Review

Molecular Genetics of Microcephaly Primary Hereditary: An Overview

Nikistratos Siskos †, Electra Stylianopoulou †, Georgios Skavdis and Maria E. Grigoriou *

Department of Molecular Biology & Genetics, Democritus University of Thrace, 68100 Alexandroupolis, Greece; nsiskos@mbg.duth.gr (N.S.); ilstylian@mbg.duth.gr (E.S.); gskavdis@mbg.duth.gr (G.S.)
* Correspondence: mgrigor@mbg.duth.gr
† Equal contribution.

Abstract: MicroCephaly Primary Hereditary (MCPH) is a rare congenital neurodevelopmental disorder characterized by a significant reduction of the occipitofrontal head circumference and mild to moderate mental disability. Patients have small brains, though with overall normal architecture; therefore, studying MCPH can reveal not only the pathological mechanisms leading to this condition, but also the mechanisms operating during normal development. MCPH is genetically heterogeneous, with 27 genes listed so far in the Online Mendelian Inheritance in Man (OMIM) database. In this review, we discuss the role of MCPH proteins and delineate the molecular mechanisms and common pathways in which they participate.

Keywords: microcephaly; MCPH; MCPH1–MCPH27; molecular genetics; cell cycle

1. Introduction

Microcephaly, from the Greek word μικροκεφαλία (mikrokephalia), meaning small head, is a term used to describe a cranium with reduction of the occipitofrontal head circumference equal, or more that two standard deviations below the mean for age, gender, and ethnicity [1–6]. Microcephaly usually reflects a small brain volume; it is presented either as an isolated finding (non-syndromic) or with additional features, such as dysostoses and short stature (for example, Seckel syndrome, Meier-Gorlin syndrome, radiosensitivity, and chromosome breakage (for example, Bloom syndrome), or diabetes (for example, Wolcott Rallison syndrome) [1,3,4]. The underlying etiology of microcephaly varies; it can be environmental, resulting, for instance, from exposure to toxic substances, or genetic [2,4]. Currently, more than 900 Online Mendelian Inheritance in Man (OMIM) phenotype entries, and approximately 800 genes with variable expressivity linked with microcephaly have been reported [1]. According to the time of diagnosis, microcephaly is classified into primary (congenital) if present at birth, or secondary, if it develops postnatally, as progressive atrophy of an initially normal brain [1,2]. Of particular interest within the genetic non-syndromic microcephaly is autosomal recessive primary microcephaly (MCPH; MicroCephaly Primary Hereditary). MCPH is a rare disease (1:10,000 to 1:250,000 live births) most frequently diagnosed in populations with consanguineous marriages [5,7–10]. The brain of MCPH patients is small, its architecture is largely normal, yet with simplified neocortical gyration [1,3–7]. Additionally, in some cases radiological studies reveal further abnormalities, for instance corpus callosum agenesis or hypoplasia, enlarged lateral ventricles, and cerebellar or brain stem hypoplasia [9–16]. Patients with MCPH usually have intellectual disability—mild to moderate retardation—their ability, however, is stable, and they can manage with daily living and safety, as well as perform simple reading and writing skills [5,15,16]. In the past 5 years, primary hereditary microcephalies inherited in an autosomal dominant manner, with clinical features as those described above, are included in the MCPH group; therefore, they are also included in the present review. MCPH is a neurodevelopmental disease with striking genetic heterogeneity; mutations in
27 genes have implicated so far, unveiling complex developmental processes that regulate mammalian brain size.

2. The Cellular Basis of MPCH

MCPH is the result of the reduction in the number of neurons in the brain. Brain neurons are derived from the neuroepithelial cells (NE) of the pseudostratified epithelium of the neural tube [17,18]. These cells, early in the development of the nervous system, undergo multiple rounds of symmetric divisions that expand the progenitor pool [17–20]. At the onset of neurogenesis, NE cells start expressing glial markers, adopt a radial glial (RG) cell fate and undergo multiple rounds of asymmetric cell divisions, in which they self-renew and generate neurons [19,20]. As neurogenesis continues, RG cells give rise also to intermediate progenitor (IP) cells, which undergo a limited number of symmetric cell divisions generating either transit-amplifying cells or neurons [18]. The small size of the brain observed in MCPH patients may arise from changes in the relative rates of symmetric and asymmetric divisions, or in the differentiation of the neuronal cells. These, in turn, may result from cell cycle dysregulation, i.e., from defects in timing and progression of mitosis or cytokinesis, in DNA replication and repair, or in maintenance of genome stability. In addition, the severity of microcephaly may, to a large extent, depend on the stage at which it arises; for instance, defects in NE self-proliferation may present with more severe phenotypes compared to cell cycle dysregulation in IP cells. Accordingly, as shown in Table 1, the 27 genes that have been so far linked to MCPH encode proteins that are key regulators of one or more molecular pathways that control the above-mentioned cellular processes.

Centrosomes are cellular structures consisting of a mature mother centriole and a less mature daughter centriole and act as platforms for multiple cellular functions [21]. From phase G1 to G2 centrosomes functions as microtubule-organizing centers (MTOC), during mitosis they mature into the spindle poles, and upon cell differentiation or cell cycle arrest, by elongating microtubules they transform into the basal bodies of the cilia [21]. Defects in centrosome biogenesis or maturation impair cell cycle progression and cell division, leading to aneuploidy, cell cycle arrest, and/or cell death [21]; in neural progenitors, these defects would result in MCPH. As already mentioned, centrosomes participate in the assembly of the mitotic spindle, which is responsible for the accurate segregation of chromosomes [22]. Three types of spindle microtubules (MTs) have been characterized within the spindle; astral MTs connect centrosomes to the cell cortex and direct the plane of division by positioning the spindle, kinetochore MTs capture condensed chromosomes, and connect them with the centrosome, and interpolar MTs interconnect the spindle poles driving the separation of the two chromosomes [22]. Defects in the organization and function of MTs, may also lead to MCPH, as mutations in proteins associated, for instance with astral MTs, which are essential for the positioning of the cleavage furrow, may affect the proportion of symmetric to asymmetric divisions of RGs. In addition to centrosome and spindle dynamics, DNA dynamics have key role in cell cycle progression [23]. During replication, cohesins associate with DNA to form the chromatids, which condense during prophase, pair in metaphase and finally segregate into the presumptive daughter cells [23]. Cellular DNA is constantly monitored for damage throughout the cell cycle; once damage is detected, repair is needed before progressing into the next stage of the cell cycle [23]. As a result, mutations in genes involved in replication, chromosomal condensation or segregation, or damage repair may affect for example the timing of the cell cycle causing delays [22], which eventually lead to reduced numbers of NEs, RGs or IPs, and hence, to MCPH.
Table 1. MCPH genes (February 2021).

| Disorder | OMIM  | Chromosomal Location | Gene                  | Protein                                                                                                                                  | Mode of Inheritance | Subcellular Localization                                      | Cellular Process (es)                                                                 |
|----------|-------|----------------------|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------|--------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------------|
| MCPH1    | 607117| 8p23.1               | MCPH1                 | Microcephalin 1 (BRCT-repeat inhibitor of hTERT, MCPH1)                                                                                    | Autosomal recessive| Nucleus Centrosome                                            | DNA damage Chromatin condensation Coupling centrosome cycle to mitosis               |
| MCPH2    | 613583| 19q13.12             | WDR62 (MCPH2)         | WDR62 (WD Repeat-containing protein 62, MCPH2)                                                                                         | Autosomal recessive| Centrosome Spindle poles                                     | Centriole biogenesis Spindle assembly and orientation                                |
| MCPH3    | 608201| 9q33.2               | CDK5RAP2 (MCPH3)      | CDK5RAP2 (CDK5 Regulatory subunit Associated Protein 2, MCPH3)                                                                          | Autosomal recessive| Pericentriolar material of the centrosome Nucleus            | Centriole biogenesis Control of spindle checkpoints Cytokinesis                      |
| MCPH4    | 609173| 15q15.1              | CASC5 (KNL1, MCPH4)  | CASC5 (CAncer Susceptibility Candidate 5, KNL1, kinetochore scaffold 1, MCPH4)                                                          | Autosomal recessive| Kinetochore                                                   | Attachment of chromatin to the mitotic apparatus Control of spindle checkpoint       |
| MCPH5    | 605481| 1q31.3               | ASPM (MCPH5)          | ASPM (Abnormal SPindle Microtubule assembly, MCPH5)                                                                                     | Autosomal recessive| Nucleus Centrosome Midbody                                   | Centriole biogenesis Spindle assembly and orientation Cytokinesis                    |
| MCPH6    | 609279| 13q12.12-q12.13      | CENPJ (SAS-4, CPAP, MCPH6) | CENPJ (CEntromere Protein J, SAS-4, CPAP, MCPH6)                                                                                    | Autosomal recessive| Centrosome                                                    | Centriole biogenesis                                                                |
| MCPH7    | 181590| 1p33                 | STIL (MCPH7)          | STIL (SCL/TAL1 Interrupting Locus, MCPH7)                                                                                            | Autosomal recessive| Centrosome                                                    | Centriole biogenesis Spindle assembly and positioning                                |
| MCPH8    | 611423| 4q12                 | CEP135 (MCPH8)        | CEP135 (CEntrosomal Protein 135, MCPH8)                                                                                               | Autosomal recessive| Centrosome                                                    | Centriole biogenesis                                                                |
Table 1. Conts.

| Disorder | OMIM   | Chromosomal Location | Gene               | Protein                                      | Mode of Inheritance | Subcellular Localization | Cellular Process (es)            |
|----------|--------|-----------------------|--------------------|----------------------------------------------|---------------------|--------------------------|----------------------------------|
| MCPH9    | 613529 | 15q21.1               | CEP152 (MCPH9)     | CEP152 (Centrosomal Protein 152, MCPH9)     | Autosomal recessive | Centrosome               | Centriole biogenesis             |
| MCPH10   | 610827 | 20q13.12              | ZNF335 (MCPH10)    | CEP152 (Centrosomal Protein 152, MCPH9)     | Autosomal recessive | Nucleus                  | Transcription remodeling          |
| MCPH11   | 602978 | 12p13.31              | PHC1 (MCPH11)      | PHC1 (PoloHomeotic like 1, MCPH11)          | Autosomal recessive | Nucleus                  | Transcription remodeling          |
| MCPH12   | 603368 | 7q21.2                | CDK6 (MCPH12)      | CDK6 (Cyclin Dependent Kinase 6, MCPH12)    | Autosomal recessive | Cytosol, Nucleus, Spindle poles, Centrosome | Cell cycle regulation            |
| MCPH13   | 117143 | 4q24                  | CENPE (MCPH13)     | CENPE (CENtromere associated Protein E, MCPH13) | Autosomal recessive | Kinetochore               | Kinetochore attachment, Control of spindle checkpoint |
| MCPH14   | 609321 | 1p21.2                | SASS6 (SAS6, MCPH14) | SASS6 (Spindle ASSEMBly abnormal protein 6 homolog, MCPH14) | Autosomal recessive | Centrosome               | Centriole biogenesis             |
| MCPH15   | 614397 | 1p34.2                | MFSD2A (MCPH15)    | MFSD2A (Major Facilitator Superfamily Domain containing 2A, MCPH15) | Autosomal recessive | Plasma membrane          | Metabolism                       |
| MCPH16   | 616062 | 12q24.33              | ANKLE2 (LEM4, MCPH16) | ANKLE2 (ANKyrin repeat and LEM domain containing protein 2, MCPH16) | Autosomal recessive | Endoplasmic reticulum, Nuclear envelope | Nuclear envelope assembly/disassembly |
| MCPH17   | 605629 | 12q24.23              | CIT (MCPH17)       | CIT (CITrOn rho-interacting serine/threonine kinase, MCPH17) | Autosomal recessive | Spindle Midbody          | Spindle assembly and orientation Cytokinesis |
| MCPH18   | 617485 | 4q21.23               | ALFY (WDFY3, MCPH19) | ALFY (Autophagy-Linked FYVE protein, WDFY3, MCPH18) | Autosomal dominant | Cytoplasm, Nucleus       | Canonical Wnt pathway            |
| Disorder | OMIM   | Chromosomal Location | Gene                        | Protein                                                                 | Mode of Inheritance | Subcellular Localization | Cellular Process (es)         |
|----------|--------|----------------------|-----------------------------|-------------------------------------------------------------------------|---------------------|--------------------------|------------------------------|
| MCPH19   | 606990 | 3q23                 | COPB2 (MCPH19)              | COPB2 (COatomer Protein complex, subunit Beta 2, MCPH19)                | Autosomal recessive | Non-clathrin vesicles   | Vesicle trafficking          |
|          |        |                      |                             |                                                                         |                     |                          | Apoptosis via the JNK/c-jun pathway |
| MCPH20   | 611279 | 1q32.1               | KIF14 (MCPH20)              | KIF14 (Kinesin Family member 14, MCPH20)                                | Autosomal recessive | Spindle poles            | Spindle assembly             |
|          |        |                      |                             |                                                                         |                     | Spindle mid-zone Midbody | Cytokinesis                |
| MCPH21   | 615638 | 12p13.31             | NCAPD2 (CNAP1 MCPH21)       | NCAPD2 (Non-SMC condensin I complex Subunit D2, Centrosomal Nek2-Associated Protein 1, MCPH21) | Autosomal recessive | Nucleus Chromatin Chromosomes | Chromatin condensation          |
| MCPH22   | 609276 | 11q25                | NCAPD3 (MCPH22)             | NCAPD3 (Non-SMC condensin II complex subunit D3, MCPH22)                | Autosomal recessive | Nucleus Chromatin Chromosomes | Chromatin condensation          |
| MCPH23   | 602332 | 2q11.2               | NCAPH (MCPH23)              | NCAPH (Non-SMC condensin I complex subunit H, MCPH23)                   | Autosomal recessive | Nucleus Chromatin Chromosomes | Chromatin condensation          |
| MCPH24   | 609264 | 12q23.2              | NUP37 (MCPH24)              | NUP37 (Nucleoforin 37, MCPH24)                                         | Autosomal recessive | Nuclear Pore Kinetochore | Nuclear Pore assembly Spindle assembly |
| MCPH25   | 618350 | 7q22.1               | MAP11 (TRAPPC14, C7orf43, MCPH25) | MAP11 (Microtubule Associated Protein 11, TRAPPC14, C7orf43, MCPH25)   | Autosomal recessive | Spindle Midbody Golgi   | Spindle assembly Cytokinesis Golgi trafficking |
| MCPH26   | 150340 | 5q23.2               | LMNB1 (LaMiN B1, MCPH26)    | LMNB1 (LaMiN B1, MCPH26)                                               | Autosomal dominant  | Nuclear Lamina Spindle   | Nuclear envelope assembly Assembly of the mitotic spindle |
| MCPH27   | 150341 | 19p13.3              | LMNB2 (LaMiN B2, MCPH27)    | LMNB2 (LaMiN B2, MCPH27)                                               | Autosomal dominant  | Nuclear Lamina Spindle   | Nuclear envelope assembly Assembly of the mitotic spindle |
3. MCPH Genes

*MCPH1*, the first MCPH gene, was identified nearly 20 years ago [24]; however, it is during the last decade with the rapid progress in next generation sequencing technology that most of the MCPH genes and variants have been characterized [7–9,24]. As shown in Table 1, nearly all MCPH genes identified so far are implicated in one or more of the processes involved in the cell cycle, having usually distinct roles at different phases and/or in different molecular pathways.

3.1. ASPM (MCPH5)

Mutations in *ASPM* are the most frequent cause of MCPH, accounting for 68.6% of the cases in consanguineous patients [10,25]. *ASPM* encodes a protein with two calponin homology domains, a microtubule-binding domain and multiple IQ calmodulin-binding motifs [26–28]. During interphase, ASPM is localized at the centriole, while in mitosis it is detected at the spindle poles [26,27,29]. In mouse telencephalic neural progenitor cells, in the absence of functional ASPM spindle positioning is altered and divisions shift from symmetrical to asymmetrical instead of, however, cell cycle arrest [30], suggesting that ASPM at the spindle poles acts by maintaining symmetric divisions [30,31]. Notably, ASPM expression in the embryo is downregulated with the onset of neurogenic divisions [30,31]. Moreover, mice expressing *ASPM* variants, similar to those identified in patients with MCPH, exhibit mild microcephaly, and can be rescued by the introduction of a transgene encoding the wild type human protein [31]. Mutations in the *Drosophila* homolog of *ASPM* cause severe reduction of brain size due to defects in mitosis and increased apoptosis in the developing neuroepithelium [32]; similarly, knockdown of the zebrafish homolog leads to head size reduction [33]. More recently, ASPM has been implicated in centriole biogenesis [27,34]. During interphase, ASPM localizes at the proximal end of the mother centriole as component of a protein complex that is essential for centriole duplication; in mouse brain, lack of functional ASPM impairs centriole duplication leading to a reduction in the number of centrosomes and cilia in the cells [27].

3.2. WDR62 (MCPH2)

*WDR62* encodes a protein with a WD40 domain, a JNK (c-Jun N-terminal Kinase) docking domain, and a MKK7 binding domain. Mutations in *WDR62* are the second most frequent cause of MCPH, accounting for approximately 10% of the cases [10,35,36]. During mitosis, WDR62 is localized at the spindle poles [35]. Following bipolar spindle formation, WDR62 has a key role in stabilizing the spindle poles; in the absence of WDR62, the distribution of PCM components such as γ-tubulin or pericentrin is altered [37]. Moreover, during mitosis, WDR62 is a substrate of JNK active at the centrosome; phosphorylated WDR62 is required for the organization of the mitotic spindle [37,38]. ASPM interacts with WDR62; this interaction is mediated by CEP63, a protein linked to Seckel syndrome, characterized by severe microcephaly with intellectual disability and short stature [34]. Knockdown of either *Wdr62* or *JNK* using shRNA in neural progenitor cells of the rat telencephalon result in spindle misorientation, asymmetric divisions and, thus, in premature differentiation to neurons [39]. These defects are rescued by expressing human WDR62 protein but not MCPH-associated WDR62 mutant proteins [39]. Moreover, in mice, neural progenitor cells lacking functional WDR62 exhibit delayed mitotic progression leading to cell death and therefore, to small brain size [40]. In zebrafish, knockdown of WDR62 using morpholinos impairs mitotic progression causing increased cell death, resulting thus, in significant reduction in head size [41]. During interphase, WDR62 localizes at the proximal end of the mother centriole and its function is essential for the recruitment of proteins in a complex required for centriole biogenesis; in the mouse brain absence of WDR62 causes reduction of the number of centrosomes and cilia due to defects in centrosome biogenesis [34,42].
3.3. MCPH1 (MCPH1)

*MCPH1*, the first *MCPH* gene identified, encodes Microcephalin 1, a protein with three BCRT (BRCA1 C-terminus) domains; the N-terminal BRCT domain interacts with the SWI–SNF complex, while the C-terminal BRCT domains mediate MCPH1 interactions with phosphorylated peptides [43]. Mutations in *MCPH1* are the third most frequent cause of MCPH accounting for approximately 8% of the cases [9]. Microcephalin 1 has various roles in the cell cycle. It is required for the localization of the checkpoint kinase1 (Chk1); in the absence of Microcephalin 1, untimely entry to mitosis is observed leading to defects in the organization of the spindle and/or chromosome misalignment associated with premature chromosome condensation [43–46]. Microcephalin 1 acts as a transcriptional repressor of hTERT and interacts with the anaphase-promoting complex via Cdc27 linking transcription with cell cycle progression [43,45,47]. In addition, it interacts with histone H2AX and is involved in the DNA damage repair mechanisms acting as tumor suppressor [43,45,48–50].

In mice, knockout of *Mcph1* leads to defects in chromosome condensation, deficiencies in DNA repair, spindle misorientation and, eventually, to a reduction of the brain size mimicking the human disease [51]. In the neuroprogenitors of these mice, the lack of Microcephalin 1 leads to a shift from symmetric to asymmetric divisions, reducing the neuroprogenitor pool and, as a result, the number of neurons in the brain [51]. In *Drosophila*, mutations in the homolog of *MCPH1* are embryonic lethal with mutant embryos exhibiting abnormal centrosomes, asynchronous nuclear and centrosomal cycles, defective spindle and premature chromosome condensation, leading to mitotic arrest [52,53].

3.4. CDK5RAP2 (MCPH3)

*CDK5RAP2* gene encodes a protein-regulator of CDK5 activity that interacts with γ-tubulin promoting microtubule nucleation in the pericentriolar material of the centrosome [54–56]. Recruitment of CDK5RAP2 depends on pericentrin and WDR62 [34,56]. During mitosis, CDK5RAP2 and pericentrin engage CEP192 to the spindle poles; this interaction can direct spindle formation in the absence of centriole [57,58]. Moreover, CDK5RAP2 acting as transcription activator, regulates the expression of *BUBR1* and *MAD2*, two genes implicated in the control of spindle checkpoints [58]. CDK5RAP2 is expressed in neural progenitor cells; mutant mice lacking CDK5RAP2 exhibit mild microcephaly [59–61]. Furthermore, at the cellular level, in the neuroprogenitors of these mice, premature cell cycle exit and increased apoptosis are observed due to the disrupted structure of the centrosomes and the presence of multipolar spindles [59–61].

3.5. CASC5 (KNL1, MCPH4)

*CASC5* encodes a scaffold protein of the kinetochore that is essential for the attachment of chromatin to the mitotic apparatus and interacts with BUB1 and BUBR1 to control the spindle assembly checkpoint [62,63]. Reduction in CASC5 levels leads to chromosome misalignment [63]. In cells derived from MCPH patients, CASC5 localizes at the metaphase plane, as well as in the cytoplasm; in these cells, the percentage of mitotic and apoptotic cells is high, probably due to mitotic delay and DNA damage [64].

3.6. CENPJ (SAS-4, CPAP, MCPH6)

*CENPJ* encodes a protein that consists of phosphorylation domains, five coiled-coil domains and a C-terminal domain with 21 G-box repeats and a leucine zipper motif [65]. CENPJ is localized in the centrosome and is required for centriole integrity and duplication [27,65]. It binds MTs and interacts with several other centrosomal proteins implicated in MCPH [27,66,67]. CENPJ is also involved in ciliogenesis; lack of functional CENPJ in neural progenitor cells results in delayed cilia disassembly and cell-cycle re-entry, promoting, thus, differentiation [68]. The phenotype of mice with targeted inactivation of *Cenpj* exhibit several of the clinical findings observed in patients suffering not only from MCPH, but also from Seckel syndrome [69,70].
3.7. STIL (MCPH7)

STIL is an oncogene encoding a protein that does not share any known structural motifs with other proteins. It is localized to the centrioles with a role in centriole biogenesis and spindle pole positioning [71,72]. STIL interacts with several other centrosomal proteins implicated in MCPH [27,66,67,71]. Upregulation of STIL leads to the generation of supernumerary nascent centrioles localized close to the parental centriole, a phenotype resembling the effect of the overexpression of two other genes, namely SASS-6 and PLK4 [73]. Moreover, STIL is required for proper positioning of the mitotic spindle and, hence, mitotic progression [71,74]. Knockout of STIL in mice or its ortholog sil in zebrafish, leads to embryonic lethality—cells lack centrioles and cilia; however, the cause of lethality is not clear and may be related to other roles of STIL, for instance, in hedgehog signaling [74,75]. Interestingly, STIL has recently been shown to have a role in the generation and regeneration of dopaminergic neurons [76].

3.8. CEP135 (MCPH8)

CEP135 encodes a protein with a coiled-coil structure that in vitro interacts with tubulin, protofilaments, and microtubules [77,78]. It is localized at the centrosome and has a role in centriole biogenesis and integrity, interacting during different phases of the cell cycle with several proteins, including the products of MCPH genes, SASS6 and CENPJ [79–81]. In both vertebrates and invertebrates, absence of CEP135 results in various structural defects of the centrioles [82,83].

3.9. CEP152 (MCPH9)

CEP152 encodes a protein with several coiled-coil domains that is localized at the centrosome [84–86]. Mutations in CEP152 are a rare cause of MCPH; however, they are a frequent cause of Seckel syndrome [16,84,85]. CEP152 interacts with several proteins during the cell cycle; interestingly, CEP152 interacts with another protein linked with Seckel syndrome, namely CEP63, to cooperatively generate an initial complex that self-assembles into a higher-order cylindrical structure that recruits downstream components for centriole duplication [87]. In addition, CEP152 interacts with CINP, a protein implicated in DNA damage response and genome maintenance; thus, it is also involved in the regulation of cell cycle checkpoints [88]. Loss of CEP152 results in the formation of monopolar spindles, chromosomal instability and, subsequently, mitotic defects [89].

3.10. ZNF335 (MCPH10)

ZNF335 encodes a zinc finger protein, of the H3K4 methyltransferase complex, localized in the nucleus, with roles in chromatin remodeling and transcriptional regulation [90,91]. ZNF335 is expressed in neural progenitor cells and prevents early cell cycle exit by regulating the levels of REST/NRSF, a key regulator of the proliferation and neuronal differentiation [91]. Mutations of ZNF335 cause severe microcephaly associated with neuronal disorganization and small brain size, therefore its characterization as MCPH gene has been questioned [7]. In mice, conditional knockout of Znf335 in the nervous system results in severely reduced cortical size, premature differentiation, and impaired neuronal morphogenesis [91].

3.11. PHC1 (MCPH11)

PHC1 encodes a component of the Polycomb complex and is localized in the nucleus [92]. PHC1 interacts with H2A and is required for its ubiquitination; cells derived from MCPH11 patients are characterized by defects in DNA repair, at baseline and following irradiation, implicating PHC1 in DNA damage repair [92,93]. Additionally, PHC1 interacts with Geminin, a protein that inhibits DNA replication; in cells that lack PHC1 geminin degradation is impaired, leading to abnormal cell cycle [93]. In Pch1, knockout mice anteroposterior patterning as well as heart and skeleton development are affected [94].
Furthermore, *Pch1* is required for the activity of hematopoietic stem cells in a gene dosage-dependent manner [95,96].

3.12. CDK6 (MCPH12)

CDK6 is a member of the CDK family that includes proteins that have key role in cell cycle progression in G1 and S phase. During interphase, CDK6 is localized in the cytosol and in the nucleus; however, it is also found at the centrosomes and at the spindle poles [97]. Patient-derived cells exhibit reduced cell proliferation, while defects in the organization of the MTs and of the mitotic spindles, as well as supernumerary centrosomes are observed [97]. Mice lacking Cdk6 develop normally [98]; however, given that at the onset of neurogenesis in the developing mouse cortex, transcription factor Pax6 directly represses *Cdk6* increasing the length of the G1 phase [99], the human phenotype may reflect novel functions of CDK6 related to the development of the primate telencephalon [100].

3.13. CENPE (MCPH13)

*CENPE* encodes a kinesin-like motor protein that is expressed during the cell cycle with the highest levels at G2/M phase [101]. CENPE is required for capturing spindle MTs and attachment to the kinetochores [102,103]. Defects in the organization of spindle MTs and delayed progression of the mitosis, leading to abnormal exit from mitosis have been described in MCPH13 patient-derived cell lines as well as in medulloblastoma [101,104]; the presence of binucleate cells of unequal size suggests that, in the absence of CENPE, chromosome segregation and cytokinesis fails [101,104]. Knockout of *Cenpe* in mice is embryonic lethal, however, in conditional mutants, severe mitotic chromosome misalignment and chromosome segregation fails, in accordance with the observations in human cells [105,106].

3.14. SASS6 (SAS-6, MCPH14)

*SASS6* encodes a protein with a coiled-coil domain that is expressed in the cells from G2 until mitosis [107,108]. At the onset of centriole duplication, SASS6 is recruited to the centriole and acts as a scaffolding component and during centriole biogenesis it interacts with several proteins [80,86,87,109,110]; overexpression of SASS6 leads to centrosome duplication [111,112].

3.15. MFSD2A (MCPH15)

*MFSD2A* encodes a fatty acid transporter critical for blood–brain–barrier (BBB) function that is expressed in the endothelium of the BBB and in neural stem cells [113]. MFSD2A transports molecules that are required for neurogenesis but are not synthesized in the brain, such as docosahexaenoic acid (DHA) [113–116]. Interestingly, mice lacking functional Mfsd2A in the BBB develop initially DHA deficiency in the brain and then mild microcephaly [116,117].

3.16. ANKLE2 (LEM4, MCPH16)

*ANKLE2* encodes a protein with ankyrin repeat and a LEM domain that is localized in the endoplasmic reticulum and the nuclear envelope and is essential for their integrity [118]. It was first characterized in *Drosophila* in a mutagenesis screen aiming to identify mutants with neurodevelopmental abnormalities [119]. Subsequently, the human homolog was used to search the exome database of the Baylor-Hopkins Center for Mendelian Genomics and an individual was identified bearing variants in *ANKLE2* [118–120]. In *Drosophila*, Ankle2 is essential for the segregation of cell fate determinants during the asymmetric divisions of neuroblasts; in *Ankle2* mutants, neuroblasts self-renewal is abolished and the larvae exhibit small brains [118–120]. Notably, the NS4A protein of ZIKA virus targets ANKLE2 pathway causing environmental microcephaly [118].
3.17. CIT (MCPH17)

CIT encodes two major isoforms that bind to the active form of the small GTPase RhoA; CIT-K is the largest and is specifically expressed in neural progenitor cells in the developing brain, while CIT-N, which lacks the kinase domain, is expressed in post mitotic neurons [121]. Mice lacking isoform CIT-K, but expressing normal levels of CIT-N, develop microcephaly as a result of cytokinesis defects and DNA double strand breaks that activate P53 [122,123]. CIT-K is localized at the spindle poles during metaphase and later at the mid-body [121] and is required for its structural integrity as well as to maintain active RhoA [124]. Fibroblasts isolated from MCPH17 patients do not show any defects, however when patient derived iPSCs are differentiated to neural stem cells, cytokinesis failure followed by apoptosis is observed [125].

3.18. ALFY (WDFY3, MCPH18)

ALFY encodes a phosphatidylinositol 3-phosphate-binding protein, which acts as scaffold protein to facilitate selective autophagy-mediated removal of aggregated intracellular proteins and clearance of mitochondria via mitophagy; a dominant missense mutation of ALFY, has been linked to microcephaly through clearance of DVL3, a target of the canonical Wnt signaling pathway [126]. In Drosophila, expression of the human mutant allele leads to small brain, recapitulating the human phenotype [126]. In the developing mouse brain, ALFY is expressed in the neocortex regulating the proliferation of neural progenitor cells; lack of ALFY product results in the expansion of the radial glial cell population as the number of asymmetric divisions is reduced [127]. Notably, several recessive mutations of ALFY have been linked with macrocephaly underlying the opposing effects that the expression levels of this gene have on brain development [128].

3.19. COPB2 (MCPH19)

COPB2 encodes a subunit of the Golgi coatomer complex required for the retrograde trafficking from the Golgi complex to the endoplasmic reticulum [129]. Hypomorph mutations of COPB2 have been linked to microcephaly; however, mice homozygous for patient variants are normal, probably reflecting species differences [130]. Recently, COPB2 silencing has been shown to inhibit cell proliferation, inducing apoptosis via the JNK/c-jun pathway in a cancer cell line [131].

3.20. KIF14 (MCPH20)

KIF14 encodes a member of the kinesin-3 motor family that is localized at the mitotic spindle, at the spindle midzone to sustain its structure and at the midbody where it acts together with CIT-K to promote cytokinesis [132]. Knockout mice lacking kif14 product exhibit severe microcephaly [133]; recently, exome-sequencing analysis identified microcephaly-causing variants of KIF14 [134]. In mitotic cells derived from MCPH20 patients, neither KIF14 nor CIT-K were detected at the midbody resulting in cytokinesis failure [134].

3.21. NCAPD2 (CNAP1, MCPH21)

NCAPD2 encodes a subunit of condensin I required for the compaction of chromosomes following the breakdown of the nuclear envelope [135]. NCAPD2 contains a nuclear localization signal required also for chromosome targeting and a HEAT repeat, a repetitive array of amphiphilic α-helices used in protein-protein interactions [136]. The interaction between NCAPD2 and the phosphorylated histone H3 targets condensin I on the chromosomes [137]. Lack of functional NCAPD2 affects mitotic chromosome assembly and resolution of sister chromatids [138]. Moreover, NCAPD2 interacts with rootletin, regulating centrosome cohesion and reducing DNA damage caused during centriole splitting [138]. Recently, hypomorphic NCAPD2 mutations leading to decatenation defects that impair chromosome segregation during mitosis have been linked with microcephaly [139,140].
3.22. NCAPD3 (MCPH22)

NCAPD3 encodes a subunit of condensin II, which is required for the early stage of axial shortening during prophase [135]. NCAPD3 contains a HEAT repeat; phosphorylation of NCAPD3 by the mitotic kinase Cdk-1 is required to timely initiate chromosome condensation during prophase [141]. Hypomorphic NCAPD3 mutations leading to decatenation defects that impair chromosome segregation at mitosis have been linked with microcephaly [139]. Fibroblasts derived from patients with NCAPD3 mutations exhibit impaired chromosome segregation [139,142].

3.23. NCAPH (MCPH23)

NCAPH encodes a subunit of condensin I member of the kleisin family of proteins that binds to ATP-binding cassette (ABC)-transporter-like ATPase domains [135]. NCAPH binds directly to DNA and is required for the stable interaction of the condensin complexes with the chromosomes [143]. One patient homozygous for a missense mutation in NCAPH has been reported [139]; patient-derived fibroblasts showed impaired chromosome segregation followed by abnormal recovery from condensation [139].

3.24. NUP37 (MCPH24)

NUP37 encodes one of the components of the nuclear pore subcomplex Nup107-160 required for the assembly of a functional nuclear pore complex [144]. During mitosis, Nup107-160 is essential for the nucleation of MTs on mitotic kinetochores and spindle assembly [144]. So far, one missense mutation in NUP37 has been described by exome sequencing in a family; patient fibroblasts have fewer nuclear pore complexes, altered structure of the nuclear envelope and decreased cellular proliferation rate [145].

3.25. MAP11 (TRAPPC14, C7orf43, MCPH25)

MAP11 encodes a component of the vesicle tethering complex TRAPP II that functions in late Golgi trafficking as a membrane tether [146]. MAP11 is dispensable for TRAPPII activity in Golgi trafficking, but it is essential for the preciliary vesicle trafficking to the mother centriole during ciliogenesis [146]. Recently, a nonsense mutation in MAP11 was shown to cause microcephaly; in addition, knockout of the gene in zebrafish resulted in microcephaly [147]. In SH-SY5Y cells, MAP11 associates with α-tubulin; this association is observed both during mitosis at the spindle as well as later, in cytokinesis at the midbody [147]. Knockdown of MAP11 in SH-SY5Y cells reduces cell proliferation [147].

3.26. LMNB1 (MCPH26) and LMNB2 (MCPH27)

LMNB1 and LMNB2 encode two related components of the nuclear lamina with coiled-coil domains that form filaments and interact with various proteins [148]. In addition, LMNB1 and LMNB2 associate with the mitotic spindle; dominant negative mutant proteins that disrupt the organization of the filaments, impair the formation of the mitotic spindle [149]. Recently, dominant mutations in LMNB1 and LMNB2 have been shown to cause primary microcephaly [150,151]; the analysis of these variants in HeLa cells revealed defects in the formation of the nuclear envelope [150]. Interestingly, mice lacking Lmnb1 or Lmnb2 exhibit neuronal migration defects as well as reduced number of neuronal cells in the cerebral cortex [152,153].

4. Cellular Processes and Molecular Pathways in MCPH

In the past 10 years, advances in next generation sequencing technologies and bioinformatics have accelerated the identification of novel genes and variants associated with MCPH (Table 1). Interestingly, in several cases, only a single patient (NCAPD2, NCAPH), or very few patients (MAP11, NUP37, COPB2, CENPE) with variants in one MCPH gene have been identified, underlining the effectiveness of the current approaches in rare disease diagnosis [154]. The discovery and analysis of novel MCPH genes and variants is of paramount
importance for the understanding not only the pathological mechanisms leading to the disease, but also the mechanisms underlying normal human brain development.

The majority of MCPH cases (approximately 80%) are caused by mutations in *ASPM* or *WDR62*; both proteins are localized at the centrosome and the spindle poles indicating that brain size is particularly vulnerable to mutations that affect these structures. Interestingly, at the centrosome, not only the localization but also the roles of *ASPM* and *WDR62* are closely linked; *ASPM* interacts with *WDR62* and they both participate along with *CEP63* in a protein complex that is required for centriole duplication [27,155]. Moreover, the products of eight other *MCPH* genes have key roles in centriole biogenesis. More specifically, at the proximal end of the parental centriole, *WDR62* recruits *CEP63*; in the absence of *WDR62*, centrosomal localization of *CEP63* is impaired [27]. The next step involves the recruitment of *ASPM* by *WDR62* and of *CEP152* by *CEP63* [27,155]. *CEP152–CEP63* interaction triggers the engagement of the kinase Plk-4, which autophosphorylates and subsequently phosphorylates *STIL*, which in turn recruits *SASS6* [155–159]. *SASS6* and *STIL* oligomerize and form the core of the daughter centriole, engaging two other *MCPH* proteins, *CEP135* and *CENPJ*, essential for microtubule nucleation and lengthening of the centriole [86,108,155,156,160,161]. *ASPM* and *WDR62* are also localized at the pericentriolar material where other proteins, for instance *CDK5RAP2* and pericentrin, are recruited, and microtubules start assembling, as the daughter centriole is forming [30,162,163]. Additionally, *CDK5RAP2* is essential for centriole and centrosome cohesion as in its absence, centriole splitting is detected resulting in supernumerary centrosomes [57]. Microcephalin 1 recruits Chk1 to the centrosome where it acts to ensure the coupling of the centrosome cycle with mitosis [44].

One striking feature of the majority of the *MCPH* proteins is that they have overlapping roles in cellular functions and usually operate in more than one pathways that influence the cell cycle; hence, several *MCPH* genes discussed above, have also key roles in spindle structure and function. *ASPM* is involved in spindle assembly and orientation recruiting CIT-K and dynein–dynactin at the poles, *WDR62* has a key role in stabilizing the spindle poles through protein–protein interactions, and *CDK5RAP2* supports γ-tubulin induced microtubule nucleation in the pericentriolar material scaffold [29,35,37,38,58,144,164,165]. Furthermore, other *MCPH* proteins operate at the spindle; *NUP37* as part of the Nup107-160 complex promotes its assembly through microtubule nucleation while *CDK6* also functions at the spindle poles [144,166]. Moreover, *LMNB1* and *LMNB2* are essential for mitotic spindle assembly by organizing into a matrix-like network that depends on RanGTP [149]. *CASC5* forms the scaffold of the kinetochore for the microtubule attachment to the centromere, controlling also the spindle assembly checkpoint, while *CENPE* is required for the attachment of the spindle microtubules to the kinetochore; both *CENPE* and *CASC5* are also implicated in the spindle assembly checkpoint [62,63,103,167,168]. During anaphase, *ASPM* interacts with CIT-K and, along with *CDK5RAP2* and *CASC5*, they localize at the midbody; *KIF14* is engaged by CIT-K and along with *MAP11* promote cytokinesis [27,132,169–171].

Besides centriole biogenesis and spindle dynamics during the cell cycle, several *MCPH* proteins are implicated in pathways related with DNA dynamics; the recently identified as *MCPH* proteins, *NCPAD2*, *NCPAD3*, and *NCPAH* are all involved in chromosome condensation as components of the condensin complexes [135]. Moreover, Microcephalin 1 acts as regulator of chromosome condensation that inhibits condensin II, interacting both with *NCAPD3* and *NCAPG2* subunits [172]. Microcephalin 1 and PHC1 are also implicated in DNA damage repair; *PCH1* and *ZNF335* have a role in chromatin remodeling and transcription [91,92]. Finally, the (de)phosphorylation of nuclear envelope proteins by ANKLE2 regulates the affinity between the nuclear envelope and the chromatin during the cell cycle [154].

Despite the progress that has been made in *MCPH* molecular genetics, only in the 50% of the patients has the gene causing mutation been identified; these observations along with the complex genetic interactions described above raise the possibility of an oligogenic model of inheritance with variable expressivity and/or incomplete penetrance. Notably, while *Aspm*−/− mice exhibit mild reduction in brain volume, this phenotype is enhanced if the animal is heterozygous for a loss-of-function mutation in *Wdr62*; furthermore, lack of both *Aspm* and
Wdr62 leads to embryonic lethality [27]; similar results were obtained in zebrafish [173]. In-depth analysis of high-throughput DNA sequencing data revealed that patients with MCPH carry a significant burden of variants in 75 genes (MCPH genes included) and identified cases of digenic inheritance [173]. These results pave the way for new approaches in the diagnosis of MCPH by exploiting the molecular pathways that have been identified.

In this review, we focused on the genes involved in non-syndromic primary microcephaly; therefore, genes involved in syndromes associated with primary microcephaly were not discussed. Nevertheless, it is worth noting that CENPJ and CEP152 have been also linked with Seckel syndrome (Table 1), while CEP63, which has been primarily implicated in Seckel syndrome, is also linked to MCPH. Moreover, CENPE has been also linked to microcephalic primordial dwarfism [70,85,101,157]. Given that microcephalies are rare diseases with digenic or oligogenic modes of inheritance in some cases, it is possible that several genes will finally be linked with more than one type of microcephaly, highlighting, thus, the common molecular pathways and pathological mechanisms involved in a spectrum of disorders, including MCPH and related conditions.

Author Contributions: N.S. writing—original draft preparation, E.S. writing—original draft preparation, G.S. writing—review and editing M.E.G. writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Abuelo, D. Microcephaly Syndromes. Semin. Pediatr. Neurol. 2007, 14, 118. [CrossRef] [PubMed]
2. Opitz, J.M.; Holt, M.C. Microcephaly: General considerations and aids to nosology. J. Craniofac. Genet. Dev. Biol. 1990, 10, 175. [PubMed]
3. Hanzlik, E.; Gigante, J. Microcephaly. Children 2017, 4, 47. [CrossRef] [PubMed]
4. Von der Hagen, M.; Pivarcsi, M.; Liebe, J.; von Bernuth, H.; Didonato, N.; Hennernann, J.B.; Buhrer, C.; Wieczorek, D.; Kaindl, A.M. Diagnostic approach to microcephaly in childhood: A two-center study and review of the literature. Dev. Med. Child Neurol. 2014, 56, 732. [CrossRef] [PubMed]
5. Woods, C.G.; Bond, J.; Enard, W. Autosomal recessive primary microcephaly (MCPH): A review of clinical, molecular, and evolutionary findings. Am. J. Hum. Genet. 2005, 76, 717. [CrossRef]
6. Von der Hagen, M. Diagnostic approach to primary microcephaly. Neuropediatrics 2017, 48, 133–134. [CrossRef]
7. Duerincka, S.; Abramowicz, M. The genetics of congenitally small brains. Semin. Cell. Dev. Biol. 2018, 76, 76. [CrossRef]
8. Alcantara, D.; O’Driscoll, M. Congenital Microcephaly. Semin. Cell. Dev. Biol. 2017, 71, 721. [CrossRef] [PubMed]
9. Zaqout, S.; Morris-Rosendahl, D.; Kaindl, A.M. Autosomal Recessive Primary Microcephaly (MCPH): An Update. Orphanet. J. Rare Dis. 2017, 48, 135. [CrossRef]
10. Muhammad, F.; Mahmood Baig, S.; Hansen, L.; Sajid Hussain, M.; Anjum Inayat, I.; Aslam, M.; Anver Qureshi, J.; Kirst, E.; Wajid, M.; et al. Compound heterozygous ASPM mutations in Pakistani MCPH families. Am. J. Med. Genet. A 2009, 149, 926. [CrossRef]
11. Passemand, S.; Titomanlio, L.; Elmaleh, M.; Afenjar, A.; Alessandri, J.L.; Andria, G.; de Villemeur, T.B.; Boespflug-Tanguy, O.; Burglen, L.; Del Guadice, E.; et al. Expanding the clinical and neuroradiologic phenotype of primary microcephaly due to ASPM mutations. Neurology 2009, 73, 962. [CrossRef]
12. Issa, L.; Mueller, K.; Seufert, K.; Kraemer, N.; Rosenkotter, H.; Ninnemann, O.; Buob, M.; Kaindl, A.M.; Morris-Rosendahl, D.J. Clinical and cellular features in patients with primary autosomal recessive microcephaly and a novel CDK5RAP2 mutation. Orphanet J. Rare Dis. 2013, 8, 59. [CrossRef] [PubMed]
13. Yu, T.W.; Mochida, G.H.; Tischfield, D.J.; Sgai, S.K.; Flores-Sarnat, L.; Sergi, C.M.; Topçu, M.; McDonald, M.T.; Barry, B.J.; Felie, J.M.; et al. Mutations in WDR62, encoding a centrosome-associated protein, cause microcephaly with simplified gyri and abnormal cortical architecture. Nat. Genet. 2010, 42, 1015. [CrossRef] [PubMed]
14. Desir, J.; Cassart, M.; David, P.; Van Bogaert, P.; Abramowicz, M. Primary microcephaly with ASPM mutation shows simplified cortical gyration with antero-posterior gradient pre- and post-natally. Am. J. Med. Genet. A 2008, 146, 1439. [CrossRef]
15. Roberts, E.; Hampshire, D.J.; Springell, K.; Pattison, L.; Crow, Y.; Jafari, H.; Corry, P.; Kabani, G.; Mannon, J.; Rashid, Y.; et al. Autosomal recessive primary microcephaly: An analysis of locus heterogeneity and phenotypic variation. J. Med. Genet. 2002, 39, 718–721. [CrossRef]
16. Barbelanne, M.; Tsang, W.T. Molecular and Cellular Basis of Autosomal Recessive Primary Microcephaly. *Biomed. Res. Int.* 2014, 547986. [CrossRef]  
17. Paridaen, J.T.; Huttner, W.B. Neurogenesis during development of the vertebrate central nervous system. *EMBO Rep.* 2014, 15, 351. [CrossRef] [PubMed]  
18. Farkas, L.M.; Huttner, W.B. The cell biology of neural stem and progenitor cells and its significance for their proliferation versus differentiation during mammalian brain development. *Curr. Opin. Cell. Biol.* 2008, 20, 707. [CrossRef]  
19. Noctor, S.C.; Flint, A.C.; Weissman, T.A.; Dammerman, R.S.; Kriegstein, A.R. Neurons derived from radial glial cells establish radial units in neocortex. *Nature* 2001, 409, 714. [CrossRef]  
20. Noctor, S.C.; Flint, A.C.; Weissman, T.A.; Wong, W.S.; Clinton, B.K.; Kriegstein, A.R. Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. *J. Neurosci.* 2002, 22, 3161. [CrossRef] [PubMed]  
21. Vertii, A.; Hehnly, H; Doxsey, S. The Centrosome, a Multitalented Renaissance Organelle. *Cold Spring Harb. Perspect. Biol.* 2016, 8, a025049. [CrossRef]  
22. Prosser, S.L.; Pelletier, L. Mitotic spindle assembly in animal cells: A fine balancing act. *Nat. Rev. Mol. Cell Biol.* 2017, 18, 187. [CrossRef] [PubMed]  
23. McIntosh, J.R. Mitosis. *Cold Spring Harb. Perspect. Biol.* 2016, 8, a023218. [CrossRef] [PubMed]  
24. Jackson, A.P.; Eastwood, H.; Bell, S.M.; Adu, J.; Toomes, C.; Carr, I.M.; Roberts, E.; Hampshire, D.J.; Crow, Y.J.; Mighell, A.J.; et al. Identification of microcephalin, a protein implicated in determining the size of the human brain. *Am. J. Hum. Genet.* 2002, 71, 136. [CrossRef] [PubMed]  
25. do Carmo Avides, M.; Glover, D.M. Abnormal spindle protein, Asp, and the integrity of mitotic centrosomal microtubule organizing centers. *Science* 1999, 283, 1733. [CrossRef] [PubMed]  
26. Higgins, J.; Midgley, C.; Bergh, A.M.; Bell, S.M.; Askham, J.M.; Roberts, E.; Binns, R.K.; Sharif, S.M.; Bennett, C.; Glover, D.M.; et al. Human ASPM participates in spindle organisation, spindle orientation and cytokinesis. *BMC Cell Biol.* 2010, 11, 85. [CrossRef]  
27. Jayaraman, D.; Kodani, A.; Gonzalez, D.M.; Mancias, J.D.; Mochida, G.H.; Vagnoni, C.; Johnson, J.; Krokan, N.; Harper, J.W.; Reiter, J.F.; et al. Microcephaly proteins Wdr62 and Aspm define a mother centriole complex regulating centriole biogenesis, apical complex, and cell fate. *Neuron* 2016, 92, 813. [CrossRef]  
28. Gai, M.; Bianchi, F.T.; Vagnoni, C.; Verni, F.; Bonaccorsi, S.; Pasquero, S.; Berto, G.E.; Sgrò, F.; Chiorto, A.A.; Annaratone, L.; et al. ASPM and CITK regulate spindle orientation by affecting the dynamics of astral microtubules. *EMBO Rep.* 2017, 18, 1870. [CrossRef] [PubMed]  
29. Zhong, X.; Liu, L.; Zhao, A.; Pfeifer, G.P.; Xu, X. The abnormal spindle-like, microcephaly-associated (ASPM) gene encodes a centrosomal protein. *Cell Cycle* 2005, 4, 1227. [CrossRef] [PubMed]  
30. Fish, J.L.; Kosodo, Y.; Enard, W.; Pääbo, S.; Huttner, W.B. Aspm specifically maintains symmetric proliferative divisions of neuroepithelial cells. *Proc. Natl. Acad. Sci. USA* 2006, 103, 10438. [CrossRef] [PubMed]  
31. Pulvers, J.N.; Bryk, J.; Fish, J.L.; Wilsch-Bräuninger, M.; Arai, Y.; Schreier, D.; Naumann, R.; Vogt, J.; et al. Mutations in mouse Aspm (abnormal spindle-like microcephaly associated) cause not only microcephaly but also major defects in the germline. *Proc. Natl. Acad. Sci. USA* 2010, 107, 16595. [CrossRef] [PubMed]  
32. Rujano, M.A.; Sanchez-Pulido, L.; Pennetiier, C.; Le Dez, G.; Basto, R. The microcephaly protein Asp regulates neuroepithelium morphogenesis by controlling the spatial distribution of myosin II. *Nat. Cell. Biol.* 2013, 15, 1294. [CrossRef] [PubMed]  
33. Kim, H.T.; Lee, M.S.; Choi, J.H.; Jung, J.Y.; Ahn, D.G.; Yeo, S.Y.; Choi, D.K.; Kim, C.H. The microcephaly gene asp is involved in brain development in zebrafish. *Biochem. Biophys. Res. Commun.* 2011, 409, 640. [CrossRef] [PubMed]  
34. Jayaraman, D.; Bae, B.I.; Walsh, C.A. The Genetics of Primary Microcephaly. *Annu. Rev. Genom. Hum. Genet.* 2018, 19, 177–200. [CrossRef] [PubMed]  
35. Nichols, A.K.; Khurshid, M.; Désir, J.; Carvalho, O.P.; Cox, J.J.; Thornton, G.; Kausar, R.; Ansar, M.; Ahmad, W.; Verloes, A.; et al. WDR62 is associated with the spindle pole and is mutated in human microcephaly. *Nat. Genet.* 2010, 42, 1010. [CrossRef]  
36. Pervaiz, N.; Abbasi, A.A. Molecular evolution of WDR62, a gene that regulates neocorticogenesis. *Meta. Gene.* 2016, 9, 1. [CrossRef]  
37. Bogoyevitch, M.A.; Yeap, Y.Y.C.; Qu, Z.; Ngoei, K.R.; Yip, Y.Y.; Zhao, T.T.; Heng, J.J.; Ng, D.C.H. WD40-repeat protein 62 is a JNK-phosphorylated spindle pole protein required for spindle maintenance and timely mitotic progression. *J. Cell Sci.* 2012, 125, 5096. [CrossRef] [PubMed]  
38. MacCorkle-Chosnek, R.A.; VanHooser, A.; Goodrich, D.W.; Brinkley, B.R.; Tan, T.H. Cell cycle regulation of ε-Jun N-terminal kinase activity at the centrosomes. *Biochem. Biophys. Res. Commun.* 2001, 289, 173. [CrossRef]  
39. Xu, D.; Zhang, F.; Wang, Y.; Sun, Y.; Xu, Z. Microcephaly associated protein WDR62 regulates neurogenesis through JNK1 in the developing neocortex. *Cell Rep.* 2014, 6, 104. [CrossRef]  
40. Chen, J.F.; Zhang, Y.; Wilde, J.; Hansen, K.C.; Lai, F.; Niswander, L. Microcephaly disease gene Wdr62 regulates mitotic progression of embryonic neural stem cells and brain size. *Nat. Commun.* 2014, 5, 3885. [CrossRef]  
41. Novorol, C.; Burkhartd, J.; Wood, K.J.; Iqbal, A.; Roque, C.; Coutts, N.; Almeida, A.D.; He, J.; Wilkinson, C.J.; Harris, W.A. Microcephaly models in the developing zebrafish retinal neuroepithelium point to an underlying defect in metaphase progression. *Open Biol.* 2013, 3, 130065. [CrossRef]  
42. Sgourdou, P.; Mishra-Gorur, K.; Saotome, I.; Henagariu, O.; Tuysuz, B.; Campos, C.; Ishigame, K.; Giannikou, K.; Quon, J.L.; Sestan, N.; et al. Disruptions in asymmetric centrosome inheritance and WDR62-auora kinase B interactions in primary microcephaly. *Sci. Rep.* 2017, 7, 43708. [CrossRef] [PubMed]
Brain Sci. 2021, 11, 581

43. Pulvers, J.N.; Journiac, N.; Arai, Y.; Nardelli, J. MCPH1: A window into brain development and evolution. Front. Cell. Neurosci. 2015, 9, 92. [CrossRef]

44. Gruber, R.; Zhou, Z.; Sukchev, M.; Joerss, T.; Frappart, P.O.; Wang, Z.Q. MCPH1 regulates the neuroprogenitor division mode by coupling the centrosomal cycle with mitotic entry through the Chk1-Cdc25 pathway. Nat. Cell Biol. 2011, 13, 1325. [CrossRef]

45. Liu, X.; Zhou, Z.W.; Wang, Z.Q. The DNA damage response molecule MCPH1 in brain development and beyond. Acta Biochim. Biophys. Sin. (Shanghai) 2016, 48, 678. [CrossRef]

46. Lin, S.Y.; Rai, R.; Li, K.; Xu, Z.X.; Elledge, S.J. BRIT1/MCPH1 is a DNA damage responsive protein that regulates the Brca1-Chk1 pathway, implicating checkpoint dysfunction in microcephaly. Proc. Natl. Acad. Sci. USA 2005, 102, 15105. [CrossRef] [PubMed]

47. Singh, N.; Wiltshire, T.D.; Thompson, J.R.; Mer, G.; Couch, F.J. Molecular basis for the association of microcephalin (MCPH1) protein with the cell division cycle protein 27 (Cdc27) subunit of the anaphase-promoting complex. J. Biol. Chem. 2012, 287, 2854. [CrossRef]

48. Singh, N.; Basnet, H.; Wiltshire, T.D.; Mohammad, D.H.; Thompson, J.R.; Héroux, A.; Botuyan, M.V.; Yaffe, M.B.; Couch, F.J.; Rosenfeld, M.G.; et al. Dual recognition of phosphoserine and phosphotyrosine in histone variant H2A.X by DNA damage response protein MCPH1. Proc. Natl. Acad. Sci. USA 2012, 109, 14381. [CrossRef]

49. Xu, X.; Lee, J.; Stern, D.F. Microcephalin is a DNA damage response protein involved in regulation of CHK1 and BRCA1. J. Biol. Chem. 2004, 279, 34091. [CrossRef]

50. Cicconi, A.; Rai, R.; Xiong, X.; Broton, C.; Al-Hiyasat, A.; Hu, C.; Dong, S.; Sun, W.; Garbarino, J.; Bindra, R.S.; et al. Microcephalin 1/BRIT1-TRF2 interaction promotes telomere replication and repair, linking telomere dysfunction to primary microcephaly. Nat. Commun. 2020, 11, 5861. [CrossRef] [PubMed]

51. Zhou, Z.W.; Tapias, A.; Bruhn, C.; Gruber, R.; Sukchev, M.; Wang, Z.Q. DNA damage response in microcephaly development of MCPH1 mouse model. DNA Repair (Amst) 2013, 12, 649. [CrossRef]

52. Brunk, K.; Vernay, B.; Griffith, E.; Reynolds, N.L.; Strutt, D.; Ingham, P.W.; Jackson, A.P. Microcephalin coordinates mitosis in the Drosophila embryo. J. Cell. Sci. 2007, 120, 3578. [CrossRef]

53. Rickmyre, J.L.; Dasgupta, S.; Oei, D.L.; Keel, J.; Lee, E.; Kirschner, M.W.; Wadell, S.; Lee, L.A. The Drosophila homolog of MCPH1, a human microcephaly gene, is required for genomic stability in the early embryo. J. Cell Sci. 2007, 120, 3565–3577. [CrossRef]

54. Fong, K.W.; Choi, Y.K.; Rattner, J.B.; Qi, R.Z. CDK5RAP2 is a pericentriolar protein that functions in centrosomal attachment of the gamma-tubulin ring complex. Mol. Biol. Cell. 2008, 19, 115. [CrossRef]

55. Barrera, J.A.; Kao, L.R.; Hammer, R.E.; Seemann, J.; Fuchs, J.L.; Megraw, T.L. Cdk5rap2 regulates centrosome function and chromosome segregation in mice. Dev. Cell. 2010, 18, 913. [CrossRef]

56. Buchman, J.J.; Tseng, H.C.; Zhou, Y.; Frank, C.L.; Xie, Z.; Tsai, L.H. Cdk5rap2 interacts with pericentrin to maintain the neural progenitor pool. J. Cell Sci. 2020, 122, e202006085. [CrossRef]

57. Watanabe, S.; Meitinger, F.; Shioua, A.K.; Oegema, K.; Desai, A. Centriole-independent mitotic spindle assembly relies on the PCNT-CDK5RAP2 pericentriolar matrix. J. Cell. Biol. 2020, 219, e202006101. [CrossRef]

58. Zhang, X.; Liu, D.; Lv, S.; Wang, H.; Zhong, X.; Liu, B.; Wang, B.; Liao, J.; Li, J.; Pfeifer, G.P.; et al. CDK5RAP2 is required for spindle checkpoint function. Cell Cycle 2009, 8, 1206–1216. [CrossRef]

59. Barrera, J.A.; Kao, L.R.; Hammer, R.E.; Seemann, J.; Fuchs, J.L.; Megraw, T.L. Cdk5rap2 regulates centriole engagement and cohesion in mice. Dev. Cell. 2010, 18, 913. [CrossRef]

60. Buchman, J.J.; Tseng, H.C.; Zhou, Y.; Frank, C.L.; Xie, Z.; Tsai, L.H. Cdk5rap2 interacts with pericentrin to maintain the neural progenitor pool in the developing neocortex. Neuron 2010, 66, 386–402. [CrossRef]

61. Lazzaraga, S.B.; Margossian, S.P.; Harris, M.H.; Campagna, D.R.; Han, A.P.; Blevins, S.; Mudhbhary, R.; Barker, J.E.; Walsh, C.A.; Fleming, M.D. Cdk5rap2 regulates centrosome function and chromosome segregation in neuronal progenitors. Development 2010, 137, 1907. [CrossRef] [PubMed]

62. Cheevesan, I.M.; Chappie, J.S.; Wilson-Kubalek, E.M.; Desai, A. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell 2006, 127, 983. [CrossRef] [PubMed]

63. Genin, A.; Desir, J.; Lambert, N.; Biervliet, M.; Van Der Aa, N.; Pierquín, G.; Killian, A.; Tosi, M.; Urbina, M.; Lefort, A.; et al. Kinetochore KMN network gene CASC5 mutated in primary microcephaly. Hum. Mol. Genet. 2012, 21, 5306. [CrossRef]

64. Szczepanski, S.; Hussain, M.S.; Sur, I.; Altmüller, J.; Thiele, H.; Abdullah, U.; Waseem, S.S.; Moawia, A.; Nürnberg, G.; Noegel, A.A.; et al. A novel homozygous splicing mutation of CASC5 causes primary microcephaly in a large Pakistani family. Hum. Mol. Genet. 2016, 135, 157. [CrossRef] [PubMed]

65. Hung, L.Y.; Tang, C.J.; Tang, T.K. Protein 4.1 R-135 interacts with a novel centrosomal protein (CPAP) which is associated with the gamma-tubulin complex. Mol. Cell. Biol. 2020, 20, 7813. [CrossRef] [PubMed]

66. Cottee, M.A.; Muschakil, N.; Wong, Y.L.; Johnson, C.M.; Johnson, S.; Andreeva, A.; Oegema, K.; Lea, S.M.; Raff, J.W.; van Breugel, M. Crystal structures of the CPAP/STIL complex reveal its role in centriole assembly and human microcephaly. Elife 2013, 2, e01071. [CrossRef]

67. Bond, J.; Roberts, E.; Springell, K.; Lazzaraga, S.B.; Scott, S.; Higgins, J.; Hampshire, D.J.; Morrison, E.E.; Leal, G.F.; Silva, E.O.; et al. A centrosomal mechanism involving CDK5RAP2 and CENP| controls brain size. Nat. Genet. 2005, 37, 353. [CrossRef]

68. Gabriel, E.; Wason, A.; Ramani, A.; Goot, L.M.; Keller, P.; Pozniakovsky, A.; Poser, I.; Noack, F.; Telugu, N.S.; Calegari, F.; et al. CPAP promotes timely cilium disassembly to maintain neural progenitor pool. EMBO J. 2016, 35, 803. [CrossRef]
McIntyre, R.E.; Lakshminarasimhan Chavali, P.; Ismail, O.; Carragher, D.M.; Sanchez-Andrade, G.; Forment, J.V.; Fu, B.; Del Castillo Velasco-Herrera, M.; Edwards, A.; van der Weyden, L.; et al. Disruption of mouse Cenpj, a regulator of centriole biogenesis, phenocopies centrosome abnormalities. *PloS Genet.* 2012, 8, e1002221. [CrossRef]

Al-Dosari, M.S.; Shaheen, R.; Colak, D.; Alkuraya, F.S. Novel CENP] mutation causes Seckel syndrome. *J. Med. Genet.* 2010, 47, 411. [CrossRef]

Kitagawa, D.; Kohlmaier, G.; Keller, D.; Strnad, P.; Balestra, F.R.; Fluckiger, I.; Gonczy, P. Spindle positioning in human cells relies on proper centriole formation and on the microphaly proteins CPAP and STIL. *J. Cell. Sci.* 2011, 124, 3884. [CrossRef] [PubMed]

Arquint, C.; Sonnen, K.F.; Stierhof, Y.D.; Nigg, E.A. Cell-cycle-regulated expression of STIL controls centriole number in human cells. *J. Cell Sci.* 2012, 125, 1342. [CrossRef]

Kleylein-Sohn, J.; Westendorf, J.; Le Clech, M.; Habedanck, R.; Stierhof, Y.D.; Nigg, E.A. Plk4-induced centriole biogenesis in human cells. *Dev. Cell* 2007, 13, 190. [CrossRef]

Pfaff, K.L.; Straub, C.T.; Chiang, K.; Bear, D.M.; Zhou, Y.; Zon, L.I. The zebrafish cassiopeia mutant reveals that SIL is required for mitotic spindle organization. *Mol. Cell. Biol.* 2007, 27, 5887. [CrossRef] [PubMed]

David, A.; Liu, F.; Tibelius, A.; Vulprecht, J.; Wald, D.; Rothermel, U.; Ohana, R.; Seitel, A.; Metzger, J.; Ashery-Padan, R.; et al. Lack of centrioles and primary cilia in STIL(−/−) mouse embryos. *Cell Cycle* 2014, 13, 2859. [CrossRef]

Li, L.; Liu, C.; Carr, A.L. STIL: A multi-function protein required for dopaminergic neural proliferation, protection, and regeneration. *Cell. Death Discov.* 2019, 5, 90. [CrossRef]

Hussain, M.S.; Baig, S.M.; Neumann, S.; Nürnberg, G.; Farooq, M.; Ahmad, I.; Alef, T.; Hennies, H.C.; Technau, M.; Altmüller, J.; et al. A truncating mutation of CEP135 causes primary microcephaly and disturbed centrosomal function. *Am. J. Hum. Genet.* 2012, 90, 871. [CrossRef]

Kraatz, S.; Guichard, P.; Obbineni, J.M.; Olieric, N.; Hatzopoulos, G.N.; Hilbert, M.; Sen, I.; Missimer, J.; Gonczy, P.; Steinmetz, M.O. The Human Centriolar Protein CEP135 Contains a Two-Stranded Coiled-Coil Domain Critical for Microtubule Binding. *Structure* 2016, 24, 1358. [CrossRef]

Kim, K.; Lee, S.; Chang, J.; Rhee, K. A novel function of CEP135 as a platform protein of C-NAP1 for its centriolar localization. *Exp. Cell Res.* 2008, 314, 3692. [CrossRef]

Lin, Y.C.; Chang, C.W.; Hsu, W.B.; Tang, C.J.; Lin, Y.N.; Chou, E.J.; Wu, C.T.; Tang, T.K. Human microcephaly protein CEP135 binds to hSAS-6 and CPAP, and is required for centriole assembly. *EMBO J.* 2013, 32, 1141. [CrossRef] [PubMed]

Carvalho-Santos, Z.; Machado, P.; Alvarez-Martins, I.; Gouveia, S.M.; Jana, S.C.; Duarte, P.; Amado, T.; Branco, P.; Freitas, M.C.; Silva, S.T.; et al. BLD10/CEP135 is a microtubule-associated protein that controls the formation of the flagellum central microtubule pair. *Dev. Cell* 2012, 23, 412. [CrossRef]

Inanç, B.; Pütz, M.; Lalar, P.; Dockezy, P.; Kuriyama, R.; Gergely, F.; Morrison, C.G. Abnormal centrosomal structure and duplication in Cep135-deficient vertebrate cells. *Mol. Biol. Cell.* 2013, 24, 2645. [CrossRef] [PubMed]

Roque, H.; Wainman, A.; Richens, J.; Kozyrska, K.; Franz, A.; Raff, J.W. Drosophila Cep135/Bld10 maintains proper centriole structure but is dispensable for cartwheel formation. *J. Cell Sci.* 2012, 125 Pt 23, 5881. [CrossRef]

Andersen, J.S.; Wilkinson, C.J.; Mayor, T.; Mortensen, P.; Nigg, E.A.; Mann, M. Proteome characterization of the human centrosome by protein correlation profiling. *Nature* 2003, 426, 570–574. [CrossRef]

Guernsey, D.L.; Jiang, H.; Hussin, J.; Arnold, M.; Bouyakdan, K.; Perry, S.; Babineau-Sturk, T.; Beis, J.; Dumas, N.; Evans, S.C. Mutations in centrosomal protein CEP152 in primary microcephaly families linked to MCPH4. *Am. J. Hum. Genet.* 2010, 87, 40. [CrossRef]

Gartenmann, L.; Vicente, C.C.; Wainman, A.; Novak, Z.A.; Sieber, B.; Richens, J.H.; Raff, J.W. Drosophila Sas-6, Ana2 and Sas-4 self-organise into macromolecular structures that can be used to probe centriole and centrosome assembly. *J. Cell Sci.* 2020, 133, jcs244574. [CrossRef] [PubMed]

Kim, T.S.; Zhang, L.; Il Ahn, J.; Meng, L.; Chen, Y.; Lee, E.; Bang, J.K.; Lim, J.M.; Ghihrlando, R.; Fan, L.; et al. Molecular architecture of a cylindrical self-assembly at human centrosomes. *Nat. Commun.* 2019, 10, 1151. [CrossRef] [PubMed]

Kalay, E.; Yigit, G.; Aslan, Y.; Brown, K.E.; Pohl, E.; Bicknell, L.S.; Kayserilli, H.; Li, Y.; Tüysüz, B.; Nürnberg, G.; et al. CEP152 is a genome maintenance protein disrupted in Seckel syndrome. *Nat. Genet.* 2011, 43, 23. [CrossRef] [PubMed]

Dzhindzhev, N.S.; Yu, Q.D.; Weiskopf, K.; Tzolovsky, G.; Cunha-Ferreira, I.; Riparbelli, M.; Rodrigues-Martins, A.; Bettencourt-Dias, M.; Callaini, G.; Glover, D.M. Asterless is a scaffold for the onset of centriole assembly. *J. Cell Sci.* 2011, 124, 190. [CrossRef] [PubMed]

Garapaty, S.; Xu, C.F.; Trojer, P.; Mahajan, M.A.; Neubert, T.A.; Samuels, H.H. Identification and characterization of a novel nuclear protein complex involved in nuclear hormone receptor-mediated gene regulation. *J. Biol. Chem.* 2009, 284, 7542. [CrossRef] [PubMed]

Yang, Y.J.; Baltus, A.E.; Mathew, R.S.; Murphy, E.A.; Evrory, G.D.; Gonzalez, D.M.; Wang, E.P.; Marshall-Walker, C.A.; Barry, B.J.; Murn, J. Microcephaly gene links trithorax and REST/NRSF to control neural stem cell proliferation and differentiation. *Cell* 2012, 151, 1097. [CrossRef] [PubMed]

Awad, S.; Al-Dosari, M.S.; Al-Yacoub, N.; Colak, D.; Salih, M.A.; Alkuraya, F.S.; Poizat, C. Mutation in PHC1 implicates chromatin remodeling in primary microcephaly pathogenesis. *Hum. Mol. Genet.* 2013, 22, 2200. [CrossRef]

Ohtsubo, M.; Yasunaga, S.; Ohno, Y.; Tsumura, M.; Okada, S.; Ishikawa, N.; Shirao, K.; Kikuchi, A.; Nishitani, H.; Kobayashi, M.; et al. Polycomb-group complex 1 acts as an E3 ubiquitin ligase for Geminin to sustain hematopoietic stem cell activity. *Proc. Natl. Acad. Sci. USA* 2008, 105, 10396. [CrossRef] [PubMed]
94. Takihara, Y.; Tomotsune, D.; Shirai, M.; Katoh-Fukui, Y.; Nishii, K.; Motaleb, M.A.; Nomura, M.; Tsujiya, R.; Fujita, Y.; Shibata, Y.; et al. Targeted disruption of the mouse homologue of the Drosophila polyhomeotic gene leads to altered anteroposterior patterning and neural crest defects. Development 1997, 124, 3673. [CrossRef] [PubMed]

95. Ohta, H.; Sawada, A.; Kim, J.Y.; Tokimasa, S.; Nishiguchi, S.; Humphries, R.K.; Hará, J.; Takihara, Y. Polycomb group gene rae28 is required for sustaining activity of hematopoietic stem cells. J. Exp. Med. 2002, 195, 759. [CrossRef] [PubMed]

96. Tokimasa, S.; Ohta, H.; Sawada, A.; Matsuda, Y.; Kim, J.Y.; Nishiguchi, S.; Hará, J.; Takihara, Y. Lack of the Polycomb-group gene rae28 causes maturation arrest at the early B-cell development stage. Exp. Hematol. 2001, 29, 93. [CrossRef]

97. Hussain, M.S.; Baig, S.M.; Neumann, S.; Peche, V.S.; Szczepanski, S.; Nürnberg, G.; Tariq, M.; Jameel, M.; Khan, T.N.; Fatima, A. CDK6 associates with the centrosome during mitosis and is mutated in a large Pakistani family with primary microcephaly. Hum. Mol. Genet. 2013, 22, 5199. [CrossRef]

98. Malumbres, M.; Sotillo, R.; Santamaría, D.; Galán, J.; Cerezo, A.; Ortega, S.; Dubus, P.; Barbacid, M. Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. Cell 2004, 118, 493. [CrossRef]

99. Mi, D.; Carr, C.B.; Georgala, P.A.; Huang, Y.T.; Manuel, M.N.; Jeanes, E.; Niisato, E.; Sansom, S.N.; Livesey, F.J.; Theil, T.; et al. Pax6 exerts regional control of cortical progenitor proliferation via direct repression of Cdk6 and hypophosphorylation of pRb. Neuron 2013, 78, 269. [CrossRef]

100. Vaid, S.; Huttner, W.B. Transcriptional Regulators and Human-Specific/Primate-Specific Genes in Neocortical Neurogenesis. Int. J. Mol. Sci. 2020, 21, 4614. [CrossRef]

101. Mirzaa, G.M.; Vitre, B.; Carpenter, G.; Abramowicz, I.; Gleeson, J.G.; Paciorkowski, A.R.; Cleveland, D.W.; Dobyns, W.B.; O'Driscoll, M. Mutations in CENPE define a novel kinetochore-centromeric mechanism for microphalic primordial dwarfism. Hum. Genet. 2014, 133, 1023. [CrossRef] [PubMed]

102. Gudimchuk, N.; Vitre, B.; Kim, Y.; Kiyatkin, A.; Cleveland, D.W.; Ataullakhanov, F.I.; Grishchuk, E.L. Kinetochore kinase CENP-E is a processive bi-directional tracker of dynamic microtubule tips. Nat. Cell Biol. 2013, 15, 1079. [CrossRef] [PubMed]

103. Yu, K.W.; Zhong, N.; Xiao, Y.; She, Z.Y. Mechanisms of kinesin-7 CENP-E in kinetochore-microtubule capture and chromosome alignment during cell division. Biol. Cell 2019, 111, 143–160. [CrossRef] [PubMed]

104. Iegiani, G.; Gai, M.; Di Cunto, F.; Pallavicini, G. CENPE Inhibition Leads to Mitotic Catastrophe and DNA Damage in Medulloblastoma. Cells Cancers 2021, 13, 1028. [CrossRef] [PubMed]

105. Putkey, F.R.; Cramer, T.; Morphew, M.K.; Silk, A.D.; Johnson, R.S.; McIntosh, J.R.; Cleveland, D.W. Unstable Kinetochore-Microtubule Capture and Chromosomal Instability Following Deletion of CENPE. Dev. Cell 2002, 3, 351. [CrossRef]

106. Weaver, B.A.A.; Bonday, Z.Q.; Putkey, F.R.; Kops, G.J.P.L.; Silk, A.D.; Cleveland, D.W. Centromere-associated protein-E is essential for the mammalian mitotic checkpoint to prevent aneuploidy due to single chromosome loss. J. Cell Biol. 2003, 162, 551. [CrossRef]

107. Vladar, E.K.; Stearns, T. Molecular characterization of centriole assembly in ciliated epithelial cells. J. Cell Biol. 2007, 178, 31. [CrossRef]

108. Keller, D.; Orpinell, M.; Olivier, N.; Wachsmuth, M.; Mahen, R.; Wyss, R.; Hachet, V.; Ellenberg, J.; Manley, S.; Göncz, P. Mechanisms of HsSAS-6 assembly promoting centriole formation in human cells. J. Cell Biol. 2014, 204, 697–712. [CrossRef]

109. Khan, M.A.; Rupp, VM.; Orpinell, M.; Hussain, M.S.; Altmüller, J.; Steinmetz, M.O.; Enzinger, C.; Thiele, H.; Höhne, W.; Nürnberg, G.; et al. A missense mutation in the PISA domain of HsSAS-6 causes autosomal recessive primary microcephaly in a large consanguineous Pakistani family. Hum. Mol. Genet. 2014, 23, 5940. [CrossRef]

110. Zhang, Y.; Li, H.; Pang, J.; Peng, Y.; Shu, L.; Wang, H. Novel SAS6 compound heterozygous mutations in a Chinese family with primary autosomal recessive microcephaly. Clin. Chim. Acta. 2019, 491, 15. [CrossRef]

111. Leidel, S.; Delattre, M.; Cerutti, L.; Baumer, K.; Göncz, P. SAS-6 defines a protein family required for centrosome duplication in, C. elegans and in human cells. Nat. Cell Biol. 2005, 7, 115. [CrossRef] [PubMed]

112. Strand, P.; Leidel, S.; Vinogradova, T.; Euteneuer, U.; Khodjakov, A.; Göncz, P. Regulated HsSAS-6 levels ensure formation of a single procentriole per centriole during the centrosome duplication cycle. Dev. Cell 2007, 13, 203. [CrossRef] [PubMed]

113. Wong, B.H.; Silver, D.L. Mfsd2a: A Physiologically Important Lysolipid Transporter in the Brain and Eye. Adv. Exp. Med. Biol. 2020, 1276, 223. [CrossRef]

114. Nguyen, L.N.; Ma, D.; Shui, G.; Wong, P.; Cazenave-Gassiot, A.; Zhang, X.; Wenk, M.R.; Goh, E.L.K.; Silver, D.L. Mfsd2a is a transporter for the essentialomega-3 fatty acid docosahexaenoic acid. Nature 2014, 509, 503. [CrossRef]

115. Guemez-Gamboa, A.; Nguyen, L.N.; Yang, H.; Zaki, M.S.; Kara, M.; Ben-Orman, T.; Akizu, N.; Rosti, R.O.; Rosti, B.; Scott, E.; et al. Inactivating mutations in MFSD2A cause omega-3 fatty acid transport in brain, cause a lethal microcephaly syndrome. Nat. Genet. 2015, 47, 809. [CrossRef]

116. Alakbarzade, V.; Hameed, A.; Quek, D.Q.; Chioza, B.A.; Baple, E.L.; Cazenave-Gassiot, A.; Nguyen, L.N.; Wenk, M.R.; Ahmad, A.Q.; Sreekantan-Nair, A.; et al. A partially inactivating mutation in the sodium-dependent lysophosphatidylcholine transporter MFSD2A causes a non-lethal microcephaly syndrome. Nat. Genet. 2015, 47, 814. [CrossRef]

117. Chan, J.P.; Wong, B.H.; Chin, C.F.; Galam, D.L.A.; Foo, J.C.; Wong, L.C.; Ghosh, S.; Wenk, M.R.; Cazenave-Gassiot, A.; Silver, D.L. The lysolipid transporter Mfsd2a regulates lipogenesis in the developing brain. PLoS Biol. 2018, 16, e2006443. [CrossRef] [PubMed]

118. Link, N.; Chung, H.; Jolly, A.; Withers, M.; Tepe, B.; Arenkel, B.R.; Shah, P.S.; Krogen, N.J.; Aydin, H.; Geckinli, B.B.; et al. Mutations in ANKLE2, a ZIKA Virus Target, Disrupt an Asymmetric Cell Division Pathway in Drosophila Neuroblasts to Cause Microcephaly. Dev. Cell 2019, 51, 713. [CrossRef]
119. Yamamoto, S.; Jaiswal, M.; Charrng, W.L.; Gambin, T.; Karaca, E.; Mirzaa, G.; Wiszniewski, W.; Sandoval, H.; Haelterman, N.A.; Xiong, B.; et al. A Drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases. Cell 2014, 159, 200. [CrossRef]

120. Posey, J.E.; O’Donnell-Luria, A.H.; Chong, J.X.; Harel, T.; Jhangiani, S.N.; Coban Akdemir, Z.H.; Buyske, S.; Pehlivan, D.; Carvalho, C.M.B.; Baxter, S.; et al. Insights into genetics, human biology and disease gleaned from family based genomic studies. Genet. Med. 2019, 21, 798. [CrossRef]

121. Bianchi, E.T.; Gai, M.; Berto, G.E.; Di Cunto, F. Of rings and spines: The multiple facets of Citron proteins in neural development. Small GT Proteins 2020, 11, 122–130. [CrossRef]

122. Di Cunto, F.; Imanisro, S.; Hirsch, E.; Broccoli, V.; Bulfone, A.; Miglieli, A.; Atzori, C.; Turco, E.; Triolo, R.; Dotto, G.P.; et al. Defective neurogenesis in citron kinase knockout mice by altered cytokinesis and massive apoptosis. Neuron 2000, 28, 115. [CrossRef]

123. Bianchi, F.T.; Tocco, C.; Pallavicini, G.; Liu, Y.; Verni, F.; Merigliano, C.; Bonaccorsi, S.; El-Assawy, N.; Priano, L.; Gai, M.; et al. Citron Kinase Deficiency Leads to Chromosomal Instability and TP53-Sensitive Microphagocytosis. Cell Rep. 2017, 18, 1674. [CrossRef]

124. McKenzie, C.; Bassi, Z.L.; Debski, J.; Gottardo, M.; Callaini, G.; Dadlez, M.; D’Avino, P.P. Cross-regulation between Aurora B and Citron kinase controls midbody architecture in cytokinesis. Open Biol. 2016, 6, 160019. [CrossRef]

125. Li, H.; Bielas, S.L.; Zaki, M.S.; Ismail, S.; Farfara, D.; Um, K.; Rosti, R.O.; Scott, E.C.; Tu, S.; Chi, N.C.; et al. Biallelic Mutations in WDFY3 in mice alters cerebral cortical neurogenesis reflecting aspects of the autism pathology. Nat. Commun. 2015, 4, 692. [CrossRef]

126. Kadir, R.; Harel, T.; Markus, B.; Perez, Y.; Bakhrat, A.; Cohen, I.; Volodarsky, M.; Feintsein-Linial, M.; Chervinski, E.; Zlotogora, J.; et al. ALFY-Driven Cytokinesis Promotes Efficient Cytokinesis. PLoS Genet. 2016, 12, e1005919. [CrossRef]

127. Orosco, L.A.; Ross, A.P.; Cates, S.L.; Scott, S.E.; Wu, D.; Sohn, J.; Rosti, R.O.; Scott, E.C.; Tu, S.; Chi, N.C.; et al. Biallelic Mutations of WDFY3 variants cause neurodevelopmental disorders and opposing effects on brain size. Brain 2019, 142, 2617. [CrossRef]

128. Le Duc, D.; Giulivi, C.; Hiatt, S.M.; Napoli, E.; Panoutsopoulos, A.; Harlan De Crescenzo, A.; Kotzaeridou, U.; Syrbe, S.; Anagnostou, E.; Azage, M.; et al. Pathogenic WDFY3 variants cause neurodevelopmental disorders and opposing effects on brain size. Brain 2019, 142, 2617. [CrossRef]

129. Styers, M.L.; O’Connor, A.K.; Grabski, R.; Cormet-Boyak, E.; Sztl, E. Depletion of beta-COP reveals a role for COP-I in compartmentalization of secretory compartments and in biosynthetic transport of caveolin-1. Am. J. Physiol. Cell Physiol. 2008, 294, C1485. [CrossRef]

130. DiStasio, A.; Driver, A.; Sund, K.; Donlin, M.; Muraleedharan, R.M.; Pooya, S.; Kline-Fath, B.; Kaufman, K.M.; Prows, C.A.; et al. Copb2 is essential for embryogenesis and hypomorphic mutations cause human microcephaly. Hum. Mol. Genet. 2017, 26, 4836. [CrossRef]

131. Wang, Y.; Xie, G.; Li, M.; Du, J.; Wang, M. COPB2 gene silencing inhibits colorectal cancer cell proliferation and induces apoptosis via the JNK/c-Jun signaling pathway. PLoS ONE 2020, 15, e0240106. [CrossRef]

132. Gruneberg, U.; Neef, R.; Li, X.; Chan, E.H.; Chalamalasetty, R.B.; Nigg, E.A.; Barr, F.A. KIF14 and Citron kinase act together to promote efficient cytokinesis. J. Cell Biol. 2006, 172, 363. [CrossRef]

133. Fujikura, K.; Setsu, T.; Tanigaki, K.; Abe, T.; Kiyonari, H.; Terashima, T.; Sakisaka, T. Kif14 mutation causes severe brain malformation and hypomyelination. PLoS ONE 2013, 8, e53490. [CrossRef] [PubMed]

134. Moawia, A.; Shaheen, R.; Rasool, S.; Waseem, S.S.; Ewida, N.; Budde, B.; Kawalia, A.; Motameny, S.; Khan, K.; Fatima, A.; et al. Mutations of KIF14 cause primary microphagocytosis by impairing cytokinesis. Ann. Neurol. 2017, 82, 562. [CrossRef] [PubMed]

135. Shintomi, K.; Hirano, T. The relative ratio of condensin I to II determines chromosome shapes. Genes Dev. 2011, 25, 1464. [CrossRef]

136. Watrin, E.; Legagneux, V. Contribution of hCAP-D2, a non-SMC subunit of condensin I, to chromosome and chromosomal protein assembly. Genes Dev. 2014, 28, 293–299. [CrossRef]

137. Di Cunto, F.; Imarisio, S.; Hirsch, E.; Broccoli, V.; Bulfone, A.; Miglieli, A.; Atzori, C.; Turco, E.; Triolo, R.; Dotto, G.P.; et al. Defective neurogenesis in citron kinase knockout mice by altered cytokinesis and massive apoptosis. Neuron 2000, 28, 115. [CrossRef]

138. Kirschner, M.W.; Mitchison, T.J. The forces that pull and push chromosomes during mitosis. Cell 1986, 46, 293–299. [CrossRef]

139. Aobe, S.; Natsui, K.; Arata, T.; Ooi, Y.; Nakamura, T.; Hirota, T. The initial phase of chromosome condensation requires Cdk1-mediated phosphorylation of the CAP-D3 subunit of condensin II. Genes Dev. 2011, 25, 863. [CrossRef]

140. D’Ambrosio, C.; Kelly, G.; Shirahige, K.; Uhlmann, F. Condensin-dependent rDNA decatenation introduces a temporal pattern to chromosome segregation. Curr. Biol. 2008, 18, 1084–1089. [CrossRef]

141. Kschenk, M.; Merkel, F.; Bist, S.; Metz, J.; Rybin, V.; Hassler, M.; Haering, C.H. Structural Basis for a Safety-Belt Mechanism That Anchors Condensin to Chromosomes. Cell 2017, 171, 588. [CrossRef]
144. Mishra, R.K.; Chakraborty, P.; Arnaoutov, A.; Fontoura, B.M.; Dasso, M. The Nup107-160 complex and gamma-TuRC regulate microtubule polymerization at kinetochores. *Nat. Cell Biol.* 2010, 12, 164. [CrossRef]

145. Braun, D.A.; Lovric, S.; Schapiro, D.; Schneider, R.; Marquez, J.; Asif, M.; Hussain, M.S.; Daga, A.; Widmer, E.; Rao, J.; et al. Mutations in multiple components of the nuclear pore complex cause nephrotic syndrome. *J. Clin. Investig.* 2018, 128, 4313. [CrossRef]

146. Cuenca, A.; Insinna, C.; Zhao, H.; John, P.; Weiss, M.A.; Lu, Q.; Walia, V.; Specht, S.; Manivannan, S.; Stauffer, J. The C7orf43/TRAPPC14 component links the TRAPP11 complex to Rab8α for preciliary vesicle tethering at the mother centriole during ciliogenesis. *J. Biol. Chem.* 2019, 294, 15418. [CrossRef] [PubMed]

147. Perez, Y.; Bar-Yaacov, R.; Kadir, R.; Wormser, O.; Shleif, I.; Birk, O.S.; Flusser, H.; Birnbaum, R.Y. Mutations in the microtubule-associated protein MAP11 (C7orf43) cause microcephaly in humans and zebrafish. *Brain* 2019, 142, 574. [CrossRef] [PubMed]

148. Gruenbaum, Y.; Foissner, R. Lamins: Nuclear intermediate filament proteins with fundamental functions in nuclear mechanics and genome regulation. *Annu. Rev. Biochem.* 2015, 84, 131. [CrossRef] [PubMed]

149. Tsai, M.Y.; Wang, S.; Heidinger, J.M.; Shumaker, D.K.; Adam, S.A.; Goldman, R.D.; Zheng, Y. A mitotic lamin B matrix induced by RanGTP required for spindle assembly. *Science* 2006, 311, 1887. [CrossRef]

150. Cristofoli, F.; Moss, T.; Moore, H.W.; Devriendt, K.; Flanagan-Steet, H.; May, M.; Jones, J.; Roelens, F.; Fons, C.; Fernandez, A.; et al. De novo variants in LMNB1 cause pronounced syndromic microcephaly and disruption of nuclear envelope integrity. *Am. J. Hum. Genet.* 2020, 107, 753. [CrossRef]

151. Parry, D.A.; Martin, C.A.; Greene, P.; Marsh, J.A.; Blyth, M.; Cox, H.; Donnelly, D.; Greenhalgh, L.; Greville-Heygate, S.; Genomics England Research Consortium. Heterozygous lamin B1 and lamin B2 variants cause primary microcephaly and define a novel laminopathy. *Genet. Med.* 2021, 23, 408. [CrossRef] [PubMed]

152. Gupta, A.; Tsai, L.H.; Wynshaw-Boris, A. Life is a journey: A genetic look at neocortical development. *Nat. Rev. Genet.* 2002, 3, 342. [CrossRef] [PubMed]

153. Wynshaw-Boris, A. Lissencephaly and LIS1: Insights into the molecular mechanisms of neuronal migration and development. *Clin. Genet.* 2007, 72, 296. [CrossRef]

154. Asencio, C.; Davidson, I.F.; Santarella-Mellwig, R.; Ly-Hartig, T.B.N.; Mall, M.; Wallenfang, M.R.; Mattaj, I.W.; Gorjánácz, M. Coordination of kinase and phosphatase activities by Lem4 enables nuclear envelope reassembly during mitosis. *Cell* 2012, 150, 122. [CrossRef]

155. Kodani, A.; Yu, T.W.; Johnson, J.R.; Jayaraman, D.; Johnson, T.L.; Al-Gazali, L.; Sztriha, L.; Partlow, J.N.; Kim, H.; Krup, A.L.; et al. Plk1-dependent recruitment of gamma-tubulin complexes to mitotic centrosomes involves forces as a multiarm ensemble. *Biocell Open* 2020, 5, 2110004. [CrossRef]

156. Schmidt, T.J.; Kleylein-Sohn, J.; Westendorf, J.; Le Clech, M.; Lavoie, S.B.; Stierhof, Y.D.; Nigg, E.A. Control of centriole length by CPAP and CP110. *Curr. Biol.* 2009, 19, 1005. [CrossRef] [PubMed]

157. Sir, J.H.; Barr, A.R.; Nicholas, A.K.; Carvalho, O.P.; Khurshid, M.; Sossick, A.; Reichelt, S.; D’Santos, C.; Woods, C.G.; Gergely, F. A coordinated kinase and phosphatase activity by Lem4 enables nuclear envelope reassembly during mitosis. *Cell* 2012, 150, 122. [CrossRef]

158. Kim, T.S.; Park, J.E.; Shukla, A.; Choi, S.; Murugan, R.N.; Lee, J.H.; Ahn, M.; Rhee, K.; Bang, J.K.; Kim, B.Y.; et al. Hierarchical recruitment of Plk4 and regulation of centrosomal biogenesis by two centrosomal scaffolds, Cep192 and Cep152. *Proc. Natl. Acad. Sci. USA* 2013, 110, E4849. [CrossRef]

159. Sonnen, K.F.; Gabryjoczky, A.M.; Anselm, E.; Nigg, E.A.; Stierhof, Y.D. Human cep192 and cep152 cooperate in plk4 recruitment and centriole duplication. *C. Cell* 2013, 126, 3223. [CrossRef]

160. Pelletier, L.; O’Toole, E.; Schwager, A.; Hyman, A.A.; Muller-Reichert, T. Centriole assembly in *Caenorhabditis elegans*. *Nature* 2006, 444, 619. [CrossRef]

161. Rogala, K.B.; Dynes, N.J.; Hatzopoulos, G.N.; Yan, J.; Pong, S.K.; Robinson, C.V.; Deane, C.M.; Gönczy, P.; Vakonakis, I. The *Caenorhabditis elegans* protein SAS-5 forms large oligomeric assemblies critical for centriole formation. *Elife* 2015, 4, e07410. [CrossRef]

162. Haren, L.; Stearns, T.; Lüders, J. Plk1-dependent recruitment of gamma-tubulin complexes to mitotic centrosomes involves multiple PCM components. *PLoS ONE* 2009, 4, e9796. [CrossRef]

163. Wueseke, O.; Zwicker, D.; Schwager, A.; Wong, Y.L.; Oegema, K.; Jülicher, F.; Hyman, A.A.; Woodruff, J.B. Polo-like kinase phosphorylation determines *Caenorhabditis elegans* centrosome size and density by biasing SPD-5 toward an assembly-competent conformation. *Biol. Open* 2016, 5, 1431. [CrossRef]

164. Hamill, D.R.; Severson, A.F.; Carter, J.C.; Bowerman, B. Centrosome maturation and mitotic spindle assembly in *C. elegans* require SPD-5, a protein with multiple coiled-coil domains. *Dev. Cell* 2002, 3, 673. [CrossRef]

165. Okumura, M.; Natsume, T.; Kanemaki, M.T.; Kiyomitsu, T. Dynemin–dynactin–NuMA clusters generate cortical spindle-pulling forces as a multiarm ensemble. *Elife* 2018, 7, e36559. [CrossRef] [PubMed]

166. van der Voet, M.; Berends, C.W.H.; Perreault, A.; Nguyen-Ngoc, T.; Gönczy, P.; Vidal, M.; Boxem, M.; van den Heuvel, S. NuMA-related LIN-5, ASPM-1, calmodulin and dynemin promote meiotic spindle rotation independently of cortical LIN-5/GPR/Ga. *Nat. Cell Biol.* 2009, 11, 269. [CrossRef] [PubMed]

167. Ciossani, G.; Overlack, K.; Petrovic, A.; Huis Int Veld, P.J.; Koerner, C.; Wohlgemuth, S.; Maffini, S.; Musacchio, A. The kinetochore proteins CENP-E and CENP-F directly and specifically interact with distinct BUB mitotic checkpoint Ser/Thr kinases. *J. Biol. Chem.* 2018, 293, 10084–10101. [CrossRef]

168. Mao, Y.; Desai, A.; Cleveland, D.W. Microtubule capture by CENP-E silences BubR1-dependent mitotic checkpoint signaling. *J. Cell Biol.* 2005, 170, 873–880. [CrossRef]
169. D’Avino, P.P.; Savoian, M.S.; Glover, D.M. Mutations in sticky lead to defective organization of the contractile ring during cytokinesis and are enhanced by Rho and suppressed by Rac. *J. Cell Biol.* 2004, 166, 61. [CrossRef]

170. Bassi, Z.I.; Audusseau, M.; Riparbelli, M.G.; Callaini, G.; D’Avino, P.P. Citron kinase controls a molecular network required for midbody formation in cytokinesis. *Proc. Natl. Acad. Sci. USA* 2013, 110, 9782. [CrossRef]

171. Arora, K.; Talje, L.; Asenjo, A.B.; Andersen, P.; Atchia, K.; Joshi, M.; Sosa, H.; Allingham, J.S.; Kwok, B.H. KIF14 binds tightly to microtubules and adopts a rigor-like conformation. *J. Mol. Biol.* 2014, 426, 2997. [CrossRef] [PubMed]

172. Yamashita, D.; Shintomi, K.; Ono, T.; Gavvovidis, I.; Schindler, D.; Neitzel, H.; Trimborn, M.; Hirano, T. MCPH1 regulates chromosome condensation and shaping as a composite modulator of condensin II. *J. Cell Biol.* 2011, 194, 841. [CrossRef] [PubMed]

173. Duerinckx, S.; Jacquemin, V.; Drunat, S.; Vial, Y.; Passemand, S.; Perazzolo, C.; Massart, A.; Soblet, J.; Racapé, J.; Desmyter, L.; et al. Digenic inheritance of human primary microcephaly delineates centrosomal and non-centrosomal pathways. *Hum. Mutat.* 2020, 41, 512. [CrossRef] [PubMed]