Advanced Review

Genetic causes of microcephaly and lessons for neuronal development

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The study of human developmental microcephaly is providing important insights into brain development. It has become clear that developmental microcephalies are associated with abnormalities in cellular production, and that the pathophysiology of microcephaly provides remarkable insights into how the brain generates the proper number of neurons that determine brain size. Most of the genetic causes of 'primary' developmental microcephaly (i.e., not associated with other syndromic features) are associated with centrosomal abnormalities. In addition to other functions, centrosomal proteins control the mitotic spindle, which is essential for normal cell proliferation during mitosis. However, the brain is often uniquely affected when microcephaly genes are mutated implying special centrosomal-related functions in neuronal production. Although models explaining how this could occur have some compelling data, they are not without controversy. Interestingly, some of the microcephaly genes show evidence that they were targets of evolutionary selection in primates and human ancestors, suggesting potential evolutionary roles in controlling neuronal number and brain volume across species. Mutations in DNA repair pathway genes also lead to microcephaly. Double-stranded DNA breaks appear to be a prominent type of damage that needs to be repaired during brain development, yet why defects in DNA repair affect the brain preferentially and if DNA repair relates to centrosome function, are not clearly understood.

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INTRODUCTION

Abnormal brain development resulting in intellectual disability is frequently associated with microcephaly (small head). In most cases, microcephaly is equivalent to microencephaly (small brain) and we will be using the terms interchangeably. In a study of children at 7 years old, of those with a head circumference 2 to 3 standard deviations below the mean, 10% had an intelligence quotient (I.Q.) <70 (2 standard deviations below the mean) while only 14% had an I.Q. >100, (where I.Q. of 100 is mean). With head circumferences <3 standard deviations below the mean, 51% had an I.Q. <70 while none were above average.[1]

Microcephaly can be developmental resulting from abnormalities of proper development or degenerative with normal development and subsequent loss of cells. Microcephaly vera (true microcephaly), sometimes called primary microcephaly, is a group of autosomal recessive diseases of brain development that results in intellectual disability, but not other neurological abnormalities. These patients were thought to have no significant brain malformations other than a small brain (Figure 1), but now it is clear that the phenotypes are not completely uniform, and that there is a continuum between patients that have microcephaly with normal gyral pattern, and microcephaly associated with other malformation.[2–4]

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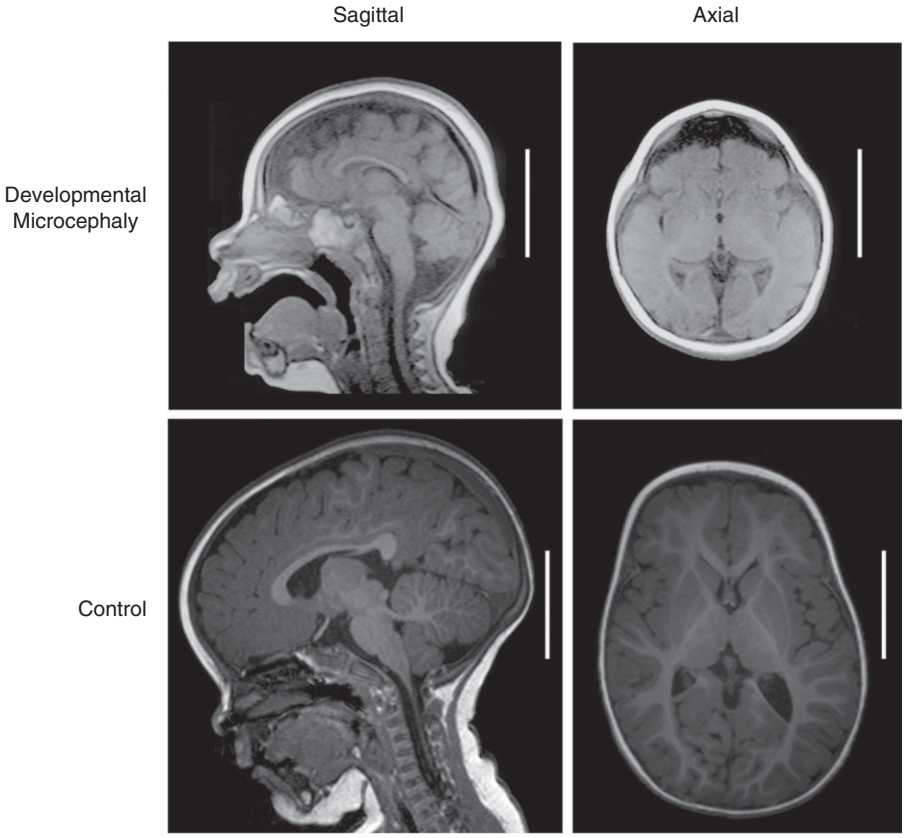


FIGURE 1 | MRI images from two 12-month-old children, the top with developmental microcephaly and bottom with a head circumference in normal range. The images are T1 weighted sequence in mid-sagittal and axial planes. Note the dramatic reduction in brain volume with the relative preservation in structure and size of facial features. The images are scaled to the same size (scale bar is 5 cm).

Other forms of microcephalies that are consistently associated with abnormal brain structure, for instance microlissencephaly (small, smooth brain), are interpreted as reflecting a gene's requirement for both producing the proper number of neurons and subsequent stages of neuronal development. To understand the causes of microcephaly, one must understand the basic processes of brain growth and neuronal proliferation, where many advances have been made in the field over the past two decades.

Nearly all neurons in the cerebral cortex have completed proliferation by mid-gestation and almost none are generated after birth,[5] although glial genesis and brain volume continues to grow until adulthood (Figure 2(a)). This is because the vast majority of cerebral volume is made up of neuropil (glial processes, axons, dendrites, etc.). Therefore, brain volume can increase without changing the number of neurons as a child develops, during which the brain acquires and prunes connections. The dramatic increase in brain size in a child during their first year of life is thought therefore to reflect predominantly an increase in neuronal processes, and an increase in the number and size of glial cells that invest them (Figure 2(b)). Head circumference, a reasonable relative proxy for brain size, continues to increase with age, though the head grows as much in the first year of life as it does over the next 17 years. This ongoing head growth, due presumably to increases in cellular processes and glial cells, relates to why children with developmental microcephaly appear to worsen as they age. The microcephalic head grows more slowly than normal and does not reflect loss of brain volume (Figure 2(a)). This is because the volume of neuropil is dependent upon the number of neurons present.[6] Therefore, severe developmental microcephaly is nearly always caused by deficiencies in the number of neurons.

We will be discussing the development of glutamatergic (excitatory) neurons of the cerebral cortex because they make up the majority of neurons in the cerebral cortex. For information regarding the development of the inhibitory GABAergic neuron and the first-born neurons of the cerebral cortex the reader is referred to other excellent recent reviews.[13–17] To understand the pathogenesis of the human diseases, we will discuss studies from various animal models,

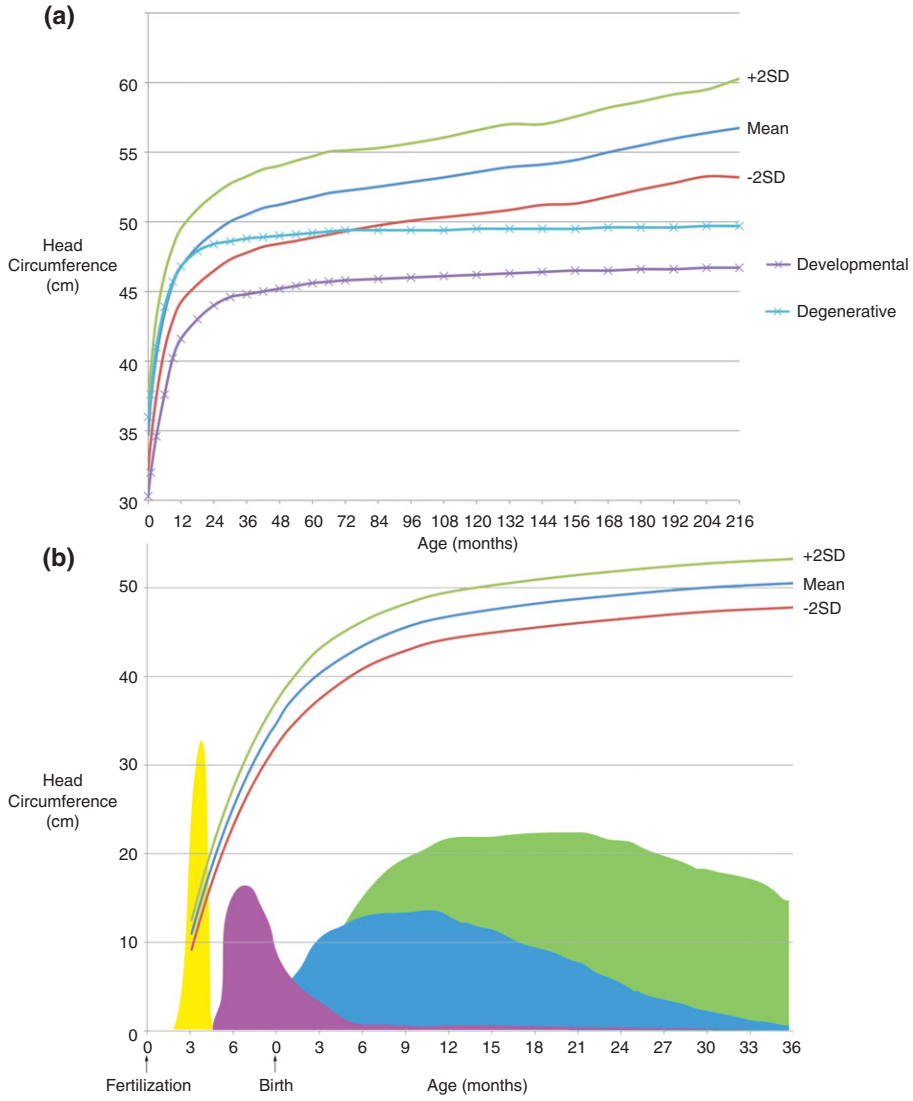


FIGURE 2 | (a) Examples of head circumference growth curves for boys aged 0 (birth) to 18 years (in months). The mean with 2 standard deviations above (+2 SD) and below (−2 SD) the mean to illustrate normal growth patterns. Note the very rapid expansion in head circumference during the first year of life. The purple line with Xs shows a child with a developmental microcephaly that starts below the normal growth curves. The head growth remains below the normal curves and follow its own trajectory with a shallower slope due to diminished brain growth potential. Clinically, these children often gain milestones more slowly compared to other children and the developmental potential eventually plateaus. However, there is no developmental milestone loss unless there are additional complications, neurological or otherwise. An example of a child with a degenerative condition leading to microcephaly is shown in light blue with Xs. Note that the child starts within the normal range then starts to cross percentile curves during the mid to late first year of life. Clinically, the child may gain early developmental milestones such as smiling, rolling over, and sitting without support, but then loses them as the neurodegenerative process proceeded. (b) Pre and post-natal brain growth from approximately 13 weeks post-fertilization to 36 months after being born at full term (prenatal ages in green and post-natal ages in black). Also shown are qualitative illustrations of the approximate timing of cerebral cortical development of neurons (yellow)[7], astrocytes (purple),[8] myelination (blue),[9] and synapses (green).[10] Developmental processes are demonstrating the relative temporal peak of each process with arbitrary units for the *Y*-axis while no attempt is made to show the relative contribution to brain volume. The exact extent of each process is not represented as each phenomenon (except for neurogenesis) continues at some level into adolescence. Comparisons between processes are very difficult because different methods were used for each type of study. In addition, some studies measure the density of phenomena in a growing volume further complicating extrapolation. The growth curves are a mathematic composite of fetal[11] and childhood[12] data and are intended for illustrative purposes not clinical use.

particularly mice due to the ability to manipulate their genetics, with the assumption that mammals with larger brains show many of the same processes. However, we will note when there are limitations in these models to determine the pathogenesis in humans due to differences in development.

Like the rest of the central nervous system, the cerebral cortex starts as pseudostratified epithelium, with cells initially proliferating symmetrically with every cell division, producing two 'daughter' progenitors, and hence increasing the number of cells exponentially.[18] The nuclei move up and down within the pseudostratified epithelium replicating their DNA at the pial surface and mitosis occurring at the ventricular surface[19] (Figure 3(a)). In mice, the founding pool of precursor cells in the ventricular zone at E11 (embryonic day 11, i.e., 11 days after conception) is an important determinant of eventual brain size. Targeted deletion of genes required for apoptosis greatly enlarges the cerebral cortex due to excessive precursor cells at the start of neurogenesis (the time in which neurons are generated), not from lack of apoptosis later in development.[20–23] Therefore, there is control in the eventual number of mature neurons even prior to neurogenesis. However, little else is understood concerning the regulation the precursor pool at this stage of development.

In mice, cells dividing within the ventricular zone during neurogenesis are dynamic in their proliferative characteristics. These factors include cell cycle length, which along with the period of neurogenesis determine the number of cell divisions that occur. Once neurogenesis begins, the control of the fate of dividing neuronal precursors appears to be critical in defining the ultimate size of the cortex. In the simplest models, during neurogenesis as precursors divide, there are three possibilities for the fate of progeny: two additional precursors, one precursor and one differentiated neuron or two differentiated neurons. If a cell proliferates symmetrically, producing two precursors every cell division, after five cell divisions one cell has produced 32 precursor cells. If a cell proliferates asymmetrically, producing one precursor and one differentiated neuron every cell division, after five cell divisions one cell has produced six cells (five neurons and one precursor). There are actually additional possible fates, such as neuronal committed with limited proliferation potential,[24] but the concept is similar. Therefore, control over proliferative versus differentiative fate is critical. In mice, there are progressively longer cell cycle times and the ratio of cells committed to proliferation versus differentiation appears to be precisely controlled (reviewed in Refs 25,26). These authors found that they could account for the number of neurons produced in a mouse by accounting for progenitor pool size, the number of cell cycles, and the ratio of cells proliferating to differentiating neurons (quiescent/proliferative or q/p). Critically, they found that the q/p ratio changed radically over the period of mouse development.[26]

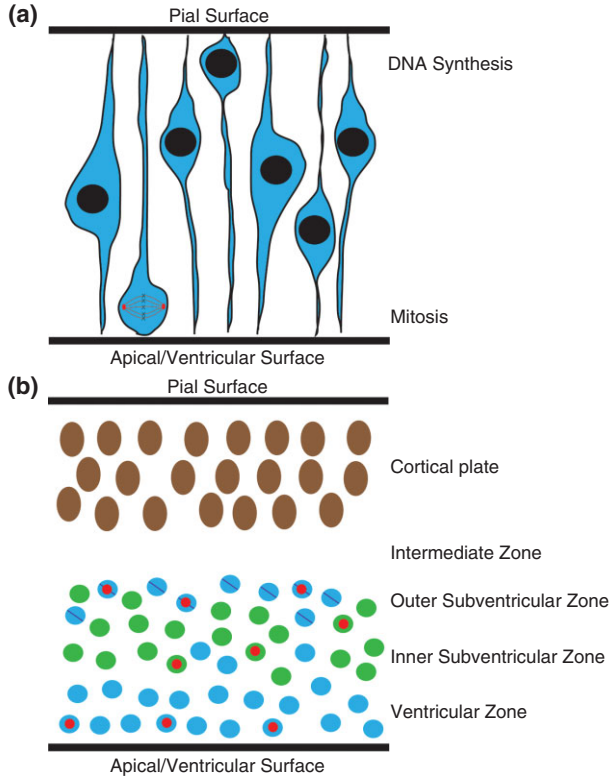


FIGURE 3 | (a) Illustration of pseudostratified epithelium of the developing cerebral cortex prior to neurogenesis. It has the superficial appearance of layers due to nuclei being scattered from the ventricular to pial surface. However, cells are apparently uniform in character and all retain contacts with both the ventricular and pial surfaces. Within each cell, nuclei move upward toward the pial surface where they synthesize DNA and downward to the pial surface where they divide in mitosis. This pattern of nuclei movement is retained within the ventricular zone later in development described in (b). Prior to neurogenesis, nearly all cells are uncommitted progenitors and the proliferative pool exponentially. (b) A diagram of the layering found during neurogenesis to emphasize the proliferative pools. Within the ventricular zone (represented by plain blue circles), uncommitted progenitors divide at the apical surface (dividing cells marked with red dot (indicative of the mitotic marker phospho-histone H3). Some of the progeny will become committed but retain limited proliferative capacity, start to express the marker TBR2, and move the inner subventricular zone, marked in green and also referred to intermediate precursors. They also divide (red dots, phospho-histone H3), but remain in the subventricular zone and do not have a connection with the apical surface. Cells within the outer subventricular zone (blue circles with purple slash) have many of the same markers of the ventricular zone, are uncommitted, have extensive proliferative capacity, but do not have connection with the apical surface, unlike the ventricular zone cells. The intermediate zone is cell sparse and the cortical plate consists of differentiated neurons that will become the cerebral cortex.

According to their modeling data, the q/p ratios are important because if too many neurons differentiate too early, there will be fewer neurons at the end of neurogenesis with microcephaly as a result.

It is now clear that there are several types of progenitor cells in the cerebral cortex with different proliferative capacities and complicated lineage relationships that have not been completely determined. For example, a significant fraction of cells committed to a neuronal lineage migrate away from the ventricular surface to the subventricular layer and begin to change their expression profile, becoming *TBR2*+[27–31] (Figure 3(b)). These cells go through limited, probably symmetrical, proliferation to produce differentiated neurons. Moreover, recent evidence suggests that mammals with gyri (folds) in the cerebral cortex and larger brains have an additional source of dividing. Progenitors that are presumably derived from the ventricular zone form a layer above the committed *TBR2*+ cells in the subventricular zone forming an outer subventricular proliferative zone[24,32,33] (Figure 3(b)). These cells appear to have extensive proliferative capacity and may be an important source of neurons in larger brained mammals. They appear to be able to undergo multiple cell symmetric divisions producing more precursors prior to differentiation. These outer subventricular cells are very much reduced in number in mouse brain compared to brains of gyrencephalic mammals,[34] and do not form a distinct outer subventricular layer as they do in larger brains. Initial evidence hints that these outer subventricular zone cells may ultimately be seen to play a critical role in cerebral cortical proliferative capacity in mammals with gyri, but the extent of their role remains to be definitively determined.

MICROCEPHALY GENES

There were originally seven genetic loci for microcephaly vera, all of which have now been connected to single genes: *MCPH1*,[35] *ASPM*,[36] *CDK5RAP2*,[37] *CENPJ*,[37] *STIL*,[38] *WDR62*,[2–4] and *CEP152*.[39] A few additional loci recently have been identified including *CEP63*[40] and a new locus potentially identified but no gene mutation found.[41] Remarkably, all of these microcephaly genes encode proteins associated with the centrosome or centrosomal-related activities. Centrosomes play multiple critical roles in cellular function including during mitosis where they are associated with microtubules and involved in formation of the mitotic spindle. However, they also are involved in coordinating microtubules in other cellular processes such as migration (for review of neuronal aspects see Refs 42,43) and primary cilia formation.

Centrosomes have a complicated structure and life cycle in dividing cells[44] (Figure 4). Interestingly, microcephaly proteins appear to play various roles in the centrosome and centriole life cycle.[45] After mitosis, the core centrioles remain loosely tethered together and CDK5RAP2 appears to be important in this activity.[46] STIL appears to interact machinery to form the core centriole.[47,48] In addition, STIL may play a role in the control of entry into mitosis as well[49] CEP152 is needed for centrosome duplication and appears to interact with CEP63.[40,50–52] After the centrioles are duplicated, they are elongated and CENPJ is involved in this activity.[53–55] MCPH1 has been implicated in both centrosomal activity and DNA repair. However, as discussed below both sets of literature can partially reconciled if MCPH1 helps link DNA repair signaling with centrosomal activity. ASPM appears to play a role in mitotic spindle orientation during mitosis.[56,57] So while there is a unifying theme that microcephaly vera genes play some role in centrosomal activity, they do not appear to play an identical role in centrosomal function. However, in future, studies could show a unified function in these genes. Therefore, the nature of the deficit for each mutation and how it results in microcephaly must be determined.

There is evidence of general centrosomal dysfunction associated with *Cdk5rap2* with some increase in apoptosis in the developing cerebral cortex.[58] In addition, there was evidence of alteration of the q/p fraction that is consistent with fewer neurons produced.[58] Similar defects have also been found in *Aspm*[56] and Nde1.[59] Finally, a microcephaly locus whose gene mutation remains unidentified appears to have chromosomal segregation abnormalities implying a potential centrosomal function as well.[41] Interestingly, chromosome copy number abnormalities have been reported in the normal developing rodent brain.[60–62] This could indicate that chromosome segregation dysfunction is common even in the absence of mutations in centrosomal genes, but the role and extent of this phenomenon remains to be determined. Interestingly, many genes that are associated with lissencephaly (smooth brain) result from neuronal migration abnormalities associated with centrosomes or microtubules as well including *PAFAH1B1 (AKA LIS1)*,[63] *DCX*,[64–66] *RELN*,[67] *TUBA1A*,[68] and *NDE1*.[69,70] This is not surprising since centrosomal function is not limited to cell division, but also plays a critical role in neuronal migration as well.[42,43] Critically, there is experimental evidence that some of the genes associated with lissencephaly can also alter neurogenesis, supporting a similar mechanism as the microcephaly genes.[59,71]

Mutations in some genes cause a combination of both microcephaly and somatic growth deficiencies, exemplified by Seckel syndrome. Interestingly, a centrosomal gene has been associated with Seckel,

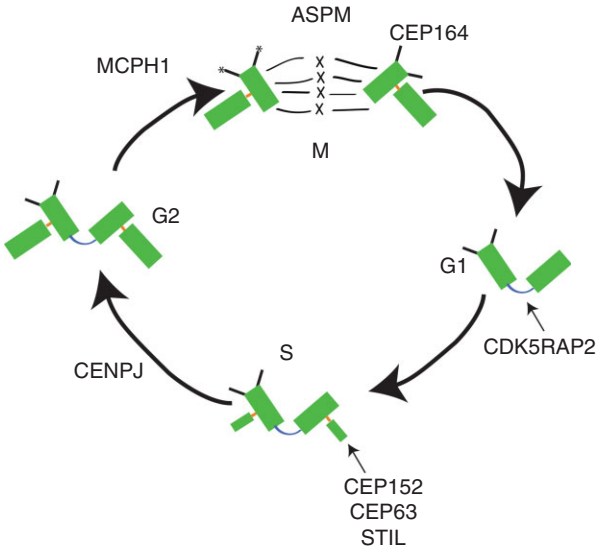


FIGURE 4 | A cartoon schematic of centriole biogenesis in dividing cells (see the excellent review in Ref 44 for further details). The potential roles of microcephaly-related genes are noted. After M phase, in G1 two centrioles (large green boxes) are attached via a linker that contains CDK5RAP2. At the G1–S transition and only once during a cell cycle, a new centriole (small green box) is formed adjacent to the parental centriole (larger green box). CEP152 is required for this process and it interacts with CEP63. Centriole duplication potentially involves STIL via its interaction with SAS6. The new centriole remains attached to the older centriole (small orange line). The new centriole elongates during S-phase and CENPJ is potentially involved this activity. MCPH1 may play a role in cell-cycle checkpoint control at the G2/M transition. ASPM may play an important role in mitotic spindle orientation during M phase. The alterations of spindle orientation play an important role in cell fate decisions invertebrates and may play a role in mammalian cortical development. Each cell inherits one centrosome, but the centrioles have different levels of maturity as it takes 1.5 cell cycles for a centriole to fully mature as it accumulates distal appendages (black lines) and other maturation markers (more mature has asterisks). The differences in maturity between centrioles have potentially important biological consequences.

PCNT[72] that when mutated causes abnormalities in the activation of the DNA repair gene, *ATR*, also mutated in Seckel syndrome.[73] In addition, *CEP152* has been associated with both microcephaly and Seckel syndrome as well.[39,74] However, microcephaly is often found without significant somatic growth defects.

POTENTIAL MECHANISMS OF MICROCEPHALY GENES

Why microcephaly is usually associated with mutations in centrosomal proteins is not certain, but there are several intriguing possibilities. It could be simply a result of mitotic dysfunction leading to deficiencies in cellular proliferation. However, as stated above, developmental microcephaly generally does not have profound somatic growth abnormalities. Alternatively, neurons could be more sensitive to cell cycle dysfunction resulting in apoptosis. Excessive cell death was seen in mouse models for *CDK5RAP2* and *MCPH1*, but it was not obvious that the levels of cell death could account for the microcephaly alone.[49,58] Therefore, microcephaly associated centrosomal dysfunction appears to have brain-specific pathophysiological mechanisms.

One potential mechanism to regulate cell proliferation and differentiation is the differential inheritance of signaling molecules that alter progenitor fate. Differential segregation of the evolutionarily conserved Par3 (Bazooka), Par6, and aPKC complex plays a critical in Drosophila cell fate determination.[75–77] The ventricular zone of the developing mammalian cerebral cortex appears to be an excellent candidate for sharing such a mechanism as well. There is a dramatic asymmetric localization of factors at the apical surface, the apical membrane complex, including PARD3 (homolog of Par3), PARD6A (homolog of Par6), and PRKCA (homolog of aPKC) while CTNNB1 (β-catenin) is adjacent but slightly more basilar.[75] There is significant evidence that this complex plays an important role in cortical development as well. Alterations in the pattern of expression of *Pard3*,[78,79] *Pard6a*,[78] *Ctnnb1*,[80] and *Mpp5* (AKA *Pals1*)[81] (another component of the apical membrane complex), all alter proliferative patterns of cortical progenitors. However, how the apical membrane complex controls the fate decisions in not certain in mammals.

Whereas in flies and worms, asymmetrical cell fate is regulated by the asymmetrical inheritance of apical proteins, this is less clear in the cerebral cortex. It was first noted in ferrets that dividing precursors within the ventricular zone usually divide within a plane that bisects the cell, leaving two cells with apparently equal distribution of the apical membrane complex and a smaller percentage dividing in a skewed plane leaving unequal distribution.[18] In this context, symmetric cell divisions has the additional connotation of precursors dividing in a plane that is perpendicular to the ventricular surface so the apical membrane complex is inherited equally (symmetrically) (Figure 5). This occurs at the same time most dividing cells produced two progenitors and the minority had asymmetric cell division resulting in one precursor and one neuron. This observation was confirmed in mouse.[82–84] However, others have found that the differences in cleavage plane are not as

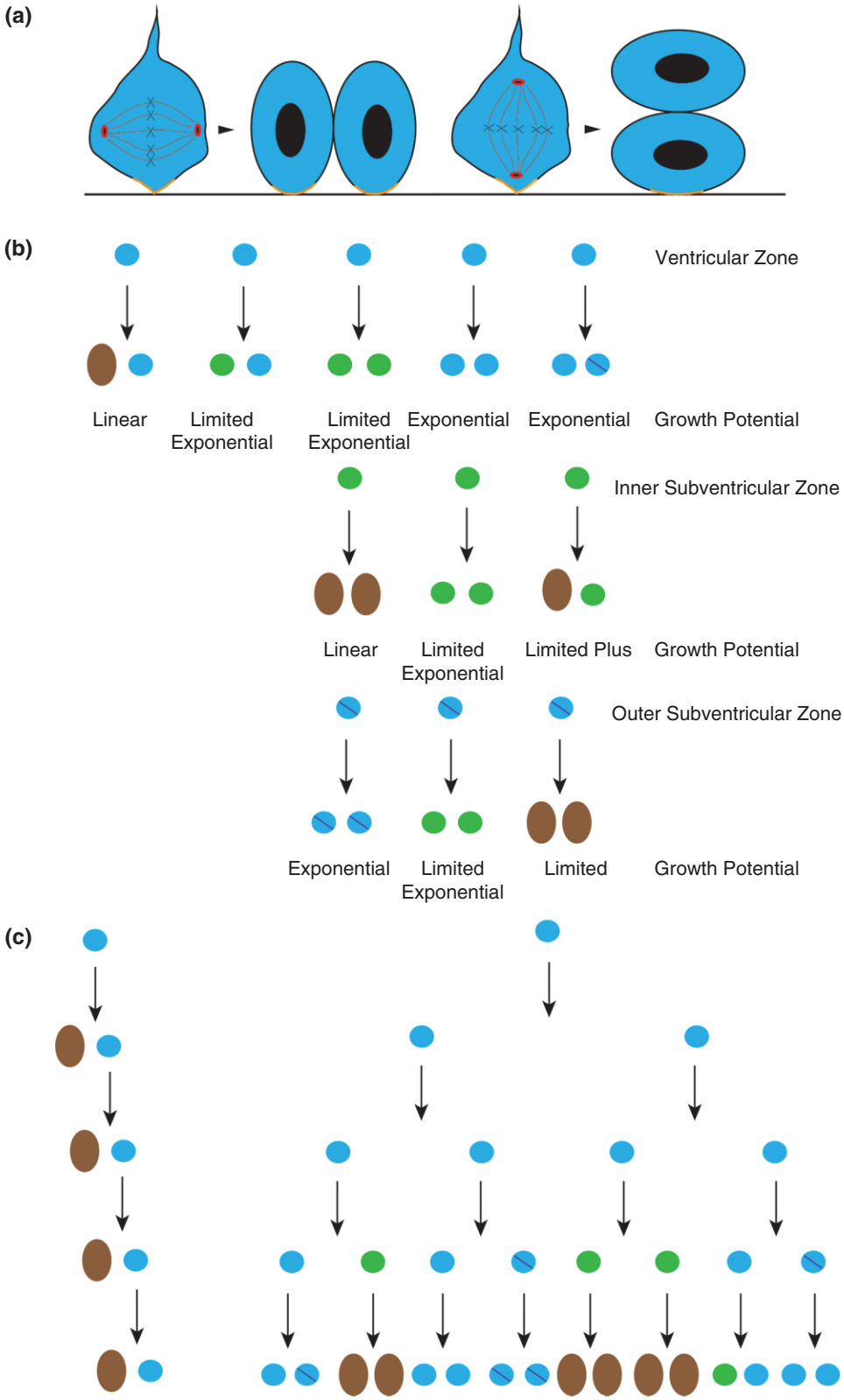
great as other have reported.[85,86] Interestingly, some studies have suggested that altering cleavage plane orientation alters where neurons proliferate, but not their proliferative fate.[87] Other studies have suggested that that alteration of cleavage plane generates more neurons by shunting the neuronally committed progeny into the subventricular zone for additional rounds of proliferation prior to differentiation.[88]

Although the apical complex may regulate some aspects of cell fate for apical progenitors, the apical membrane complex is not even present within the subventricular zone, as these cells are not epithelial and do not have a connection with the ventricular surface. Although intermediate progenitors within the inner subventricular zone—such as TBR2-positive cells—appear to show limited cell production capacity,[28–30] the outer subventricular zone cells appear to have extensive proliferative capacity and also do not have an apical membrane complex, since they do not have an apical process in connection with the ventricular surface.[24,33,85] As most studies of microcephaly genes have been carried out in mice, which have a minute outer subventricular zone, these studies have provided limited understanding of the role that the outer subventricular zone could be playing in microcephaly in humans. Animal models with gyri, such as ferret, may be required to determine the role of microcephaly genes in this cell type.[34] Every proliferative neuronal precursor has various potential fates (Figure 5(b)) and the choices made will greatly influence the final number of neurons produced (Figure 5(c)). Centrioles duplicated in dividing cells are born at different times and have different levels of maturity[44,89,90] (Figure 4). One daughter cell will inherit the older, 'maternal' centriole while the other the less mature, 'daughter' centriole. Interestingly, a recent study has implicated a newly discovered microcephaly protein, CEP63, as localizing preferentially to the mother centriole, but the implications of this finding remain to be determined.[40] One group has been found that cells in the developing cerebral cortex that inherit the mother centriole tend to remain proliferating, whereas those with the daughter centriole more frequently differentiate into neurons.[91] However, it is unknown what accounts for these phenomena mechanistically. Intriguingly, cells that inherit the mother centriole develops a primary cilia faster, than cells that inherit the daughter centriole.[92] While the mechanism for these phenomena is also unclear, it could be related to addition of CEP164 to the mother centriole at the G2/M transition that plays a role in primary cilia formation.[93]

An intriguing hypothesis for ASPM suggests that it might influence WNT signaling.[94,95] Buchman et al. found that they could rescue the loss of ASPM with over expression of CTNNB1 (β-catenin) that is downstream of WNT.[96,97] However, it was previously found that over expression of CTNNB1 resulted in excessive proliferation.[80] Therefore, it is difficult to distinguish between ASPM interacting with WNT resulting in decrease in CTNNB1 or rescue of decreased proliferation by CTNNB1 by a different mechanism. Interestingly, cilia function has been tied to both neurological disease and WNT signaling.[98,99] If the microcephaly-related abnormalities in centrosomal activity could be tied to abnormal primary cilia formation or function, then it is possible that abnormal signaling through the primary cilia accounts for some microcephaly phenotype. However, it should be noted that there are many clinical differences between the microcephaly centrosomal diseases and the human diseases associated with ciliopathies.[100,101]

MICROCEPHALY GENES AND BRAIN EVOLUTION

The potential mechanisms of the evolutionary expansion in brain size in humans compared to other primates have been of interest for many decades. Recent work has indicated that the human is similar to other primates in terms of the number of neurons to volume ratio, with the implication that differences in brain volumes result from differences in neuronal number.[6] As discussed above, there are several potential strategies that could allow humans to have more neurons than other primates: such as starting with more precursors at the start of neurogenesis via more proliferation or reduced apoptosis of the precursor pool, additional rounds of proliferation during neurogenesis, or alterations in the proliferative/differentiative (q/p) ratio during neurogenesis. Since mutations in microcephaly genes alter this process in pathological states, they were looked to as candidates to play a role in controlling cell number in evolution as well. One method to identify genes that play a role in evolution is to compare the relative number of nucleotide changes in protein coding regions that change amino acids (non-synonymous) to those that do not (synonymous) across species.[102] The synonymous changes control for the frequency of nucleotide changes in a particular gene and the genetic distance between species while the non-synonymous changes potentially reflect adaptation that may have been selected for in evolution. Interestingly, numerous genes associated



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with microcephaly may have had positive selection in primates and/or humans including *MCPH1*, *ASPM*, *CDK5RAP2*, *CENPJ*, and *CEP63*[40,103–109] (reviewed in Refs 110,111). The changes in microcephaly genes that appear to be selected for in evolution could produce larger brains by producing more neurons secondary to altering q/p fraction or by a different mechanism such as shunting cells into other proliferative pools such as the inner or outer subventricular zones (Figure 5(b) and (c)). Microcephaly genes are not unique in the potential primate evolutionary selection, as genes with many different biochemical functions also may have shown increased potential positive selection in primates and/or humans.[112–117] However, this selection in recent human evolutionary lineages does not seem to be present in all genes expressed in the human brain.[118,119] In addition, microcephaly genes could even play a role in normal variation of head size and neurological diseases not associated with microcephaly.[120,121]

DNA DAMAGE REPAIR AND MICROCEPHALY

DNA damage responses and repair pathways along with the maintenance of genomic integrity are related to microcephaly genes in multiple aspects. Defects in the maintenance of genomic integrity can range from the alteration of a single nucleotide to errors in chromosomes segregation during mitosis. It is useful conceptually to separate different aspects of these cellular functions although it is sometimes difficult to do experimentally. DNA, while a superficially simple structure of four bases pairing appropriately, connected by deoxyribose sugars and phosphodiester bonds in very long strands forming anti-parallel helices, can be damaged in many ways and the requirement for DNA's integrity is clear. Not surprisingly, different repair mechanisms exist for different types of damage. First, DNA damage such as double-strand DNA breaks must be recognized followed by DNA damage response activation (Figure 6). This often includes arrest of the cell cycle presumably to allow completion of DNA repair so that damage is not propagated either through DNA replication or mitosis.[122] In addition, if the amount of DNA damage is severe enough, programmed cell death pathways are activated. Finally, the appropriate enzymatic proteins for a particular repair pathway are recruited and coordinated to repair the damage. DNA damage response pathways are frequently disrupted in cancer probably because it encourages accumulation of mutations and prevents cell cycle arrest or programmed cell death when DNA damage does occur. In addition, disruptions in cell cycle control encourage both uncontrolled proliferation as well as reducing DNA repair by not allowing time for repair processes. On the other hand, aberrant sorting of chromosomes due to defects in the mitotic machinery has different mechanisms even though also common in cancer.[123] However, recent work may indicate that DNA repair and aberrant chromosome segregation maybe more closely linked than previously thought.[124]

MCPH1 has been implicated in both centrosomal and DNA repair activity and provides an excellent illustration of the difficulties of distinguishing between different aspects of genomic integrity maintenance. Microcephaly from mutations in MCPH1 is associated with premature condensation of chromosomes in G2.[131,132] *MCPH1* defective cells have impaired G2 arrest after DNA damage from ionizing radiation, MCPH1 localizes to sites of double strand breaks, and the gene is mutated in certain types of cancer potentially implicating DNA damage repair as a central function.[133–137] The first published mouse models of *Mcph1* mutations did not have defects in neuronal development.[138,139] However, a

FIGURE 5 | (a) Illustration of different planes of cell division in the ventricular zone with an emphasis on the retention of the apical membrane complex (orange). When a dividing cell has centrosomes aligned so that the cleavage plane is perpendicular to the ventricular surface, the apical membrane complex is shared equally between progeny. When the centrosomes are aligned so that the cleavage plane is parallel to the ventricular surface, one cell inherits the apical membrane complex while the other does not. Since the apical membrane complex takes up a very small area of the ventricular surface, it has been hypothesized that slight deviations from a perpendicular cleavage plane may be enough to cause unequal distribution in progeny. (b) Illustration of potential progeny derived from different proliferative zones along with the growth potential. For instance, only linear growth occurs when a ventricular zone cell (blue circle) divides to produce one neuron (brown oval) and one uncommitted precursor. However, when two uncommitted precursors (in either ventricular zone (blue circle) or outer subventricular zone (blue circle with purple slash)) cell growth can be exponential. When the progeny include inner subventricular zone cells (green circle), there can be extensive expansion compared to producing neurons directly. However, true exponential growth is not possible because these neuronally committed cells divide a limited number of times before differentiating into neurons. The outer subventricular zone cells appear to be comparable to the proliferative capacity of the ventricular zone; however, it is not fully clear what cells it can produce or how these decisions are controlled. Presumably, fate decisions in this group can significantly alter the final number of neurons in the brain. (c) An example of the number of cells produced in four divisions from one ventricular zone cell with linear growth versus combined exponential, limited exponential, and linear growth demonstrating how neuronal number could be controlled though control of cell fate. Note, neurons (brown) no longer divide, but move out of the ventricular zone and migrate to the cortical plate.

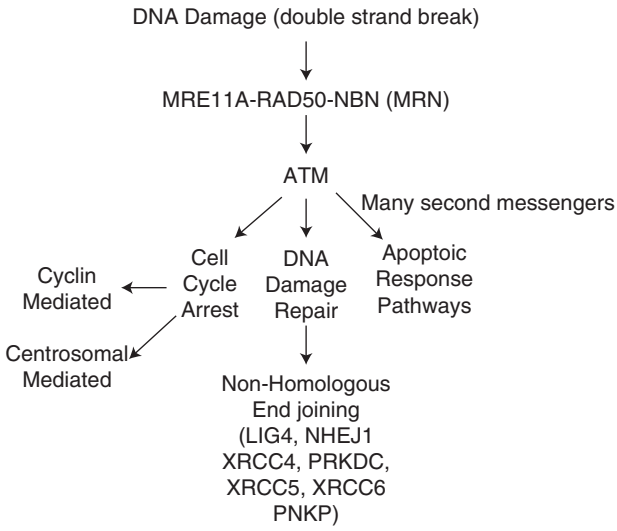

FIGURE 6 | Schematic of DNA damage response to double strand breaks. After a double-strand break occurs ATM is activated via interaction with the MRN (MRE11A-RAD50-NBN (NBS1) complex. After ATM is activated it establishes a very broad cascade of second messengers (including the MRN complex) to perform a variety of cellular functions. Progression of the cell cycle is arrested. This is thought to help prevent the propagation of the DNA damage and allow time for repair. Arrest of the cell cycle involves interaction with the cyclin-dependent kinase machinery and potentially the centrosome as well. In addition, apoptotic response pathways related to TP53 (p53) are activated permitting the cell to initiate programmed cell death if the level of DNA damage is severe, but much remains to be determined as to how these decisions are made.[125] Finally, the proteins that will perform the DNA repair are attracted to the site of damage break. Abnormalities in genes involved in the NHEJ pathway leads to microcephaly in humans including *LIG4*, *NHEJ*, and *PNKP* and an analogous phenotype in mice *Lig4*,[126,127] *Xrcc4*,[128] *Xrcc6* (*Ku70*)/*Xrcc5* (*Ku80*),[129] or *Prkdc* (*DNA-PKcs*).[130]

more recent mouse model has microcephaly due to premature differentiation of neurons.[140] This was thought to be secondary to premature entry into mitosis and chromosomal alignment difficulties via disrupted cell cycle control of the CHEK1–CDC25C (AKA CHK1–CDC25) pathway. Therefore, *MCPH1* neurological pathophysiology is likely from abnormal cell cycle control associated with centrosome function and not due to inability to DNA repair directly. MCPH1 may play an important role linking DNA damage response to centrosomal function and/or an additional role in DNA repair. This supports the conclusion that proliferative versus differentiated fate control may play the critical role in microcephaly in *MCPH1* patients. In addition, *CEP152* patient fibroblasts develop aneuploidy in culture indicating there are abnormalities in the control of chromosome segregation.[74] Therefore, CEP152 likely plays an important role in genome integrity but not necessarily DNA repair and it is unclear if this gene plays any role in cancer.

Abnormalities in DNA repair do result in several different neurological phenotypes including developmental microcephaly as well as cancer and immunodeficiency. The broad reasons why abnormalities in DNA repair lead to cancer and immunodeficiencies are reasonably well understood even though many details remain to be determined.[141–144] However, why the brain is so sensitive to abnormalities in DNA repair remains a mystery. The human neurological diseases associated with abnormalities in DNA repair can be broken down into three categories, microcephaly,[73,145–153] white and gray matter degeneration[154] or ataxia-associated degenerative conditions[155–160] (reviewed in Refs 161,162).

Some DNA repair-associated microcephaly disorder genes are involved in the early steps of DNA repair signaling activation, including *NBN* (AKA *NBS1*), *RAD50*, *MRE11A*, and *ATR*.[73,145–147,151,153] MRE11A, RAD50, and NDN form the MRN complex that helps regulate ATM while ATR is a paralog of ATM[163,164] (Figure 6). Proteins from the MRN complex also involved more directly in the repair of DNA and other signaling functions.[165,166] Interestingly, mutations in ATM cause the ataxia-associated degenerative disease, ataxia-telangiectasia, not a developmental microcephaly. To make matters even more complex, *MRE11* mutations can cause either an ataxia-telangiectasia-like degenerative disease or a developmental microcephaly.[153,156] Perhaps, the difficulty of interpreting the neurological phenotypes in relation to specific DNA repair pathways should not be surprising since both ATM and ATR play central roles in DNA repair signaling pathways and can phosphorylate over 700 proteins in response to DNA damage.[167] Even though mutations in *NBN* and *ATR* both cause microcephaly, *ATR* also cause somatic growth restriction.[73,145–147] Recent work suggests that the microcephaly associated with *NBN* and *ATR* mutations may have different pathogenetic mechanisms.[168] Better insight into specific pathways of DNA repair can be gained focusing on proteins downstream of these early signaling molecules.

Mutations in *LIG4* or *NHEJ1* result in developmental microcephaly and both genes play a critical role in NHEJ in which double strand breaks are repaired by trimming DNA ends, bringing the ends together and ligating the strands.[148–150,169] NHEJ can be used in any part of the cell cycle, but generally introduces some sequence changes to the DNA while completing repairs. A different double-strand break pathway, homologous

recombination, is only available in late S-phase and in G2.[170,171] This is because homologous recombination uses a duplicated strand as a template to create an exact replacement of the broken strand. Interestingly, mice in which NHEJ genes have been knocked out show significant and sometimes massive cell death in differentiated neurons around mid-gestation that is analogous to the human microcephaly phenotype.[126–130,172] This means apoptosis during neurogenesis is likely an important mechanism of microcephaly in humans in these diseases although other mechanisms remain a possibility as well. The apoptosis is most likely secondary to cell death programs activated by the lack of repair of double strand breaks mediated though ATM and/or TP53 (formerly known as p53).[172–175] Homologous recombination appears to be required for murine brain development as determined via targeted deletion of *Xrcc2* or *Brca2*.[175,176] Interestingly, it appears that homologous recombination is required for precursors during proliferation and NHEJ is needed after differentiation.[175] This may be because homologous recombination can compensate for the loss of NHEJ until the cells differentiate, permanently entering G1 where homologous recombination is no longer available.

Analogous to the association between abnormalities in NHEJ and microcephaly, the combined gray–white neurodegeneration typified by Cockayne syndrome is associated with abnormalities in nucleotide excision repair.[154] The DNA repair diseases associated with ataxia do not have a clear, single DNA repair pathway defect pathway identified yet even though there is significant overlap of their neurological phenotypes. There is considerable neurological phenotypic overlap within a group of DNA repair diseases, but very little phenotypic overlap between the DNA repair diseases cause that microcephaly, Cockayne, or ataxia. This suggests the likely correlation between DNA repair deficiency pathway and neurological phenotype; NHEJ pathway way for developmental microcephaly, nucleotide excision repair for Cockayne-like diseases and a yet to be determined pathway for ataxia diseases. *PNKP* can play a role in multiple repair pathways and mutations cause both microcephaly and seizures.[152,177,178] PNKP's role in NHEJ likely explains the microcephaly phenotype.[152,178] However, seizures are not a very common feature of other microcephaly vera disorders or DNA repair associated microcephaly implying that the seizures may be secondary to defects in additional DNA repair pathways. Interestingly, mice with brain-specific defects in the base excision repair pathway appear to have defects in interneurons and seizure-like activity and PNKP can be used in this pathway as well.[152,177,179]

Diseases that lead to developmental microcephaly have given significant insight into the genes required for normal brain development. However, pathophysiological mechanisms of microcephaly reviewed here generate many new questions. Why do mutations centrosomal genes that are used in all dividing cells generally result in a brain specific phenotype? Is there a unifying mechanism between the centrosomal genes? Fate decisions during neurogenesis are clearly important for determining cell final cell number. However, in which cell types are these decision made? There is tantalizing data suggesting that centrosomal genes are playing a role in these decisions and may have over the course of evolution. How they may be helping control cell fates remains to be definitively determined. In addition, the DNA repair pathways that lead to microcephaly are used in all cells, but the brain is sensitive to their perturbation while most other tissues develop normally (with the exception of lymphocytes). The reasons why the brain is sensitive to loss of DNA repair genes remain a mystery. It is not clear if neuroblasts have more DNA breaks than other cells during their development and if so, what that source might be. Alternatively, neurons may be more sensitive to the presence of DNA damage found in all dividing cells and activate programmed cell death pathways when repair pathways are disrupted. Distinguishing between these hypotheses is difficult experimentally. However, it does appear that defects in specific DNA repair pathways lead to specific phenotypes whether that is NHEJ in microcephaly, nucleotide excision repair in Cockayne, or an as of yet to be determined pathway in ataxia.

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