Review

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Tubulin-related cortical dysgeneses: microtubule dysfunction underlying neuronal migration defects

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The fine tuning of proliferation and neurogenesis, neuronal migration and differentiation and connectivity underlies the proper development of the cerebral cortex. Mutations in genes involved in these processes are responsible for neurodevelopmental disorders, such as cortical dysgeneses, which are usually associated with severe mental retardation and epilepsy. Over the past few years, the importance of cytoskeleton components in cellular processes crucial for cortical development has emerged from a body of functional data. This was reinforced by the association of mutations in the *LIS1* and *DCX* genes, which both encode proteins involved in microtubule (MT) homeostasis, with cerebral cortex developmental disorders. The recent discovery of patients with lissencephaly and bilateral asymmetrical polymicrogyria (PMG) carrying mutations in the α- and β-tubulin-encoding genes *TUBA1A* and *TUBB2B* further supports this view, and also raises interesting questions about the specific roles played by certain tubulin isotypes during the development of the cortex.

Genetic basis of cortical dysgeneses

Genetic studies in humans and mice have identified a spectrum of mutations in genes involved in a large array of crucial processes (e.g. cell proliferation, cell adhesion, cell migration, chemoattraction and repulsion, post-translational modifications and dynamics of the cytoskeleton) that often disrupts the development of the cerebral cortex (Box 1 and Figure I) and can lead to severe cortical malformations. The heterogeneity of developmental disorders affecting the cortex recapitulates its development, because impairments in the different steps are associated with specific features [1–3]. For instance, early genetic assaults in proliferation and neuronal production might lead to microcephaly (genes involved in these disorders will be discussed later). Following neurogenesis (Box 1), the disruption of neuronal migration resulting from genetic mutations represents a major cause of cortical dysgeneses and encompasses a large variety of malformations. Many of these genes encode important effectors that modulate cytoskeletal dynamics during the migration of neuronal cells. The importance of the cytoskeleton in neuronal migration has emerged from a body of functional and genetic studies. This has been gleaned from the contribution of actin and its associated proteins, such as Filamin A, because mutations in the *FLNA* (*Filamin A*) gene impairs the migration of neurons out of the germinal zone and leads to nodular periventricular heterotopia (Box 1). In addition, mutations in *DCX* and *LIS1* genes [which encode microtubule-associated proteins (MAPs)] were shown to be associated with a large spectrum of neuronal migration

Glossary

Agyria: a smooth brain with the complete absence of gyration.
Corpus callosum: a white matter structure that connects the left and right hemispheres containing fibers from callosal projections neurons of layers II/III and IV.
ENU-mutagenesis screen: N-ethyl N-nitrosourea (ENU) is an alkylating agent considered a powerful mutagen. When administrated to male mice intraperitoneally, it can induce a point mutation at a given random locus every 700 gametes.
Gyration: a pattern of brain folds at the surface of the cortex.
***In utero* electroporation**: a method used to deliver plasmid DNA into the developing cortex. The DNA solution is injected into the lateral ventricles and the subsequent high voltage discharge across the embryonic head allows the transfection of cells located in the ventricular zone. This method can be combined with RNAi.
Intermediate zone: an heterogeneous compartment that lies between the proliferative zones and the post-migratory cells above.
Internal capsule: an area of white matter located at the striatocortical junction. It contains corticothalamic axonal fibers from projection neurons of cortical layer V and thalamocortical fibers in its anterior limb, as well as corticospinal and sensory fibers in its posterior limb.
Lateral and medial ganglionic eminences (LGE, MGE): structures transiently located in the ventral telencephalon, in which precursors of GABAergic cells (interneurons) are located.
Meninges: the meninges consist of three distinct mesenchymal cell layers, named pia, arachnoid and dura, from the inner to the outer. The innermost layer produces the basement membrane that covers the surface of the cortex.
Nucleation: a process required to initiate the *de novo* formation of microtubules and that relies on the presence of γ-tubulin in the microtubule organizing center usually located in the centrosome.
Pachygyria: a brain with a simplified pattern of gyration and thick folds.
Perisylvian: regions located close to the Sylvian fissure (also named lateral sulcus), which separates the frontal, parietal and temporal lobes.
Pial basement membrane (or glia limitans): the outside membrane located at the surface of the cortex, lying directly under the meningeal pia matter. It is constituted by radial glia endfeet attached to a basal lamina, made of extracellular matrix components.
Subcortical laminar heterotopia: a group of neuronal cells abnormally located in the white matter, below the cortex and displaying a band-shape.
Telencephalon: the most anterior region of the developing vertebrate central nervous system. The dorsal and ventral parts of the telencephalon become the cortex and the basal ganglia of the brain, respectively.
Type II lissencephaly: a neurodevelopmental disorder, usually associated with severe congenital muscular dystrophies, which encompasses three different syndromes such as muscle-eye-brain disease (MEB), Fukuyama-type muscular dystrophy (FCMD) and Walker-Warburg syndrome (WWS). These syndromes are all associated with a massive neuronal overmigration into the meningeal space, beyond the pial basement membrane.

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disorders. These genes were previously shown to be involved in type I lissencephaly, which is a congenital malformation affecting gyral patterning and cortical lamination that encompasses a large range of cortical abnormalities, extending from agyria to pachygyria, either posterior- (*LIS1*-associated pattern) or anterior-predominant (*DCX*-associated pattern). Thus, the term lissencephaly refers to a 'smooth' aspect of the brain surface. Clinically, patients often demonstrate moderate to severe mental retardation and refractory epilepsy [1,3]. Until recently, only four genes were associated with human type I lissencephaly: *LIS1* (or *PAFAH1B1*, *platelet-activating factor acetylhydrolase*, *isoform Ib*), *DCX* (*Doublecortin*), *ARX* (*aristaless-related homeobox*) and *RELN* (*reelin*) [4–10]. Functional studies revealed that the LIS1 protein (also known as PAFAH1B1) is involved in the regulation of the molecular motor complex formed by Dynein and Nudel [2] and that Doublecortin is a MAP that both induces nucleation and stabilizes MTs by linking adjacent tubulin protofilaments [6,11–14]. The genetic suppression in mice of *Dcx* or *Lis1* is associated with neuronal migration defects [15–20]. Another group of cortical dysgeneses, called type II lissencephaly, was shown to be associated with pial basement membrane defects and an overmigration of neuronal cells into the leptomeningeal space. The pial surface of the cortex is limited by a basement membrane, which is a specialized structure of extracellular matrix serving as an anchor for radial glial endfeet (Figure I in Box 1). Type II lissencephaly is characterized by a defective basement membrane in which breaches are formed. This further allows the migrating neurons to pass beyond the pial basement membrane and settle within the meningeal space. To date, several mutated genes [*fukutin, fukutin-related protein* (*FKRP*), *O-mannosyltransferase 1* (*POMT1*), *O-mannosyltransferase 2* (*POMT2*) *and O-mannose b-1,2-N-acetylglucosaminyltransferase* (*POMGnT1*)], known to encode proteins that ensure post-translational modifications of transmembrane and extracellular matrix components, have been associated with type II lissencephaly [1]. Altogether, these data suggest that these proteins are involved in the development and maintenance of the basement membrane.

Despite the significant progresse over the past few years, many cases of cortical dysgeneses are still unexplained. The identification of further genes is important not only for the transfer to the clinic and genetic counseling but also to better understand the physiopathology of human cortical dysgeneses. Moreover, genetic data are also valuable for neurobiologists to unravel the functions of key proteins during the development of the cortex. Here, we review the recent progresses made towards the comprehension of the forms of cortical dysgenesis associated with mutations in tubulin genes. On one hand, human genetic investigations carried out on patients with unexplained abnormalities of cortical development has led to the description of two new syndromes associated with mutations in two genes encoding specific isotypes of α- and β-tubulin, respectively. These findings have uncovered the possibility that at least a few tubulin isotypes might play specific roles during the development of the cortex. They also suggest that these isotypes are probably not interchangeable, a concept that is still a matter of debate. On the other hand, there is emerging evidence demonstrating that MT dynamics are involved in the regulation of neuronal differentiation and migration. These recent findings open new avenues for further investigation to understand better the normal and pathologic development of the cortex.

Mutations in α-tubulin 1A are associated with type I lissencephaly

Despite the known implication of several MAPs in cortical development, tubulins and MTs (Figure I in Box 2) have only recently been reported as crucial protagonists of neuronal migration processes. Keays and colleagues (2007) found lamination defects in both the isocortex and hippocampus of a mutant mouse strain generated by N-ethyl N-nitrosourea (ENU) mutagenesis. Further genetic investigations revealed that the mutant strain carries a heterozygous missense mutation in the autosomal *tuba1a* gene (NM_011653) that encodes an α-tubulin subunit. The neuroanatomical similarities between the *tuba1a* mutant and *Dcx* deficient mice [17], and that

Box 1. Development of the cerebral cortex

The mammalian cerebral cortex is characterized by a six-layered laminar structure. The complexity of its cytoarchitecture and connectivity reflects the complexity of the tasks that the cortex has to ensure, namely cognitive functions, sensory perception and consciousness. Its proper development above the ventricular vesicles at the rostral ends of the neural tube relies on the succession of finely tuned processes widely studied in rodents, monkeys and humans, that is, proliferation and neurogenesis, neuronal migration and connectivity [75–77]. Projection neurons and interneurons are the basic neuronal components of the cortex and originate either from the dorsal ventricular zones or ganglionic eminences of the basal forebrain, respectively [78] (Figure I in Box 1).

The onset of active neurogenesis, at approximately E33 in the human dorsal telencephalon [79] and E10 in mice [80], follows a period of proliferative symmetrical divisions and is marked by the first asymmetrical divisions of radial glial cells in the periventricular neuroepithelium [81] (Figure I in Box 1). This type of mitosis produces one daughter cell that remains a progenitor and one post-mitotic cell that will give rise to a pyramidal neuron or a glial cell either directly or indirectly through an intermediate progenitor cell (IPC) [81–88]. The pool of IPCs further contributes to the formation of the subventricular zone (SVZ). Successive generations of neurons further migrate radially along radial glia fibers towards the surface of the cortex through the entire cortical plate (CP) and settle in superficial regions as soon as GW7–8 in human and non-human primates and E14 in mice. The younger neurons bypass their predecessors and form the CP in an ''inside-out'' manner [89,90] (Figure I in Box 1). In rodents, cortical interneurons are born primarily outside the neocortex, in the ventral telencephalon and cortical hem [91]. Thus, they have to migrate a long distance, for example from the medial ganglionic eminences tangentially towards the dorsal telencephalon, to insert into the cortex [91,92]. At the end of their journey, some interneurons move towards the ventricular zone before migrating radially to their final destination into the CP [90]. Unlike in rodents, one subpopulation of human interneurons originates from a final mitosis of progenitors located in the dorsal ventricular zone [93–95]. Finally, a further aspect of the corticogenesis is the development of cortical connectivity of projection neurons that requires proper axonal growth and guidance towards cortical or subcortical targets, that is, located in structures including the thalamus, brainstem or spinal cord [96].

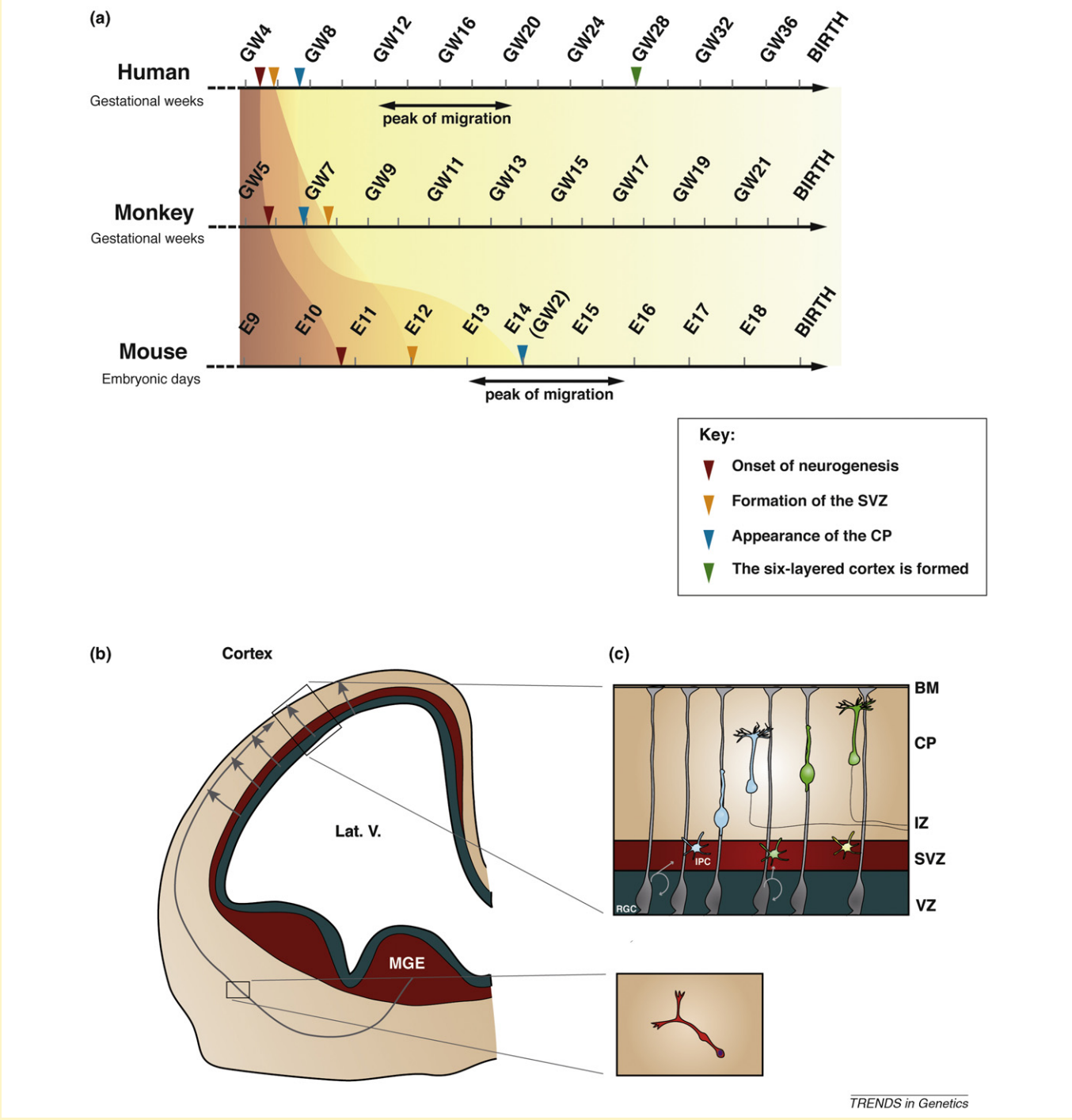

Figure I. Development of the mammalian cerebral cortex. (a) Relative timelines of conserved cortical development hallmarks in human, primate and mouse. The chronologic representation starts with the neural tube closure that occurs around GW4, GW5 and E9 in human, primate and mouse, respectively. A period of proliferation of neuroepithelial cells present at the ventricular surface precedes the onset of neurogenesis. Once the first neurogenic mitoses occur (around GW5–6 in human and primate and E10–11 in mouse), the cortical thickness increases with the formation of the SVZ. Early post-mitotic neurons migrate radially towards the surface of the cortex and give rise to a transitory preplate. The latter will be replaced by the CP, as successive waves of migrating neurons settle and form the cortical layers. The peak of neuronal migration occurs between GW12 to GW20 and E13 to E16 in human and mouse, respectively. **(b)** Schematic representation of the developing rodent cortex. The central nervous system is mainly composed of two types of neurons: inhibitory interneurons and excitatory projection neurons. They originate in the medial ganglionic eminence in the ventral telencephalon and in the dorsal telencephalon in the ventricular zone of the cortex, respectively. Post-mitotic interneurons have to migrate upwards following a path that is tangential to the surface of the cortex. Post-mitotic projection neurons migrate radially to reach their final location and further settle within the cortical thickness following an inside-out pattern. The younger neurons settle in lower layers, whereas the latter neurons integrate in higher layers. **(c)** Insets depicting migrating cells from the (b) panel at a higher magnification. The upper inset illustrates neurogenic asymmetrical divisions of radial glial cells (RGC, in gray) in the ventricular zone that subsequently give rise either to one progenitor and one multipolar IPC located within the SVZ or directly to post-mitotic neurons [87]. Post-mitotic projections neurons are also born from symmetrical mitosis of the IPC and further adopt a bipolar shape to migrate radially towards the CP along the RGC processes. These processes extend towards the cortical surface and are attached to the pial basement membrane. The lower inset shows a tangentially migrating interneuron displaying a characteristic dynamic shape, splitting, extending and retracting branches at its leading edge.

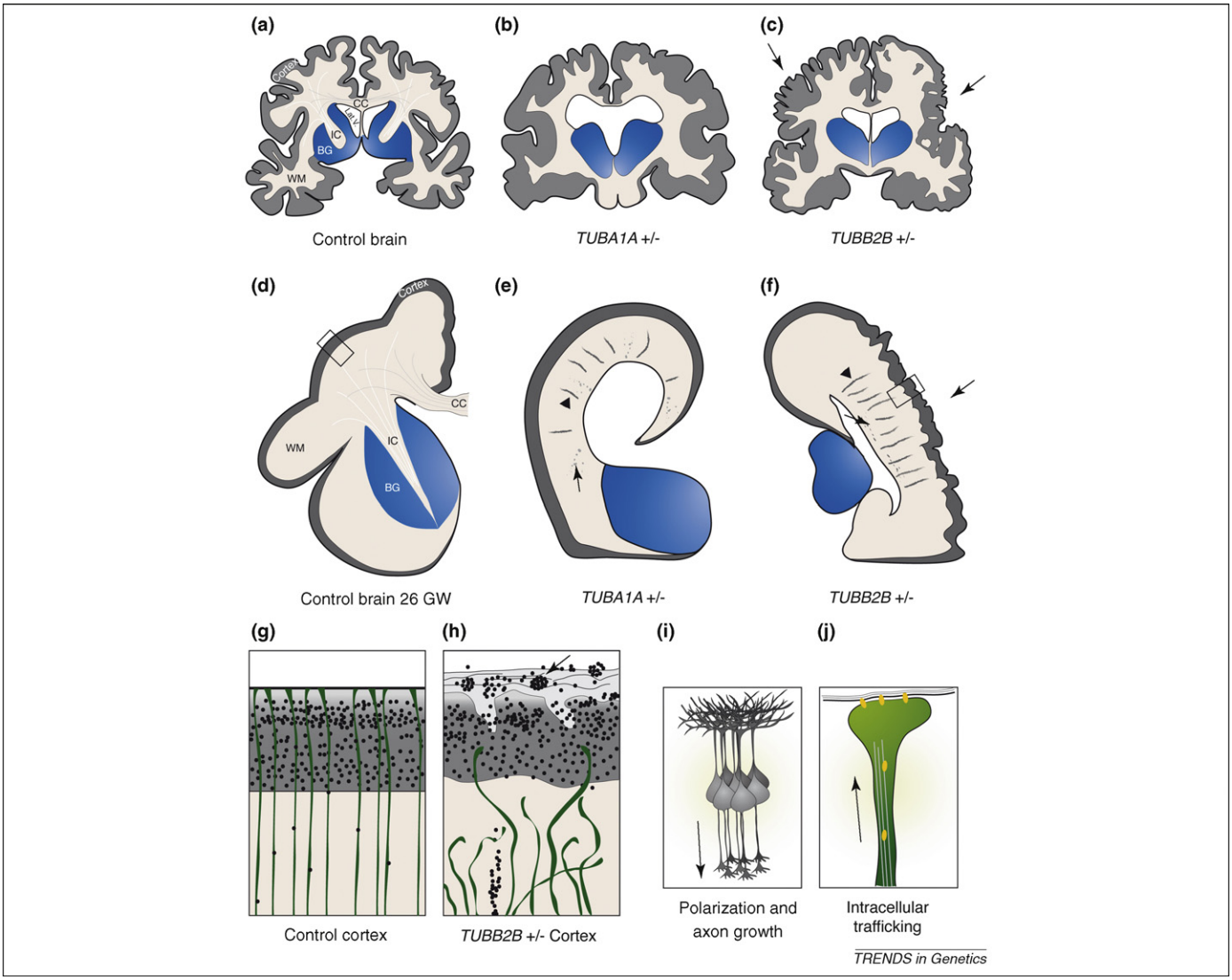

Figure 1. **Representation of TrCD associated with mutations in *TUBA1A* and *TUBB2B* genes.** **(a–f)** Schematic representations of coronal sections of adult **(a–c)** or GW26 fetal brains **(d-f)**, either control **(a,d)**, with a *TUBA1A* mutation **(b,e)** or with a *TUBB2B* mutation **(c,f)**. The cortex is depicted in gray, the white matter in light pink and the basal ganglia in blue. Normal brains exhibit two hemispheres linked by crossing axonal tract bundles forming the corpus callosum. Internal capsules are made up by corticofugal or thalamocortical fibers. The *TUBA1A* and *TUBB2B*-related cortical dysgeneses are associated with corpus callosum abnormalities (thinner than control, or absent) and basal ganglia dysgenesis (absence of an internal capsule, leading to the fusion of the basal ganglia) [23,35]. Radial and nodular neuronal heterotopia in the white matter are also present in the fetal brains (depicted in e,f by arrowheads and arrows, respectively). Taken together, these features are indicative of neuronal migration disorders related to *TUBA1A* and *TUBB2B* mutations. However, the gyral patterning abnormalities are different. *TUBA1A* mutations lead to type I lissencephaly that is characterized by a thickened and disorganized cortex and lateral ventricle enlargement. *TUBB2B* mutations are causative of bilateral asymmetrical PMG. Zones of PMG (depicted by large arrows in c,f) are characterized by an increased number of brain folds and the disorganization of the cortex. **(g,h)** Schematic representation of the developing CP in control **(g)** and pathologic situation **(h)**. A mutation in the *TUBB2B* gene leads to a striking disorganization of radial glial processes (h, in green), which are perpendicularly oriented towards the cortical surface in the control situation **(g)**. These defects are associated with a disorganization of the layering of the cortex (compare h to g). Neurons are abnormally settled within the CP thickness and can even migrate beyond the pial basement membrane into the meningeal space (arrow). **(i–j)** Illustrations of processes that could be impaired in *TUBA1A* and *TUBB2B*-related disorders. Defects in MT-based functions during polarization and axonal growth could contribute to the neuronal heterotopia and axon tract disruptions, leading to corpus callosum and basal ganglia dysgeneses **(i)**. The proper MT-based intracellular transport of key proteins (i.e. of GPR56 or β1-integrin [36,109]) involved in the development and maintenance of pial basement membrane integrity could be affected when the β-tubulin 2B is mutated. Thus, breaches could form at the surface of the cortex and further allow the migration of neurons into the meningeal space.

Doublecortin is a MAP [11], led the authors to identify and associate *de novo* mutations in *TUBA1A* with type I lissencephaly [21,22]. Recently, the clinical features of *TUBA1A*-related cortical dysgeneses, which are commonly associated with a striking perisylvian or posterior predominant pachygyria, have been refined [23] (Figure 1). However, in addition to microcephaly, rare occurrences of agyria and subcortical heterotopia demonstrate that *TUBA1A*-related lissencephaly could encompass a large spectrum of cortical abnormalities. This abnormal gyral pattern is combined with dysgenesis of the anterior limb of the internal capsule to give a dysmorphic aspect to the basal ganglia. This combination of gyral abnormalities and dysmorphic basal ganglia might constitute the specific features associated with *TUBA1A* mutations. Moreover, other extracortical defects often include complete to partial agenesis of the corpus callosum and mild to severe cerebellar hypoplasia [23]. These findings have been confirmed in a second patient panel, and the frequency of *TUBA1A* mutations in a lissencephaly panel has been estimated to be ~4% [24]. The analysis of several fetal cases, aged 23 GW to 35 GW, have revealed the presence of lissencephaly

characterized by variable cortical thickness and abnormal lamination patterns. In all fetal cases, the cortical laminar organization is disrupted, with the cortex being either thick and four-layered or thin, immature and two-layered [22,25] (Figure 1). The hippocampus is hypoplastic and disorganized. The subcortical and periventricular white matter was found to contain abnormal heterotopic cells either poorly differentiated or strongly expressing pyramidal neuron markers (MAP2, DCX) or interneuron markers (calbindin, calretinin) [25], showing that pyramidal neurons and interneurons might be equally affected. Taken together, these observations suggest that the development of cortical abnormalities is based on defective neuronal migration and differentiation. Reminiscent of the imaging features observed in living patients, a hypoplastic cerebellum and brainstem were also observed. Moreover, axonal tracts in the white matter were found to be abnormally developed in most fetal cases with aberrant pathways leading to internal capsule and/or corpus callosum abnormalities (Figure 1). These features suggest that neuronal axonal guidance and/or growth defects, in addition to early neuronal differentiation abnormalities, are likely to be involved in the pathogeny of *TUBA1A*-related cortical dysgeneses [25].

Mutations in β-tubulin 2B cause asymmetrical bilateral PMG

PMG is characterized by a disorganized cortical lamination and the presence of multiple small, partially fused gyri separated by shallow sulci that produce an irregular cortical surface [3]. Several subtypes of PMG are recognized based on differences in extension and topography, and on their association with a neuromotor impairment, cognitive deficits and epilepsy of variable severity [26]. The pathogenesis of PMG is poorly understood, although some experimental data has suggested that the disruption of processes regulating late neuronal migration and cortical organization could underlie some forms of PMG [3,27]. Etiologies of PMG are likely to be heterogeneous, with extrinsic *in utero* causes – mostly vascular causes – and some genetic origins. To date, six human genes associated with PMG have been identified, including two genes implicated in several familial cases: *GPR56* (*G-protein-coupled receptor 56*) in bi-frontoparietal PMG [28] and *SRPX2* (*sushi-repeat-containing protein, X-linked 2*) in bilateral perisylvian PMG [29]. For the remaining genes, the association between mutations and PMG was reported in only a few cases: *PAX6* (*paired box gene 6*) in PMG associated with aniridia [30], *TBR2 (EOMES, eomesodermin homolog*) [31], *KIAA1279 and NHEJ1* (*nonhomologous end-joining factor 1*) in diffuse PMG [32,33] and *RAB3GAP* (*RAB3 GTPase-activating protein*) in frontal PMG [34]. Recently, a candidate gene strategy was used to identify five *de novo* mutations in the β-tubulin 2B encoding gene *TUBB2B*, that are associated with anteriorly predominant asymmetrical PMG, in four living patients and one fetus [35]. All living patients share asymmetrical PMG, corpus callosum hypogenesis or agenesis and dysmorphic basal ganglia, with no visible anterior arm of the internal capsule (Figure 1). Neuropathological analyses conducted on the fetal case revealed a disorganized layering of both cortical hemispheres, a typical PMG on the left side with presence of ectopic clusters of neurons in both hemispheres and heterotopia in the white matter. Moreover, the radial processes of radial glial cells were strikingly disorganized, not perpendicularly orientated towards and not attached to the pial basement membrane (Figure 1). The radial process disorganization and presence of breaches in the *glia limitans* are likely to be the consequences of a common defect underlaid by MT dysfunction in radial glial cells that remains to be deciphered. Breaches in the basement membrane at the surface of the cortex associated with the presence of ectopic clusters of neurons beyond this membrane were reported for the first time to our knowledge in association with a PMG syndrome in human. It is worth mentioning that ectopic neurons in the leptomeningeal spaces have so far only been reported as a typical feature of type II lissencephaly. Moreover, as will be discussed later, the same presence of ectopic neurons associated with breaches in the pial surface were also observed in the knockout model of *Gpr56*-related PMG [36]. Thus, further investigations have to confirm the idea brought up by these new findings and indicate that PMG and type II lissencephaly could share some features.

Specific developmental and imaging features of *TUBA1A*- and *TUBB2B*-related forms of cortical dysgeneses in living patients include a spectrum ranging from bilateral agyria to perisylvian/posterior pachygyria for the former or subcortical laminar heterotopia, and bilateral asymmetrical PMG for the latter as the only striking and constant cortical hallmarks of each tubulin-related developmental disease. However, the common features (i.e. cortical abnormalities, genetic transmission, absence of apparent white matter density abnormalities, dysmorphic basal ganglia and corpus callosum dysgenesis) help direct the diagnosis towards a tubulin-related disorder. The discovery of lissencephaly and PMG-associated mutations in α-tubulin 1A and β-tubulin 2B reinforces the notion that proper MT function is crucial for migrating neurons during cortical development.

Overlapping features of tubulin-related cortical disorders

What are the common and specific roles of these tubulins during cortical development? Overlapping characteristics most probably result from impaired mechanisms related to the same MT function. The cortical dysplasia (abnormal thickness and organization) associated with the presence of heterotopic neurons in the white matter, and the observations in living patients of gyral pattering abnormalities support the classification of tubulin-related cortical dysgeneses (TrCD) as neuronal migration disorders [3,27]. However, only neuropathological approaches enable the accurate investigation of migration defects. Analyses of fetuses with mutations in *TUBA1A* revealed the presence of numerous ectopic cells, supporting the evidence for migration defects. Such abnormalities were also reported in *TUBB2B*-related PMG in one fetal case analyzed to date. The presence of radial columnar neuronal heterotopia in the white matter, nodular heterotopic neuron clusters in both hemispheres and heterotopia in the cerebellum [35] confirms the contribution of defective neuronal migration in the pathogenesis of these tubulin-related conditions. These results are also supported by neurohistopathological

investigations carried out on *tuba1a* mutant mice, which show a severe lamination defect in the hippocampus and a discrete wave-like perturbation in layers II/III and IV of the visual cortex [21]. In addition, knockdown of the *tubb2b* expression performed on rat embryos using RNAi and *in utero* electroporation led to an arrest of migrating neurons within the intermediate zone [35]. These functional analyses undertaken using animal models further confirmed that both *TUBA1A* and *TUBB2B* are necessary for proper radial migration. However, further investigations are needed to test their respective contributions to tangential migration, which might also be involved as suggested by recent studies in mouse models of *DCX*- and *LIS1*-related lissencephaly [16,37] and by observations in fetuses with either *DCX* or *LIS1* mutations [1,25,38]. In *TUBA1A* fetuses, abnormally voluminous ventral germinal zones were found to correlate with a reduced number of interneurons in the cortex, suggesting either a delayed migration or a delayed proliferation of neuronal cells [25]. However, the second possibility is less likely according to the expression pattern of *TUBA1A,* which seems to be restricted to post-mitotic neurons [22,39].

Previous studies using RNAi delivered by *in utero* electroporation to knockdown the *Dcx* gene in mouse embryonic cortices showed a disruption of radial migration [40,41]. Reduced expression of the MTs stabilizing MAP Doublecortin [13,14] led to an accumulation of multipolar neurons in the intermediate zone of the cortex, similar to the formation of the subcortical laminar heterotopia observed in human patients [40]. These results are consistent with the idea that the local stabilization of MTs is required for neuronal polarization and to induce the formation of the future axon from one neurite out of many extending and retracting neurites during the multipolar stage (Box 3) [42]. Taken together, these observations support the idea that the proper regulation of MT dynamics during polarization is crucial for the transition from a multipolar to a bipolar shape, which is a prerequisite for the initiation of radial migration [43,44]. Biochemical investigations revealed that the different disease-associated mutations in α-tubulin 1A and β-tubulin 2B are associated with a spectrum of folding and heterodimerization defects, leading in the most severe cases to loss of function of the mutant tubulin [35,45]. Disruptions in the interactions with crucial molecular chaperones of the folding pathway (Figure I in Box 2) are responsible for the observed defects. Because tubulin isotype expression is temporally and spatially regulated [22,35,46], and because the different isotypes might be needed to form specific sets of MTs with specific dynamic characteristics that carry out unique functions [47–52], one can speculate that the loss of function of the mutant tubulin could disrupt the homeostasis of MT dynamics and thereby perturb the polarization process, leading to neuronal migration abnormalities. In addition to the migration defects, another striking hallmark of the pathology associated with *TUBA1A* and *TUBB2B* mutations is the presence of dysmorphic basal ganglia associated with an absence of the anterior arm of the internal capsule and corpus callosum dysgenesis [21–23,25,35]. The development and guidance of axons from commissural and corticofugal projection neurons rely on both intrinsic – the polarization and regulation of MT dynamics seem to be of major importance – and extrinsic factors or guiding cells. Genetic inactivations of specific cortical or thalamic genes can disrupt this process, giving rise to internal capsule or callosal dysgeneses. Indeed, the genetic deletion of the serine/threonine kinase (*LKB1)* in the pyramidal neurons of the cortex, using a murine conditional mouse strain bearing a conditional allele ($LKB1^{F}$) that generates a null allele upon Cre-mediated recombination, prevents the formation of axons in cortical pyramidal neurons, specifically showing an absence of axons in the internal capsule and corpus callosum [53]. Moreover, the genetic deletion of the murine *APC* (encoding adenomatous

Box 2. Tubulins and MTs

MTs are key structural components involved in cell cycle, cell morphology, cell motility and intracellular trafficking. They are polarized and dynamic polymers that consist of head-to-tail-associated heterodimers of α- and β-tubulins (Figure I in Box 2). These isoforms of tubulin are the most commonly conserved throughout evolution and belong to a large family of proteins named α-, β-, γ-, δ-, ε-, ζ- and η-tubulins. Genes encoding α-, β- and γ-tubulins exist in all eukaryotes, however, genes for δ-, ε-, ζ- and η-tubulins do not have a ubiquitous distribution in eukaryotic organisms [97]. The α- and β-tubulins are also characterized by the existence of different isotypes whose expressions are spatially and temporally regulated [46,98]. The functional relevance of these numerous isoforms is a point of major interest discussed in the main text. The α/β tubulin heterodimer is formed by the association of one α- and one β-tubulin subunit through a complex folding and heterodimerization pathway, which involves a series of molecular chaperones, whose function is to ensure the assembly of the α/β tubulin heterodimer [99] (Figure I in Box 2). These heterodimers further form protofilaments that bind laterally to form, in mammalian cells, a 13 protofilament cylindric MT in association with other proteins, such as MAPs [100]. The GTP hydrolysis controls MT behavior balancing between shrinkage and elongation at each extremity, leading to the so-called apparent dynamic instability of MTs [101]. The '+' β-tubulin-capped extremity undergoes either elongation through a polymerization process or shrinkage through a depolymerization process. During the elongation, this growing end of MTs incorporates heterodimers containing GTP-bound β-tubulin, which will further hydrolyze the GTP in GDP. The depolymerization process is characterized by the rapid loss of GDP-bound tubulin heterodimers [100]. However, extrinsic mechanisms known as MT-regulating proteins also contribute to the control of MT behavior. MT-stabilizing proteins (classical MAPs, STOPs, DCX), MT-polymerizing proteins (CRMPs) and MT-depolymerizing proteins, MT-severing proteins (katanin, spastin) and tubulin-regulating proteins (stathmin, TBCB) [58] exert a tight control of the neuronal MT network.

In addition to the genetic diversity of tubulins, a second level of complexity is provided by the extensive array of reversible post-translational modifications of tubulins and MTs [60]. Acetylation, tyrosination and detyrosination, polyglycylation and polyglutamylation, phosphorylation and palmitoylation constitute a code whose origins and deciphering are still under investigation. With the exception of acetylation, these modifications occur on the tubulin carboxy-terminal extremity located on the outer surface of the MT [102] and, thus, could be involved in the regulation of interactions with MAPs. According to their post-translational modification pattern, the MTs are subdivided into different populations that display similar spatial distributions and tubulin half lives. For instance, acetylated MTs are stable [103] and located in less dynamic cellular compartments (i.e. the axons of neurons).

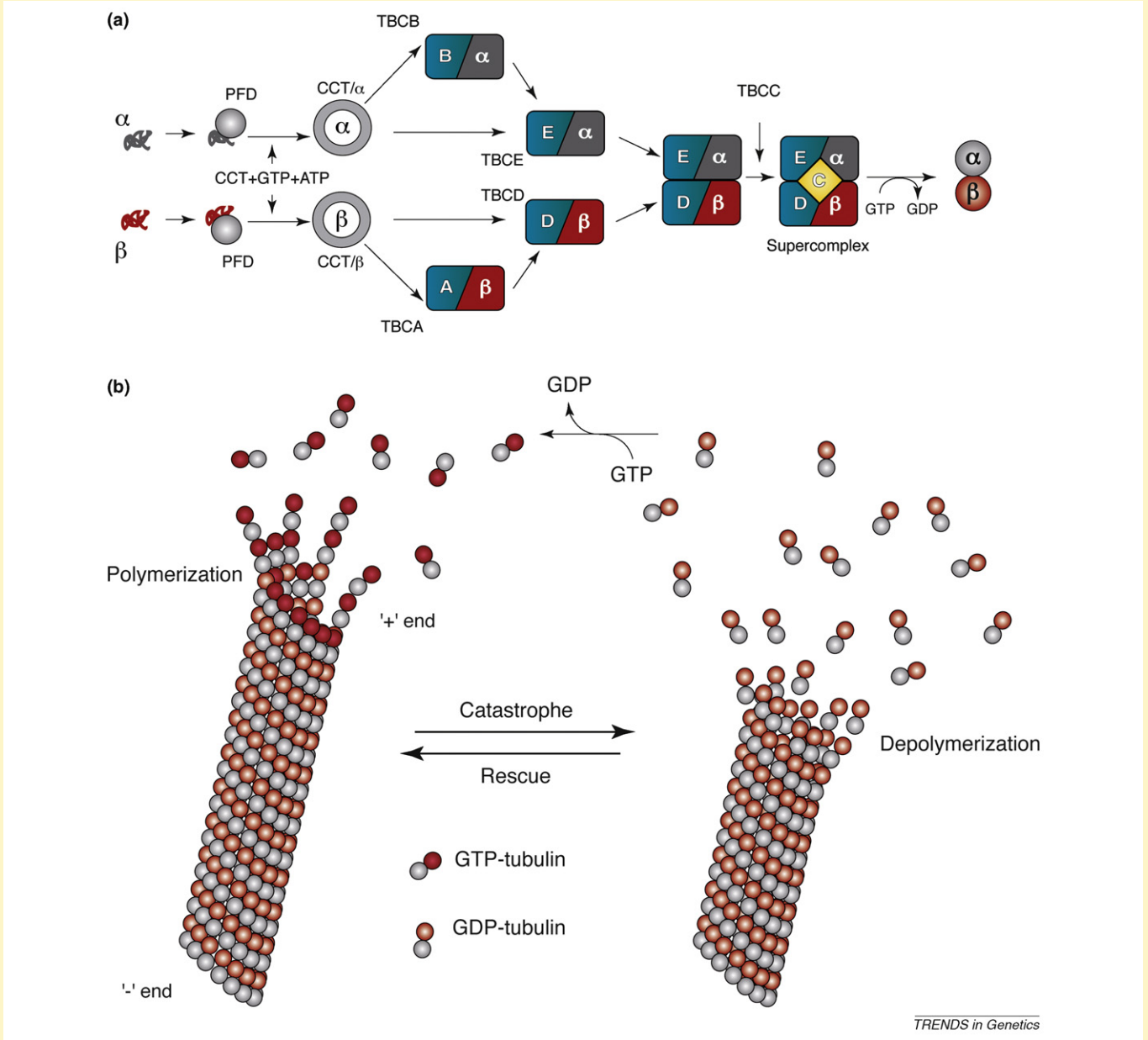

Figure I. Tubulins and MTs. (a) The tubulin folding pathway [107,108] involves a series of molecular chaperones whose function is to facilitate the assembly of the α/β-tubulin heterodimer. Newly translated α-tubulin (α) and β-tubulin (β) polypeptides are first captured and stabilized by prefoldin (PFD), which acts as a shuttling protein to deliver its bound target protein to the cytosolic chaperonin (CCT), with which it interacts. CCT generates folding intermediates via one or more cycles of ATP binding and hydrolysis. These intermediates then interact with a set of downstream TBCs. Two TBCs (TBCB and TBCE) capture CCT-generated α-tubulin intermediates in which the encapsulating GTP-binding pocket (the N-site) is already formed, producing TBCB/α-tubulin (B/α) and TBCE/α-tubulin (E/α) co-complexes. Two others (TBCA and TBCD) capture and stabilize CCT-generated β-tubulin intermediates forming TBCA/β-tubulin (A/β) and TBCD/β-tubulin (D/β) co-complexes. The TBCA/β-tubulin co-complex can act as a donor of its target protein to TBCD. TBCD/β-tubulin (D/β) and TBCE/α-tubulin (E/α) converge to form a supercomplex with TBCC (C-D/β–E/α). Interaction with TBCC (C) results in the triggering of GTP hydrolysis by β-tubulin. This reaction acts as a switch to signal the release of newly formed GDP-bound α/β heterodimers (light red β-tubulin), which are then competent (following spontaneous exchange with GTP at the E-site) for incorporation into MTs. Several cortical dysgeneses-associated mutations in α- or β-tubulin were shown to disrupt this pathway [21,35,45]. **(b)** Upon an exchange of GDP against GTP, the GTP-bound heterodimer (dark red β-tubulin) can undergo a round of polymerization at the growing '+' end of the MT (rescue phase). During or soon after its incorporation, the β-tubulin hydrolyzes its bound GTP into GDP. This induces the depolymerization of GDP-bound heterodimers from the '+' end at a very rapid state (catastrophe phase).

polyposis poli protein), which binds to the highly dynamic plus end of MTs to stabilize them [54], or the inactivation in mice of both *Dcx* and *Dclk* (*Doublecortin-like kinase* encodes the most similar protein to Doublecortin, which also contains MT-binding domains) are associated with the absence or prominent defects of growth, orientation and organization of corticothalamic and thalamocortical fibers tracts through the internal capsule [55] and with the absence of a corpus callosum [56,57]. This could be because of impaired polarization and early differentiation, or to axon elongation and growth cone defects [58]. Finally, disrupted neuronal migration could also explain the axonal tract disruption in tubulin-related cortical dysgeneses. Indeed, recent work conceptualized the idea that lateral ganglionic eminence-derived migrating interneurons provide a supporting and permissive environment for pioneering thalamocortical axons [59]. However, further investigations are needed to establish whether the impaired migration of *TUBA1A* and *TUBB2B*-mutated interneurons also contributes to fiber tract defects.

Gyral patterning and cytoarchitectonic specific features of tubulin-related cortical disorders

Although recent implications of *TUBA1A* and *TUBB2B* genes in developmental malformations of the cerebral cortex have opened new avenues to understanding the pathogenesis of these cortical dysgeneses and the neurodevelopmental processes underlying them, functional data explaining the observed differences are lacking. However, several hypotheses can be formulated to account for these differences. First, the discrete differences in the spatial and temporal expression patterns of these genes could contribute to the specific cortical phenotypes. Second, slight variations in the functions of α-tubulin 1A and β-tubulin 2B (i.e. their dynamic properties or interacting proteins) might be responsible for the phenotypic divergences. Abnormalities in specific patterns of post-translational modifications [60] associated with α-tubulin 1A and β-tubulin 2B could also lead to neuronal migration defects. Recent data showing that the loss of MT acetylation disrupts neuronal radial migration support the importance of specific post-translational modifications [61].

However, it is accepted that the pachygyria–agyria spectrum of diseases results from neuronal migration defects and that PMG is classified among disorders of late migration [3,27]. Nevertheless, it is difficult to reconcile late migration defects as a major event in the pathogeny of PMG because *TUBB2B* dysfunction is implicated in a phenotype that associates PMG with heterotopic neuronal cells in the white matter. Heterotopia is reminiscent of the histopathological phenotype observed in cases with *TUBA1A* mutations. Regarding the specific disruption of the perpendicular orientation of radial glia processes observed in the *TUBB2B* fetal case [35], one might suspect that the protein plays a crucial role in radial glia process development or maintenance. Indeed, MTs are known to be involved in the polarization of radial glial cells [55,62]. The disorganization of radial processes is associated with the presence of ectopic neurons beyond breaches in the pial basement membrane. This observation is highly informative in light of data generated from the mouse model of *GPR56* mutated in bi-frontoparietal polymicrogyria (BFPP). *GPR56* encodes a G-protein-coupled adhesion receptor that is highly expressed in radial glial cells and enriched in glial endfeet [28,36]. *Gpr56* mutant mice present the same phenotype of radial glia process abnormalities and breaches in the basement membrane. Thus, the absence of Gpr56 in glial endfeet is likely to be involved in the disruption of the pial basement membrane, leading to neuronal overmigration [36]. To date, in the absence of neuropathological data for patients with BFPP, the presence of neuroglial ectopia in humans cannot be confirmed. However, it is becoming clear that radial glial cells play a crucial role in the development of the pial basement membrane, the disruption of which in *Gpr56* and *TUBB2B*-mutated cases is associated with the overmigration of neurons within the meninges. Several mutations in *GPR56* in humans impair the intracellular trafficking of the protein and result in the absence of the protein at the membrane [63]. Thus, we propose that the absence of a fully functional β-tubulin 2B in MTs could affect the intracellular trafficking and/or recycling of GPR56 towards the glial endfeet membrane, leading to pial membrane breaches. Finally, another specific feature of the *TUBB2B*-related developmental disorder is the asymmetrical pattern of PMG lesions [35]. The causes of this asymmetry are still unexplained; however, several familial cases of unilateral right predominant PMG have recently been reported [64]. Thus, the converging evidence of pathologic asymmetries and the well-described physiologic brain asymmetry associated with lateralization and handedness [65] open the field to further investigations that could help understand the development of functional and anatomical brain asymmetries. Chang and colleagues propose that left–right differentially expressed genes could be involved in these asymmetrical PMG syndromes [64]. In light of recent results, we think that the concept of asymmetrical molecular patterning of the brain [65] has to be extended to genes acting downstream of morphogens and transcription factors. Indeed mutations in a β-tubulin encoding gene, despite its wide expression in both central and peripheral embryonic nervous systems, are associated with a left predominant bilateral PMG [35]. These results add a further layer of complexity, suggesting that hemisphere-specific functions or partners of β-tubulin 2B-containing MTs are associated with the development of a polymicrogyric gyral pattern rather than the tubulin itself.

Moving beyond lissencephaly and PMG: other tubulin-related neurological disorders

MTs and MT-related proteins are involved in a wide array of cellular processes, not just in neuronal migration and differentiation. Thus, other MT-related neurological diseases that do not specifically affect tubulins have been described. Although these conditions are poorly understood, they are not thought to involve neuronal migration disorders. Indeed, a 12 bp deletion in the *TBCE* gene leads to a human disease named hypoparathyroidism, mental retardation and facial dysmorphism (HRD syndrome or Sanjad/Sakati syndrome) [66,67]. *TBCE* encodes the ubiquitous tubulin-specific chaperone (TBC) E, which contributes to α- and β-tubulin folding and heterodimerization (Figure I in Box 2). Moreover, the presence of a hypoplastic thin corpus callosum in six HRD patients has been recently reported [68]. These observations support the idea that tubulins and MT homeostasis is crucial not only for proper neuronal migration but also for axonal growth. Further investigations are needed to evaluate the severity of fiber tract disruptions in HRD patients, especially in the internal capsule. Interestingly, a homozygous missense mutation in the same gene (*TBCE*) has been found to be associated with a progressive form of neuropathy in mice [66,67].

Studies of the congenital forms of human microcephaly have led to the identification of several genes that influence brain size through the regulation of neural progenitor divisions. Mutations in the *ASPM* (*abnormal spindle-like microcephaly-associated*) gene [69] are the most common causes of congenital microcephaly. This gene encodes a MT-associated protein specifically expressed in progenitors within the ventricular zone. In the mutant *Drosophila*, the progenitor cells stop dividing in metaphase and thereby cannot proceed to asymmetric cell division. The

Box 3. Polarity and MT plasticity

Neurons are highly specialized cells with a characteristic polarized morphology composed of a cell body and extended neurites that can be several centimeters in length. In the cerebral cortex, neuronal polarization occurs when neurons begin radial migration in the intermediate zone. IPCs or young post-mitotic neurons, upon cell cycle exit through the asymmetric division of RGCs, display multipolar morphology where multiple neurites rapidly extend and retract from the cell body [87]. They then adopted a bipolar morphology with a leading process (oriented towards the surface of the cortex) and a trailing process (oriented towards the ventricle) from which projection neurons will acquire their dendrite and axon polarity, respectively [89,104,105]. The mechanism leading to polarization requires a tight regulation of the actin cytoskeleton through phosphoinositide 3-kinase (PI3K), the Rho family of small GTPases, the Par complex and their downstream effectors [104]. Local MT assembly and stabilization in one neurite is also required to promote the axonal fate of this neurite, as supported by elegant experiments using a photoactivatable form of the MT-stabilizing drug taxol to promote the stabilization of MTs in a restricted neurite [42]. During the multipolar step, local stabilization of MTs in one neurite is important to induce the formation of the future axon. Radially migrating projection neurons display a rather simple bipolar morphology, whereas migrating interneuron precursors are typically bipolar with several leading processes orientated in different directions [92,106] (Figure I in Box 1). During the long migration from their origin in the ventral telencephalon towards the cortex, interneurons might have to undergo directional changes. Rather than changing the orientation of their leading process, they do this by extending new branches in new directions. MTs are concentrated in the leading processes and around the nucleus of migrating neurons [106]. It makes sense, therefore, that morphological changes associated with neuronal migration are dependent on MT rearrangement and the regulation of the neuronal cytoskeleton.

absence of the functional protein has been shown to disrupt spindle organization through defective binding and assembly of MTs at spindle poles. *CDK5RAP2* (encoding cyclin-dependent kinase 5 regulatory-associated protein 2) and *CENPJ* (encoding centromere-associated protein J) can regulate or enhance the assembly of MTs from the microtubule organizing center (MTOC) [70]. Taken together, these genetic investigations have provided convincing evidence that proper MT functions are required at every step of cortical development. That means either during proliferation and neurogenesis, as shown for the congenital microcephaly proteins, or during post-mitotic neuronal migration and differentiation, as shown by MAP or tubulin-related lissencephaly and PMG.

Mechanistic overview and concluding remarks

Recent genetic, molecular, cellular and neurobiology results have shed considerable light on TrCD. The tubulin genes, *DCX* and *LIS1* have been implicated in several neuronal migration disorders. We propose that both α-tubulin 1A and β-tubulin 2B are crucial effectors of neuronal polarization during the transition from the multipolar to bipolar stage, and during axonal growth and guidance (Figure 2). Haploinsufficiency, or the dysfunction of α-tubulin 1A or β-tubulin 2B, can induce changes in the dynamics or stability of MTs with potential consequences for the polarization and elongation of axons and growth cone mobility. Defects in those processes could result in migration defects and neuronal heterotopia, corpus callosum and internal capsule abnormalities. Moreover,

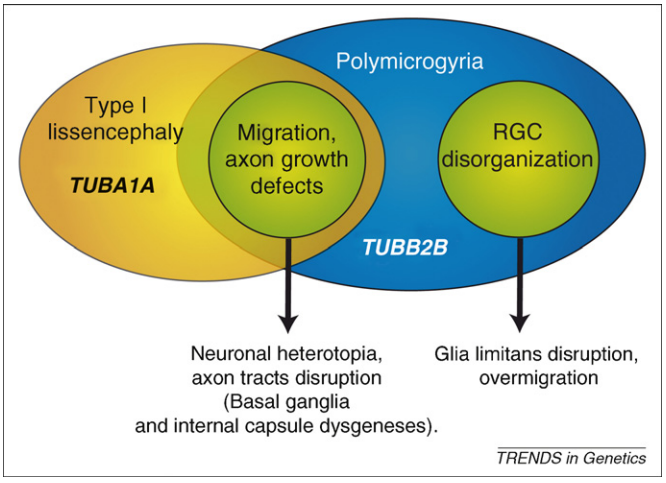

Figure 2. Intermingled and specific cellular defects could cause tubulin-related brain cortex dysgeneses. We propose that the gyral patterning abnormalities, corpus callosum and basal ganglia dysgeneses associated with mutations in the α-tubulin encoding gene *TUBA1A* could be affected by the alteration of MT-based functions (polarization for instance), resulting in defective neuronal migration and axonal growth. These defects are also observed in cases of PMG with mutations in the β-tubulin encoding gene *TUBB2B*. The functional links that exist between the two genes further support the idea that the two disorders might share pathophysiological mechanisms. Although specific features of *TUBB2B*-related forms of PMG, i.e. disorganized processes of radial glial cells and overmigration of neurons into the meningeal spaces that might be explained by specific functional properties carried out by the β-tubulin 2B, could contribute to gyral patterning abnormalities.

deciphering the role of tubulins as single molecules and in complexes with Doublecortin and LIS1 is of special interest. Their contributions to the regulation of dynamic processes (vesicle and membrane fusion and recycling, vesicle transport, cell–cell adhesion) at the leading edge and growth cone of migrating neuronal cells need to be further investigated.

On the basis of the common functions of α- and β-tubulins and in the current state of knowledge, gyral patterning discrepancies are more complex to understand. We hypothesize that *TUBA1A*-related agyria–pachygyria could be because of migration defects, leading to an abnormal lamination and scattered or clustered ectopic neurons. By contrast, *TUBB2B*-related PMG could be a combination of the aberrant migration of postmitotic neurons and earlier defects in progenitor cells, as suggested by the disrupted organization of radial processes and the presence of breaches in the pial basement membrane. Moreover, because intermediate progenitor cells (IPCs) and neurons originate from radial glia, the consequences of the disrupted morphology and function of the progenitors on the specification of their progeny has to be further considered to understand the differences in cortical layering and gyral abnormalities.

Finally, we want to integrate into this discussion about TrCD growing evidence of the regulation of neuronal migration by neurotransmitters [71]. Dopamine-dependant signaling can induce cytoskeleton rearrangement through the activation of D_1- or D_2-like receptors, which promote or delay the tangential migration of interneurons, respectively [72]. A recent study has shown that the upregulation of the potassium/chloride (K^+/Cl^-) exchanger KCC2 (also called solute carrier family 12, Member 5; SLC12A5) in migrating interneurons induces a shift in GABA responsiveness from a motogenic to a stop signal

through a modulation in voltage-gated Ca^{++} channel activation [73]. Interestingly, evidence suggests that the calcium acts as a second messenger and further leads to acute effects through the modification of MT stability [74]. Therefore, we need to bear in mind that in addition to defective intrinsic cellular processes, mutated tubulins with altered dynamic properties could also affect the responsiveness of migrating neurons to extracellular cues such as neurotransmitters-mediated signaling.

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