes progressive restriction of its fate potential and serially gives rise to all neuronal subtypes and glia along the way (64). A newer, competing theory posits that there are distinct subtypes of fate-restricted progenitors that are each programmed to yield a different neuronal subtype (49). The evidence for both theories was obtained primarily in mice and needs to be compared against other species with a large, gyrified cerebral cortex in order to settle this debate.

THE IMPORTANCE OF SYMMETRIC AND ASYMMETRIC CELL DIVISIONS DURING NORMAL NEOCORTICAL DEVELOPMENT

Another concept critical to the genetics of microcephaly is that, in the mammalian ventricular neuroepithelium, the symmetry of cell division of progenitors is critical in determining the fate of the daughter cells (**Figure 2**). Symmetric division of a stem cell yields two daughter stem cells with identical pluripotency as well as identical capacity to self-renew. Asymmetric divisions, by contrast, result in the formation of one stem cell and one fate-restricted daughter cell (123). Early in neurogenesis, neuroepithelial progenitors undergo symmetric divisions that lead to an exponential increase in the size of the progenitor pool (32, 148). The symmetric proliferative phase is followed by an asymmetric phase of neurogenesis, when the apical VRG cells divide asymmetrically to yield one daughter VRG cell and one IP cell, ORG cell, or postmitotic neuron (32, 71). The neuronal daughter cell then migrates along radial glial fibers toward its appropriate laminar destination in the cortex, while the progenitor continues to divide in the VZ (132).

The timing of the transition from symmetric to asymmetric cell divisions among early NPCs is crucial in facilitating the adequate expansion of the progenitor pool and ensuring the eventual generation of a sufficiently large brain. This switch from symmetric to asymmetric divisions may be quantified by measuring the cell cycle exit fraction—the ratio of cells that leave the cell cycle to cells that continue to divide as progenitors (Q/P ratio) over the course of one cell division (165). Misexpressing β-catenin in mouse NPCs, which has the effect of reducing the cell cycle exit fraction and delaying the transition to asymmetric divisions, has been demonstrated to result in a larger cerebral cortex (33). On the other hand, prematurely raising the cell cycle exit fraction—effectively switching from symmetric to asymmetric divisions earlier in neurogenesis—diminishes the progenitor pool, generating a smaller neocortex (30, 45). For example, RNAi knockdown of *Wdr62* in mice and rats caused premature cell cycle exit and premature differentiation of NPCs, respectively (20, 180).

Asymmetric progenitor divisions result in an unequal distribution of several components between the two daughter cells, suggesting the hypothesis of asymmetric inheritance of cell fate determinants. Numb is a particularly well-known example of a protein required for cell fate determination that is asymmetrically inherited in the developing nervous system of *Drosophila* (90, 152, 163, 171) as well as in the mouse neocortex (189). Likewise, Notch1 is selectively inherited during asymmetric divisions by the basal daughter cell, which goes on to assume a more differentiated or neuronal fate (32). Interestingly, Numb inhibits Notch signaling in neuronal cell fate determination (51, 162). Similarly, *Minibrain/Dyrk1A* mRNA is asymmetrically inherited by one of the daughter cells when neuroepithelial progenitors start to undergo asymmetric divisions in chick and mouse embryos (65). These observations are consistent with a mechanism whereby asymmetric inheritance of key components from the mother cell leads to differences in fate between the two daughter cells.

Asymmetric inheritance of cell fate determinants may be consistent with a role for mitotic spindle orientation in asymmetric cell divisions. If the mitotic spindle is oriented such that the eventual cleavage plane is horizontal, it is plausible that the daughter cell located more apically

(near the ventricular surface) might selectively retain determinants of progenitor cell fate, at the expense of the more basally located daughter cell. Time-lapse microscopy in ferret brain sections from embryonic day 29 (E29) revealed that vertical cleavage planes (oriented perpendicular to the ventricular surface) generated two progenitor cells, while horizontal cleavage planes (oriented parallel to the ventricular surface) yielded an apical daughter cell that continued to proliferate and a basal daughter cell that differentiated into a neuron (32). In these asymmetric divisions with a horizontal cleavage plane, Notch1 is selectively inherited by the basal daughter cell. Thus, changes in the orientation of the mitotic spindle apparatus might represent a mechanistic explanation underlying the switch from symmetric to asymmetric cell division.

Nde1 and *Cdk5rap2* mutant mouse models of microcephaly support the mitotic spindle hypothesis. Human *NDE1* mutations cause an extremely severe form of microcephaly with lissencephaly (4, 12). *Nde1* mutant mice have a profound microcephaly phenotype, as well as spindle orientation defects within dividing progenitors. In addition, the cell cycle exit fraction between E14.5 and E15.5 increases; apoptosis increases only slightly, and a cell fate change leads to the generation of more neurons in the deep cortical layers at the expense of superficial-layer neurons (45). *CDK5RAP2* mutations cause autosomal recessive MCPH in humans (22). Similarly to *Nde1* mutant mice, *Cdk5rap2* mutant mice exhibit spindle orientation defects, along with early cell cycle exit and the ensuing loss of superficial-layer neurons. In contrast to *Nde1* mutant mice, however, *Cdk5rap2* mutant mice show only a modest increase in deep-layer neurons and a significant rise in cell death (107). Importantly, while both mouse models suggest an association between changes in mitotic spindle orientation and premature cell cycle exit, neither definitively establishes causality.

Despite circumstantial evidence for the role of mitotic spindle orientation in brain development, there is a considerable controversy as to whether changes in spindle orientation actually cause progenitor cell divisions to become asymmetric. The answer is not clear from analysis of normal cortical progenitors. Contrary to previous work, time-lapse microscopy of neocortical progenitors in the rat revealed that the orientation of the cleavage plane did not necessarily predict the fates of the daughter cells (133). VRG cells tended to divide with their cleavage planes oriented vertically regardless of whether the divisions were symmetric or asymmetric (133).

Initially, several studies implicated Aspm in the regulation of mitotic spindle orientation, but this is now a matter of some debate. In vitro loss-of-function studies in *Drosophila* embryo extracts showed that *Asp*, the homolog of *ASPM*, is required for proper organization of γ-tubulin ring complexes and microtubule organizing center activity (37, 176). Knockdown of *ASPM* in cultured human U2OS osteosarcoma cells led to spindle orientation defects (73). An *Aspm* RNAi knockdown study using in utero electroporation in mice similarly suggested defects in spindle orientation, although the absence of both a scrambled shRNA control and testing for apoptosis makes the results difficult to interpret (48). Moreover, analysis of *Aspm* mutant mice revealed no changes in either spindle orientation or the ratio of symmetric to asymmetric cell divisions in the germinal zones (144).

The data on spindle orientation and *WDR62* are similarly equivocal. One study showed that phosphorylation of WDR62 by PLK1 helped maintain proper orientation of the mitotic spindle in human cells; homozygous *WDR62* missense mutations increased spindle angle (117). The one limitation of this study is that it was based on analysis of conventional two-dimensional cell culture, which does not fully recapitulate the three-dimensional stem cell niche in vivo. By contrast, analysis of *Wdr62* exon 21 gene-trap mice ($Wdr62^{-/-}$) and $Wdr62^{-/-};Aspm^{+/-}$ mice using both two-dimensional microscopy and a three-dimensional en face technique in flat-mount cortex (82) showed that the mitotic spindle orientation in the mutants was not significantly different from that of controls in anaphase (81). These findings suggest that, although the spindle orientation hypothesis is appealing, it does not explain the microcephaly in these animal models.

THE MOTHER CENTRIOLE HYPOTHESIS

One alternative hypothesis besides spindle orientation that could explain how the symmetry of progenitor cell divisions is controlled involves the mother centriole, or asymmetric inheritance of centrosomes. Centrosomes duplicate at G_1/S phase, resulting in a cell containing two daughter centrioles and two mother centrioles that are not equivalent; one of the mother centrioles (the so-called grandmother centriole) was the mother centriole in the prior cell cycle, whereas the other mother centriole was the daughter centriole in the prior cell cycle (129). After cell division, the cell inheriting the grandmother centriole is able to form a primary cilium earlier than the cell inheriting the younger mother centriole (5). In short, there exists an asymmetry not only between mother and daughter centrioles within an individual centrosome but also between centrosomes containing differently aged mother centrioles in a dividing cell.

An emerging theory in stem cell biology is that this inherent asymmetry between the two centrosomes is critical in determining whether a given daughter cell will retain the potency of the mother cell and continue to proliferate or will begin to differentiate into a transit-amplifying cell or neuron. In the male germline of *Drosophila*, the asymmetric inheritance of centrosomes determines the fates of the two daughter cells (183). Studies in mice have suggested that it is the asymmetry between the two differently aged maternal centrioles in a dividing progenitor cell that seems to be critical to the maintenance of stem cells; specifically, the daughter cell that inherits the older mother (grandmother) centriole is maintained as a progenitor in the VZ, whereas the other daughter cell, which inherits the younger mother centriole, tends to leave the VZ and differentiate (177). Note that this is the opposite of what occurs in *Drosophila* neuroblasts, where the older mother centriole segregates with the more basally located, differentiated daughter neuroblast, and the younger mother centriole segregates with the apically located neural stem cell. Live cell imaging in *Drosophila* neuroblasts revealed that the mitotic spindle does get misaligned in *wdr62* mutants but eventually realigns with the apical–basal axis, suggesting that the cell is capable of detecting and fixing spindle orientation defects. However, this correction fails to prevent errors in asymmetric centrosome inheritance, as *wdr62* mutant flies occasionally showed the older mother centriole segregating with the apical neuroblast (149). The *Drosophila* study added further support to the model of asymmetric centrosome inheritance but also did not establish a causal relationship between asymmetric segregation of centrosomes and microcephaly.

In addition to the asymmetric inheritance of centrosomes, the alternative splicing of *Ninein* further substantiates the role of the maternal centriole in the regulation of NPC cell fate (187). *NINEIN* (see **Table 3**) is a gene linked to Seckel syndrome, a form of microcephalic dwarfism in humans (34). RNAi knockdown of *Ninein*, which localizes to mother centrioles, abrogates the asymmetric inheritance of centrosomes, resulting in precocious loss of progenitors from the VZ in mice (177). A recent study showed that *Ninein* is alternatively spliced, with one splice isoform expressed in NPCs and the alternative splice isoform expressed in neurons (187). The NPC-specific isoform of Ninein localizes to the mother centriole, whereas the neuronal isoform localizes to the cytoplasm. Overexpressing neuronal *Ninein* drove the differentiation of NPCs into neurons. The centrosomal protein CEP170, which is dependent on Ninein for its localization, interacts with the alternatively spliced exon 18 of *Ninein*, which is specific to NPCs. Rbfox regulates alternative splicing of *Ninein* by promoting the neuronal, noncentrosomal isoform at the expense of the NPC-specific, centrosomal isoform, leading to differentiation of the NPCs into neurons. Thus, the alternative splicing of *Ninein* that affects its centrosomal localization and interacting partners represents a novel mechanism involving the mother centriole that controls cell fate in the developing brain.

Given the role of the mother centriole in the formation of primary cilia, one possible explanation for proliferation defects in some models of microcephaly may have to do with the regulation

of primary cilia themselves. Primary cilia are nonmotile sensory organelles consisting of nine microtubule doublets surrounded by a ciliary membrane that projects from the surface of quiescent cells (53). The mother centriole is a critical component of the primary cilium; the basal body, which nucleates the cilium, consists of the mother centriole and its associated appendage proteins, and the distal appendages dock the basal body to the cell membrane (24). As centrioles are also part of the mitotic spindle, it is believed that cilia need to be resorbed before mitosis begins (91). Nde1 localizes to the mother centriole and is thought to promote cell cycle reentry by negatively regulating ciliogenesis (88). Therefore, loss of function of Nde1 and similar proteins may prevent normal cell cycle progression, leading to mitotic arrest. Human mutations in *KATNB1*, which encodes the noncatalytic, regulatory p80 subunit of the microtubule-severing enzyme katanin, cause severe microlissencephaly reminiscent of the *NDE1* phenotype (74, 116). Mouse embryonic fibroblasts from *Katnb1*-null mice show centriole overduplication and supernumerary cilia, suggesting that katanin p80 negatively regulates the number of centrioles and cilia in a cell (74). Loss of the *KATNB1* ortholog *kat80* disrupts asymmetrically dividing neuroblasts in the *Drosophila* optic lobe neuroepithelium, resulting in supernumerary centrosomes, mitotic spindle abnormalities, delays in cell cycle progression, and ultimately fewer neurons (116). The centriole overduplication phenotype associated with *Nde1* and *Katnb1* contrasts with the centriole underduplication phenotype seen in association with several other microcephaly genes, including *ASPM*, *CENPJ*, *WDR62*, and *CEP63* (81). These findings on Nde1 and Katnb1 function also suggest that the primary cilium may mediate defects in neurogenesis in at least some models of microcephaly and that this may partly explain the importance of the mother centriole in cerebral cortical development.

Contrary to prior work indicating that primary cilia usually disassemble completely before mitosis, some studies have recently suggested that remnants of the ciliary membrane linked to the older centrosome can be asymmetrically inherited between the two daughter cells during cell division. In an asymmetric progenitor cell division, the grandmother centriole and the ciliary membrane remnant linked to this centriole are preferentially inherited by the daughter cell that is destined to remain an apical progenitor cell. This daughter cell also gives rise to a primary cilium more rapidly in the next cell cycle. The association between centrosomes and ciliary membrane is not static but decreases over the course of neurogenesis. Initially, both centrosomes stain positive for ciliary remnants, likely reflecting de novo capture of Golgi-derived ciliary membrane vesicles; this stage coincides with symmetric proliferative divisions of neuroepithelial or apical progenitors. In the middle stage, when VRG cells undergo asymmetric divisions to self-renew and also yield basal progenitors or neurons, only one of the two centrosomes in a dividing cell has an associated ciliary remnant. In the final stage, when the progenitors undergo symmetric transit-amplifying or neurogenic divisions, ciliary remnants are dissociated from both centrosomes (140). Thus, the gradual dissociation of ciliary remnants from centrosomes coincides with the differentiation of cells from apical progenitors to basal progenitors and neurons.

One might predict that, in the absence of a microcephaly-associated protein, NPCs may undergo premature dissociation of ciliary membrane from centrosomes, resulting in a failure to inherit the ciliary membrane asymmetrically. Such progenitors would be expected to lose stem cell character and give rise to basal progenitors or neurons prematurely. The studies by Jayaraman et al. (81) in *Wdr62* and *Aspm* mutant mice represent the first genetic test of this model of asymmetric ciliary membrane inheritance in a model of microcephaly. They found an early increase in noncentrosomal Arl13b staining, signifying premature dissociation of ciliary remnants from centrosomes, at E12.5 in $Wdr62^{-/-}$ mice, or two full days earlier than wild-type mice. This premature dissociation could help explain the precocious generation of $Tbr2^{+}$ IP cells at the expense of $Sox2^{+}$, $Pax6^{+}$ apical VRG cells in $Wdr62^{-/-}$ and $Wdr62^{+/-};Aspm^{-/-}$ mice that was described above. Their findings are consistent with a model in which the association between the maternal

centriole and ciliary remnants, mediated by the maternal centriole proteins Wdr62 and Aspm, controls the symmetry of progenitor cell division and maintains neural stem cell fate. If true, this model could at least partially explain the observed cell fate change from apical to basal progenitors in mice with mutations in *Wdr62* and *Aspm* that deplete the very progenitors with the greatest potential for self-renewal.

We propose an alternative mechanism for the cell fate changes seen in *Wdr62* and *Aspm* mutants based on the gene-dose-dependent disruption of apical polarity complex proteins in these mutants. The apical polarity complex is essential for the maintenance of VRG cells in the VZ (87), likely by mediating proproliferative signals from the embryonic cerebrospinal fluid that bathes the ventricular surface (102). Jayaraman et al. (81) found a reduction in the localization of the apical complex proteins Pals1 and aPKCζ at the apical surface that was relatively mild in $Aspm^{-/-}$ embryos, intermediate in severity in $Wdr62^{+/-};Aspm^{-/-}$ embryos, and most dramatic in $Wdr62^{-/-}$ embryos. The more severe the loss of apical complex proteins was, the greater the associated disruption of the ventricular surface and epithelial organization was. This trend in the severity of the phenotype paralleled the gene dose dependence in the severity of the microcephaly, centriole duplication defects, and CENPJ/CPAP/SAS-4 levels in these mutants. Overall, this loss of apical complex proteins and associated disruption of the epithelial lining helps explain the precocious delamination of progenitors from the germinal zones and the apical-to-basal cell fate changes in these mutants. This model would also explain the assorted structural malformations, such as polymicrogyria, and especially schizencephaly and periventricular heterotopia, that are commonly observed with human *WDR62* mutations (18, 186) and occasionally with *ASPM* mutations as well (141).

The nature of the link between centrosomes and the apical polarity complex, in particular, is not entirely clear and warrants future study. In support of the apical complex model, a study of *Drosophila* neural stem cells showed that the wild-type apical centrosomes remained close to the apical cortex, whereas basal centrosomes lost their apical association and moved through the cytoplasm, maturing near the basal cortex. *Wdr62* mutant apical centrosomes behaved similarly to basal centrosomes, moving away from the apical cortex to the basal cortex (149). Members of the Par family of apical membrane constituents are asymmetrically segregated between daughter cells during asymmetric progenitor cell divisions (98). Of these, Par3, Par6, and aPKC associate with centrosomes (9, 78, 93). Asymmetric localization of several evolutionarily conserved members of the planar spindle-positioning pathway is critical in mediating the Par complex–dependent spindle orientation in neuroepithelial progenitors (159). Members of this pathway in mammals include LGN/AGS3 (Pins in *Drosophila*, GPR-1/2 in *Caenorhabditis elegans*) and NuMA (Mud in *Drosophila*, LIN-5 in *C. elegans*). In particular, expression of LGN and NuMA in a belt in the lateral cell cortex is required for appropriate spindle orientation and maintenance of symmetric divisions in neuroepithelial progenitors (97, 143). In *C. elegans*, the apical complex component LIN-5 binds ASPM-1 (172). Regardless, it is becoming increasingly clear that a key subset of MCPH proteins, including WDR62 and ASPM, are essential not only for centriole biogenesis but also for normal localization of the apical complex and maintenance of apical–basal polarity, suggesting links among centrioles, centrosomes, the apical complex, and the maintenance of cell fate in the developing brain.

Identification and characterization of MCPH genes have revealed how VRG cells are maintained at the molecular level. MCPH proteins are not randomly distributed but instead are localized to a few organelles, including the centrosome, suggesting common mechanisms regulating the VRG pool. However, many enthralling problems remain to be solved:

- What roles do MCPH proteins play in other NPC types, such as ORG cells, IP cells, and interneuron progenitors? Do MCPH proteins control VRG cells uniformly, or is there a

spatial/temporal specificity? By extension, how is a microcephalic brain different from a normal brain, other than its small size?

- How do MCPH proteins, which are expressed in multiple types of stem cells, explain brain-specific microcephaly? What is their NPC-specific role?
- What is the meaning of strong evolutionary changes in MCPH proteins that correlate with brain size in placental mammals, including primates (11, 122)? Do those changes affect how many times VRG cells divide symmetrically in the VZ?

Solving these problems will help answer some of the tantalizing questions in human brain development and evolution.

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Errata

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