Autosomal Recessive Primary Microcephaly: Not Just a Small Brain

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Microcephaly or reduced head circumference results from a multitude of abnormal developmental processes affecting brain growth and/or leading to brain atrophy. Autosomal recessive primary microcephaly (MCPH) is the prototype of isolated primary (congenital) microcephaly, affecting predominantly the cerebral cortex. For MCPH, an accelerating number of mutated genes emerge annually, and they are involved in crucial steps of neurogenesis. In this review article, we provide a deeper look into the microcephalic MCPH brain. We explore cytoarchitecture focusing on the cerebral cortex and discuss diverse processes occurring at the level of neural progenitors, early generated and mature neurons, and glial cells. We aim to thereby give an overview of current knowledge in MCPH phenotype and normal brain growth.

Keywords: MCPH genes, microcephaly, brain, intellectual disability, neuronal differentiation, animal models, brain malformation

INTRODUCTION

Microcephaly is clinically defined by a significant reduction of the occipito-frontal head circumference (OFC) of more than two (microcephaly) or three (severe microcephaly) SDs below the mean for a given sex, age, and ethnicity (von der Hagen et al., 2014). The prevalence of microcephaly ranges between 1.5 and 8.7 per 10,000 births in Europe and the United States, respectively (Cragan et al., 2016; Morris et al., 2016). However, 15%–20% of children with developmental delay have microcephaly (Sassaman and Zartler, 1982; Watemberg et al., 2002; Aggarwal et al., 2013). Depending on the time of appearance, microcephaly can be classified as primary/congenital or secondary/postnatal (Passemand et al., 2013; Woods and Parker, 2013; Zaqout et al., 2017). It has been suggested that the primary causes of microcephaly lead to a reduction in the number of generated neurons, while the secondary causes mainly affect the dendritic complexity and synaptic formations (Woods, 2004). Primary microcephaly is by definition present at birth, and it can be caused by environmental and/or genetic factors (Zaqout et al., 2017; Alcantara and O’Driscolll, 2014; Kaindl et al., 2010). Various environmental factors such as infections, toxins, radiation, or alcohol result in primary microcephaly. The recent identification of epidemic infections with the Zika virus as a cause for primary microcephaly has highlighted this rare condition as a key topic in neuroscience to understand normal brain development (Kleber de Oliveira et al., 2016; Subramanian et al., 2019). This condition is an addition to the genetic prototype of isolated primary microcephaly, autosomal recessive primary microcephaly (microcephaly primary hereditary (MCPH)).

MCPH is a group of rare heterogeneous neurodevelopmental disorders characterized by intellectual disability and a significant reduction in the brain volume reflected by a reduction in
the head circumference already at birth (Kaindl et al., 2010; Zaqout et al., 2017; Sleazak et al., 2021). The reduction in brain volume in MCPH cases affects disproportionately the neocortex, though without obvious changes in the cortical organization (Kaindl et al., 2010; Kraemer et al., 2011; Jayaraman et al., 2018). The increasing use of whole-exome sequencing (WES) has uncovered a growing number of novel and disease-causing MCPH variants (Boycott et al., 2013). Simultaneously, further radiological and postmortem studies expand the spectrum of brain malformations reported in individuals with MCPH. The prevalence of MCPH differs from 1:10,000 in populations with a high rate of consanguineous marriage to 1:250,000 in the general population (Van Den Bosch, 1959; Cox et al., 2006). In consanguineous families, most MCPH diagnosed cases reveal homozygous variants in the disease-causing gene. However, compound heterozygous variants are increasingly discovered in MCPH patients, raising the importance of using advanced and accurate diagnosis methods for such cases (Jean et al., 2020).

Currently (December 2021), twenty-eight MCPH-related genes have been identified and tagged sequentially as MCPH1–MCPH28 (MCPH; OMIM phenotypic series: PS251200; Siskos et al., 2021) (Table 1). Still, more genetic loci are expected to exist given the fact that approximately 62% of western Europeans/North Americans and 25% of Indians/Pakistani families diagnosed with MCPH fail to show linkage to any of the MCPH loci (Verloes et al., 1993; Kaindl et al., 2010; Sajid Hussain et al., 2013). Most MCPH gene variants are nonsense, frameshift, or splice site-affecting variants leading to a production of non-functional, truncated proteins (Kaindl et al., 2010; Barbelanne and Tsang, 2014; Jean et al., 2020). Most of the MCPH genes encode centrosomal and/or pericentriolar matrix (PCM) proteins that are, in turn, ubiquitously expressed (Kaindl et al., 2010; Hussain et al., 2013; Barbelanne and Tsang, 2014). It is therefore not surprising to find that many MCPH proteins are involved in centriole biogenesis including organization, maturation, and distribution (Subramanian et al., 2014; Jean et al., 2020). Furthermore, MCPH proteins play crucial roles in microtubule dynamics, mitotic spindle formation, DNA damage responses, Wnt signaling, transcriptional regulation, and cell cycle checkpoint control (Kraemer et al., 2011; Mahmood et al., 2011; Jayaraman et al., 2018; Jean et al., 2020). Disruption of one or more of these functions during cortical neurogenesis adversely affects neuronal progenitor proliferation, differentiation, and survival leading to a severe reduction in the total number of generated neurons reflected by the microcephaly phenotype. Being highly conserved among species, ongoing research on MCPH animal models deems to be an important key for understanding the pathomechanisms behind microcephaly as well as the role of MCPH proteins during normal brain development (Gilbert et al., 2005; Woods et al., 2005; Zaqout et al., 2017). Although microcephaly found in MCPH patients simulates an evolutionary retrogression of the brain size (McHenry, 1994), human brain evolution cannot be attributed solely to the protein-coding sequences of MCPH genes (Pervaiz et al., 2021). Therefore, it has been hypothesized that complex conditional effects of human-specific coding and non-coding regulatory changes in MCPH only assist this evolution process (Pervaiz et al., 2021).

Classically, radiological investigations of patients with MCPH fail to show severe brain malformation except for simplified neocortical gyration. However, the increasing number of reported MCPH-linked mutations reveals that further deformities in brain architecture might occur (Table 2). The overall aim of this review is to explore the various effects of MCPH disease-causing genes on the cytoarchitecture of the cerebral cortex.

### NORMAL CORTICOGENESIS

MCPH arises principally from a decreased production of neurons due to defects in progenitor proliferation, differentiation, and/or apoptosis during critical stages of brain development. Hence, it is important to briefly review the normal process of cortical neurogenesis before discussing the multiple facets of MCPH protein functions in maintaining a smooth running of this process.

Before the neurogenesis journey begins, the neural stem cells represented by neuroepithelial progenitors (NE) at the ventricular zone (VZ) undergo initial expansion in number through symmetrical cell divisions (Homem et al., 2015). Once the antiproliferative gene Tis21 starts to be expressed, NE cells begin to switch from proliferative division to neuronal division (Götz and Huttner, 2005). Simultaneously, NE cells transform gradually into more fate-restricted progenitors known as radial glial cells (RGCs) as an indication for their glial gene expressions (Götz and Huttner, 2005; Mori et al., 2005; Subramanian et al., 2019). RGCs possess apical processes attaching to the ventricular surface and basal processes reaching the basement membrane (future pial surface) (Homem et al., 2015). RGCs expand their number and exhibit a much higher number of asymmetrical cell divisions as compared with NE cells (Subramanian et al., 2019). During cell expansion, RGC nuclei show a characteristic interkinetic nuclear migration (INM) synchronized with the cell cycle phases during proliferation (Kosodo et al., 2011). The RGC nuclei migrate toward the basal side of the developing cortex during G1 phase and remain there during S phase before they migrate apically during G2 phase and proceed with M-phase once they reach the ventricular surface (Kosodo et al., 2011; Miyata et al., 2014). This pattern of migration during early neurogenesis requires functional microtubules and actin filaments (Götz and Huttner, 2005). It has been proposed that INM allows RGC rapid proliferation while maintaining their dense packing and determines cell fate through signaling gradients along their migration pathway (Götz and Huttner, 2005; Baye and Link, 2007; Del Bene et al., 2008). It is therefore very likely to find defects in neurogenesis involving RGC expansion and neuronal cell fate decisions when INM is disrupted (Latasa et al., 2009). Intriguingly, INM shows differences between species and might affect the total number of the generated neurons and thence the brain size (Okamoto et al., 2021).
| Locus | Protein | Gene | Location | OMIM | Model organisms | Generation method | Key findings | Ref |
|-------|---------|------|----------|------|-----------------|-------------------|-------------|-----|
| MCPH1 | Microcephalin 1 | MCPH1 | 8p23.1 | 607117 | Xenopus Fly Rodent | 1. Drosophila in vitro expression cloning (DICE) 2. Deletion of the mcph1 gene by imprecise excision of a P-element 3. Mcph1-knockout mice (deletion of exon 4–5) | 1. DMC1PH1 is a substrate of anaphase-promoting complex (APC) 2. Lethal phenotype due to mitotic arrest, uncoordinated centrosome, and nuclear cycles 3. Premature increase in asymmetrical neural progenitor cell (NPC) divisions, uncoupled mitosis and centrosome cycle, misoriented mitotic spindle alignment 4. Shorter survival rates, defected mitotic chromosome condensation | Hainline et al. (2014) Brunk et al. (2007) Gruber et al. (2011) |
|       |         |      |          |      |                 | 4. Mph1-knockout mice (gene trap) 5. Brit1-knockout mice (gene targeting) | 5. Hypersensitive to γ-irradiation, defective DNA repair, infertility, meiotic defects | Liang et al. (2010) |
| MCPH2 | WD-repeat-containing protein 62 | WDR62 | 19q13.12 | 613583 | Fish Rodent Human cerebral organoid | 1. Morpholino-mediated knockdown of wdr62 2. Wdr62-knockout mice (gene trap) 3. Wdr62-knockout mice (gene trap) 4. SHRNA knockdown of Wdr62 in rats (in utero electroporation) 5. SIRNA knockdown of Wdr62 in mice (in utero electroporation) 6. Wdr62-knockout mice (Wdr62<sup>-/-</sup>; homologous recombination followed by germline transmission) 7. WDR62<sup>-/-</sup> cerebral organoids (mutant Human pluripotent stem cell (hPSC) lines; CRISPR-Cas9) | 1. Reduction in head and eye size, prometaphase delay, increased apoptosis 2. Abnormalities in asymmetric centrosome inheritance, neuronal migration delays, altered neuronal differentiation, prometaphase delay, infertility 3. Mitotic arrest, cell death, reduced thickness of upper cortical neuronal layers, dwarfism 4. Premature differentiation of NPCs, abnormal spindle formation, and mitotic division 5. Spindle orientation defects, delayed mitotic progression, reduced NPC proliferation, increased cell cycle exit 6. Mild microcephaly, reduced NPC number, impaired mitosis, increased apoptosis, increased cilium length 7. Reduced organoid size, reduced outer radial glial cell (oRGC) proliferation, impaired mitosis, increased NPC vertical division, premature differentiation, increased apoptosis, increased cilium length | Novorol et al. (2013) Sgourdou et al. (2017) Chen et al. (2014) Xu et al. (2014) Bogoyevitch et al. (2012) Zhang et al. (2019a) |
| MCPH3 | Cyclin-dependent kinase 5 regulatory subunit-associated protein 2 | CDK5RAP2 | 9q33.2 | 608201 | Fly Rodent Human cerebral organoid | 1. Centrosomin (cnn) knock-out flies (chemical mutagenesis) 2. Hertwig’s anemia mouse (inversion of exon 4 of Cdk5rap2) 3. shRNA knockdown of Cdk5rap2 in mouse (in utero electroporation) 4. RNAi knockdown of CDK5RAP2 (co-electroporating green fluorescent protein (GFP) with shRNA and patient-derived cerebral organoids) | 1. Nuclear cleavage defects, microtubule organization defects, abnormal mitotic spindle formation, Disconnections between centrioles and PCM 2a. Fewer total neurons with special reduction in upper cortical neurons, abnormal spindle formation, and mitotic division, defective mitotic spindle orientation, premature cell cycle exit, increased cell death 2b. Reduced dendritic complexity of layer 2/3 pyramidal neurons, increased spine density, shifted excitation—inhibition balance toward excitation 3. Premature differentiation of NPCs, reduced proliferation, increased cell cycle exit 4. Premature neural differentiation, increased NPC oblique, and vertical divisions | Megraw et al. (1999) Lucas and Raft (2007) Lizaraga et al. (2013) Zaqout et al. (2019) Buchanan et al. (2010) Lancaster et al. (2013) |
| MCPH4 | Kinetochore scaffold 1 | KNL1 | 15q15.1 | 609173 | Rodent | 1. Conditional Knl1 knockout in mouse brain | 1. Impaired NPC proliferation, missetegrated chromosomes, DNA damage and p53 activation, rapid and robust apoptosis | Shi et al. (2019) |
| Locus  | Protein                          | Gene          | Location | OMIM   | Model organisms                  | Key findings                                                                 | Ref                      |
|--------|---------------------------------|---------------|----------|--------|----------------------------------|----------------------------------------------------------------------------|--------------------------|
| MCPH5  | Abnormal spindle-like, microcephaly-associated protein | ASPM          | 1p31.3   | 605481 | Fish                             | 2a. Reduction in head and eye size, prometaphase delay, increased apoptosis  | Novorol et al. (2013)   |
|        |                                 |               |          |        | Ry                               | 2b. Reduction in head and eye size, mitotic arrest, increased apoptosis      | Kim et al. (2011a)       |
|        |                                 |               |          |        | 3. Mutagenesis (x-irradiation)    | 2. High-mitotic index, metaphase arrest, mitotic and meiotic non-disjunction, hemi-spindles formation | Gonzalez et al. (1990)   |
|        |                                 |               |          |        | 4. Mutagenesis (recombinant chromosomes) | 4a. Arrested mitotic cycle at metaphase, high frequency of polyploid cells, defected sex chromosome disjunction | Ripoll et al. (1985)    |
|        |                                 |               |          |        |                                  | 4b. Disrupted microtubule-organizing centers, failure of cytokinesis         | Riparbelli et al. (2002) |
|        |                                 |               |          |        | Rodent                           | 5. Centrosome detachment, altered cleavage plane orientation, increased non-NE fate, increased neuron-like fate | Fish et al. (2008)       |
|        |                                 |               |          |        |                                  | 6. Mild microcephaly, midbody localization defects, Major germine defects    | Pulvers et al. (2010)    |
|        |                                 |               |          |        | 7. Aspm-knockout mouse (removal of exons 2 and 3) | 7. Much thicker layer I and thinner layer VI cortical neurons, aberrant expression of Tbr1 and Satb2 in the subplate | Fujimori et al. (2014)   |
|        |                                 |               |          |        | Ferret                           | 8. Aspm germline knockout ferret                                           | Johnson et al. (2018)    |
|        |                                 |               |          |        | Human cerebral organoid           | 9. RNAi knockdown of ASPM (co-electroporating GFP with shRNAs) and patient-derived cerebral organs | Li et al. (2017)         |
| MCPH6  | Centromeric protein J            | CENPJ         | 13q12.2  | 609279 | Rodent                           | 1. Morphologically normal, no detectable centrioles or centrosomes, lack of cilia, early postnatal lethality | Basto et al. (2006)      |
|        |                                 |               |          |        |                                  | 2. Centriole loss, reduced binding affinity of the DSas-4 and Ana2 interaction | Cottey et al. (2013)     |
|        |                                 |               |          |        | 3. Conditional Cenpj knockout in mouse brain | 4. Microcephaly, dwarfism, skeletal abnormalities, increased levels of DNA damage, and apoptosis | McIntyre et al. (2012)   |
|        |                                 |               |          |        | 4. Cenpj-knockout mice (cassette insertion between exons 4 and 5) | 3a. Long cilia and abnormal cilia disassembly, uncompleted cell division, reduced cell proliferation, increased apoptosis | Ding et al. (2019)      |
| MCPH7  | SCL/TAL1-interrupting locus protein | STIL          | 1p33     | 181590 | Rodent                           | 1. Reduction in head and eye size, prometaphase delay, increased apoptosis  | Novorol et al. (2013)   |
|        |                                 |               |          |        |                                  | 2. Embryonic lethality, high mitotic index, highly disorganized mitotic spindles, lack of centrosomes, increased apoptosis | Plaef et al. (2007)      |
|        |                                 |               |          |        | 3. Stil-knockout mouse (removal of exons 3–5) | 3a. Embryonic lethality, defected neural folding, randomization of left-right asymmetry, impaired response to Sonic 3a. Hedgehog (SHH) signaling Lack of centrioles and primary cilia | Izraeli et al. (1999)    |
|        |                                 |               |          |        | Protopzoa                        | 2. Cassiopea (csp) mutant zebrabfish                                       | Imaizumi et al. (2008)   |
|        |                                 |               |          |        | 3. SIRNA knockdown of bld10 in Paramaecium | 2. Basal-body defects                                                      | Hiraki et al. (2007)     |
|        |                                 |               |          |        | 4. bld10-knockout flies (transposon insertion) | 3. Abnormal basal body assembly                                            | Jerka-Dziadosz et al. (2010) |
|        |                                 |               |          |        | Fish                             | 4a. Disrupted localization of the inner and outer centriole components      | Roug et al. (2012)       |
|        |                                 |               |          |        | 2. bld10 null mutants (series of truncations) | 4b. Short centrioles and basal bodies, immotile sperm, infertility         | Mottier-Pave and Megraw, (2009) |
|        |                                 |               |          |        | 3. Abnormal basal body assembly   | 4c. Lack of singlet microtubules and disassembly of central microtubule pair | Carvalho-Santos et al. (2012) |
| MCPH8  | Centrosomal protein 135 kD       | CEP135        | 4q12     | 611423 | Alga                             | 1. Lack of basal bodies, disorganized mitotic spindles and cytoplasmic microtubules, abnormal cell division, and slow growth | Matsuura et al. (2004)   |
|        |                                 |               |          |        |                                  | 2. bld10 null mutants (series of truncations) | 2. Basal-body defects | Hiraki et al. (2007) |
|        |                                 |               |          |        | Protozoa                        | 3. Abnormal basal body assembly                                            | Jerka-Dziadosz et al. (2010) |
|        |                                 |               |          |        | 4. bld10-knockout flies (transposon insertion) | 4a. Disrupted localization of the inner and outer centriole components     | Roug et al. (2012)       |

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| Locus | Protein | Gene | Location | OMIM | Model organisms | Generation method | Key findings | Ref |
|-------|---------|------|----------|------|-----------------|-------------------|--------------|-----|
| MCPH9 | Centrosomal protein 152kD | CEP152 | 15q21.1 | 613529 | Fly | 5. RNAi knockdown in bld10 mutant flies | 5. Spindle alignment and centrosome segregation defects, perturbed centrosome asymmetry, mispositioned microtubule-organizing centers (MTOCs) | Singh et al. (2014) |
|       |                                   |       |          |      |                 | 1. astereless (asl1, asl2, asl3) mutant flies (P-element-mediated transformation) | 1. Defect in PCM stabilization and centrosome segregation, reduced microtubule nucleation, severe defects in mitotic spindle assembly | Varmark et al. (2007) |
|       |                                   |       |          |      |                 | 2. astereless (asl1, asl2, asl3) mutant flies (P-element-mediated transformation) | 2. Lack of centrioles, basal bodies, and cilia | Blachon et al. (2008) |
|       |                                   |       |          |      | Fish | 3. Morpholino-mediated knockdown of cep152 | 3. Curly tail (ciliary defects) | |
| MCPH10 | Zinc finger protein 335 | ZNF335 | 20q13.12 | 610827 | Rodent | 1. Znf335-knockout mice (gene trap) | 1. Early embryonic lethality | Yang et al. (2012) |
|       |                                   |       |          |      |                 | 2. Conditional Znf335 knockout in mouse brain (flanked promoter and exon1/2) | 2. Lack all cortical structure and cortical neurons, enlarged ventricles | |
|       |                                   |       |          |      |                 | 3. shRNA knockdown of Znf335 in mice (in utero electroporation) | 3. Disrupted NPC proliferation, premature differentiation, abnormal cell Rgs orientation, disorganized dendritic outgrowth, lack of apical dendritic process | |
|       |                                   |       |          |      | Fish | 3. Morpholino-mediated knockdown of cep152 | 3. Curly tail (ciliary defects) | |
| MCPH11 | Polyhomeotic-like 1 protein | PHTC1 | 12p13.31 | 602978 | N/A | 1. Cdk6 knockout mice (removal of 1st coding exon) | 1. Develop normally, slight hematopoiesis defect | Malumbres et al. (2004) |
| MCPH12 | Cyclin-dependent kinase 6 | CDK6 | 7p21.2 | 603368 | Rodent | 1. Cdk6-knockout mice | 1. Develop normally, slight hematopoiesis defect | Malumbres et al. (2004) |
| MCPH13 | Centromeric protein E | CENPE | 4q24 | 117143 | Fly | 1. Mutations in cnp-em gene (P-element-mediated disruption) | 1. Embryonic lethality, defects in metaphase chromosome alignment | Yuzi et al. (2000) |
|       |                                   |       |          |      | Rodent | 2. Conditional and complete Cenpe gene disruptions in mouse | 2. Early developmental arrest, mitotic chromosome misalignment | Putkey et al. (2002) |
| MCPH14 | SAS-6 centriolar assembly protein | SAS6 | 1p21.2 | 606321 | Worn | 1. RNAi knockdown of sas-6 in Caenorhabditis elegans | 1. Abnormal centrosome duplication cycle | Leidel et al. (2005) |
|       |                                   |       |          |      | Fish | 2. cellular atoll (cea) mutant zebrafish | 2. Defects in nuclear division, mitotic spindle formation, and centrosome duplication | Yabe et al. (2007) |
|       |                                   |       |          |      | Fly | 3. sas-6-knockout flies | 3. Lack of cohesion between centrioles | Rodrigues-Martins et al. (2007) |
| MCPH15 | Major facilitator superfamily domain-containing protein 2A | MFSD2A | 1p34.2 | 614397 | Rodent | 1. Morpholino-mediated knockdown of mfds2a | 1. Embryonic lethality before neural maturation, disrupted blood-brain barrier (BBB) integrity | Guerez-Gambboa et al. (2015) |
|       |                                   |       |          |      | Fish | 2. Mfsd2a-knockout mice (gene targeting) | 2a. Increased plasma lysophosphatidylcholine (LPC) 2b. Reduced body weight and length, increased energy expenditure, increased BAT β-oxidation, increased ataxic movement | Berger et al. (2012) |
|       |                                   |       |          |      |       | 3. Mfsd2a-knockout mice (gene trap) | 3. Leaky BBB, dramatic increase in central nervous system (CNS) endothelial cell-vascular transcytosis | Nguyen et al. (2014) |
|       |                                   |       |          |      |       | 4. Mfsd2a-endothelial-specific knockout mice | 4. Reduced neuronal arborization and decreased dendrite length | Chan et al. (2018) |
| MCPH16 | ANKLE2 | 12q24.33 | 616082 | N/A | Worn | 1. and75 mutant worms (missense mutation in the kem-4L open reading) | 1. Abnormal nuclear morphology | Asencio et al. (2012) |

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| Locus | Protein | Gene | Location | OMIM | Model organisms | Generation method | Key findings | Ref |
|-------|---------|------|----------|------|----------------|------------------|-------------|-----|
| MCPH17 | Citron rho-interacting serine/threonine kinase | CIT | 12q24.23 | 605629 | Fly | dck2 knockout (EMS chemical mutagenesis) | 1. Defective in both neuroblast and spermatocyte cytokinesis, abnormal F actin and anillin rings | Naim et al. (2004) |
| | | | | | | | 2. Reduced brain size, dygenesis of neocortex, hippocampus, cerebellum, and retina, increased apoptosis, seizures, tremor, impaired coordination, and premature death | Roberts et al. (2000) |
| | | | | | | 2b. Reduced brain size, cytokinesis failure, binucleated cells | Sarkisian et al. (2002) |
| | | | | | | 2c. Decrease in the number of interneurons, hypertrophied soma and dendritic arbors of interneurons, increased apoptosis, cytokinesis failure, binucleated cells | Sarkisian et al. (2001) |
| | | | | | | 3. Depletion of microglia in the olfactory bulb, hippocampus, and cerebellum, increased apoptosis, abnormal cytokinesis, tremor and severe ataxia, reduced life span due to lethal epilepsy | Di Cunto et al. (2000) |
| MCPH18 | WD repeat and FYVE domain-containing 3 | WDFY3 | 4q21.23 | 617485 | Fly | 1. Blue cheese (bchs) knockout flies (P-element-mediated disruption) | 1a. Extensive neurodegeneration, premature adult death, formation of protein aggregates, neuronal apoptosis | Finley et al. (2003) |
| | | | | | | | 1b. Morphological abnormalities in motor neurons, increased apoptosis, reduced endolysosomal vesicles mobility | Lim and Kraut, (2009) |
| | | | | | | 2. hALFY mutant flies (single point mutation) | Kadir et al. (2018) |
| | | | | | | 3. Disconnected mutant mice (Wdfy3del512); spontaneous nonsense mutation in exon 59 of 67 of Wdfy3) | Orosco et al. (2014) |
| | | | | | | 3. Perinatal lethality, enlarged frontal cortical aspects, tangential expansion but lateral thinning of the neocortical neuroepithelium, focal cortical dysplasia, abnormal ganglionic eminences, enlarged ventricles, reduction in the size of the olfactory bulbs | Napoli et al. (2018) |
| | | | | | | 4. Wdfy3-knockout mice (Wdfy3del12; gene targeting) | Napoli et al. (2021) |
| | | | | | | 5. Wdfy3-haploinsufficiency mice (Wdfy3del12; gene targeting) | Le Duc et al. (2019) |
| | | | | | | 5b. Decreased mitochondrial localization at synaptic terminals, decreased synaptic density, defective brain glycophagy, cerebellar hypoplasia | Napoli et al. (2021) |
| | | | | | | 5c. Macrocephaly, defects in motor coordination and associative learning, downregulation of the Wnt signaling pathway | Le Duc et al. (2019) |

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### TABLE 1 (Continued) List of microcephaly primary hereditary (MCPH) genes and related animal/organism models.

| Locus | Protein | Gene | Location | OMIM | Model organisms | Generation method | Key findings | Ref |
|-------|---------|------|----------|------|----------------|------------------|--------------|-----|
| MCPH20 | Kinesin family member 14 | KIF14 | 1p32.1 | 611279 | Fish | 1. kif14 mutant zebrafish (sa241465 mutant line Ketteleborough et al. (2013))<br>2. Mutations in the Kif14 gene<br>3. Laggard (lag) mutant mice (spontaneous mutation, disruption within exon 5 of Kif14) | 1. Microcephaly, eye defects, body curvature, cardiac edema, glomerular cysts, high mitotic index, ciliopathy-like phenotypes<br>2. Semi-lethality, abnormal cell cycle progression, failure of cytokinesis, rough eyes, missing bristles, abnormal abdominal cuticles<br>3. Small head, tremor, ataxic gait, severe hypomyelination in the CNS, disrupted cytoarchitecture in the neocortex, hippocampus, and cerebellar cortex, increased apoptosis during late neurogenesis | Reilly et al. (2019) |
| MCPH21 | Non-SMC condensin I complex subunit D2 | NCAPD2 | 12p13.31 | 615638 | Rodent | 1. Ncaph2 condensin II mutant mice (Ncaph2<sup>lox/lox</sup>; ENu chemical mutagenesis)<br>2. Morpholino-mediated knockdown of nup107, nup85, or nup133 | 1. Isolated T-lymphocyte developmental defect, reduced brain size, increased anaphase DNA bridge formation in apical NPCs, impaired chromosome segregation<br>2. Developmental anomalies, early lethality | Gosling et al. (2007), Martin et al. (2016) |
| MCPH22 | Non-SMC condensin II complex subunit D3 | NCAPD3 | 11q25 | 609276 | Rodent | 1. Morphinol-mediated knockdown of nup107, nup85, or nup133<br>2. nup107 or nup85 knockout in zebrafish (CRISPR-Cas9) | 1. Disrupted glomerulogenesis<br>2. Developmental anomalies, early lethality | Braun et al. (2018) |
| MCPH23 | Non-SMC condensin I complex subunit H | NCAPH | 2q11.2 | 602332 | Xenopus | 1. Disrupted glomerulogenesis | 1. Microcephaly, decreased neuronal density, lack of upper cortical layers, nuclear blebs in embryonic neurons, increased apoptosis, asymmetric distribution of Lmnb2<br>2. Very small cortex, low neuronal density, absence of cerebellar foliation, impaired neuronal migration, altered cleavage plane orientation, increased cell cycle exit<br>3. Defects in lungs, diaphragms, and brains, thinner cerebral cortex, disorganized cortical layers, impaired neuronal migration, altered cleavage plane orientation, increased cell cycle exit<br>4. Perinatal lethality, impaired neuronal migration, layering defects in the cerebral cortex and hippocampus, absence of cerebellar foliation, absence of a discrete Purkinje cell layer, elongated nuclei in cortical neurons | Vergnes et al. (2004), Coffinier et al. (2011), Coffinier et al. (2010), Coffinier et al. (2011) |
| MCPH24 | Nucleoporin 37 | NUP37 | 12q23.2 | 600264 | Xenopus | 1. Disrupted glomerulogenesis<br>2. Developmental anomalies, early lethality | Perez et al. (2019), Cuenca et al. (2019) |
| MCPH25 | Trafficking protein particle complex subunit 14 | TRAPP14 | 7q22.1 | 618350 | Fish | 1. Map17 knockout in zebrafish (CRISPR-Cas9)<br>2. Morpholino-mediated knockdown of c7orf43 | 1. Microcephaly, decreased neuronal proliferation<br>2. Curved bodies, small eyes, ciliogenesis defects | Fujikura et al. (2013), Perez et al. (2019), Cuenca et al. (2019) |
| MCPH26 | Lamin B1 | LMNB1 | 5q32.3 | 150340 | Rodent | 1. Lmnb<sup>B1</sup>-knockout mice (Lmnb<sup>B1</sup><sup>fl/fl</sup>; gene trap)<br>2. Forebrain-specific Lmnb<sup>B1</sup>-knockout mice (Emx1-Cre Lmnb<sup>B1</sup><sup>fl/fl</sup>)<br>3. Lmnb<sup>B1</sup>/Lmnb<sup>B2</sup>-knockout mice (Lmnb<sup>B1</sup><sup>−/−</sup>Lmnb<sup>B2</sup><sup>−/−</sup>; gene targeting)<br>4. Lmnb<sup>B2</sup>-knockout mice (Lmnb<sup>B2</sup><sup>−/−</sup>; gene targeting) | 1a. Perinatal lethality, abnormal lung development and bone ossification, abnormal skull and skull shape<br>1b. Perinatal lethality, absence of the cortical layering with reduced number of neurons, absence of lamination in the hippocampus, absence of cerebellar foliation, impaired neuronal migration, reduced NPC proliferation, solitary nuclear bleb in cortical neurons<br>2. Very small cortex, low neuronal density, lack of upper cortical layers, nuclear blebs in embryonic neurons, nuclear membrane ruptures, increased apoptosis, asymmetric distribution of Lmnb2<br>3. Defects in lungs, diaphragms, and brains, thinner cerebral cortex, disorganized cortical layers, impaired neuronal migration, altered cleavage plane orientation, increased cell cycle exit<br>4. Perinatal lethality, impaired neuronal migration, layering defects in the cerebral cortex and hippocampus, absence of cerebellar foliation, absence of a discrete Purkinje cell layer, elongated nuclei in cortical neurons | Coffinier et al. (2011), Coffinier et al. (2010), Coffinier et al. (2011), Coffinier et al. (2011) |

(Continued on following page)
Asymmetrical division of an RGC at the ventricular surface generates a self-renewing RG daughter cell and either a postmitotic neuron or a basal progenitor (intermediate progenitor cell (IPC)) (Homem et al., 2015; Subramanian et al., 2019). It has been earlier believed that the asymmetrical division of RGCs is principally driven by a change in mitotic spindle positions leading to a shift of the cleavage plane orientation from perpendicular (vertical) to parallel (horizontal) relative to the ventricular surface (Chenn and McConnell, 1995; Zhong et al., 1996; Kosodo et al., 2004; Fietz and Huttner, 2011; Homem et al., 2015). However, further investigations revealed that the rate of asymmetrical divisions in RGCs is not necessarily altered by the orientation of the cleavage plane (Morin et al., 2007; Konno et al., 2008; Postiglione et al., 2011). The asymmetrical RGC fate might be affected by inheriting centrioles with different maturity and primary cilium (Wang et al., 2009; Goetz and Anderson, 2010; Paridaen et al., 2013). It has been also shown that alterations in RGC cycle length control the shift from self-renewing divisions to neurogenic divisions (Calegari and Huttner, 2003; Calegari et al., 2005; Pilaz et al., 2009; Arai et al., 2011). Furthermore, it has been proposed that Notch signaling triggers neurogenic cell fate either by its distinct apicobasal gradient during INM or through asymmetric inheritance of endosomes positive for Sara (Smad anchor for receptor activation) (Del Bene et al., 2008; Nerli et al., 2020). This latter is achieved by targeting signaling endosomes to the central spindle by the action of plus-end kinesin motor (Klp98A) (Derivery et al., 2015).

Unlike RGCs, IPCs lack the connection with the ventricular surface and settle mainly in the subventricular zone (SVZ) basal to the VZ (Rakic, 2009; Homem et al., 2015; Subramanian et al., 2019; Heide and Huttner, 2021). IPCs undergo some proliferative divisions and terminate by generating two cortical neurons (Noctor et al., 2004; Pontious et al., 2008; Rakic, 2009). Asymmetrical divisions of RGCs in many mammals, especially in primates, yield an additional generation of a special type of basal progenitors known as basal RGCs (outer RGCs (oRGCs)) (Homem et al., 2015). Compared with IPCs, the oRGCs show a much higher proliferative capacity, which amplifies the total number of generated neurons and contribute to the characteristic folded cerebral cortex observed in primates, especially in humans (Reillo et al., 2011; Fietz et al., 2010; Hansen et al., 2010; Betizeau et al., 2013; Heide and Huttner, 2021). The newly established 3D in vitro human brain organoid model exhibits a considerable number of oRGCs (Lancaster et al., 2013; Zhang et al., 2019a).

Postmitotic cortical neurons are generated from both VZ and SVZ neural progenitors in an inside-out manner by which later-born neurons (superficial layers IV–VI) bypass earlier-born neurons (deep layers VI–V) (Molyneaux et al., 2007; Leone et al., 2008). This pattern of neural generation is under spatiotemporal, cell cycle, and competency precise controls of neural progenitor fates (Kohwi and Doe, 2013). Eventually, six neural layers are created in the developing cerebral cortex, and the neurons start the formation of their distinctive dendrites, axons, and functional synapses. The fully developed cortical network contains 80% glutamatergic excitatory neurons produced by VZ and SVZ neural progenitors located in the dorsal telencephalon and 20% GABAergic inhibitory neurons that originated from the medial and caudal ganglionic eminence (Marín, 2013; Costa and Müller, 2014). The terminal number of generated cortical neurons is affected not only by the original number of neural progenitors but also by their starting and ending proliferation points and their cell lineage (Homem et al., 2015).

At the final stage of neurogenesis, RGCs lose their neuronal lineage and the connection with the apical surface switching to glial cell generators (Malatesta et al., 2000; Qian et al., 2000). Cortical astrocytes are firstly detected followed by oligodendrocytes, and the number of both glial cells is hugely expanded postnatally (Qian et al., 2000). Glial cells induce the development of white matter and axonal outgrowth by producing myelin and forming astrocytic branches (Subramanian et al., 2019). Taken together, forming a normal cerebral cortex requires highly organized spatiotemporal control for the neural progenitor populations to generate different neuronal and glial subtypes. Any defect during this process can lead to a major impact on brain development.

### BRAIN PHENOTYPE IN INDIVIDUALS WITH MICROCEPHALY PRIMARY HEREDITARY

The morphological changes in the brain structure of MCPH individuals have been mainly identified by radiological studies. Most MCPH cases show a reduction in brain volume associated with a simplified neocortical gyration pattern. However, the increased number of reported mutations and the ongoing neuroimaging of MCPH individuals reveal further brain malformations (Table 2). Some of these structural changes point toward the causative MCPH gene (e.g., the association between malformations of basal ganglia and mutations in gene encoding zinc finger-335 protein (ZNF335; MCPH10) (Sato et al.,

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**TABLE 1 | (Continued) List of microcephaly primary hereditary (MCPH) genes and related animal/organism models.**

| Locus      | Protein                        | Gene          | Location | OMIM     | Model organisms | Key findings                                                                 | Ref                     |
|------------|--------------------------------|---------------|----------|----------|-----------------|-------------------------------------------------------------------------------|------------------------|
| MCPH28     | Ribosomal RNA processing 7 homolog A | RRP7A         | 22q13.2  | 619449   | Fish            | 1. mp7a mutant zebrafish (sa11429 mutant line (Kettleborough et al., 2013))   | Fanoq et al. (2020)    |
|            |                                |               |          |          |                 | 5. Forebrain-specific Lmnb2− knockout mice (Emx1-Cre Lmnb2fl/fl)              | Coffnier et al. (2011)  |

Continued...
## TABLE 2 | Brain malformations associated with microcephaly primary hereditary (MCPH) additional to microcephaly.

| Locus | Gene | Brain malformations | Key findings | Refs |
|-------|------|---------------------|--------------|------|
| MCPH1 | MCPH1 | Corpus callosum abnormalities | Mild hypoplasia | Hosseini et al. (2012) |
|       |       |                     | Agenesis of the genu | Neftiu et al. (2002) |
|       |       | Pachygyria | + | Neftiu et al. (2002), Trimbom et al. (2004) |
|       |       |                     | Thinning of fronto-parietal and temporal gyri | Hosseini et al. (2012) |
|       |       | Heterotopia | Neopallial neuronal heterotopia (ventricular, infratentorial, and subependymal) | Neftiu et al. (2002), Trimbom et al. (2004) |
|       |       | Frontal lobe hypoplasia | + | Trimbom et al. (2004) |
|       |       | Ventricular system abnormalities | Dilatation of lateral ventricles (dorsal and temporal), dilated external liquor space | Neftiu et al. (2002) |
|       |       | Myelination/white matter abnormalities | Slight retardation of myelination of cerebral medullary layer | Neftiu et al. (2002) |
|       |       |                      | Diffuse pachgyria, Severe pachgyria | Farag et al. (2010) |
|       |       |                      | Widespread polymicrogyria | Neftiu et al. (2002), Bhat et al. (2011), Bastaki et al. (2016), Slezak et al. (2021) |
|       |       |                      | Polymicrogyria in the right hemisphere | Sgourdou et al. (2017), Zombor et al. (2019) |
|       |       |                      | Bilateral polymicrogyria | Sgourdou et al. (2017), Zombor et al. (2019) |
|       |       |                      | Extensive polymicrogyria in the left cerebral hemisphere | Sgourdou et al. (2017), Zombor et al. (2019) |
|       |       |                      | Extensive areas of polymicrogyria in the right frontal lobe | Sgourdou et al. (2017), Zombor et al. (2019) |
|       |       | Abnormal Sylvian fissure | Under-opercularization | Bilgävar et al. (2010), Poulton et al. (2014) |
|       |       |                      | Widened Sylvian fissure | Banerjee et al. (2016) |
|       |       |                      | Open-Sylvian fissures | Banerjee et al. (2016) |
|       |       | Schizencephaly | + | Banerjee et al. (2016) |
|       |       |                      | Narrow right temporoparietal open lip schizencephaly, right temporoparietal open lip schizencephaly | Banerjee et al. (2016) |
|       |       |                      | Suspected schizencephaly in the right parietal lobe | Banerjee et al. (2016) |
|       |       |                      | Closed schizencephaly in the right cerebral hemisphere | Banerjee et al. (2016) |
|       |       | Heterotopia | Subcortical heterotopia, bilateral band heterotopia in the posterior frontal and parietal lobes | Banerjee et al. (2016) |
|       |       |                      | Band heterotopias | Banerjee et al. (2016) |
|       |       | Hemispherical asymmetry | Asymmetric microcephalic hemispheres | Bilgävar et al. (2010), Zambor et al. (2019) |
|       |       |                      | Volume loss worse on the left than the right cerebral hemisphere | Banerjee et al. (2016) |
|       |       |                      | Volume loss worse on the right than the left cerebral hemisphere | Banerjee et al. (2016) |
|       |       | Frontal lobe hypoplasia | + | Banerjee et al. (2016) |
|       |       | Hippocampal abnormalities | Dysmorphic | Banerjee et al. (2016) |
|       |       |                      | Simplified hippocampal gyration | Banerjee et al. (2016) |
|       |       | Infra- and supratentorial abnormalities | Unilateral cerebellar hypoplasia, unilateral brainstem atrophy | Banerjee et al. (2016) |
|       |       |                      | Cerebellar hypoplasia | Banerjee et al. (2016) |
|       |       | Ventricular system abnormalities | Dilated ventricles | Banerjee et al. (2016) |
|       |       |                      | Prominent extra-axial cerebrospinal fluid | Banerjee et al. (2016) |
|       |       |                      | Light expansion of bilateral brain ventricles, obvious expansion of the fourth ventricle | Banerjee et al. (2016) |
|       |       |                      | Asymmetrical enlargement of the ventricles, dilated Virchow-Robin spaces | Banerjee et al. (2016) |
|       |       |                      | Posterior horn-dominant enlargement of the lateral ventricles | Banerjee et al. (2016) |
|       |       | Thickened gray matter | + | Banerjee et al. (2016) |
|       |       |                      | Diffuse thickening of the cortex | Banerjee et al. (2016) |
|       |       |                      | Mildly thickened cortex (1.5 mm) | Banerjee et al. (2016) |
|       |       | Blurred gray-white matter junction | Loss of gray-white junction | Banerjee et al. (2016) |
|       |       |                      | Ill-defined gray and white matter pattern | Banerjee et al. (2016) |
|       |       |                      | Indistinct gray-white matter border in certain areas | Banerjee et al. (2016) |
|       |       |                      | Gray-white matter blurring involving the left parietooccipital cortex | Banerjee et al. (2016) |

(Continued on following page)
| Locus | Gene | Brain malformations | Key findings | Ref |
|-------|------|---------------------|--------------|-----|
| MCPH3 | CDK5RAP2 | Corpus callosum abnormalities | Hypoplasia | Abbas et al. (2016) |
|       |       |                     | Agenesia/hypogenesis | Issa et al. (2013) |
|       |       | Hypothalamic abnormalities | Infrahypothalamic adhesion | Nassar et al. (2003) |
|       |       |                  | + | Abbas et al. (2016) |
|       |       | Myelination/white matter abnormalities | Bilateral enhancement in the white matter (white matter disorder) | |
| MCPH4 | KNL1 | Infrafantorial abnormalities | Cerebellar vermis hypoplasia | Saadi et al. (2016) |
|       |       | Ventricular system abnormalities | Wide cyst in the posterior fossa communicating with an expanded fourth ventricle | |
| MCPH5 | ASPM | Corpus callosum abnormalities | Thin corpus callosum | Passemand et al. (2009), Saadi et al. (2009), Létard et al. (2018) |
|       |       | Agenesia of splenium | | Passemand et al. (2009) |
|       |       | Agenesia of rostrum | Partial agenesis | Abd-El-Hamid et al. (2016) |
|       |       | Hypoplasia | | | Létard et al. (2018) |
|       |       | Partial agenesis | | | Shielem et al. (2019) |
|       |       | Pachygyria | Temporal pachygyria | Saadi et al. (2009) |
|       |       | Polymicrogyria | Extensive unilateral parietal polymicrogyria from the frontal pole to the occipital pole | Passemand et al. (2009) |
|       |       | Polymicrogyria | Extensive bilateral posterior polymicrogyria | Létard et al. (2018) |
|       |       | Frontal lobe hypoplasia | Severe hypoplasia of the frontal lobes | Saadi et al. (2009) |
|       |       | Frontal lobes are short and hypoplastic | | Desir et al. (2008) |
|       |       | Infrafantorial abnormalities | Mild asymmetric cerebellar hypoplasia | Passemand et al. (2009) |
|       |       | Cerebellar vermis and/or hemispheres hypoplasia | | Abd-El-Hamid et al. (2016), Létard et al. (2018) |
|       |       | Elongated superior cerebellar peduncles | | Létard et al. (2018) |
|       |       | Relatively small pons | | Abd-El-Hamid et al. (2016) |
|       |       | Thin brain stem | | Aniss et al. (2013), Létard et al. (2018) |
|       |       | Ventricular system abnormalities | Occipital horns of the lateral ventricles enlarged | Passemand et al. (2009) |
|       |       | Dysembryopic frontal ventricles | | Passemand et al. (2009) |
|       |       | Enlarged lateral ventricles and occipitomegaly | | Abd-El-Hamid et al. (2016) |
|       |       | Large pachygyric cyst communicating with lateral ventricle | | |
|       |       | Small midline cyst | | Létard et al. (2018) |
|       |       | Ventricular enlargement | | |
|       |       | Arachnoid cyst in the posterior fossa | | |
|       |       | Enlarged V-V-R spaces | | |
|       |       | Enlarged subarachnoid spaces, mega cisterna magna | | |
|       |       | Myelination/white matter abnormalities | Reduced white matter | Abd-El-Hamid et al. (2016) |
|       |       | Myelination delay | | Létard et al. (2018) |
| MCPH6 | CENPJ | NA | Partial agenesis of the corpus callosum | Mouden et al. (2019), Shielem et al. (2019) |
| MCPH7 | STIL | Corpus callosum abnormalities | Short dysmorphic corpus callosum | Kiasar et al. (2016) |
|       |       | Hypoprosopagnosia | Lobar hypoprosopagnosia | Kiasar et al. (2016), Mouden et al. (2019) |
|       |       | Frontal lobe hypoplasia | Disproportionately short frontal lobes, continuity of the right and left frontal lobes at the level of the basal ganglia and lateral ventricles | Kiasar et al. (2016) |
|       |       | Infrafantorial abnormalities | Atrophy of the vermis | Cheng et al. (2009) |
|       |       | Ventricular system abnormalities | Cerebellar hypoplasia, hypoplasia | Mouled et al. (2019) |
|       |       | Absence of ventricular frontal horns | | Mouled et al. (2019) |
|       |       | Absence of occipital lobe and a large unilateral temporal and occipital fluid cavity communicating | | Mouden et al. (2013) |
|       |       | Small third ventricle, enlarged lateral ventricles posteriorly | | Kiasar et al. (2015) |
|       |       | Large pachygyric cyst replacing most of the posterior right hemisphere | | Cheng et al. (2009) |
|       |       | Dilatation of the fourth ventricle | | Cheng et al. (2009) |
|       |       | Blurred gray-white matter junction | | Kiasar et al. (2015) |
|       |       | Myelination/white matter abnormalities | Diffuse severe reduction of the white matter volume | |
| MCPH8 | CEP135 | Heterotopia | Bilateral nodular heterotopia in the parieto-occipital regions | Bamborschke et al. (2010) |
| MCPH9 | CEP152 | Corpus callosum abnormalities | Severe hypogenesis | Shielem et al. (2019) |

(Continued on following page)
| Locus   | Gene   | Brain malformations associated with microcephaly primary hereditary (MCPH) additional to microcephaly. | Key findings                                                                                     |
|---------|--------|----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| MCPH10  | PA7C11 | Polymicrogyria + Ventricular system abnormalities                                                             | Inter-hemispheric cyst at left aspect of the falx continuous with the third ventricle                           |
| MCPH11  | ZNF335 | Corpus callosum anomalies                                                                                      | Corpus callosum abnormalities                                                                                                           |
| MCPH12  | PHF1   | Lissencephaly/agyria                                                                                           | Anterior agyria and a posterior simplified gyral pattern                                          |
| MCPH13  | CRYPE  | Infratentorial abnormalities                                                                                    | Absent basal ganglia                                                                        |
| MCPH14  | SA09   | Lissencephaly/agyria                                                                                           | Diffuse severely simplified gyral pattern with virtually no gyri over the frontal lobe        |
| MCPH15  | MRPLA  | Corpus callosum abnormalities                                                                                    | Partial agenesis of the corpus callosum                                                         |
| MCPH16  | ANPEZ  | Infratentorial abnormalities                                                                                    | No gyral or sulcal development                                                                                                      |
| MCPH17  | CYF    | Lissencephaly/agyria                                                                                           | Partial agenesis of the corpus callosum                                                         |
| MCPH18  | WFP17  | Corpus callosum abnormalities                                                                                    | Partial agenesis of the corpus callosum                                                         |
| MCPH19  | O2M2   | Corpus callosum abnormalities                                                                                    | Partial agenesis of the corpus callosum                                                        |
| MCPH20  | HN4    | Lissencephaly/agyria                                                                                           | Partial agenesis of the corpus callosum                                                         |

*Continued on following page*
The increasing number of reported brain malformations in MCPH individuals widens its pathogenesis spectrum. This indicates that the disruption of MCPH proteins not only is affecting the generation of neurons but could additionally affect neuronal differentiation, migration, dendritic and axonal outgrowth, and synaptogenesis. This is understandable given the fact that MCPH proteins are highly expressed in various neuroprogenitor organelles, especially the centrosome. In the following sections, we will discuss the consequences of MCPH mutations on brain development.

**ACCIDENTS DURING THE BRAIN DEVELOPMENT JOURNEY IN MICROCEPHALY PRIMARY HEREDITARY**

Studying the molecular mechanisms behind the pathogenesis of MCPH is very limited in humans. In fact, MCPH genes are highly conserved among different species (Woods et al., 2005; Gilbert et al., 2005), and this led to the discovery of several MCPH animal models mimicking the human phenotype (Table 1). Therefore, most of our current knowledge on the role of MCPH proteins in brain development is enriched through extensive studies on MCPH animal models. However, the pronounced difference of the human brain compared with most of the studied MCPH animal models establishes a new research direction toward 3D in vitro human brain organoid systems in studying the pathogenesis of microcephaly (Muzio and Consalez, 2013; Gabriel et al., 2020). The remarkable presence of oRGCs in this model opens the door for deeper insights into their role during the course of this disease in humans (Lancaster et al., 2013; Zhang et al., 2019a). As many MCPH proteins share overlapped functions, we saw to categorize them according to their major role(s) rather than discussing each one individually (Figure 1).

**Dysfunctional Centrosome**

Almost one-third of MCPH mutations occur in centrosomal or mitotic spindle proteins. Defective centrosomes can affect cell cycle progression and cell division, leading to abnormal chromosomal numbers, cell cycle arrest, and apoptosis (Barbelanne and Tsang, 2014). It has been proposed that alterations of the cleavage plane orientation during NE proliferation increase asymmetric cell divisions (Buchman and Tsai, 2007; Knoblich, 2008; Zhong and Chia, 2008). This, in turn, leads to an early consumption of progenitor cells at the expense of a premature generation of neurons with ultimately reduced number, thence a smaller brain (Fish et al., 2006; Buchman et al., 2010; Kaindl et al., 2010; Lizarraga et al., 2010). In this notion, several MCPH mouse models show a shift in the cleavage plane orientation during NE proliferation increase asymmetric cell divisions (Buchman and Tsai, 2007; Knoblich, 2008; Zhong and Chia, 2008). This evidence has been also supported by human brain organoid models (Lancaster et al., 2013; Zhang et al., 2019a). Intriguingly, most of the MCPH fly models with defected centrosomal proteins exhibit normal brain size (Basto et al., 2006; Lucas and Raff, 2007; Roque et al., 2012), indicating that changes in the cleavage plane
orientation might only have a minor impact on brain growth. Alternatively, flies could have compensatory mechanisms bypassing the effect of the misoriented cleavage plane (Nigg and Raff, 2009). On the other hand, further studies discovered MCPH mouse models with the microcephaly phenotype, though unaffected cleavage plane (Pulvers et al., 2010; Marjanović et al., 2015). Furthermore, depletion of some other MCPH centrosomal proteins in mice does not affect brain growth at all (Malumbres et al., 2004).

The neural progenitor cell (NPC) symmetrical proliferation speed is frequently reduced in mutated MCPH genes, which encode centrosomal proteins (Lizarraga et al., 2010; Gruber et al., 2011; Sgourdou et al., 2017; Ding et al., 2019). This is much obvious toward the end of neurogenesis (Lizarraga et al., 2010; Gruber et al., 2011; Sgourdou et al., 2017) when the later-born neurons (superficial layers II–IV) start to be generated. Together with the premature generation of neurons, this explains why superficial cortical neurons are the most affected in most MCPH models (Lizarraga et al., 2010; Chen et al., 2014). This is in line with postmortem pathological analysis described in a case of WD-repeat-containing protein 62 gene (WDR62; MCPH2) mutation (Yu et al., 2010). In addition, MCPH proteins are important for the normal distribution of cells between cortical zones. Knockout of abnormal spindle-like, microcephaly-associated gene (Aspm) in ferret increases the number of generated oRGCs, affecting the RGC overall proliferative capacity (Johnson et al., 2018). Likewise, knockdown of the cyclin-dependent kinase five regulatory subunit-associated protein two gene (Cdksrap2) in a mouse model alters the distribution of progenitor pool leading to more generation of basal progenitors (Buchman et al., 2010). By contrast, somatosensory cortical layer VI has been reported to be thinner in an Aspm knockout mouse model (Fujimori et al, 2014). Indeed, several in vivo and in vitro studies including human brain organoid

FIGURE 1 | Major roles of microcephaly primary hereditary (MCPH) proteins in brain development. The increased number of discovered MCPH proteins expands the pathomechanism spectrum to include several cellular components. Centrosome Functions: the proteins of this group regulate proper centrosomal functions to balance the transition between neural progenitor cell (NPC) proliferation and differentiation by controlling cell cycle progression and cell cycle exit fraction. Nuclear Envelope Integrity: the proteins of this group affect the proper spindle alignment and cell fate determinants during NPC proliferation and protect radial glial cell (RGC) nuclei from mechanical stress injury during INM. Kinetochore Structure: the proteins of this group assure the correct alignment of chromosomes during mitosis. Mitotic Spindle Dynamics: the proteins of this group regulate the spindle dynamics and cell division. Chromatin Structure: the proteins of this group regulate gene expression during neurogenesis and assure proper DNA damage repair. Cytokinesis: the proteins of this group control the terminal step in the cell cycle, which leads to a physical separation between the daughter cells. Autophagy: the proteins of this group facilitate the removal of cytosolic protein aggregates and maintain mitochondrial homeostasis. Intracellular trafficking: the proteins of this group control the cellular retrograde trafficking from the Golgi to the endoplasmic reticulum. Fatty Acid Metabolisms: the proteins of this group affect the postnatal neuronal morphogenesis, which requires a normal lipogenesis process. Ribosomal RNA Biogenesis: the proteins of this group regulate ribosomal RNA processing and affect primary cilia resorption. Please refer to (Table 1) for full protein names. *MCPH1 is also involved in chromatin structure. **CENPJ is also involved in kinetochore structure. ***LMNB1 and LMNB2 are also involved in mitotic spindle dynamics.
models revealed that MCPH centrosomal genes balance the transition between NPC proliferation and differentiation by controlling cell cycle progression and cell cycle exit fraction (Buchman et al., 2010; Lizarraga et al., 2010; Bogoyevitch et al., 2012; Lancaster et al., 2013; Hainline et al., 2014; Zhang et al., 2019a). This explains, respectively, the reduced proliferation and premature neuronal differentiation detected in the respective MCPH animal models. Furthermore, using the conditional knockout mouse model, it has been shown that centromeric protein J (Cenpj) regulates NPC cell cycle progression by regulating cilium disassembly during neurogenesis (Ding et al., 2019). Similarly, depletion of WDR62 and centrosomal-P4.1-associated protein (CPAP) in human cerebral organoids impairs the cilium disassembly and cell cycle progression (Gabriel et al., 2016; Zhang et al., 2019a).

It has been reported that mutations in genes encoding MCPH centrosomal proteins alter the maturation and cellular number of centrosomes (Pfaff et al., 2007; Rodrigues-Martins et al., 2007; Yabe et al., 2007; Megraw et al., 2011; Hussain et al., 2012; McIntyre et al., 2012; Hussain et al., 2013; Arquint and Nigg, 2014). This, in turn, might affect the proper distribution of chromosomes to daughter cells leading to spindle instability and mitotic delay or arrest at metaphase checkpoint (Ripoll et al., 1985; Gonzalez et al., 1990; Megraw et al., 1999; Riparbelli et al., 2002; Matsuura et al., 2004; Pfaff et al., 2007; Lizarraga et al., 2010; Kim et al., 2011a; Vitale et al., 2011; Novorol et al., 2013; Chen et al., 2014). In most of these cases, such defect triggers the apoptotic cascade leading to cellular loss (Pfaff et al., 2007; Lizarraga et al., 2010; Vitale et al., 2011; McIntyre et al., 2012; Novorol et al., 2013; Chen et al., 2014). Remarkably, increased apoptosis of NPCs—associated with or without proliferation/differentiation defects—contributes to the microcephaly phenotype by depleting the neural stem cell pool (Izraeli et al., 1999; Pfaff et al., 2007; Lizarraga et al., 2010; Gruber et al., 2011; McIntyre et al., 2012; Sgourdou et al., 2017; Zhang et al., 2019a; Ding et al., 2019). Intriguingly, neuronal populations also seem to be vulnerable to apoptosis during later stages of development (Lizarraga et al., 2010).

Apparently, the impact of mutated MCPH centrosomal genes in brain development not only is restricted to NPC proliferation and differentiation phase but also exceeds it to affect neuronal migration, dendritic and axonal outgrowth, and synaptogenesis. The presence of gray matter heterotopia, polymicrogyria, lissencephaly, and pachygyria in several MCPH conditions points toward impaired neuronal migration (Table 2) (Leventer et al., 2010; Yu et al., 2010). The signs of this impairment have been reported in postmortem histopathological MCPH samples and various MCPH animal models (Yu et al., 2010; Buchman et al., 2011; Xu et al., 2014). Besides that, an interesting role of Cdk5rap2 in regulating dendritic development and synaptogenesis in superficial cortical layer II/III has been reported (Zaqout et al., 2019). The contribution of dendritic complexity deficits in the microcephaly phenotype points toward a progressive nature during the MCPH course (van Dyck and Morrow, 2017).

**Defective Chromatin Structure**

Chromatin structure is a golden stone for gene expression regulation during neurogenesis. Mutations in genes encoding chromatin-linked proteins expand the pathomechanism spectrum of the MCPH. Microcephalin (BRCT/BRIT1) mutated cells taken from MCPH1 individuals and mouse models displayed premature chromosome condensation (PCC) associated with a high frequency of prophase-like cells and defective DNA damage repair (Jackson et al., 2002; Neitzel et al., 2002; Liang et al., 2010; Trimborn et al., 2010). This feature has been considered as a diagnostic marker for individuals with MCPH1 gene mutations (Jackson et al., 2002).

In flies, mcph1 mutants display embryonic lethality due to mitotic arrest and uncoordinated centrosome/nuclear cycles in early syncytial cell cycles (Bunk et al., 2007). Likewise, mutations in gene encoding *polyhomeotic-like one protein* (*PHC1, MCPH11*) are associated with aberrant DNA damage repair (Awad et al., 2013). In addition, *PHC1* mutations disturb the expression of Nanog and the ubiquitination of histone H2A, in which the former maintains pluripotency and the latter affects the cell cycle progression by the accumulation of Geminin (Awad et al., 2013; Chen et al., 2021).

Complete ablation of Znf335 gene in mice is embryonically lethal, but conditional knockout leads to a reduction in the cortical size affecting the forebrain much severely (Yang et al., 2012). This has been attributed to disruptions in NPC proliferation, cell fate, and neuronal differentiation (Yang et al., 2012). Consistently, postmortem histopathological studies on brain samples taken from ZNF335 patients reveal a severe reduction in the neuronal number associated with abnormalities in neuronal morphogenesis, migration, and polarity (Yang et al., 2012).

Mutations in genes encoding condensin complex proteins NCAPD2, NCAPD3, and NCAPH have been linked to MCPH21, 22, and 23, respectively (Martin et al., 2016). One of the hallmarks of hypomorphic Ncaph2 mice is the formation of a chromatid bridge in apical NPCs (Martin et al., 2016). These bridges result from failed sister chromatid disentanglement leading to chromosome segregation errors and aneuploidy (Martin et al., 2016). Subsequently, NPCs undergo a reduced cell proliferation and an increased apoptosis without obvious alterations in spindle orientations or cell fat (Martin et al., 2016).

### Deformed Kinetochore Proteins

Mutations in genes encoding kinetochore scaffold one protein (KNL1, previously known as CASC5) and centromere-associated protein E (CENPE) have been linked to MCPH4 (Jamieson et al., 1999; Genin et al., 2012; Mirzaa et al., 2014; Saadi et al., 2016; Szczechanski et al., 2016; Zarate et al., 2016). Proper function of proteins associated with centromeric kinetochore assures the correct alignment of chromosomes during mitosis; else, spindle assembly checkpoint (SAC) is activated and suspends the mitotic progression (Cleveland et al., 2003; Musacchio and Salmon, 2007; Santaguida and Musacchio, 2009; Hori and Fukagawa, 2012).

Conditional knockout of *Kn11* in mouse cortical NPCs results in DNA damage due to chromosomal segregation errors (Shi et al., 2019). This triggers a p53-dependent apoptotic cascade and leads to massive loss of NPCs and microcephaly (Shi et al., 2019). Similar to MCPH centrosomal proteins, the progressive loss of NPCs in *Kn11* conditional knockout mice affects mainly the later-born neurons (superficial layers II–IV) (Shi et al., 2019). CENPE facilitates the transition from metaphase to anaphase during the cell cycle (Yen et al., 1991). Disruption of Cenpe function in mice
and *Drosophila* leads to early embryonic lethality due to chromosomal instability (Yucel et al., 2000; Putkey et al., 2002).

**Interruption of Fatty Acids Uptake Into the Brain**

Proper brain development and function require essential omega-3 fatty acids, which need to be obtained from the circulation via specific transporters (Alakbarzade et al., 2015). Sodium-dependent lysophosphatidylcholine (LPC) transporter (MFSD2A) is exclusively expressed in the endothelium of the blood–brain barrier (BBB) and a major transport facilitator for docosahexaenoic acid (DHA) (Ben-Zvi et al., 2014; Nguyen et al., 2014; Alakbarzade et al., 2015; Guemez-Gamboa et al., 2015). Depending on the transporter residual activity, mutations in *MFSD2A* (*MCPH15*) gene is associated with either a progressive microcephaly syndrome or a much lethal phenotype (Berger et al., 2012; Alakbarzade et al., 2015; Guemez-Gamboa et al., 2015). The progressive feature associated with the milder form of this disease raises the possibility that LPC transportation is continuously required for membrane biogenesis in the brain (Alakbarzade et al., 2015; Scala et al., 2020). Endothelial-specific deletion of *Mfsd2a* in mice leads to a microcephaly phenotype accompanied by a reduction in neuronal arborization and dendritic length (Chan et al., 2018). Interestingly, neuronal loss detected in *Mfsd2a* knockout mice was restricted to cerebellar Purkinje cells and hippocampal CA1 and CA3 regions (Nguyen et al., 2014). Taken together, these data demonstrate that, unlike other MCPHs, Mfsd2a deficiency affects the postnatal neuronal morphogenesis, which requires a normal lipogenesis process (van Deijk et al., 2017; Ziegler et al., 2017; Chan et al., 2018). Notably, variable degrees of white matter reduction have been also reported in *MCPH15* individuals (Alakbarzade et al., 2015; Harel et al., 2018; Scala et al., 2020). Further studies are required to assess to which extent do white matter deficits contribute to the microcephaly phenotype (Huang and Li, 2021).

**Altered Nuclear Envelope**

Mutations in several genes encoding nuclear envelope components have been recently linked to MCPH conditions. Ankyrin repeat- and lem domain-containing protein 2 (Ankle2) is localized to the endoplasmic reticulum and nuclear envelope (Link et al., 2019). *Drosophila dAnkle2* mutant larvae show a reduction in the brain size due to impaired nuclear envelope integrity, which eventually affects proper spindle alignment and cell fate determinants during NPC proliferation (Link et al., 2019). Another study, however, suggests that the reduction in *Drosophila dAnkle2* mutant NPCs cells is due to defects in proliferation and massive apoptosis rather than an alteration in asymmetrical cell division (Yamamoto et al., 2014). In this line, *Caenorhabditis elegans* ANKLE2 ortholog protein (LEM-4L) plays a critical role in mitosis by facilitating nuclear envelope reassembly during mitotic exit (Asencio et al., 2012). Individuals with mutations in genes encoding various nuclear pore complex proteins (NPC) are diagnosed with severe forms of nephrotic syndrome (Braun et al., 2018). However, mutations in NPC subunit component nucleoporin 37 (NUP37) also exhibit intellectual disability and MCPH (Braun et al., 2018). More recently and yet to be linked to a specific OMIM MCPH number, mutations in NUP85 subunit are associated with a reduction in brain volume, delayed myelination, agenesis of the corpus callosum, gray matter heterotopia, and frontal lobe cortical malformation (Ravindran et al., 2021). Fibroblasts derived from NUP85 individuals are characterized by reduced cell viability, proliferation rate, abnormal mitotic spindle apparatus, and altered cytoskeletal protein expressions (Ravindran et al., 2021). As most of the studies performed in viable animal models with NPC defects focused on the nephrotic phenotype, further investigations to understand their effects on brain growth are still warranted.

**Defective Cytokinesis**

Cytokinesis is the terminal step in the cell cycle, which leads to a physical separation between the daughter cells. Defects in this process frequently result in the formation of binucleated cells, aneuploidy, chromosomal instability, cell cycle arrest, and apoptosis (Li et al., 2016a). Notably, the elevated number of binucleated cells—including pyramidal and Purkinje cells—is considered as a key feature for cytokinesis failures (Di Cunto et al., 2000; Roberts et al., 2000; Reilly et al., 2019). Citron rho-interacting kinase (CIT) midbody protein has important roles in cytokinesis, and its defect leads to MCPH17 in humans (Li et al., 2016a; Basit et al., 2016; Harding et al., 2016; Shaheen et al., 2016). Postmortem histopathological analysis of brain samples taken from *MCPH17* individuals reveals a thickened neocortex with disorganized layers and unmyelinated white matter with scattered neurons (Harding et al., 2016). In addition, the cerebellar cortex and hippocampus show dysplastic and hypoplastic features, and Purkinje cells exhibit a simplified dendritic tree where many of them are multinucleated (Harding et al., 2016). Studies conducted in *Cit* knockout rodent models reveal that NPCs undergo massive apoptosis due to interrupted cytokinesis (Di Cunto et al., 2000; Roberts et al., 2000). In these models, binucleated neurons have been detected in several brain
and spinal cord regions; however, apoptosis seems to be more pronounced in the cerebral cortex, granular layers of cerebellum, hippocampus, and olfactory bulb (Di Cunto et al., 2000; Sarkisian et al., 2002). In addition, the high rate of cell death reported at the ganglionic eminence reduces the total number of generated interneurons (Sarkisian et al., 2001; Di Cunto et al., 2000). Consistent with human brain findings, cerebellar Purkinje cells are disorganized and show underdeveloped dendritic complexity (Di Cunto et al., 2000). The presence of disorganized cortical layering and scattered neurons in the white matter should raise the possibility of an abnormal migration process even though it is yet to be confirmed by further studies.

The role of CIT in cytokinesis requires a proper function of kinesin family 14 (KIF14) microtubule motor protein (Makrythanasis et al., 2018). It is then unsurprising to realize that mutations in KIF14 also lead to MCPH by a common mechanism as CIT mutations (Moawia et al., 2017; Makrythanasis et al., 2018; Reilly et al., 2019). This is supported by several studies conducted in various animal models with depleted KIF14 homologs (Ohkura et al., 1997; Fujikura et al., 2013; Reilly et al., 2019). Mutations of Drosophila KIF14 homolog, also known as kinesin-like protein at 38B (KLP38B), affect the cell cycle progression due to cytokinesis failure (Ohkura et al., 1997). In the same notion, Laggard mice (lag), an animal model for KIF14, are characterized by microcephaly, cortical dysgenesis, and severe hypomyelination as a consequence of massive apoptosis during late neurogenesis (Fujikura et al., 2013). Consequently, Cux1-positive upper cortical neurons are much reduced in number, and some of them are displaced (Fujikura et al., 2013). Similar to Cit knockout models, lag mice show scattered cerebellar Purkinje cells with simplified dendritic trees pointing toward abnormalities in neuronal migration and neurite formation (Fujikura et al., 2013).

**Disturbed Autophagy and Mitochondrial Dynamics**

MCPH18 is caused by mutations in gene encoding WD repeat and FYVE domain-containing 3 (WDFY3), also known as Autophagy-
Linked FYVE (ALFY) (Kadir et al., 2016). Normally, this scaffolding protein facilitates the removal of cytosolic protein aggregates, which, in turn, maintains mitochondrial homeostasis (Kadir et al., 2016; Napoli et al., 2018; Napoli et al., 2021). Wdfy3 is highly expressed in RGCs, and its loss of function prevents the transition from symmetrical proliferative divisions to asymmetrical differentiative divisions by altering the Wnt signaling cascade (Tacchelly-Benites et al., 2013; Oroscó et al., 2014; Kadir et al., 2016). The imbalance in NPCs mode of cell division leads to regional differences in neocortical thickness and opposing phenotypes of micro- and macrocephaly (Oroscó et al., 2014; Le Duc et al., 2019). On the other hand, the disruption of mitochondrial dynamics in Wdfy3 mutant mice decreases the synaptic density, alters the synaptic plasticity, and probably affects dendritic development (Napoli et al., 2018; Napoli et al., 2021). Remarkably, proteins involved in GABAergic neurotransmission are downregulated in Wdfy3 mutant mice (Napoli et al., 2021). Furthermore, Wdfy3 plays a role in neural migration during early neurogenesis (Oroscó et al., 2014).

Interrupted Intracellular Trafficking

Coatamer Protein Complex Subunit Beta 2 (COPB2) controls the cellular retrograde trafficking from the Golgi to the endoplasmic reticulum (Orči et al., 1993; Letourneur et al., 1994). Interestingly, mutations in COPB2 interrupt brain growth and lead to MCPH19 (DiStasio et al., 2017). While the complete loss of Copb2 is incompatible with life, partial loss of Copb2 in mice interferes with the growth of the brain (DiStasio et al., 2017). This has been associated with increased cell death and a high number of proliferative cells positive for phosphorylated histone H3 (pH3) (DiStasio et al., 2017). Still, further studies are necessitated to dissect the exact role of Copb2 in controlling brain size.

Disturbed Mitotic Spindle Dynamics

Trafficking protein particle complex subunit 14 (TRAPPC14)—also known as microtubule-associated protein 11 (MAP11)—is localized to mitotic spindles and interacts with α-tubulin regulates the spindle dynamics and cell division (Perez et al., 2019). Recently, TRAPPC14 mutations have been linked to MCPH25 in human and microcephaly phenotypes in the zebrafish model (Perez et al., 2019). This has been mainly attributed to a decreased brain cell proliferation due to altered spindle dynamics affecting the mitotic progression and probably the cytokinesis, however, without increased apoptosis (Perez et al., 2019). In addition, TRAPPC14 has been implicated in ciliogenesis and cilia stability, which, in turn, could affect brain growth (Cuenca et al., 2019).

Defective Ribosome Biogenesis

The most recently diagnosed MCPH28 cases have been linked to a mutation in Ribosomal RNA Processing seven Homolog A (RRP7A) (Farooq et al., 2020). It is known that mutations in genes involved in ribosome biogenesis are associated with neurodevelopmental defects together with other abnormalities (Hetman and Slomnicki, 2019). The encoded RRP7A protein shows high expression in RGCs and cellular localizations at the centrosome, primary cilium, and nucleolus (Farooq et al., 2020). Depletion of RRP7A alters ribosomal RNA processing and affects primary cilia resorption, causing a delay in S-phase entry and progression (Farooq et al., 2020). Mutated rrp7a zebrafish embryos display a reduction in the expression pattern of some proliferation and neural differentiation markers, while TUNEL assay analysis indicates increased apoptosis (Farooq et al., 2020). These findings might result from defective rrp7a functions at the level of centrosome and/or primary cilia. MicroRNA processing has been identified as a contributing factor in temporal fate specification (Kohwi and Doe, 2013). Thus, dysregulated ribosomal RNA processing with subsequent nucleolar stress establishes a new insight into MCPH pathomechanisms.

MICROCEPHALY PRIMARY HEREDITARY VERSUS INFECTION-INDUCED MICROCEPHALY

After describing the genetic component of microcephaly, we here shed some light on some infectious agents associated with microcephaly. Particularly Toxoplasma gondii, cytomegalovirus, rubella virus, and syphilis, but also the herpes simplex virus, HIV, and Zika virus, have been reported in children born with microcephaly. The severity of microcephaly depends not only on the type of the infectious agent but much importantly on the gestational age when an infection occurs (Devakumar et al., 2018). It has been shown that neural progenitors are targeted by these pathogens; however, the mechanisms by which most of these infections lead to microcephaly are not fully understood (Devakumar et al., 2018). Nevertheless, the epidemic infections with the Zika virus and its association with congenital microcephaly triggered extensive research in this field. Several studies based on various in vitro and in vivo models point toward NPC cell cycle arrest or an increase in cell death upon the infection with the Zika virus (Adams Waldorf et al., 2016; Li et al., 2016b; Cugola et al., 2016; Garcez et al., 2016; Tang et al., 2016; Wu et al., 2016). Intriguingly, several MCPH genes including Mph1, Aspm, Cdk5rap2, Stil, and Cep135 are downregulated in brain tissues extracted from Zika-infected mice (Li et al., 2016b; Wu et al., 2016). This raises the possibility that infection-induced microcephaly might alter brain growth via altering the expression of various MCPH genes. However, the direct impact of infectious agents on the pathogenesis of microcephaly cannot be ruled out.

CONCLUSION

The journey during brain growth and development is impeded at specific points with crucial steps (Figure 2). Minor defects at any of these developmental points result in various neurodevelopmental disorders including MCPH. The earlier the insult during the journey, the greater the impact on brain growth. The major obstacle is faced during the rapid NPC proliferation just before the commencing generation of neurons. Either decreased proliferation or increased NPCs apoposis depletes the neuronal stem cell pool and ultimately leads to a smaller number of generated neurons. Obviously, most MCPHs are caused by mutations in centrosomal proteins. Hence,
dysfunctional centrosome alters NPC proliferation, cell cycle progression, and cell fate determination. In addition, the resulting aneuploidy and increased DNA damage response associated with some mutated MCPH genes trigger apoptosis. The accelerated number of discovered MCPH genes expands the pathomechanism spectrum of this disease beyond the centrosomal component. Similarly, the simultaneous generation of animal models mimicking the human MCPH phenotype provides a strong platform for future studies to dissect further molecular mechanisms behind the microcephaly phenotype. This will also expand our knowledge of normal brain growth and evolution.

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AUTHOR CONTRIBUTIONS

SZ wrote the initial manuscript draft and generated the figures and tables. AK revised the manuscript. Both authors approved the final article.

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