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Malformations of Cerebral Cortex Development: Molecules and Mechanisms

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Abstract

Malformations of cortical development encompass heterogeneous groups of structural brain anomalies, associated with complex neurodevelopmental disorders, and diverse genetic and non-genetic etiologies. Recent progress in understanding the genetic basis of brain malformations has been driven by extraordinary advances in DNA sequencing technologies. For example, somatic mosaic mutations that activate mTOR signaling in cortical progenitor cells during development are now recognized as the cause of hemimegalencephaly and some types of focal cortical dysplasia. Also, research on brain development has begun to reveal the cellular and molecular basis of cortical gyrification and axon pathway formation, so disorders involving these processes may be better understood. New neuroimaging techniques with improved resolution have enhanced our ability to characterize subtle malformations, such as those associated with intellectual disability and autism. In this review, we broadly discuss cortical malformations and focus on several in which genetic etiologies have elucidated pathogenesis.

Keywords

Microcephaly; Hemimegalencephaly; Focal Cortical Dysplasia; Polymicrogyria; Lissencephaly; Hippocampal Dysgenesis

1. INTRODUCTION

The past few years have been marked by unprecedented progress in the rapid identification of genes and signaling pathways that cause different types of cortical malformations. Concurrently, improved resolution and new techniques in neuroimaging have facilitated the recognition of increasingly subtle malformations, associated with disorders such as focal epilepsy. Recent studies have also revealed novel mechanisms of cortical development relating to processes such as brain gyrification, especially in humans. Neuropathological

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studies have also played an important part in recent progress, as genetic and histological studies of patient brain specimens are critical to diagnosis and research. The purpose of this review is to integrate these advances in brain development, genetics, and malformations in an updated perspective, focusing on disorders (such as cortical overgrowth disorders) where progress has led to the greatest new insights.

2. GENERAL FEATURES OF CORTICAL MALFORMATIONS

2.1. Impact of cortical malformations

Corticogenesis is a complex sequential process that, in humans, leads to the formation of a layered cortex with consistent gyral patterns and axonal connections. Broadly, corticogenesis can be divided into three partially overlapping stages, consisting of cell proliferation, neuronal migration, and postmigrational cortical organization (1). Disruption of any of the steps may result in malformations of cortical development (MCDs), a large spectrum of disorders with varied cortical morphologies, genetic or extrinsic etiologies, related syndromes, and clinical manifestations (2,3). Epilepsy, developmental delay (DD), intellectual disability (ID), autism, and schizophrenia are among the common clinical consequences of MCDs, and they sometimes occur together in various combinations.

The definite incidence of MCDs is unknown. Up to 40% of medically refractory childhood seizures are attributable to MCDs (4), and at least 75% of patients with MCDs develop seizures (5). Some MCDs are discovered incidentally after magnetic resonance imaging (MRI) for other conditions or unrelated events, and some cases are probably never detected (6).

2.2. Causes of cortical malformations; role of *de novo* mutations

The majority of MCDs are thought to be caused by underlying genetic mutations, which disturb the encoded proteins and associated molecular pathways involved in early and/or later stages of cerebral cortex development (2). Mutations that arise *de novo*, during gametogenesis or postzygotic development, are increasingly recognized as causes of MCDs, epilepsy, ID, and autism (7–9). In addition, environmental insults, such as infection or ischemia *in utero* at different stage of brain development, or during the perinatal or postnatal period, may contribute to the development of MCDs (10,11). The time of insult during corticogenesis also seems to impact the severity of cortical malformation, with disruptions of later neurodevelopmental processes proposed to cause more severe network disruptions (12). The severity of MCDs also reflects their focal or widespread impact on cortical structure.

Some MCDs are associated with postzygotic mutations that produce somatic mosaicism, classified as either *type 1* (new heterozygous mutation; “first hit”) or *type 2* (loss of heterozygosity, on germline heterozygous background; “second hit”) mosaicism (13,14). For example, type 2 *TSC2* point mutations have been identified in lesions from tuberous sclerosis patients (15). Type 1 mosaicism is represented by some forms of hemimegalencephaly and focal cortical dysplasia (FCD), caused by activating mutations in genes such as *PIK3CA* or *AKT3* (14).

2.3. Detection of cortical malformations

Some MCDs, such as lissencephaly, polymicrogyria, or large heterotopia (e.g., subependymal nodules in tuberous sclerosis), can be detected *in utero* by fetal ultrasound or MRI (16). However, many MCDs are detected postnatally or during the first year of life, depending on the severity of the malformation (2). Although neuroimaging is the clinical cornerstone of MCD detection, cytogenetic and genetic studies, including next generation (deep) sequencing, as well as neuropathology, represent essential tools for the modern diagnosis of these disorders.

2.4. Classification of cortical malformations

Since MCDs are complex disorders involving multiple etiologies and neurodevelopmental processes, classification has always been challenging and, to some degree, incomplete. Traditionally, MCDs have been classified into disorders of neuronal and glial proliferation or apoptosis; disorders of cell migration; disorders of postmigrational development; and malformations caused by metabolic disorders, peroxisomal disorders, or sublobar dysplasia (12,17) (Table 1). However, classifications continue to evolve with the identification of new types of MCDs and causative genes, and with ongoing advances in the understanding of brain development (6,18,19).

Classification schemes increasingly incorporate the genetic basis of MCDs as an important organizing principle (2,6). Human genetic studies have identified hundreds of genes associated with one or more types of MCDs, which are involved in various cellular activities, such as cell-cycle regulation and apoptosis, cytoskeletal structure and function, basement-membrane function, neuronal migration, and intracellular metabolic activities.

For instance, micrencephalies, although sharing a common phenotype, are genetically heterogeneous. Underlying genes are involved in cell cycle progression and checkpoint regulation (*MCPH1*, *CDK5RAP2*, *CENPJ*); mitotic spindle formation (*WDR62*, *NDE1*); and centrosome duplication and maturation (*CDK5RAP2*, *NDE1*) (20,21). In addition, mutated genes involved in microtubule formation (*TUBA1A*, *TUBB2B*, *TUBB3*, *TUBG1*) are identified not only in primary microcephalies, but also in other MCDs, including lissencephaly, microlissencephaly, pachygyria, and various examples of polymicrogyria (22). Microtubule-associated proteins (MAPs), including LIS1, DCX, DYNC1H and KIF5C are associated with cytoskeleton development and are identified in patients with severe microcephaly, diverse lissencephalies, and subcortical band heterotopia (23). Postmigrational microcephalies (2), usually developing during the first two postnatal years, are associated with a congenital variant of Rett syndrome caused by *FOXG1* mutations (24), and with pontocerebellar hypoplasia secondary to *CASK* gene deletion (25).

3. DEVELOPMENT OF THE CEREBRAL CORTEX

Research on cortical development has progressed rapidly in the last five years, and in particular, has been marked by the extension of cellular and molecular research from experimental animals (especially mice) to human developing brain. These advances have

been especially relevant to understanding the morphogenesis of gyri and sulci, and carry important implications for disorders of gyrus formation, such as pachygyria.

Classic studies of cortical development from previous centuries showed that cell proliferation occurs mainly adjacent to the embryonic ventricles; that new neurons migrate from the periventricular zones towards the pial surface, to form the cortical plate; that cortical networks are organized in columns, derived from corresponding “radial unit” progenitors; that cortical layers are generated “inside-out,” with deep layers formed earlier than superficial layers; that axons and dendrites begin to grow concurrently with cell migration; and that gyri and sulci are initially generated by differential growth of developmental zones in regions of cortex (26,27). Subsequent studies have elucidated the cellular and molecular bases of these processes, and revealed additional complexities, especially in the past five years.

3.1. Projection neurons and interneurons: distinct cell types with separate origins

Until about 20 years ago, all cortical neurons, including projection neurons (long axon, glutamatergic) and interneurons (short axon, GABAergic) were thought to arise from common progenitor cells that divided at the ventricular surface of the embryonic neuroepithelium. A succession of important discoveries dramatically changed this view. The first key discovery was that interneurons are produced outside the cortical neuroepithelium, in basal forebrain compartments known as the ganglionic eminences (28,29). The interneurons, comprising about 15–20% of all cortical neurons, migrate tangentially into all cortical areas, including hippocampus, before integrating radially into cortical columns. This discovery harkened back to the observations of Ramón y Cajal, who was the first to classify cortical neurons into fundamentally different projection neurons and interneurons. The separate origins of interneurons and projection neurons have significant implications for interpreting the effects of mosaic mutations that arise during development.

3.2. Projection neurons are generated from several types of progenitor cells

Studies in the past several years have revealed that multiple types of progenitor cells are present in the developing cortex, where projection neurons are produced (Fig. 1). The primary source of projection neurons and glial cells in mammalian cortex is radial glial cells, long known as scaffolds that guide the migration of new neurons from periventricular zones to the cortical plate. The “radial glial progenitors” (RGPs), as they are now known, have the capacity to produce diverse types of neurons and glia (multipotency), and to proliferate extensively by self-renewal. These properties (neural multipotency and extensive self-renewal) mark RGPs as a special type of neural stem cells (NSCs) in the developing cortex (30,31).

A second type of cortical progenitor cells, known as intermediate progenitors (IPs), were also found to play a major role in cortical neurogenesis. The IPs are produced from RGPs, but differ from them in several important ways. First, while RGPs undergo M-phase at the ventricular surface, most IPs divide away from the ventricular surface, in not only the ventricular zone (VZ) but also the subventricular zone (SVZ), once thought to harbor only glial progenitors. Second, while RGPs have high proliferative capacity and are multipotent,

IPs have low proliferative capacity and are strictly committed to produce projection neurons. Third, while RGPs have long radial processes, IPs have only short processes. Fourth, at the molecular level, RGPs express markers of NSC identity such as Sox2 and Sox9, but IPs express markers of glutamatergic neuronal differentiation, such as Tbr2.

Since the vast majority of cortical projection neurons are generated from IPs (32), they have been proposed to influence evolutionary features of cortical development, such as the growth of cortical upper layers in higher species, or the formation of gyri and sulci by regionally localized IP proliferation (33).

3.3. Basal progenitor cells and gyrification

Most previous studies of cortical development have utilized mice, which have many advantages for research. However, since mice have small, naturally “lissencephalic” brains, their relevance for studying gyrification of the brain is limited. To understand how gyri form, and to correlate human brain malformations with mouse brain defects, studies of larger species are necessary.

Gyrification is an ancient feature of neocortex in large-brained mammals of all orders, including monotremes and marsupials (27,34). In larger mammals such as primates, the developing cortex is histologically more complex than in mice, with a vastly expanded SVZ divided into “inner” and “outer” zones (35). The increased complexity of progenitor zones was an important clue leading to key discoveries concerning the process of gyrification.

More recent studies of gyrencephalic species, including human fetal cortex in slice cultures, revealed a new type of cortical progenitor cells in the outer SVZ, known as “outer” or “basal” RGPs (oRGPs). The oRGPs were distinguished by their location in the outer SVZ of human developing cortex, and by their observed genesis of IPs and neurons (36). Unlike classic ventricular or “apical” RGPs, the oRGPs lack connections to the ventricular surface. Nevertheless, oRGPs retain the capacity to produce IPs and neurons, and they express markers of NSC identity. Also, oRGPs express some specific molecular markers, such as transcription factor HOPX. Significantly, oRGPs are enriched in mTOR signaling activity (37), which has been implicated in human MCDs, such as megalencephaly (see below).

By detaching from the ventricular surface and migrating towards the cortical plate, oRGPs maintain radial columnar organization during neurogenesis, while focally expanding the external surface area of cortex, but not the internal ventricular surface area—a key property required for gyrification (Fig. 1A). While oRGPs appear to be present in all mammals, their increased abundance in larger, gyrencephalic brains provides a putative substrate for the formation of gyri. Consistent with this idea, oRGPs become abundant shortly before the onset of gyrification, during neurogenesis of supragranular (upper-layer) neurons (38). This transition to increased oRGP abundance occurs at about 16.5 gestational weeks in humans. Concurrently, RGPs in the ventricular zone maintain contact with the ventricular surface, but lose contact with the pial surface to become “truncated” RGPs (38).

Significantly, oRGPs, and the outer IPs generated from them, are most abundant beneath cortical regions that form gyri (38,39). The amplification of oRGPs and IPs is driven in part

by fibroblast growth factor (FGF) signaling (41). The involvement of specific FGF receptors (FGFRs) in gyrification is directly relevant to disorders of human brain gyrification, such as thanatophoric dysplasia (see below). Furthermore, disorders of human brain gyrification, such as pachygyria and lissencephaly, involve defects of neurogenesis and migration that are not well modeled in mice (Fig. 1B). The cortex is invariably thin in mouse models with reduced neurogenesis, but is abnormally thick in humans with reduced neurogenesis (as occurs in lissencephaly), due to impaired gyrification and decreased cortical surface area (Fig. 1B).

3.4. Prolonged developmental neurogenesis and vulnerability

While it was long believed that human cortical neurogenesis is complete by the middle of gestation, modern research has changed this view. Recent evidence suggests that genesis of projection neurons actually continues until approximately 28 gestational weeks (41). In the dentate gyrus of hippocampus, neurogenesis continues into the postnatal period, albeit at declining levels with increasing age (42). The prolonged genesis of cortical neurons suggests vulnerabilities in fetal and postnatal life that may explain some malformations, as well as susceptibilities to factors that modify neurogenesis, such as radiation and inflammation.

3.5. Cortical area patterning and anomalies

Studies in mice and non-human primates have demonstrated that the parcellation of cortex into distinct motor and sensory regions, and ultimately the definition of functional areas, is driven by gradients of gene expression along rostrocaudal and mediolateral axes (43). Some genes associated with cortical patterning in mice, such as *Tbr1* (44) and *Fgfr3* (45), are also implicated in human MCDs. While it seems likely that disturbances of cortical arealization occur in human neurodevelopmental disorders, very little is known about these effects.

4. CORTICAL OVERGROWTH AND FOCAL CORTICAL DYSPLASIA

“Cortical overgrowth” encompasses diffuse or partial (e.g., hemispheric) excess of cortical tissue, which may or may not meet strict medical definitions of megalencephaly (>2.5 standard deviations above the mean for age and sex), but usually meets the less stringent definition of brain weight “greater than average for the age and gender of the child” (NINDS Megalencephaly Information Page). Focal cortical dysplasia (FCD) refers to more limited lesions, usually restricted to one or two lobes, in which the cortex is dysplastic, due to abnormal radial and/or tangential organization (FCD type I; FCD-I), in some cases accompanied by neuronal cytomegaly and dysplasia (FCD type II; FCD-II). Additional subtypes of FCD are also described, most relevantly the division of FCD-II into FCD-IIa, in which no balloon cells are present, and FCD-IIb, in which balloon cells are present (46). In children who undergo brain tissue resection for drug treatment-resistant focal epilepsy, FCD is the most common histopathological diagnosis (47).

In recent years, many cortical overgrowth malformations, and some types of FCD (especially FCD-II), have been linked to mutations that cause overactivation of the receptor tyrosine kinase (RTK)→phosphatidylinositol-3-kinase (PI3K)→AKT→mechanistic target of rapamycin (mTOR) signaling pathway (48–50). This signaling cascade mediates

progenitor proliferation and cell growth during development. Thus, overactivation due to genetic mutations causes hyperplasia and/or hypertrophy of the brain and sometimes other organs, depending on the specific genes and mutations.

4.1. Outline of the RTK→PI3K→AKT→mTOR signaling pathway

Many growth factors, such as insulin-like growth factor 1 (IGF-1) and most fibroblast growth factors (FGFs), activate cell proliferation and growth by signaling through RTKs. In turn, these RTKs are coupled to downstream signaling pathways, most notably the PI3K→AKT→mTOR pathway, which is critically involved in brain development. Since each kinase in the cascade has multiple downstream targets (for example, dozens of proteins are phosphorylated by activated AKT), the signaling pathway is extremely complex and resembles a network. Besides mTOR, additional downstream pathways activated by growth factor RTKs include Ras-MAPK-Erk, FOXO, primary cilium, and others (51,52). In this review, we focus on elements of this cascade that have been linked to cortical overgrowth, including megalencephaly (MEG), hemimegalencephaly (HME), and FCD (Fig. 2).

Critical outputs of this signaling pathway are mediated by the mTOR protein, a serine/threonine kinase that forms two complexes, mTORC1 and mTORC2, that mainly control cell growth/differentiation and proliferation/survival, respectively (Fig. 2). Importantly, mTORC1 is regulated synergistically by not only growth factor RTK signaling, but also by amino acids (leucine, arginine), as well as overall energy balance. Signaling to mTORC1 from growth factors is mediated through the tuberous sclerosis complex (TSC), which constitutively inhibits mTORC1 activity in the absence of growth factors. In parallel, signaling from amino acids is mediated by pathways that converge on the GATOR1 complex, comprised of DEPDC5, Nprl2, and Nprl3. The GATOR1 complex inhibits mTORC1 activity unless amino acid concentrations are sufficient to support growth. Energy stress (e.g., high AMP levels) also regulates mTORC1 activity, in part via the STRADα complex (composed of STRADα, LKB1, and MO25), which activates AMPK, a direct inhibitor of mTORC1 (53).

Thus, the TSC, GATOR1, and STRADα complexes function as parallel brakes on mTORC1 activity, which must all be released for full activation of mTORC1 (49). Mutations that impair the functions of these complexes all lead to mTORC1 activation, and cortical overgrowth or FCD (Table 2). One of the main biochemical readouts of mTORC1 activation is phosphorylation of ribosomal protein S6 to form phospho-S6 (pS6), a readily detectable marker of mTORC1 activation (Fig. 3).

Activation of mTORC2 appears to be driven mainly by growth factor RTK signaling (49, 52). Currently, the roles of mTORC2 activation in cortical overgrowth and FCD are not very clear, in part because there is no convenient assay for mTORC2 activation comparable to pS6 for mTORC1.

The association of cortical overgrowth and FCD with overactive RTK→PI3K→AKT→mTOR signaling links these disorders to tuberous sclerosis (TSC), a disorder that shares many clinical and pathologic features with FCD-IIb. Indeed, the cortical tubers in TSC can be considered a syndromic form of FCD-IIb.

4.2. Mutations of the RTK→PI3K→AKT→mTOR pathway in cortical overgrowth and FCD

A landmark in the analysis of brain overgrowth disorders was the linkage of Proteus syndrome, a disorder marked by patchy asymmetric overgrowth of somatic tissues (including hemimegalencephaly in some cases), with activating ("gain-of-function") mosaic somatic mutations in *AKT1* (54). This finding confirmed longstanding hypotheses about the medical significance of mosaic mutations, which arise during development and thus affect only a subset of somatic cells; and set the stage for a new conceptual paradigm of understanding brain overgrowth and FCD, as well as other types of brain malformations, including subcortical band heterotopia (7).

Further studies, focusing on patients with megalencephaly or hemimegalencephaly, quickly identified mutations in *AKT3*, *PIK3CA* (p110α catalytic subunit of class IA PI3K), *PIK3R2* (p85β regulatory subunit of class IA PI3K), and *MTOR*, all of which led to overactive RTK→PI3K→AKT→mTOR signaling at some level (55–57).

Indeed, variants in numerous mTOR signaling pathway genes are involved in a range of disorders affecting cortical function—including autism, developmental delay (DD), intellectual disability (ID), and epilepsy—in which sometimes only mild cortical overgrowth is detected (58–60). Depending on the type of mutation and the patient's genetic background, the degree of cortical overgrowth may range from very mild to extreme, and the degree of functional impairment may likewise vary. For example, *RHEB* variants were identified as important factors in ID, and in megalencephaly (59). Furthermore, mosaic as well as germline mutations have been detected in many PI3K→AKT→mTOR pathway genes, leading to a spectrum of brain overgrowth and FCD (Table 2).

The specific mutations in each gene are typically diverse, although hotspots are sometimes observed, such as the *AKT3* p.E17K activating mutation often found in patients with hemimegalencephaly and other disorders (61). The diversity of mutations is beyond the scope of this review, but it is worth noting that when mosaic mutations occur, different types of mutations and mosaicism are associated with different genes, depending on their functional significance in the signaling pathway. Genes that encode activators of PI3K→AKT→mTOR signaling, such as *PIK3CA*, are associated with activating ("gain of function") mutations and type 1 ("first hit") mosaicism. In contrast, genes that encode inhibitors of PI3K→AKT→mTOR signaling, such as *DEPDC5*, are associated with inactivating ("loss of function") mutations and type 2 ("second hit") mosaicism. This distinction is a reflection of the fact that overactivation of the signaling pathway can be effected by activating mutation of just one allele of an pathway-activating gene, but reduced signaling activity typically requires inactivation of both alleles of pathway-inhibiting genes.

The abundance of cells carrying mosaic mutations in activating genes such as *AKT3* has been found to vary from ~1–40% across brain regions, in disorders such as hemimegalencephaly (61,62). Variable mosaicism also seems to be the rule for inhibitors of mTOR signaling, such as *TSC1* (hamartin) and *TSC2* (tuberin). While some previous studies have failed to detect loss of heterozygosity in tubers and other tuberous sclerosis lesions (15), recent studies indicate that "second hit" mutations can be detected using deep sequencing approaches (62). The low abundance of mutant cells in some cases has raised the

question of how macroscopic lesions, such as tubers and FCD, can arise from such a small proportion of abnormal cells. Possibly, seizures may be driven by a few abnormal neurons; also, secondary effects (e.g., gliosis) and non-autonomous effects (e.g., secreted factors) may contribute. Indeed, *AKT3* overactivation results in aberrant secretion of Reelin, an important extracellular regulator of neuron migration (63).

Another important question has been whether lesions arise when mutations involve projection neurons, interneurons, or glial cells. Recent studies in mice, in which *Pik3ca* was overexpressed selectively in different lineages, indicated that cortical overgrowth occurred after overexpression in projection neurons, but not interneurons (62). Also, in lesions from human cases, mutations were always present in neurons, but only sometimes in glial cells (62). Thus, mutant cortical projection neurons seem to be necessary and sufficient for overgrowth lesions involving the PI3K→AKT→mTOR pathway. Accordingly, deep sequencing has become an important adjunct to neuropathology of resected seizure foci.

In FCD-I, mutations involving the PI3K→AKT→mTOR pathway have been detected much less frequently than in FCD-II. In two cases of FCD-I, mosaic duplications of small chromosomal segments containing *AKT3* have been detected (64,65). Also, an activating mutation of *AKT3* was documented in a case of extreme megalencephaly (the largest pediatric brain on record), in which no dysplastic cytomegalic neurons or balloon cells were present, and the pathology resembled FCD-I (61). These findings suggest that some *AKT3* mutations can cause focal or diffuse brain overgrowth without marked neuronal cytomegaly. Mutations of *NPRL2* and *DEPDC5* (components of the GATOR1 complex, which inhibits mTORC1; Fig. 2) have also been reported in FCD-I, but in both cases it was suggested that resections of dysplastic cortex were incomplete, and possibly spared regions of FCD-II (66,67).

Other genes rarely implicated in FCD-I include *ALDH7A1* (encoding the glial protein Antiquitin), also known as the causative gene of pyridoxine-dependent epilepsy (68); and *STXBP1* (syntaxin binding protein 1), a regulator of synaptic vesicle release (69). In one case, the *STXBP1* gene was furthermore found to have undergone somatic mosaic mutation (type 2 mosaicism) within the FCD-I lesion (69). However, in most FCD-I cases, mutations have not been found, and the etiology remains puzzling.

While RTKs are major activators of the PI3K→AKT→mTOR pathway, mutations involving RTKs are much less common in brain overgrowth disorders. One exception is a form of severe lethal dwarfism known as thanatophoric dysplasia. In this condition, activating mutations of *FGFR3* lead to brain overgrowth with excessive gyrification of the occipitotemporal regions, but no cytomegalic neurons (45). Since FGFR3 activates PI3K→AKT→mTOR signaling (52), this pathway presumably contributes to brain overgrowth. Interestingly, the occipitotemporal overgrowth reflects a gradient of *FGFR3* expression during development (45). Another major RTK activator of PI3K→AKT→mTOR signaling is the insulin-like growth factor-1 (IGF-1) and IGF-2 receptor IGF1R, but no mutations in the *IGF1R* gene have yet been linked to human brain overgrowth. In mice, transgenic overexpression of IGF-1 causes marked cortical overgrowth (70).

4.3. Heterogeneous pathology of FCD, hemimegalencephaly, and megalencephaly

The pathology of FCDs is heterogeneous, as mentioned above, and as encapsulated in the current classification (46,71). The key features include cortical laminar disorganization and, in FCD-II, the presence of cytomegalic dysplastic neurons, either in the absence (FCD-IIa) or presence (FCD-IIb) of eosinophilic "balloon cells." The significance of balloon cells is unknown, since mutations in the same genes have been found in FCD-IIa and -IIb (Table 2), but no genotype-phenotype correlations have emerged .

Diagnostically, pathologists can screen for mTORC1 activation in FCDs by immunohistochemical detection of pS6, which is elevated in dysplastic neurons (Fig. 3). Since glial cells exhibit elevated levels of pS6 in epilepsy unrelated to FCD or cortical overgrowth (14), and mutant glial cells are not always present in FCD but mutant neurons are (62), pS6 expression in glial cells is not diagnostically significant. Interestingly, studies have also found that FCD-II lesions exhibit abnormal differentiation, including co-expression of neuronal and glial markers in dysplastic neurons, and expression of "primitive" NSC markers, such as Nestin, in balloon cells (72–75). These findings presumably reflect the role of PI3K→AKT→mTOR signaling in regulating neuronal and glial differentiation.

As in FCD, the pathology of hemimegalencephaly and megalencephaly is likewise heterogeneous. Cytomegalic dysplastic neurons may or may not be present, as may various other features such as polymicrogyria, immature cell rests (hamartias), glioneuronal leptomeningeal heterotopia, and subcortical or periventricular heterotopia (61,72,76).

4.4. Relation of FCD to tuberous sclerosis

Tuberous sclerosis (TSC) is an autosomal dominant disorder caused by mutations in *TSC1* (hamartin) or *TSC2* (tuberin). Like forms of brain overgrowth and FCD described above, TSC is classified as an "mTORopathy" (71). Increased mTORC1 signaling, as indicated by markers such as pS6, has been detected in tubers from fetal and adult cortex (77). Accordingly, tubers are now considered to be a special type of FCD-IIb, and indeed the two lesions can be histologically indistinguishable (78,79). Stem cell proteins such as Nestin are also expressed in TSC giant cells (80), as in FCD-IIb balloon cells (72, 74, 75). Moreover, mutations in *TSC1* and *TSC2* seem to exhibit a spectrum of FCD and hemimegalencephaly (Table 2).

On the other hand, tubers are more often calcified than are FCD-IIb lesions, and more often exhibit inflammatory changes (77,80). Other somatic and brain lesions (such as subependymal nodules) also distinguish TSC from FCD-IIb (71,81).

4.5. Treatment implications of RTK→PI3K→AKT→mTOR pathway overactivation

As a kinase cascade, the RTK→PI3K→AKT→mTOR signaling pathway presents attractive targets for pharmacotherapy, to possibly seizure prevention and counter associated neurodevelopmental problems, such as intellectual disability and autism. This pathway is also important in cancer. Indeed, PI3K inhibitors and mTOR inhibitors have been approved

for clinical use (50), and everolimus, an mTOR inhibitor, has been found effective for the treatment of subependymal giant cell astrocytoma in tuberous sclerosis (82,83).

5. LISSENCEPHALY, COBBLESTONE MALFORMATIONS, AND MICRENCEPHALY

Malformations associated with abnormal cell migration are often linked to abnormal cell proliferation as well. For example, brain mass is typically reduced in classic (non-cobblestone) lissencephalies (formerly known as “type I”). Accordingly, disorders of proliferation and migration are considered together in this section. Cobblestone malformations can also cause the appearance of lissencephaly (formerly “type II”), but are mechanistically distinct. Cobblestone malformations result from defects in the pial-limiting membrane, leading to neuronal “overmigration” into the subarachnoid space. The spectrum of cobblestone malformations also includes focal leptomeningeal glioneuronal heterotopia, and more extensive lesions sometimes classified as polymicrogyria, such as bilateral opercular cobblestone cortex caused by *GPR56* mutations.

5.1. Classic and variant lissencephalies

Classic lissencephaly (LIS) is a disorder of neuronal proliferation and migration characterized most saliently by absent (agyria) or excessively wide (pachygyria) gyri, along with cortical thickening and disorganization, and misplaced or ectopic neurons in the subcortical white matter, sometimes forming band or nodular heterotopia (2,3,19,84). LIS is uncommon, with an estimated prevalence of about 12 per million births (85). Clinical manifestations are usually severe, with feeding problems, delayed motor milestones, mental retardation, or seizures before 6 months in most cases (84,85). Typical associated abnormalities of classic LIS may include enlarged lateral ventricles, hypoplasia of the corpus callosum, hypoplasia of the pyramidal tracts, and abnormal inferior olives and cerebellum (6,86). Syndromes associated with classic LIS include Miller-Dieker, Norman-Roberts, and X-linked LIS with ambiguous genitalia (XLAG). The brain size and weight are usually well below average; LIS cases that meet the strict definition of micrencephaly (≥2.5 standard deviations below average for age and gender) are designated microlissencephaly.

The cortex in LIS measures 5–10 mm or more in thickness (as compared to normal 3–5 mm), and the boundary with white matter is irregular (5,6,18). Interestingly, different laminar patterns have been observed in LIS related to different causative genes (87). While increased cortical thickness in the face of reduced brain mass may seem paradoxical, the explanation is that cortical surface area is dramatically reduced in LIS (Fig. 1B).

Many genes have been linked to LIS, and diagnosis now depends primarily on genetics along with neuroimaging. (Non-genetic forms of LIS also occur but are less common.) Most classic LIS is caused by mutations in genes encoding cytoskeletal proteins, while “variant” forms are caused by mutations involving other types of proteins, such as the transcription factor ARX, and the secreted protein Reelin (87).

Deletions and mutations in *LIS1* (officially known as *PAFAH1B1*), located on chromosome 17p13.3, account for many cases of LIS, and this was the first LIS gene to be identified (88).

Chromosomal microdeletions affecting *LIS1* plus other genes cause most cases of Miller-Dieker syndrome (89). The *LIS1* gene encodes a 45-kDA protein with dual functions, both as a regulatory subunit of platelet-activating factor acetylhydrolase 1B, and as a component of the neuronal cytoskeleton that interacts with microtubule-associated proteins, especially dynein, and is thus important for cell division and migration (90). Somatic mutations of *LIS1*, affecting only a subset of cortical neurons, cause subcortical band heterotopia (91). *Pafah1b1* mutant mice show dose-dependent disorganization of cortical layers (92).

An X-linked form of LIS in males, or of subcortical heterotopia ("double cortex") in females, is caused by mutations in the *DCX* (Doublecortin) gene on Xq22.3-q23. DCX is a microtubule-associated protein that is expressed in IPs and post-mitotic neurons. Most cases of classic LIS are associated with *LIS1*, while most cases of subcortical band heterotopia (SBH) are associated with *DCX* mutations. *LIS1*-related LIS affects posterior brain regions more severely, whereas *DCX*-related LIS is more severe in anterior cortex (19,84).

Tubulinopathies are another group of cytoskeletal disorders, caused by mutations in tubulin genes, that can cause LIS, microlissencephaly, SBH, and other MCDs (Fig. 4). Mutations in *TUBA1A*, encoding tubulin α−1A, were the first to be associated with LIS (93). Approximately 1% of patients with classic LIS have a recurrent mutation in the *TUBA1A* gene, and 30% of patients with LIS and cerebellar hypoplasia also present *TUBA1A* mutations (94). As well, other isoforms of α-, β-, and γ-tubulin, and various kinesin and dynein isoforms, have been associated with LIS, and with related MCDs including microcephaly, pachygyria, SBH, and polymicrogyria-like cobblestone malformations (6,22,94.95). Microtubules are involved in mitosis, centrosome formation, organization of intracellular structure, axon pathfinding, and protein transport, accounting for the diverse MCDs caused by tubulinopathies. Abnormal brain development in at least some tubulinopathies is caused by a dominant negative effect of heterozygous missense mutations (95).

Some forms of genetic LIS are considered "variant" because they do not directly impact cytoskeletal functions. One of these is XLAG, resulting from mutations of a transcription factor gene, *ARX*, on Xp22.3. Patients with XLAG have occipital-predominant three-layered LIS, agenesis of the corpus callosum, abnormal basal ganglia, thalamocortical tract defects, and hydrocephalus (18,19,87). Also, approximately 5–10% of patients with X-linked ID (but not XLAG) have *ARX* mutations. *ARX* mutations cause a reduction of GABAergic neurons in the basal ganglia and cerebral cortex (96), and XLAG has sometimes been considered an "interneuronopathy." However, ARX also plays important roles in cortical projection neuron development (97).

Another variant LIS is caused by mutations of *RELN*, encoding the extracellular protein Reelin, which functions in regulating both cell migration and mTOR signaling (98). Patients with autosomal recessive mutations of *RELN*, or its receptor *VLDLR* (reelinopathies), are developmentally impaired with global DD and early onset of generalized epilepsy. The MRI phenotype of reelinopathies ranges from simplified gyral pattern with mild cortical thickening, to frank LIS with substantial cortical thickening (6).

Microlissencephaly represents a heterogeneous group of rare disorders (Di Donato et al, 2017). Microlissencephaly can be caused by *RELN* mutations (Norman-Roberts syndrome). Also, as noted above, some tubulinopathies result in microlissencephaly, most often linked to *TUBA1A*, less often *TUBB2B*, *TUBB3* or *TUBG1* mutations (99). Mutations in *CIT* (encoding citron kinase, important in mitosis) have also been linked to microlissencephaly (100). Another group includes patients with autosomal recessive microcephalic osteodysplastic primordial dwarfism type 1 (MOPD-1), exhibiting predominantly anterior 3-layered microlissencephaly, glioneuronal heterotopia, hypoplasia of the corpus callosum, and mid- and hindbrain defects (101). Recently, mutations in *RNU4ATAC*, a noncoding small nuclear RNA gene involved in splicing, have been linked to MOPD-1 (102).

5.2. Cobblestone malformations

Cobblestone LIS, formerly known as LIS type II (103), results from impaired cerebral basement membrane and glia limitans formation, usually attributed to defects in the linkage of radial glia to the basement membrane. Cobblestone LIS represents the severe end of a spectrum, generally characterized by migration of neurons and glia into the subarachnoid space, conferring an irregular “lumpy-bumpy” appearance to the cortical surface (6). Cobblestone malformations are predominantly autosomal recessive disorders with cerebral, ocular and muscular abnormalities.

Different forms of severe cobblestone LIS include Walker-Warburg syndrome (WWS), muscle-eye-brain (MEB) disease, and Fukuyama congenital muscular dystrophy (FCMD). The most severe of these is the rare WWS, with an incidence of 1.2 cases per 100,000 live births (104). In contrast, FCMD, linked to *FKTN* and *FKRP* mutations and found mainly in Japanese populations, is typically much less severe clinically (105,106). The FCMD cortical phenotype ranges from nearly normal cerebral cortex with few heterotopia, to cobblestone LIS with large gaps and prominent cortical surface bumps (107).

These related disorders (WWS, MEB, FCMD) are caused by mutations in enzymes that glycosylate α-dystroglycan, encoded by genes including *POMT1*, *POMT2*, *POMGNT1*, *FKTN*, *FKRP*, and *LARGE*. Together, these diseases are known as α-dystroglycanopathies (107). Mutations in *TMEM5* and *ISPD* genes, likewise involved in dystroglycan glycosylation, were also recently detected in severe cobblestone LIS (108). Defective glycosylation of α-dystroglycan on the basal end of radial glia causes defects of radial glia adhesion to the pial basement membrane, leading to aberrant neuronal “overmigration” into the subarachnoid space (109).

Other genes important for basement membrane structure and function have also been linked to cobblestone MCDs. Mutations involving laminins or glycosyltransferases, including *LAMB1*, *LAMB2*, *LAMC3*, and *SRD5A3* have been linked to cobblestone malformations (110,111). Mutation of *GPR56*, encoding a collagen III receptor, is also associated with a cobblestone MCD, manifesting as bilateral frontoparietal polymicrogyria-like dysgenesis (112).

5.3. Micrencephaly

Micrencephaly (referring to small brain) is usually recognized by microcephaly (small head). The most severe forms, the primary micrencephalies, are caused by defects in important genes for cell division, reviewed elsewhere (113). Interestingly, less severe micrencephaly can result from defects in PI3K→AKT→mTOR signaling, such as loss-of-function mutations of *AKT3* (114). Non-genetic causes of micrencephaly include toxic, infectious, and ischemic insults, such as fetal alcohol exposure, or Zika virus infection.

6. POLYMICROGYRIA

Like lissencephaly, polymicrogyria (too many, too small gyri) has been variously applied to MCDs of exceedingly diverse etiology and histopathology. Thus, by the broadest definitions, polymicrogyria is observed in many heterogeneous genetic disorders, including brain overgrowth syndromes (Table 2); cobblestone malformations; cortex with excessive but relatively well-formed gyri (polygyria), as in thanatophoric dysplasia; and unique forms of cortical dysgenesis, as in monosomy 1p36 (115). However, definitions of polymicrogyria remain unsettled, even among neuropathologists. For example, one recent study suggested that polymicrogyria is essentially defined by fusion of the molecular layer across gyri (116), while another reported that fusion is present in fewer than one-third of cases, and that disruptions at the brain surface are the most consistent anomaly in polymicrogyria (117).

Given these discrepancies in the definition and features of polymicrogyria, and to avoid redundancy with categories such as brain overgrowth and cobblestone malformations, polymicrogyria may be considered for now as a descriptive grouping that can be applied in many circumstances, rather than as a distinct, independent class of MCDs.

7. AXON PATHWAYS DEFECTS

7.1. Agenesis of the corpus callosum: partial and complete

Corpus callosum abnormalities, which include complete and partial agenesis, as well as dysgenesis, are among the most common brain malformations, observed in isolation or associated with complex malformation syndromes (118). The estimated overall prevalence of callosal abnormalities is 3–7 per 1000 births, and reaches 3–5% in patients with various disorders of brain development (118,119). Most patients with callosal agenesis (~75%) show normal intelligence, with the remainder exhibiting some degree of ID (119).

Callosal anomalies are often diagnosed on neuroimaging studies. Initial formation of the corpus callosum depends on coordination between callosal projection neurons extending axons towards the midline, and multiple midline structures that may guide callosal axons, such as the glial wedge. This conclusion is based on observations from transgenic mice, in which defects of the corpus callosum have been associated with mutations that perturb development of neurons or the glial wedge (120–122).

The etiology of most human callosal abnormalities remains unknown, although genetic and non-genetic factors contribute. It is estimated that up to 45% of callosal abnormalities are associated with genetic defects, including 20% with chromosomal aberrations, and the

remainder with genetic syndromes caused by a single or multiple gene variants (118). Indeed, a vast number of MCDs include callosal defects, and thus implicate an equally vast number of genes in callosal development (2,19, 22,123). For example, ciliopathies such as orofaciodigital type 1 syndrome, Meckel-Gruber syndrome, Joubert syndrome, primary ciliary dyskinesia, and Bardet-Biedl syndrome often include callosal defects, and there are dozens of ciliopathy genes (124, 125).

The genetic causes of isolated callosal agenesis appear more elusive, given the lack of strong phenotypes and family studies. Nevertheless, *CDK5RAP2* (*MDPH3*), a gene linked to autosomal recessive microcephaly, was also recently linked to isolated callosal agenesis (126).

7.2. Other axon pathways

Even more elusive are genes linked to the formation or guidance of other axon pathways, such as the corticothalamic pathway. Evaluation of these axon tracts in humans requires sophisticated techniques, such as diffusion tensor imaging (DTI) by neuroradiology, and defects may not have clear phenotypes. In future studies of disorders such as ID and autism that lack definite MCDs but are linked to specific genes, it may be fruitful to study axonal organization by methods such as DTI, since brain wiring may be equally important to cellular structures in such cases.

8. CONCLUSIONS AND FUTURE DIRECTIONS

Among the most important implications of recent research, it has become clear that many genetic MCDs span multiple classification categories based on morphology and primary mechanisms such as proliferation, differentiation, and migration. Polymicrogyria, for example, spans several categories. This reflects the involvement of some genes, such as tubulins, in diverse processes from cell division to axon growth. Thus, genetic diagnoses are an increasingly important adjunct to pathology.

Adding to the challenges of classification and diagnosis, it has also become clear that some genetic MCDs, especially FCDs, can arise from type 1 or type 2 somatic mosaic mutations in many different genes. Deep sequencing of resected brain tissues, along with the application of markers such as pS6 to evaluate mTORC1 activation (Fig. 3), can help resolve uncertainties about the involved gene and pathways, as well as the type of mosaicism.

While tremendous progress has been made in studying MCDs, subtle defects, such as disorganization of axon pathways and cortical arealization in disorders such as autism, remain to be explored. While genetic studies of ID and autism have revealed the important role of *de novo* mutations, more sophisticated techniques in neuroimaging, such as DTI to detect axon defects, and functional MRI to assess cortical arealization, may help advance the phenotypic characterizations. Advances in these and other areas of MCD research will be important to drive future progress in diagnosis and treatment.

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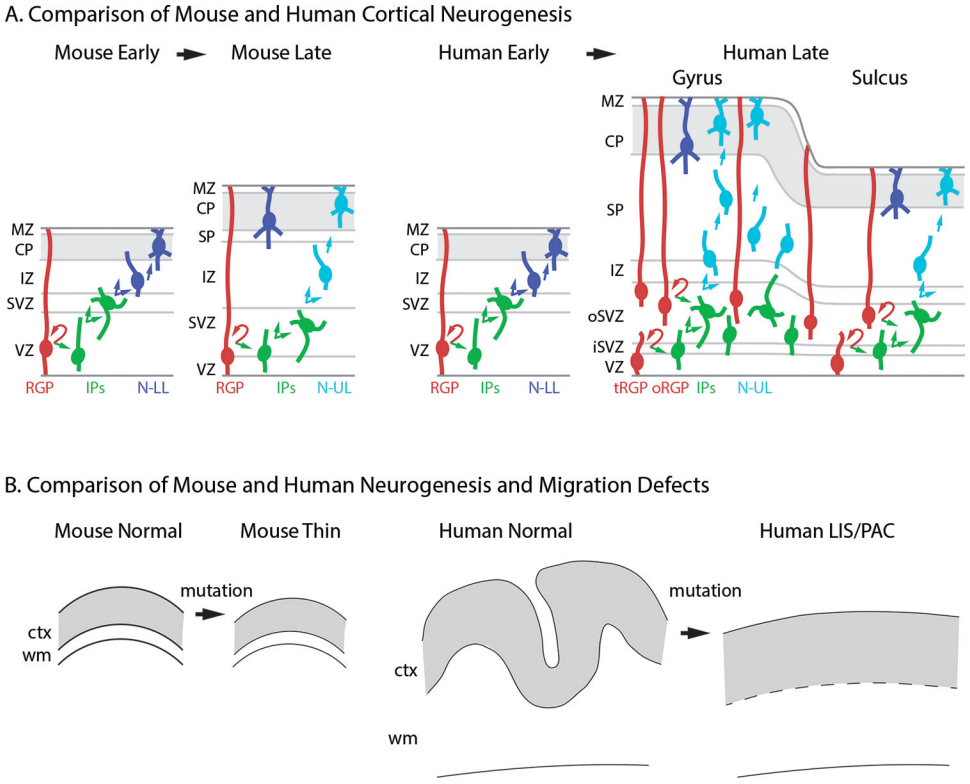

Figure 1.

Comparison of cortical development and defective neurogenesis in mice and humans. (**A**) Normal development. In mice, classic RGPs predominate during early (deep-layer) and late (superficial-layer) neurogenesis, and the cortex lacks gyri and sulci. In humans, a transition from classic RGPs to mainly oRGPs occurs between early and late neurogenesis, when gyri and sulci begin to form. The abundance of oRGPs and outer IPs is much higher beneath prospective gyri. (**B**) Reduced neurogenesis in mice leads to cortical thinning. In humans, reduced neurogenesis is usually associated with impaired gyrification (lissencephaly or pachygyria), and leads to increased cortical thickness. Abbreviations: CP: cortical plate; ctx: cortex; IP: intermediate progenitor; IZ: intermediate zone; LIS: lissencephaly; MZ: marginal zone; N-LL: neuron destined for lower layers; N-UL: neuron destined for upper layers; oRGP: outer radial glial progenitor; PAC: pachygyria; RGP: radial glial progenitor; SVZ: subventricular zone; tRGP: truncated radial glial progenitor; VZ: ventricular zone; wm: white matter.

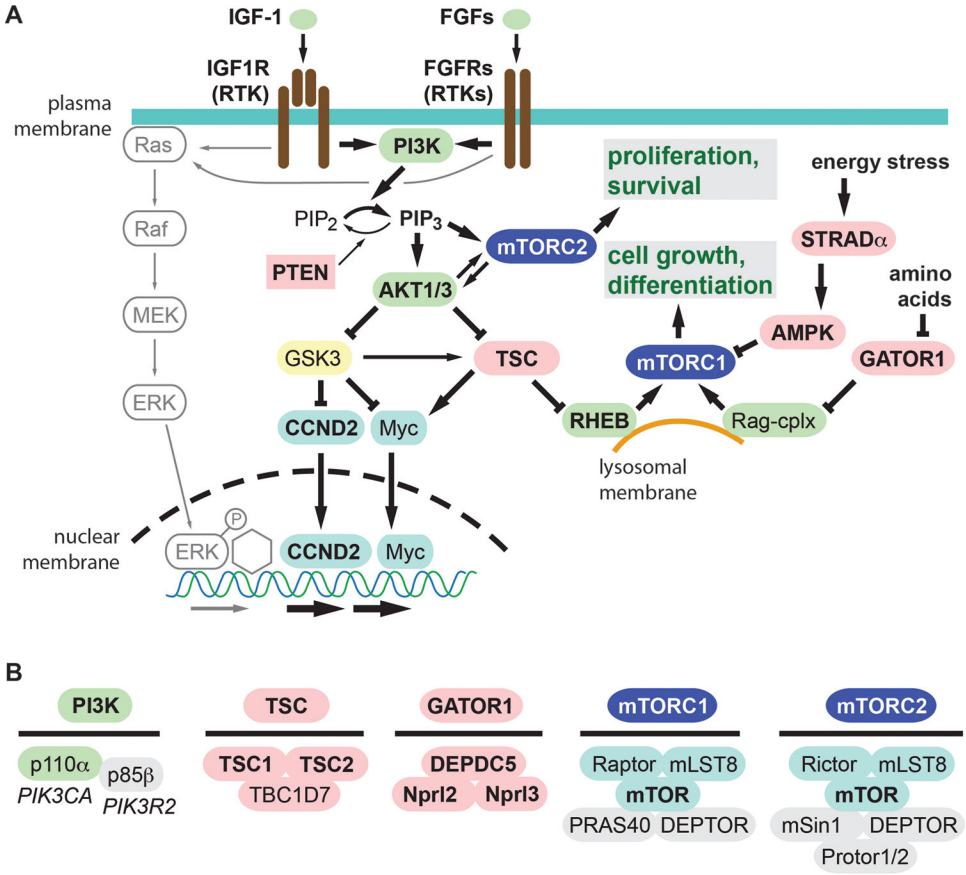

Figure 2.

The RTK-PI3K-AKT-mTOR signaling pathway. (**A**) The pathways is driven by growth factors interacting with their receptors (RTKs), as well as by intracellular metabolic factors including energy status and amino acids. Activators are colored green, and inhibitors red. Molecules known to be linked to brain overgrowth or FCD are indicated by bold type. (**B**) Subunit composition of key protein complexes linked to brain overgrowth or FCD. The genes encoding p110α (*PIK3CA*) and p85β (*PIK3R2*) are also indicated.

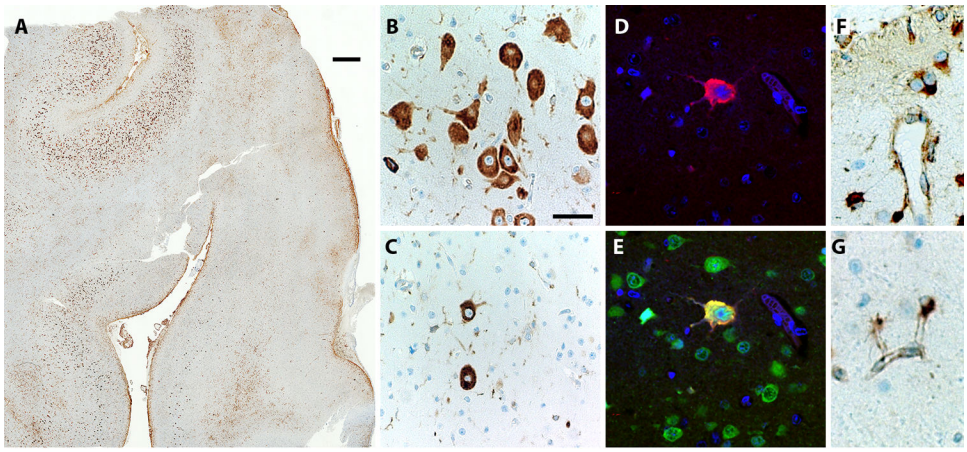

Figure 3.

Patchy mTORC1 activation in a case of FCD-IIa reflects somatic mosaicism. (**A**) Phospho-S6 immunoreactivity in cortical resection specimen at low magnification. Note local variations in the abundance of immunoreactive cells. (**B**) Higher magnification shows an area of relatively abundant dysplastic neurons with high phospho-S6 immunoreactivity. (**C**) An area of sparse dysplastic neurons. (**D, E**) Two-color immunofluorescence to detect phospho-S6 (red) and NeuN (green) demonstrates that a single dysplastic neuron with high phospho-S6 expression. (**F, G**) High expression of phospho-S6 in subpial (F) and deep-layer (G) astrocytes is not diagnostically significance, as this may be seen in all forms of epilepsy (Jansen et al., 2015). Scale bar (A): 1 mm. Scale bar (B): 50 μm for B, C; 25 μm for C–F.

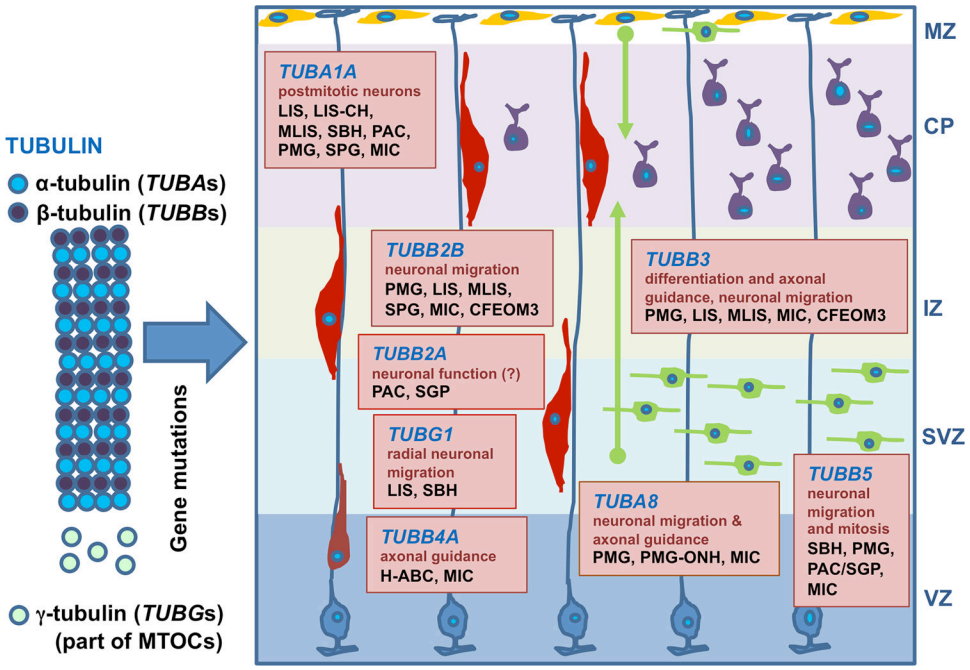

Figure 4.

Tubulinopathies affect multiple processes in cortical development, and cause heterogeneous MCDs. Boxes represent the expression, functions, and MCDs associated with *TUBA1A*, *TUBA8*, *TUBB2A*, *TUBB2B*, *TUBB3*, *TUBB4A*, *TUBB5*, and *TUBG1*. Abbreviations: CFEOM3: congenital fibrosis of extraocular muscles type 3; CP: cortical plate; H-ABC: hypomyelination with atrophy of the basal ganglia and cerebellum; IZ: intermediate zone; LIS: lissencephaly; LIS-CH: lissencephaly with cerebellar hypoplasia; MIC: congenital microcephaly; MLIS: microlissencephaly; MTOCs: microtubule organizing centers; MZ: marginal zone (Cajal-Retzius neurons in yellow); PAC: pachygyria; PMG: polymicrogyria; PMG-ONH: polymicrogyria with optic nerve hypoplasia; SBH: subcortical band heterotopia; SGP: simplified gyral pattern; SVZ: subventricular zone; VZ: ventricular zone.

Table 1.

Simplified classification of genetic MCDs

MCD group	MCD type	Morphologies	Related pathways
Disorders of proliferation, apoptosis, and/or differentiation	Microcephalies	Microcephaly, microlissencephaly Alobar, lobar, variant holoprosencephaly	Tubulinopathies, microtubule-associated proteins Decreased RTK-PI3K-AKT-mTOR Sonic hedgehog pathway Midline differentiation
	Cortical overgrowth disorders (focal and diffuse)	Megalencephaly, hemimegalencephaly, polymicrogyria, FCD-II	Overactive RTK-PI3K-AKT-mTOR
Disorders of neuronal migration	Classic lissencephaly spectrum	Smooth lissencephaly, microlissencephaly, subcortical band heterotopia	Tubulinopathies, microtubule-associated proteins Variant lissencephalies (non-cytoskeletal)
	Cobblestone malformations	Rough lissencephaly, polymicrogyria, leptomeningeal glioneuronal heterotopia	Dystroglycanopathies Other basement membrane - glia limitans interaction disorders
	Periventricular heterotopia	Nodular or linear periventricular heterotopia	Microtubule-associated proteins
	Dyslamination without cytologic dysplasia or growth abnormality	FCD-I	Overactive RTK-PI3K-AKT-mTOR Other rare forms (e.g., variant Rett syndrome)
Disorders of axon pathway formation	Isolated callosal defects	Agenesis, hypogenesis, dysgenesis of corpus callosum	Axon growth and guidance Midline differentiation
	Other isolated axon defects (putative)	Unknown	Axon growth and guidance

Table 2.

Genes linked to cortical overgrowth and FCDs[a]

Gene	Typical mutations	Syndromes	Brain[b]	M	H	I	IIa	IIb	Body	References
AKT1	postzygotic mosaic GOF	Proteus	HMEG	ND	+	ND	ND	ND	Asymmetric overgrowth affecting multiple tissues	54, 62
AKT3	de novo germline > postzygotic mosaic, GOF; or chr microdup	MPPH	DMEG, HMEG, FCD-Ib, periventricular or subcortical heterotopia	+	+	+	ND	ND	ND: *AKT3* is expressed specifically in brain	56, 57, 61, 62, 64, 65, 127
CCND2	de novo germline	MPPH	DMEG	+	ND	ND	ND	ND	Postaxial polydactyly	128
DEPDC5	heterozygous germline, postzygotic mosaic LOF	BPP[c]	HMEG, FCD-I,[d] FCD-IIa/b	ND	+	ND	+	+	ND	62, 66, 129–133
FGFR3	de novo constitutional GOF	TD	Occipito-temporal hypergyration	+	ND	ND	ND	ND	Severe dwarfism	45
MTOR	mosaic, GOF	ND	MEG, HMEG, FCD-IIa/b	+	+	ND	+	+	Pigmentary mosaicism	127, 129, 134–137
NPRL2	heterozygous germline, LOF	ND[c]	FCD-Ia,[e] FCD-IIa	ND	ND	+	+	ND	ND	62, 67
NPRL3	familial, heterozygous germline, LOF	ND[c]	FCD-IIa	ND	ND	ND	+	ND	ND	67, 138
PIK3CA	mosaic GOF	CLOVES (severe), MCAP (moderate)	DMEG, HMEG, FCD-IIa	+	+	ND	+	ND	CLOVES: lipomatous overgrowths, vascular malformations, epidermal nevi, spinal/skeletal anomalies MCAP: Asymmetric overgrowth, poly/syndactyly, capillary/ lymphatic malformations	14, 57, 62, 127
PIK3R2	de novo constitutional > postzygotic mosaic, GOF	MPPH, BPP	DMEG, HMEG	+	+	ND	ND	ND	Polydactyly	57, 62, 139, 140
PTEN	germline with postzygotic mosaic LOF	Cowden, PTEN-HTS[f]	DMEG, HMEG, FCD-IIb	+	+	ND	ND	+	Hamartomas (e.g., oral papillomatous papules)	14, 141
RHEB	de novo constitutional GOF	ND	MEG	+	ND	ND	ND	ND	ND	59
STRADA	de novo constitutional GOF	PMSE	MEG	+	ND	ND	ND	ND	Polyhydramnios	53
TSC1	germline with postzygotic mosaic LOF	TSC	Tubers, subependymal nodules, SEGA, FCD-IIa/b	ND	ND	ND	+	+	Facial angiofibromas, shagreen patch, ungual fibromas, renal angiomyolipoma, cardiac rhabdomyoma	62, 142–144
TSC2	germline with postzygotic mosaic LOF	TSC	Tubers, subependymal nodules, SEGA, HMEG, FCD-IIa	ND	+	ND	+	ND	Facial angiofibromas, shagreen patch, ungual fibromas, renal angiomyolipoma, cardiac rhabdomyoma	62, 80, 142–144

[a] Abbreviations: BPP: bilateral perisylvian polymicrogyria; chr: chromosomal; CBTE: cerebellar tonsillar ectopia; CLOVES: congenital lipomatous overgrowth, vascular malformations, epidermal nevi and scoliosis/skeletal/spinal anomalies; DMEG: dysplastic hemimegalencephaly; GOF: gain of function; H: hemimegalencephaly; HMEG: hemimegalencephaly; I: FCD-I; IIa: FCD-IIa; IIb: FCD-IIb; LOF: loss of function; microdup: microduplication; M: megalencephaly; MCAP: megalencephaly-capillary malformation; MEG: megalencephaly; MPPH: megalencephaly-polymicrogyria-polydactyly-hydrocephalus; ND: not detected; PMG: polymicrogyria; PTEN-HTS: PTEN-hamartoma tumor syndrome; SBH: subcortical band heterotopia; SEGA: subependymal giant cell astrocytoma; TD: thanatophoric dysplasia. +: reported in at least one case. Other abbreviations as in text.

[b] Hydrocephalus/ventriculomegaly, and cerebellar tonsillar ectopia/Chiari-I are common findings that accompany cortical overgrowth. Polymicrogyria (often bilateral perisylvian) and/or pachygyria are also frequent findings in megalencephaly or hemimegalencephaly. Bilateral perisylvian polymicrogyria is documented with *PIK3R2* (139) and *DEPDC5* (133) mutations. Callosal hyperplasia is documented in a minority of megalencephaly and hemimegalencephaly. Periventricular nodular heterotopia were found in patients with germline *AKT3* mutations (61), and subcortical nodular heterotopia in *AKT3* amplification due to chromosomal microduplication (64).

[c] *DEPDC5*, *NPRL2*, and *NPRL3* mutations are also associated with temporal lobe epilepsy, febrile seizures, and frontal lobe epilepsy (133).

[d] FCD-I was associated with *DEPDC5* mutation, but residual FCD-II was suspected (66).

[e] FCD-Ia has been associated with *NPRL2* mutation, but residual FCD-II was considered likely (67).

[f] PTEN-HTS is also known as Bannayan-Riley-Ruvalcaba syndrome.