Congenital microcephaly

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The underlying etiologies of genetic congenital microcephaly are complex and multifactorial. Recently, with the exponential growth in the identification and characterization of novel genetic causes of congenital microcephaly, there has been a consolidation and emergence of certain themes concerning underlying pathomechanisms. These include abnormal mitotic microtubule spindle structure, numerical and structural abnormalities of the centrosome, altered cilia function, impaired DNA repair, DNA Damage Response signaling and DNA replication, along with attenuated cell cycle checkpoint proficiency. Many of these processes are highly interconnected. Interestingly, a defect in a gene whose encoded protein has a canonical function in one of these processes can often have multiple impacts at the cellular level involving several of these pathways. Here, we overview the key pathomechanistic themes underlying profound congenital microcephaly, and emphasize their interconnected nature. © 2014 Wiley Periodicals, Inc.

KEY WORDS: cell division; mitosis; DNA replication; cilia


INTRODUCTION

Congenital microcephaly, an occipital-frontal circumference of equal to or less than 2–3 standard deviations below the age-related population mean, denotes a fundamental impairment in normal brain development [Woods and Parker, 2013]. Depending on the underlying cause, congenital microcephaly can be associated with structural brain malformations [e.g., gyrification issues, agenesis of corpus callosum, pituitary abnormalities] or secondary consequences such as craniosynostosis [Verloes et al., 2013]. Congenital microcephaly can have an environmental or genetic etiology [Gilmore and Walsh, 2013]. Cerebral cortical neurons must have developed by mid-gestation although glial cell division and consequent brain volume enlargement does continue after birth [Spalding et al., 2005]. Impaired neurogenesis is therefore most obviously reflected clinically as congenital microcephaly.

Fundamentally, neurogenesis incorporates several stages that are very susceptible to problems in the efficient and effective execution of genome maintenance, DNA replication and ultimately cell division. The developing human neuroepithelium must undergo a rapid expansion in stem cell numbers to fuel its own symmetric expansion [Rakic, 1995]. This is essential to generate enough capacity to instigate and maintain asymmetric division for neuronal differentiation, enabling the formation of the various cortical layers. Furthermore, differentiating and developing neurons must migrate to their defined locations to construct the complex architecture and laminar layered structure of the cortex [Tan and Shi, 2013; Wu et al., 2014] (Fig. 1).

What spectrum of physiological deficits underlies congenital microcephaly? Defects resulting in elevated levels of apoptosis can deplete neuroprogenitor stem and differentiating cells. Defects impacting upon efficient DNA replication can limit the capacity of the neuroepithelium to expand under its strict temporal constraints. Defects in the mitotic apparatus (e.g., microtubule spindles, centrosomes, centrioles) can lead to impaired symmetric-asymmetric
division, inappropriate cell cycle arrest and/or elevated apoptosis. Mutations in genes encoding key players in each of these biological processes have been described in patients exhibiting profound congenital microcephaly (Tables I–III). This can present as the most marked clinical feature; primary microcephaly (PM), or in association with pronounced growth restriction; as in the growing family of genetically identifiable microcephalic primordial dwarfs (MPDs), such as Seckel syndrome, microcephalic primordial dwarfism types I and II and Meier–Gorlin syndrome. Herein, we overview the genetic defects associated with severe congenital microcephaly and discuss how they contribute to the general phenomena of limiting cell division capacity and elevating levels of stem and/or neuroprogenitor cell death as mechanisms underlying impaired neurogenesis.

**MITOSIS AND MICROCEPHALY**

To date, the majority of the genetic defects identified in PM and MPDs involve genes encoding proteins that play fundamental roles in various processes that collectively enable cells to
<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>OMIM</th>
<th>Clinical presentation</th>
<th>Localization and function</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCPH1</td>
<td>Microcephalin</td>
<td>251200</td>
<td>PM. Cells exhibit premature chromosome condensation</td>
<td>Centrosome-role in DNA repair and G2-M dynamics</td>
</tr>
<tr>
<td>WDR62</td>
<td>WD-repeat containing protein 62</td>
<td>604317</td>
<td>PM</td>
<td>Mitotic spindle pole formation-scaffold for JNK pathway</td>
</tr>
<tr>
<td>CDK5RAP2/CEP215</td>
<td>Cyclin dependent kinase 5 regulatory subunit-associated protein 2</td>
<td>604804</td>
<td>PM</td>
<td>Centrosome, spindle and microtubule organizing function.</td>
</tr>
<tr>
<td>CASC5</td>
<td>Cancer susceptibility candidate 5</td>
<td>604321</td>
<td>PM</td>
<td>Kinetochore [KNM] component-spindle-assembly checkpoint</td>
</tr>
<tr>
<td>ASPM</td>
<td>Abnormal spindle-like, microcephaly associated</td>
<td>608716</td>
<td>PM</td>
<td>Microtubule associated protein-spindle organization and orientation</td>
</tr>
<tr>
<td>CENPJ/CPAP</td>
<td>Centromeric protein J</td>
<td>608393</td>
<td>PM-MPD-Seckel syndrome</td>
<td>Centriole biogenesis-cilia formation</td>
</tr>
<tr>
<td>STIL (MCPH7)</td>
<td>SCL/TAL1 interrupting locus</td>
<td>612703</td>
<td>PM</td>
<td>Centrosome-centriole biogenesis</td>
</tr>
<tr>
<td>CEP135</td>
<td>Centrosomal protein 135kDa</td>
<td>614673</td>
<td>PM</td>
<td>Centrosome-centriole biogenesis</td>
</tr>
<tr>
<td>CEP152</td>
<td>Centrosomal protein of 152 kDa</td>
<td>614852</td>
<td>PM-MPD-Seckel syndrome</td>
<td>Centrosome-centriole biogenesis and genome stability</td>
</tr>
<tr>
<td>CEP63</td>
<td>Centrosomal protein of 63 kDa</td>
<td>614728</td>
<td>Microcephaly with growth retardation-Seckel syndrome [mild]</td>
<td>Centrosome-centriole biogenesis</td>
</tr>
<tr>
<td>NDE1</td>
<td>Nuclear distribution protein nseE homolog 1</td>
<td>614019</td>
<td>Micro lisencephaly microhydrancephaly</td>
<td>Centrosome-mitotic spindle</td>
</tr>
<tr>
<td>NIN</td>
<td>Ninein</td>
<td>614851</td>
<td>MPD-Seckel syndrome</td>
<td>Centrosome function-microtubule organization</td>
</tr>
<tr>
<td>PCNT</td>
<td>Pericentrin</td>
<td>210720</td>
<td>Microcephalic Osteodysplastic Primordial Dwarfism (MOPD) II</td>
<td>Component of pericentriolar material-scaffold for signaling molecules?</td>
</tr>
<tr>
<td>BUB1B</td>
<td>BUB1 Mitotic Checkpoint Serine/Threonine Kinase B1 (BUBR1)</td>
<td>257300</td>
<td>Growth retardation-microcephaly-cancer-Mosaic Variegated Aneuploidy [MVA]</td>
<td>Kinetochore kinase-role in spindle checkpoint</td>
</tr>
<tr>
<td>CENPE</td>
<td>Centromere protein E (CENP-E)</td>
<td>117143</td>
<td>MPD</td>
<td>Microtubule capture and stabilization</td>
</tr>
<tr>
<td>KIF5C</td>
<td>Kinesin family member 5C</td>
<td>615282</td>
<td>Microcephaly and cortical malformations</td>
<td>Microtubule motor protein</td>
</tr>
<tr>
<td>KIF2A</td>
<td>Kinesin family member 2A</td>
<td>615411</td>
<td>Severe microcephaly-cortical malformations-early-onset epilepsy</td>
<td>Microtubule motor protein</td>
</tr>
<tr>
<td>KIF11</td>
<td>Kinesin family member 11</td>
<td>152950</td>
<td>Microcephaly with or without chorioretinopathy, lymphoedema and mental retardation</td>
<td>Microtubule motor protein-role in microtubule crosslinking and bipolar spindle formation</td>
</tr>
<tr>
<td>TUBG1</td>
<td>Tubulin-gamma complex associated protein 1</td>
<td>615412</td>
<td>complex cortical malformations-microcephaly (not all reported cases)</td>
<td>Structural component of the centrosome-role in microtubule nucleation</td>
</tr>
<tr>
<td>TUBB2B</td>
<td>Tubulin, beta 2B class Ib</td>
<td>610031</td>
<td>Microcephaly-spastic tetraparesis-severe intellectual disability-scoliosis</td>
<td>Microtubule component-binds to GTP</td>
</tr>
<tr>
<td>TUBA1A</td>
<td>Tubulin, alpha 1A</td>
<td>611603</td>
<td>Microcephaly-severe intellectual disability</td>
<td>Microtubule component</td>
</tr>
<tr>
<td>POC1A</td>
<td>Proteome of the centriole 1A</td>
<td>614783</td>
<td>MPD</td>
<td>Centriolar protein required for cilia formation</td>
</tr>
</tbody>
</table>

PM, primary microcephaly; MPD, microcephalic primordial dwarism. *Denotes the gene entry in OMIM.
execute precise chromosomal segregation and mitotic division [Thornton and Woods, 2009; Mahmood et al., 2011; Verloes et al., 2013][Table I]. The mitotic phase of the cell cycle involves an intricate and highly complex ballet of interactions and transactions occurring in an organized and inter-dependent fashion [Walczak et al., 2010]. These include chromosome condensation, bipolar mitotic microtubule spindle network formation and dissolution, along with chromosomal kinetochore-mediated nucleation and capture by spindle microtubules to instigate the amphitelic restraining of chromosomes for alignment at metaphase prior to segregation. The centrosome represents an important spindle microtubule organizing center [Bettencourt-Dias and Glover, 2007]. There is now an increasing list of examples of hypomorphic defects in genes encoding core components of the centrosome associated with PM and MPD (Table I and Figs. 2 and 3).

Very often, the precise roles of these proteins at centrosomes are rather opaque. For some, such as Pericentrin and γ-tubulin, these often have “structural” or “scaffold” functions attributed them [Zimmerman et al., 2004]. In most instances, descriptive impacts upon centriole and centrosome duplication, and consequently abnormalities in microtubule spindle organization, have been observed for defects in these proteins [Griffith et al., 2008; Rauch et al., 2008]. Furthermore, there is growing evidence that defects in some of these proteins have additional negative impacts upon the centrosomal localization of other centrosome proteins that have independently been identified as underlying defects of PM and/or MPD. Illustrative examples include the interplay between CEP152 and CEP63 or for CEP152 and CENPJ (CPAP) [Cizmecioglu et al., 2010; Sir et al., 2011]. These occurrences further highlight the interconnected and functional interplay between many of these proteins, explaining to some degree why defects herein present with a common clinical manifestation of congenital microcephaly.

**Centrosomes and Spindles**

The centrosome cycle is coordinated with the canonical cell cycle whereby the mother centrosome, inherited from the previous mitosis, must duplicate to generate a mother-daughter pair prior to G2-phase where they then act as a microtubule organizing center at the

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**TABLE II. Defects in the Origin Recognition Complex Core and Associated Components Underlying Meier–Gorlin Syndrome**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>OMIM</th>
<th>Clinical presentation</th>
<th>Localization &amp; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORC1</td>
<td>Origin recognition complex subunit 1</td>
<td>224690</td>
<td>Meier–Gorlin syndrome</td>
<td>Component of the pre-replication complex-initiation of DNA replication</td>
</tr>
<tr>
<td>ORC4</td>
<td>Origin recognition complex subunit 4</td>
<td>613800</td>
<td>Meier–Gorlin syndrome</td>
<td>Component of the pre-replication complex-initiation of DNA replication</td>
</tr>
<tr>
<td>ORC6</td>
<td>Origin recognition complex subunit 6</td>
<td>613803</td>
<td>Meier–Gorlin syndrome</td>
<td>Component of the pre-replication complex-initiation of DNA replication, coordination of chromosome-replication and segregation</td>
</tr>
<tr>
<td>CDT1</td>
<td>Chromatin licensing and DNA replication factor 1</td>
<td>613804</td>
<td>Meier–Gorlin syndrome</td>
<td>Component of the pre-replication complex-origin licensing factor</td>
</tr>
<tr>
<td>CDC6</td>
<td>Cell division cycle 6</td>
<td>613805</td>
<td>Meier–Gorlin syndrome</td>
<td>Component of the pre-replication complex-loading MCM complex-role in cell cycle checkpoints</td>
</tr>
<tr>
<td>Gene</td>
<td>Protein</td>
<td>OMIM</td>
<td>Clinical presentation</td>
<td>Localization &amp; function</td>
</tr>
<tr>
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<td>------------------------</td>
</tr>
<tr>
<td>ATR</td>
<td>Ataxia telangiectasia and rad3-related</td>
<td>210600</td>
<td>MPD–Seckel syndrome</td>
<td>Protein kinase-apical DDR regulator–cell cycle checkpoint activation–role in DNA replication</td>
</tr>
<tr>
<td>ATRIP</td>
<td>ATR-interacting protein</td>
<td>606605&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MPD–Seckel syndrome</td>
<td>Essential ATR partner–binds to RPA-coated ssDNA-DDR–role in DNA replication</td>
</tr>
<tr>
<td>RBBP8/CTIP</td>
<td>Retinoblastoma–binding protein 8/CTIP–interacting protein (G3P)</td>
<td>606744</td>
<td>MPD–Seckel syndrome</td>
<td>DNA DSB resection–role in ATR recruitment to DSBs–associates with BRCA1 in regulation of cell cycle checkpoints</td>
</tr>
<tr>
<td>RAD50</td>
<td>DNA repair protein RAD50</td>
<td>613078</td>
<td>NBS–like disorder; microcephaly–intellectual disability–“bird–like” face–short stature</td>
<td>Component of M–R–N complex with central role in DNA DSB repair</td>
</tr>
<tr>
<td>MRE11A</td>
<td>Double–strand break repair protein MRE11A</td>
<td>600814&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NBS–like severe microcephaly</td>
<td>Component of M–R–N complex with central role in DNA DSB repair</td>
</tr>
<tr>
<td>PNKP</td>
<td>Bifunctional polynucleotide phosphatase and kinase</td>
<td>613402</td>
<td>Microcephaly–seizures–developmental delay</td>
<td>Role in DNA repair pathways (NHEJ, BER)</td>
</tr>
<tr>
<td>CDK6</td>
<td>Cyclin–dependent kinase 6</td>
<td>603368&lt;sup&gt;a&lt;/sup&gt;</td>
<td>PM</td>
<td>Control of cell cycle and differentiation–centrosomal association in mitosis</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Breast cancer type 1 susceptibility protein</td>
<td>113705&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Microcephaly–short stature–developmental delay–cancer predisposition</td>
<td>DNA repair–cell cycle checkpoint control–maintenance of genomic stability (HRR)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Breast cancer type 2 susceptibility protein</td>
<td>600185&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MPD</td>
<td>DNA repair–cell cycle checkpoint control–maintenance of genomic stability (HRR)</td>
</tr>
<tr>
<td>LIG4</td>
<td>DNA ligase 4</td>
<td>606593</td>
<td>Ligase IV syndrome: microcephaly–dysmorphic facial features–growth retardation–skin anomalies–pancytopenia.</td>
<td>DNA DSB repair (NHEJ) and V(D)J recombination</td>
</tr>
<tr>
<td>NHEJ1</td>
<td>Non–homologous end–joining factor 1</td>
<td>611291</td>
<td>Growth retardation–microcephaly–immunodeficiency</td>
<td>DNA DSB repair (NHEJ) and V(D)J recombination</td>
</tr>
<tr>
<td>CHLR1/DDX11</td>
<td>DEAD/H (Asp–Glu–Ala–Asp/Hij) box helicase 11</td>
<td>601150&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Warsaw breakage syndrome [WABS]: microcephaly–pre– and postnatal growth retardation–abnormal skin pigmentation</td>
<td>DNA helicase–genome stability</td>
</tr>
<tr>
<td>PHC1 (MCPH11)</td>
<td>Polyhomeotic–like protein 1</td>
<td>615414</td>
<td>PM–short stature</td>
<td>Component of polycomb group (PcG) multiprotein PRC1–like complex–repression of transcription–role in chromatin remodelling and histone modification</td>
</tr>
<tr>
<td>DNA2</td>
<td>DNA replication helicase 2</td>
<td>601810&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MPD</td>
<td>Helicase–DNA repair–genome stability</td>
</tr>
<tr>
<td>XRCC2</td>
<td>X–ray repair complementing defective repair in Chinese hamster cells 2</td>
<td>600375&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Microcephaly–growth deficiency–facial nerve palsy–skeletal abnormalities</td>
<td>DNA DSB repair (HR)–genome stability</td>
</tr>
<tr>
<td>XRCC4</td>
<td>X–ray repair complementing defective repair in Chinese hamster cells 4</td>
<td>194363&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MPD</td>
<td>DNA DSB repair (NHEJ)–genome stability</td>
</tr>
<tr>
<td>NHEJ1</td>
<td>Nonhomologous end–joining factor 1. Also called XLF/Chernunos</td>
<td>611291</td>
<td>Severe combined immunodeficiency (SCID)–microcephaly–growth retardation–IR sensitivity</td>
<td>DNA DSB repair (NHEJ)–genome stability</td>
</tr>
</tbody>
</table>

PM, primary microcephaly; IR, ionizing radiation.

Figure 2. Centrioles, centrosome and mitotic microtubule organization. (A) The centriole cycle is intimately coordinated with the canonical cell cycle where centriolar division occurs during S-phase to ensure mature centrioles are available prior to the onset of mitosis. (B) A normal mitosis is depicted in the upper panel where the centrosomes are shown in black, the chromosomes in gray and the microtubule spindles as the dotted lines. Bi-polar spindle formation is an essential prerequisite to effective chromosome segregation during mitotic division. The lower panels show mitotic LCLs from a wild-type (WT) and PCNT-mutated individual. The spindles were detected using α-tubulin (green) whilst the centrosomes were stained using γ-tubulin (yellow-orange). The WT mitotic cell shows a bipolar spindle in contrast to the multipolar spindle from the PCNT-individual. (Images courtesy of Dr. Iga Abramowicz).

Figure 3. The distribution of genetic defects underlying congenital microcephaly attributable to the centrosome, spindle and kinetochore. The upper panel depicts a normal bipolar mitosis. For the centrosome panel, the proteins in black are centrosome-specific proteins whilst those in blue are spindle constituent and spindle-associated proteins associated with congenital microcephaly. For the kinetochore panel CENP-E is shown extending from the kinetochore via its large coiled-coiled region onto microtubules. The orange section of CENP-E denotes its kinesin motor domain. CCAN: constitutive centromere-associated network. KNM network: KNL1-NDC80 (CASC5)-MIND.
onset of the next mitosis [Hinchcliffe, 2001] (Fig. 2A). Currently, the working functional model to explain impaired neurogenesis in the context of a defect in a centrosomal protein is that these often result in supernumerary centrosomes, fragmented centrosomes and/or premature centriolar separation [Woods and Parker, 2013]. Consequently, these can result in defects in mitotic spindle microtubule nucleation when establishing a bipolar spindle. Very often, multipolar spindles are observed in patient-derived cell lines representing a catastrophic consequence for mitosis, chromosome segregation and cell division [Rauch et al., 2008; Issa et al., 2013] (Fig. 2B). Such outcomes can result in permanent cell cycle arrest via activation of the spindle assembly checkpoint (SAC), as well as cytokinesis failure and subsequent apoptosis [Musacchio, 2011]. All of these impacts could limit neuroepithelial stem cell maintenance and expansion, as well as disrupt the important balance between cell division and differentiation (Fig. 1).

Defects in microtubule spindle components and spindle-associated proteins represent the most frequent underlying cause of congenital microcephaly described to date (Table I). One well known example is ASPM (abnormal spindle-like microcephaly-associated protein), a spindle binding protein that localizes to the pericentriolar matrix (PCM) of the centrosome at the onset of mitosis [Bond et al., 2002]. It has been shown that defects in ASPM function can result in altered spindle pole orientation in the developing neuroepithelium, thereby disrupting the balance between symmetric and asymmetric division of neuronal stem cells [Fish et al., 2006] (Fig. 1C). Indeed this has also recently been elegantly demonstrated in the developing brain of a mouse model of Mph1 (Microcephalin), a common cause of PM in humans [Gruber et al., 2011]. Defects in microtubule and cytoskeletal constituents (e.g., α- and β-tubulin) and even microtubule interacting proteins that regulate diverse processes such as microtubule formation, stabilization and depolymerization, can also result in congenital microcephaly [Morris-Rosendahl et al., 2008; Jagln and Chelly, 2009; Romaniello et al., 2014] (Table I and Fig. 3). These defects are typically also associated with marked deficits in cortical development (e.g., lissencephaly, pachygyria, polymicrogyria) due to abnormalities in neuronal migration (e.g., KIF2A, KIF5C) [Poirier et al., 2013].

The Kinetochore and Spindles

Considering its role in microtubule capture and chromosome segregation, defects in kinetochore components as an underlying pathomechanism for congenital microcephaly are very much under-represented to date, compared to the spindle and centrosome (Table I and Fig. 3). Mutations in BUB1B cause mosaic variegated aneuploidy, an MPD associated with elevated cancer predisposition, particularly Wilms tumor [Matsura et al., 2000; Hanks et al., 2004]. BUB1B encodes BUBR1, a SAC protein that localizes to the kinetochore of lagging chromosomes during mitosis [Bolanos-Garcia et al., 2009; Kiyomitsu et al., 2011]. SAC activation ensures all kinetochores have robust amphitelic microtubule attachments prior to segregation [Rudner and Murray, 1996; Musacchio, 2011].

A defect in CASC5 was reported in three related families used to define the original MCPH4 locus [Jamieson et al., 1999; Genin et al., 2012]. CASC5 encodes a component of the KMN Complex [KNL1-Mis12 complex-Ndc80], a kinetochore localizing multi-protein complex involved in microtubule stabilization and SAC silencing [Kiyomitsu et al., 2007]. Mirzaa et al. [2014] recently described the first example of a defect in a core kinetochore component as the underlying cause of MPD. They found defects in CENPE, the gene encoding the large kinetochore protein CENP-E which plays a vital role in microtubule capture during mitosis (Fig. 3). Cells from the affected individuals exhibited multiple interconnected mitotic abnormalities. It is likely that other kinetochore-associated defects await to be identified as novel causes of congenital microcephaly disorders, as PM and/or MPD.

DNA REPLIATION, CILIA FUNCTION AND MICROCEPHALY

Compared to other cell types, cell cycle length can be remarkably short in developing neuroprogenitors [Rakic, 1995]. Since these cells need to undergo rapid and temporally restricted expansion, efficient DNA replication is fundamental to ensure normal neuronal development. In fact, even within progenitor populations there appears to be significant variation in the duration of certain cell cycle phases; most notably G1 and S-phase, depending upon the specific lineage commitment of the progenitors in question [Dehay et al., 2001; Dehay and Kennedy, 2007; Pilaz et al., 2009; Ari et al., 2011]. Therefore, genetic defects that can adversely impact upon the duration of these cell cycle phases could potentially have a dramatic effect upon the efficiency of cortical development.

Origin Licensing, G1-S Transition, DNA Replication and S-Phase Progression

Recently, defects in multiple components of the origin recognition complex (ORC), a multi-subunit complex that ‘licenses’ and thereby initiates DNA replication from mainly non-sequence specific discrete genomic regions, were identified in Meier-Gorlin syndrome (MGS).

Recently, defects in multiple components of the origin recognition complex [ORC], a multi-subunit complex that ‘licenses’ and thereby initiates DNA replication from mainly non-sequence specific discrete genomic regions, were identified in Meier–Gorlin syndrome (MGS).
replication from mainly non-sequence specific discrete genomic regions, were identified in Meier-Gorlin syndrome (MGS) [Bicknell et al., 2011a,b; Guernsey et al., 2011] (Table II). MGS is an MPD associated with additional features including endochondral ossification abnormalities [Gorlin et al., 1975; Ahmad and Teebi, 1997; Bongers et al., 2005]. With respect to ORC1-mutated MGS, specific deficiencies in DNA origin licensing, DNA replication initiation, G1-S transition and S-phase progression were catalogued in patient lymphoblastoid cell lines (LCLs), thus suggesting a pathomechanism based upon delayed DNA replication as underlying the clinical presentation [Bicknell et al., 2011b]. Furthermore, several ORC1-MGS mutations localized to the BAH (bromodomain adjacent homology) domain of ORC1 [Bicknell et al., 2011b]. This region was shown to bind histone H4-lysine-20-dimethylated (H4-K12-Me2), and since H4-K12-Me2 is enriched at two known human replication origins, it has been postulated that this domain is required for ORC-rerecruitment to origins [Noguchi et al., 2006; Kuo et al., 2012]. Collectively, these findings appear to support a model whereby a direct impact upon DNA replication kinetics, due to defects in components that initiate DNA replication, are associated with congenital microcephaly in the context of this MPD.

Recent findings suggest that there is more to this basic model. For example, pathogenic defects in MCM4, a core component of the replisome, have been identified in patients with adrenal insufficiency, growth restriction and a selective Natural Killer cell defect, although without any overt indication of congenital microcephaly [Casey et al., 2012; Gineau et al., 2012; Hughes et al., 2012]. Similarly, a polymerase-inactivating defect in the catalytic subunit (POLD1) of DNA polymerase δ, the lagging strand DNA polymerase, has been identified in an individual with a complex disorder involving lipodystrophy, deafness, hypogonadism and mandibular hypoplasia; again, without overt congenital microcephaly [Weedon et al., 2013].

Cilia-Function and S-Phase Entry

With respect to the impaired origin licensing and delayed S-phase kinetics observed in MGS, subsequent investigation provided evidence to suggest a more complex and multifaceted pathomechanism. Stiff et al. [2013] found that while MGS-causative defects were associated with reduced licensing of an ectopically supplied DNA replication origin, unexpectedly, some of these defects did not segregate with delayed S-phase progression in patient LCLs. Rather, all of these defects were associated with centriole-centrosome abnormalities, impaired ciliation and consequently cilia-dependent signaling [Stiff et al., 2013]. Interestingly, ORC1 can localize to the centrosome in a Cyclin A-dependent manner and has previously been implicated in controlling centriole and centrosome copy number via interaction with Cyclin E [Hemerly et al., 2009]. Whether other ORC components have direct roles at the centrosome and/or cilia is not clear.

Cilia formation and function are vital for coordinating cell cycle entry from G0 phase into G1-S [Heldin and Westermark, 1999; Schneider et al., 2005]. Furthermore, cilia function is intimately associated with neuronal development [Spasky et al., 2008; Han and Alvarez-Buylla, 2010; Lee and Gleeson, 2011]. Delayed G1-S transit appears to be a feature of MGS cells and this delay appears also to be dependent upon cilia-signaling [Stiff et al., 2013]. This is an intriguing result considering the importance of G1 phase length in regulating the balance between neuroprogenitor stem cell regeneration and progenitor lineage commitment via differentiation [Dehay and Kennedy, 2007; Pilaz et al., 2009]. The cilia-dependent findings in MGS originating from defects in components with canonical roles in DNA replication adds an extra layer of complexity to our understanding of the molecular and cellular impacts that converge here to adversely affect normal neuronal development (and height attainment and endochondral ossification). Additionally, several ORC components have also been implicated in multiple post-mitotic neuronal functions, suggestive of additional roles outside of DNA replication origin licensing (reviewed in Kerzendorfer et al., 2013).

Is there precedence for centrosome-based cilia dysfunction in human congenital microcephaly disorders? Defects in PCNT, encoding the centrosomal protein Pericentrin, underlie the MPD microcephalic primordial osteodysplastic dwarfism type-II (MOPD-II) [Griffith et al., 2008; Rauch et al., 2008]. Impaired PCNT function is associated with cilia-dysfunction [Miyoshi et al., 2006, 2009; Mühlhans et al., 2011]. Multiple mutations in POC1A which encodes a centriole protein, have been identified in several MPD families [Shaheen et al., 2012]. Interestingly these defects were associated with centrosome fragmentation, microtubule spindle abnormalities (e.g., multipolar spindles) as well as cilia formation and signaling defects [Shaheen et al., 2012]. In fact, ASPM has recently been associated with compromised WNT signaling which could also reflect an underlying problem in cilia function [Ponting, 2006; Buchman et al., 2011].

Therefore, it would appear that clinically relevant cilia abnormalities impacting on neurogenesis can originate from at least two routes; firstly, as a direct consequence of defects in proteins with known and/or probable roles in cilia formation which emanates from the centriolar-basal body, and secondly, from the likely secondary or indirect consequence of defects in proteins without prescribed roles in the cilium or in cilia formation, as in the case of the origin licensing components. The latter route may prove to be more widespread than currently appreciated.

THE DNA DAMAGE RESPONSE (DDR) AND MICROCEPHALY

The PI3-kinase-like family members ATM (Ataxia Telangiectasia Mutated) and ATR (Ataxia Telangiectasia and Rad3-related) are the apical protein kinases of the DNA Damage Response (DDR) [Cimprich and Cortez, 2008;
Seckel syndrome; the archetypal MPD
hypomorphic defects in neurons specifically [Lavin, 2008]. Involving the progressive loss of Purkinje results in ataxia telangiectasia [Aoki et al., 2012]. Both ATM and ATR function in controlling dormant DNA replication fork firing and activation of G1-S, intra-S and G2-M cell cycle checkpoints. One of the first bona fide substrates identified and characterized for these kinases was p53 [Banin et al., 1998]. There are now many others [Matsuoka et al., 2007; Stokes et al., 2007]. Therefore, both kinases directly signal to the apoptotic machinery via this and other routes [Roos and Kaina, 2006]. Loss of ATR function is associated with elevated replication fork collapse and consequently DSB formation, therefore triggering an ATM-dependent DDR cascade. This was elegantly demonstrated as a physiological consequence of hypomorphic ATR-ablation in a mouse model of ATR-mutated Seckel syndrome (AtrS/S) [Murga et al., 2009]. Embryos of the AtrS/S animal exhibited massively elevated spontaneous levels of replicative stress-induced DNA damage and apoptosis, even in the developing neuroepithelium [Murga et al., 2009]. The surviving animals exhibited craniofacial abnormalities (receding forehead, micrognathia), growth restriction and congenital microcephaly reminiscent of the ATR-mutated Seckel syndrome individuals, consistent with the concept of intrauterine programming [Murga et al., 2009; O’Driscoll, 2009].

Hypomorphic defects in ATR underlie Seckel syndrome; the archetypal MPD

Defective DDR, Apoptosis and Microcephaly

Conditional Cre-restricted mouse models have shown that the functional interrelationships between in the apical DDR kinases are more complex when considering brain development. For example, conditional Atr ablation was unexpectedly found to impact relatively late upon neurogenesis, and then only in certain progenitor populations [Lee et al., 2012b]. Rapid proliferation of granule neurons within the embryonic cerebellar external germ layer (EGL) occurs in response to sonic hedgehog (Shh), whose mitogenic potential is realized via cilia [Spaskey et al., 2008]. Atr-deficient EGL cells underwent p53-independent proliferation arrest, while other areas underwent p53-dependent apoptosis [Lee et al., 2012b]. Furthermore, co-incident inactivation of Atm was unexpectedly found not to exacerbate Atr loss in the brain, suggesting a non-overlapping role for each kinase in this developmental context [Lee et al., 2012b].

Topoisomerase II binding protein I (TopBP1) is essential for DNA replication and cell cycle checkpoint activation [Sokka et al., 2010]. It also plays a key role in activating ATR kinase [Kumagai et al., 2006]. Interestingly, conditional progenitor-restricted deletion of Topbp1 was found to be essential for early progenitor genome stability and survival, but not, unexpectedly, for replication per se [Lee et al., 2012a]. Furthermore, this impact was found to be p53-dependent whilst Atm-independent [Lee et al., 2012a]. Atm has previously been shown to be an important mediator of p53-dependent DSB-induced apoptosis in the nervous system [Herzog et al., 1998]. Collectively, these findings highlight the importance of the cell-specific context within the brain, along with the nature of the genomic instability (e.g., replication fork-dependent), as being fundamental to the precise impacts of defects in DDR-signaling controllers such as Atr, Atm and Topbp1, in the developing mouse brain at least.
congenital defects in DSB repair mechanisms are frequently associated with congenital microcephaly [O’Driscoll and Jeggo, 2008]. Mouse studies suggest that both pathways are essential for developmental viability in mammals; an indication that substantial levels of spontaneous DSBs can be generated during development [Symington and Gautier, 2011]. NHEJ involves direct re-ligation of DSBs, sometimes necessitating processing of damaged base and/or sugar moieties at DSB termini prior to ligation [Deriano and Roth, 2013]. This processing can potentially result in the loss of genetic material. HRR on the other-hand requires the presence of a sister chromatid to act as a template for repair via strand invasion from one end of the DSB during Holliday Junction (HJ) formation [Heyer et al., 2010] (Fig. 4B). Therefore, this pathway has been assumed to only be operational during cell cycle phases where a sister chromatid is present (i.e., late S-G2-M) [Symington and Gautier, 2011].

**Figure 4.** Defective DDR and DNA repair as a cause of congenital microcephaly. (A) Neuroanatomical images of the ATRIP-mutated Seckel syndrome individual described by [Ogi et al., 2012]. The patient is severely microcephalic [−10 SD] with evidence of an abnormally shaped pituitary (without an obvious fossa). (Images courtesy of Dr. Margaret Barrow). (B) Homologous recombination repair (HRR) from a DSB is initiated by exonuclease-mediated resection of the DSB ends. These RPA-coated single stranded overhangs are then bound by RAD51 generating a filament structure that can invade the sister chromatid to form a D-loop. The crossover point is referred to as a Holliday Junction (HJ). Template driven replication occurs and is followed by second end capture to generate a crossover containing a double HJ (dHJ). This intertwined molecule requires resolution which can occur via BLM helicase-dependent route, which generates a non-crossover product, or, a nuclease–dependent route to generate a cross-over. The nucleases that can act on this structure are termed “resolvases.” (C) An on-going DNA replication fork is shown with the parental DNA in black and the newly replicated DNA in blue. If this structure collides with a DNA single strand break (SSB) the resultant product can contain a DNA double strand break (DSB). This situation requires the coordinate action of DDR, mechanisms and distinct DNA repair pathways. The same situation can also occur if an active or moving transcription fork was to collide with a SSB. (D) Some of the damaged or modified DNA strand ends that can occur following oxidative damage to DNA. The normal end polarity of a 5′ phosphate and 3′ hydroxyl group must be restored in order to allow enzymatic ligation of a break. Topo I denotes a stabilized Topoisomerase I cleavable complex (CC) which is a normal intermediate in Topo I’s action on DNA. This enzyme normally introduces a SSB to release torsional tension ahead of on-going replication and transcription forks. These breaks are normally dealt with by the SSBR machinery.

Non-Homologous DNA End-Joining [NHEJ]

NHEJ is required for immunoglobulin and T cell receptor generation via the V(D)J and Class Switch Recombination mechanisms [Gellert, 2002]. Therefore, congenital defects in NHEJ pathway components are also frequently associated with variable immunodeficiency, ranging from variable/combined immunodeficiency to severe combined immunodeficiency [O’Driscoll and Jeggo,
To date, human defects have been identified in PRKCD encoding DNA-PKcs, DCLRE1C encoding ARTEMIS, LIG4 encoding DNA ligase IV and the genes encoding XRCC4 and XLF/LIG4 encoding DNA ligase IV and the PKcs, identified in 2006. To date, human defects have been viewed in O line defects have been described [reviewed in O’Driscoll, 2012]. Recent examples include XRCC2 in a patient exhibiting Fanconi anemia (FA), BRCA1 in a woman with early onset ovarian cancer and microcephaly, and BRCA2 in the context of MPD [Shamseldin et al., 2012; Domichek et al., 2013; Shaheen et al., 2014]. FA patients frequently exhibit congenital microcephaly although bone marrow failure and acute myeloid leukaemia development are the typically invariant features of this condition [Kee and Andrea, 2012]. The FA pathway is functionally integrated with HRR, and defects in other core HRR components have emerged clinically as FA; the classical example being BRCA2 mutations in FA individuals of the FANC-D1 complementation group [Moldovan and D’Andrea, 2009; Kim and D’Andrea, 2012]. Other examples include RAD51C (FANC-O), PALB2, encoding the BRCA2 interacting protein (FANC-N) and SLX4 (FANC-P), encoding a component of the SLX4-SLK1 HJ resolving endonuclease (Fig. 4B). Interestingly, germ-line mutations (het-
erozygous) in the HRR genes RAD51C and RAD51D have been identified in breast and ovarian cancer cohorts [Meindl et al., 2010; Loveday et al., 2011, 2012; Shaheen et al., 2014]. This suggesting that HRR defects can have a highly variable clinical presentation.

**Helicases and Nucleases**

The Bloom syndrome helicase BLM [RECQL3] plays an important role in HJ resolution, and congenital microcephaly can be marked in this condition. Warsaw breakage syndrome, a disorder of severe congenital microcephaly, growth retardation and chromosomal instability collectively reminiscent of FA and the cohesinopathies, was found to result from a mutation in another ATP-dependent DNA helicase, CHLR1 (DDX11) [van der Lelij et al., 2012]. Other examples include RAD51C (FANC-O), PALB2, encoding the BRCA2 interacting protein (FANC-N) and SLX4 (FANC-P), encoding a component of the SLX4-SLK1 HJ resolving endonuclease (Fig. 4B). Interestingly, germ-line mutations (het-
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erozygous) in the HRR genes RAD51C and RAD51D have been identified in breast and ovarian cancer cohorts [Meindl et al., 2010; Loveday et al., 2011, 2012; Shaheen et al., 2014]. This suggesting that HRR defects can have a highly variable clinical presentation.
DSB Repair Pathways in the Developing Brain: Spatial Regulation and Checkpoint Activation

As mentioned above, HRR is by its nature of requiring a homologous DNA strand as a template for repair, restricted to specific phases of the cell cycle where sister chromatids are available (i.e., S-G2-M) (Fig. 4B). Therefore, tissues with a very high replicative index would be expected to rely heavily on this pathway to repair DSBs, compared to non-replicative post-mitotic tissues. Studies using knockout embryonic models of NHEJ and HRR have suggested that both these DSB repair pathways may have distinct spatiotemporal functionality during neuronal development [Orii et al., 2006]. Defective HRR (Xrcc2)-induced apoptosis appeared to occur predominantly within the proliferating neuronal precursors of the ventricular zone (VZ) [Orii et al., 2006] (Fig. 1A). In contrast, defective NHEJ (Lig4)-induced apoptosis appeared restricted to the postmitotic differentiating neurons of the subventricular zone (SVZ) [Orii et al., 2006]. Work using ionising radiation (IR)-induced DSBs in a hypomorphic Lig4 embryonic mouse model (Lig4Y288C) has further developed this concept by providing evidence for a functional, but relatively insensitive, G2-M cell cycle checkpoint in the VZ-SVZ [Gatz et al., 2011]. This insensitive checkpoint allows cells with low numbers of DSBs to transit from the VZ-SVZ to the intermediate zone (IZ)-cortical plate (CP) region where they then die by apoptosis [Gatz et al., 2011]. Interestingly, work in somatic cells has shown that the DSB-induced G2-M cell cycle checkpoint can be quite an ineffective block to preventing cells with modest levels of DSBs from entering mitosis [Lobrich and Jeggo, 2008; O’Driscoll, 2012].

A growing body of evidence now indicates that the inability to repair DSBs because of defects in NHEJ and/or HRR clearly adversely impacts upon normal neurogenesis often resulting in congenital microcephaly [O’Driscoll and Jeggo, 2008; O’Driscoll, 2012]. There has been much speculation as to what precisely could be the origin of endogenous DSBs during neurogenesis. One suspect, because of the high oxygen consumption and metabolic rate of neurons and their supportive cells, is endogenously generated reactive oxygen species (ROS)-induced oxidative DNA damage [Caldecott, 2008]. This can result in base and ribose sugar backbone damage/modifications, abasic apurinic/apyrimidinic (AP) site formation (i.e., base loss) and even overt single strand break (SSB) formation. These lesions are rapidly dealt with by the coordinated action of the base excision repair (BER) and single strand break repair (SSBR) pathways [Caldecott, 2008]. If an active DNA replication or transcription fork encounters an SSB there is an elevated risk of consequent DSB formation (Fig. 4C).

CONCLUDING REMARKS

Congenital microcephaly has a complex underlying genetic basis, as demonstrated by the selection of defects discussed here. An inability to divide and differentiate effectively resides at the center of this and there are multiple routes through which these processes can be disrupted. The growing number of causative genetic defects described for congenital microcephaly consolidates the importance of mitotic spindle organization and centrosome stability in enabling the execution of precise chromosome segregation during mitosis. Novel causative genetic defects also provide evidence that perturbations of other cell cycle phases are also relevant to normal neurogenesis, for example, DNA replication origin licensing, G1-S entry, DNA synthesis and efficient S-phase progression. The importance and integration of functional cilia-signaling in the context of regulated cell cycle entry and progression cannot be overstated. Finally, defects in multiple components of the complex pathways that control genome stability and integrity via signal transduction and repair processes are additional important contributors to congenital microcephaly.

Without doubt, the advent of exome sequencing has greatly facilitated the identification of novel microcephaly-causing defects, further developing our understanding of the molecular basis of this abnormality. This trend is likely to continue. The consequent challenge in

Origins of Endogenous DNA Breaks in the Developing Brain

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future will be functionally validating and determining the pathogenicity of the multitude of candidate variants derived from such approaches. This issue is compounded by the multigenic occurrence of variants, which is still likely an under-appreciated and under-reported situation at present [Agha et al., 2014]. Functional cellular biology using patient-derived cell lines, model cell systems (siRNA, shRNA, cDNA complementation strategies) and even iPS from patients’ cells, will likely continue to remain the basic cornerstone approach in helping to meet this challenge. The growing use of more cost-effective and rapidly growing model organisms in this area, such as zebrafish, is effective and rapidly growing model system at present [Agha et al., 2014].

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