Rare genetic causes of lissencephaly may implicate microtubule-based transport in the pathogenesis of cortical dysplasias

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Disruption of early neural development can cause severe forms of mental retardation and epilepsy, associated with defects in cortical structure. Lissencephaly is a disorder resulting from abnormal neuronal migration. Of the six causative genes for classical lissencephaly, three, *LIS1*, *DCX*, and *TUBA1A* encode for microtubule-related proteins, indicating the importance of this pathway for neuronal migration (Table 1). The lissencephaly 1 (LIS1) protein is an adaptor for dynein, a microtubule motor protein [1]. Doublecortin (*DCX*) encodes a microtubule-associated protein (MAP).[2, 3] Finally, tubulin α1a (*TUBA1A)* is a gene that encodes an alpha tubulin subunit that is enriched during brain development.[4]

These lissencephalic syndromes clinically all share a widespread disruption of lamination in the cerebral cortex (Figure 1). Since all three genes appear to regulate microtubule-based transport, their functional relation and regulation during development is an area of active investigation with implications that may be significant for a wider cohort of patients with focal cortical dysplasias. Cortical dysplasias are a common cause of refractory epilepsy and share some of the same histological features of lissencephaly, including the dyslamination and abnormal neuronal morphology.[5] Disruption of microtubule-based pathways may lead to cortical dysplasias and the causative genes for lissencephaly are a starting point for further investigation.

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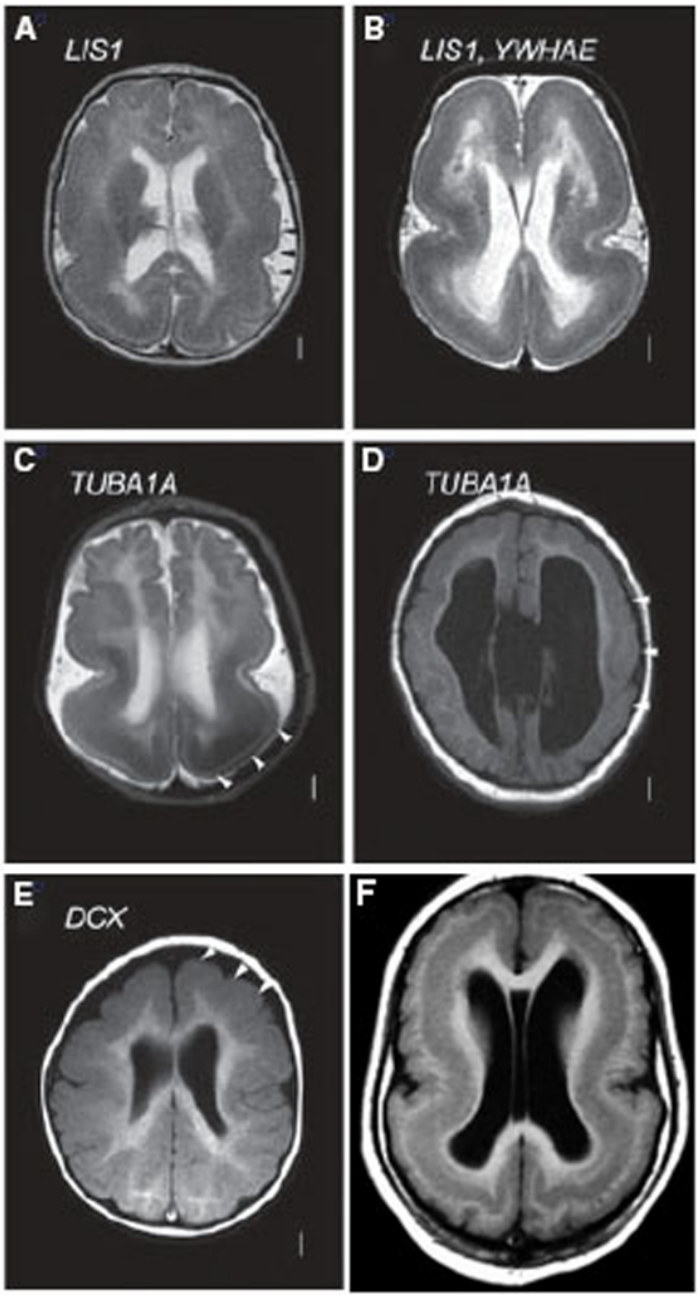

Figure 1. MRI of lissencephaly caused by microtubule pathway genes. High axial magnetic resonance images of lissencephaly (LIS) associated with mutations in microtubule related genes. Arrowheads on the axial images mark the most severely involved brain regions. **(A)** Classic LIS, with posterior more severe than anterior (p > a) gradient associated with an intragenic mutation of LIS1. **(B)** Severe classic LIS due to deletion 17p13.3 that results in loss of LIS1, YWHAE, and all of the intervening genes in a child with Miller-Dieker syndrome. **(C)** Classic LIS with a p > a gradient caused by an intragenic mutation of TUBA1A. **(D)** Moderate severity lissencephaly with cerebellar hypoplasia (LCH) with complete agenesis of the corpus callosum, large dysplastic midbrain and tectum, and severe cerebellar hypoplasia associated with another intragenic mutation of TUBA1A. **(E)** Classic LIS with anterior more severe than posterior (a > p) gradient caused by an intragenic mutation of DCX. Adapted from Epilepsia 51: Dobyns, WB. The clinical patterns and molecular genetics of lissencephaly and subcortical band heterotopia, 5-9, Copyright (2010), with permission from Wiley. Panel F shows the MRI of a 15-year-old girl with a severe subcortical band heterotopia due to a DCX mutation. Reprinted from Neurobiology of Disease, 38(2): Guerrini R, Parrini E, Neuronal migration disorders, 154-66, Copyright (2010), with permission from Elsevier.

Table 1. **Lissencephaly genetics.**

Mode of inheritance	Gene	Locus	Type	Pathway defect
X-linked or autosomal dominant:				
a. X-linked lissencephaly with abnormal genitalia	*ARX*	Xp22.1	Type 1	Transcriptional regulation
b. Isolated lissencephaly or subcortical band heterotopia	*DCX*	Xq22.3–q23	Type 1	MT-based transport
	TUBA1A	12q13.12	Type 1	MT-based transport
	LIS1	17p13.3	Type 1	MT-based transport
c. Miller-Dieker syndrome	*LIS1 + YWHAE*	17p13.3	Type 1	MT-based transport
Autosomal recessive:				
d. Lissencephaly with cerebellar hypoplasia group b	*RELN*	7q22.1	Type 1	Signaling
	VLDLR	9p24.2	Type 1	Signaling
e. Cobblestone lissencephaly				
Fukuyama congenital muscular dystrophy or Walker-Warburg syndrome	*FCMD*	9q31.2	Type 2	Matrix protein glycosylation
Muscle-eye-brain disease or Walker Warburg syndrome	*FKRP*	19q13.32	Type 2	Matrix protein glycosylation
	POMT1	9q34.13	Type 2	Matrix protein glycosylation
	POMT2	14q24.3	Type 2	Matrix protein glycosylation
Muscle-eye-brain disease	*LARGE*	22q12.3	Type 2	Matrix protein glycosylation
	POMGnT1	1p34.1	Type 2	Matrix protein glycosylation

INTRODUCTION

Normal cognitive function is dependent on proper brain development, including the coordination of multiple steps of neuronal development throughout gestation and beyond. Disruption in the early steps of neuronal development, including neuronal migration, can result in severe cognitive deficits and epilepsy. In the cortex, subset of neurons may be differentially affected, including excitatory pyramidal neurons that migrate radially from the ventricular zone, and inhibitory neurons that migrate tangentially from the lateral and medial ganglionic eminences (Figure 2A and B). As a consequence, the normal lamination of the neurons in the cortex may be altered, leading to a failure of normal circuit formation and/or the establishment of abnormal circuits leading to cognitive dysfunction and epilepsy (Figure 2C).

The phenotypic characteristics of brain malformations can be correlated with defects at certain stages in development. Cortical development is dependent on the proliferation of neural progenitors. When numbers of neural progenitors are reduced, the result may be a brain with microcephaly (“small brain”) that is otherwise normal in structure. Microcephaly can be caused by a variety of environmental factors including infection [e.g.

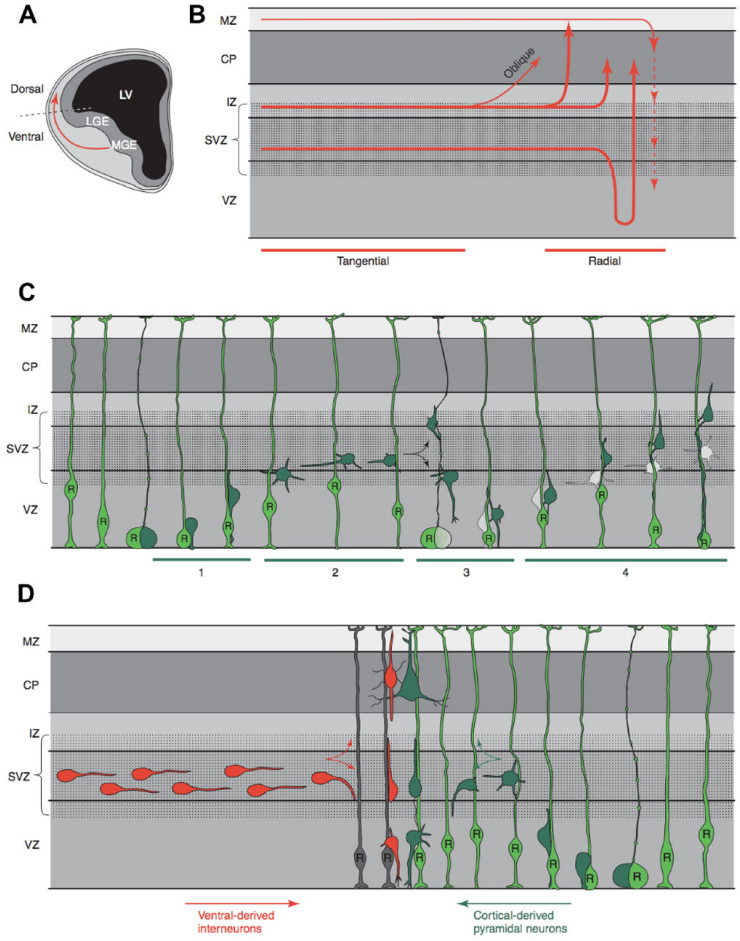

Figure 2. Neuronal migration. (A, B) Cortical interneurons derived from the ventral telencephalon and reach their final locations by migrating through specific phases. **(A)** Origin of interneurons in the ventral telencephalon. Most cortical interneurons are generated in the medial ganglionic eminence (MGE) of the ventral telencephalon and migrate across the corticostriatal junction (broken line) to enter the dorsal telencephalon. **(B)** Phases of interneuron migration within the dorsal telencephalon. Cortical interneurons arising in the ventral telencephalon migrate tangentially in the cortex, and then change direction to enter the cortical plate (CP) by following a radial or an oblique path. The broken line indicates that some interneurons have been observed to descend radially into the CP and others to continue radially to deeper lamina. Abbreviations: IZ, intermediate zone; LGE, lateral ganglionic eminence; LV, lateral ventricle; MZ, marginal zone; SVZ subventricular zone; VZ, ventricular zone. **(C)** Cortical pyramidal neurons undergo distinct phases of locomotion migration. Phase one involves radial movement of pyramidal neurons (dark green) from the site of origin at the ventricular surface to the subventricular zone (SVZ). In phase two, cells become multipolar and pause their migration in the lower intermediate zone (IZ) and subventricular zone (SVZ). Some neurons undergo phase three, which is characterized by retrograde motion toward the ventricle. Phase four is the final radial migration to the cortical plate (CP), guided by radial glial fibers. Radial glia (light green) remain mitotic, undergo interkinetic nuclear migration, and generate additional daughter cells (grey). Abbreviations: MZ, marginal zone; R, radial glial cell; VZ, ventricular zone. **(D)** Migratory patterns of interneurons and pyramidal neurons converge in the dorsal cortex. This scheme depicts the apparent convergence of the migratory patterns of interneurons (red) and pyramidal cell movements (dark green) in the cortex. Subsets of both cell types display ventricle-directed migration followed by radial movement to the cortical plate (CP). Interneurons might migrate radially along unrelated, adjacent radial glial cells (grey) to reach the cortical plate. Abbreviations: IZ, intermediate zone; MZ, marginal zone; R, radial glial cell; SVZ, subventricular zone; VZ, ventricular zone. Reprinted from *Trends in Neurosciences,* 27(7), Arnold R. Kriegstein and Stephen C. Noctor, "Patterns of neuronal migration in the embryonic cortex", Pages 392–399, Copyright (2004), with permission from Elsevier.

TOxoplasmosis, **R**ubella, **C**ytomegalovirus, **HE**rpes simplex, **S**yphilis (TORCHES)] or toxins,(e.g. alcohol)[6, 7]. Microcephaly can be genetic, caused by mutations in genes that regulate cell division resulting in defects in the expansion of progenitors[8]. Furthermore, defects in genes that are important for multiple stages of development can result in a patient with malformation with multiple features. Microcephaly can also occur in combination with a migration defect, i.e. microcephaly with pachygyria (Norman Roberts syndrome)[9], so that the malformation appears to reflect the disruption of function of the gene throughout development.

Disruptions in neuronal migration can cause a number of malformations. Lissencephaly ("smooth brain") or pachygyria ("few gyri") are malformations caused by a disorder of neuronal migration. Pachygyria and lissencephaly are often less and more severe manifestations of gene mutations causing type 1 lissencephaly including LIS1 and DCX mutations. Other variants of lissencephaly that resemble type 1 have subsequently been identified including mutations in *ARX*[10], *RELN*[11], *VLDLR*[12] and *TUBA1A*[13] (Table 1).

Type II lissencephaly, also called "cobblestone lissencephaly", is associated with Walker-Warburg and Fukuyama muscular dystrophies and is caused by defects in the basement membrane that result from mutations in glycosyl transferase enzymes (Table 1). Again, mutations in additional genes involved in dystroglycan glycosylation have been observed. [14] Cobblestone lissencephaly has a similar, but not identical appearance to polymicrogyria, which literally means "many small gyri". This malformation can be regional with variants that are perisylvian, or parietal, or predominantly bifrontal. The pathophysiology of polymicrogyria is not apparent and may be due to damage of deeper layer or early born neurons, resulting in a over folding of later born, or more superficial neurons, or may be due to an abnormal expansion of the superficial layers.

Finally, defects in axonal growth and guidance can lead to commonly observed white matter abnormalities, such as agenesis of the corpus callosum or enlarged ventricles. Interestingly, most causes of lissencephaly (non-cobblestone) are also known to be associated with defects in axon outgrowth[15], thus implying a role of the causative genes in both stages in development and closely relating the molecular pathways regulating both neuronal migration and axon outgrowth. In addition, these defects in axonal growth are likely associated with abnormal connectivity and may very generally be associated with seizure pathogenesis including in patients with lissencephaly.

IMPLICATIONS OF MICROTUBULE ASSEMBLY AND MICROTUBULE-BASED TRANSPORT FOR NEURONAL DEVELOPMENT

Not only have these specific diseases been modeled and studied, but also basic science has informed the understanding of human disease. Neuronal migration has been studied extensively in the mouse even prior to identification of causative genes for human syndromes. Prior to identification of *RELN* as a causative gene for lissencephaly, work on mouse the reeler mouse identified striking neuronal migration defects. [16] The cdk5 mouse

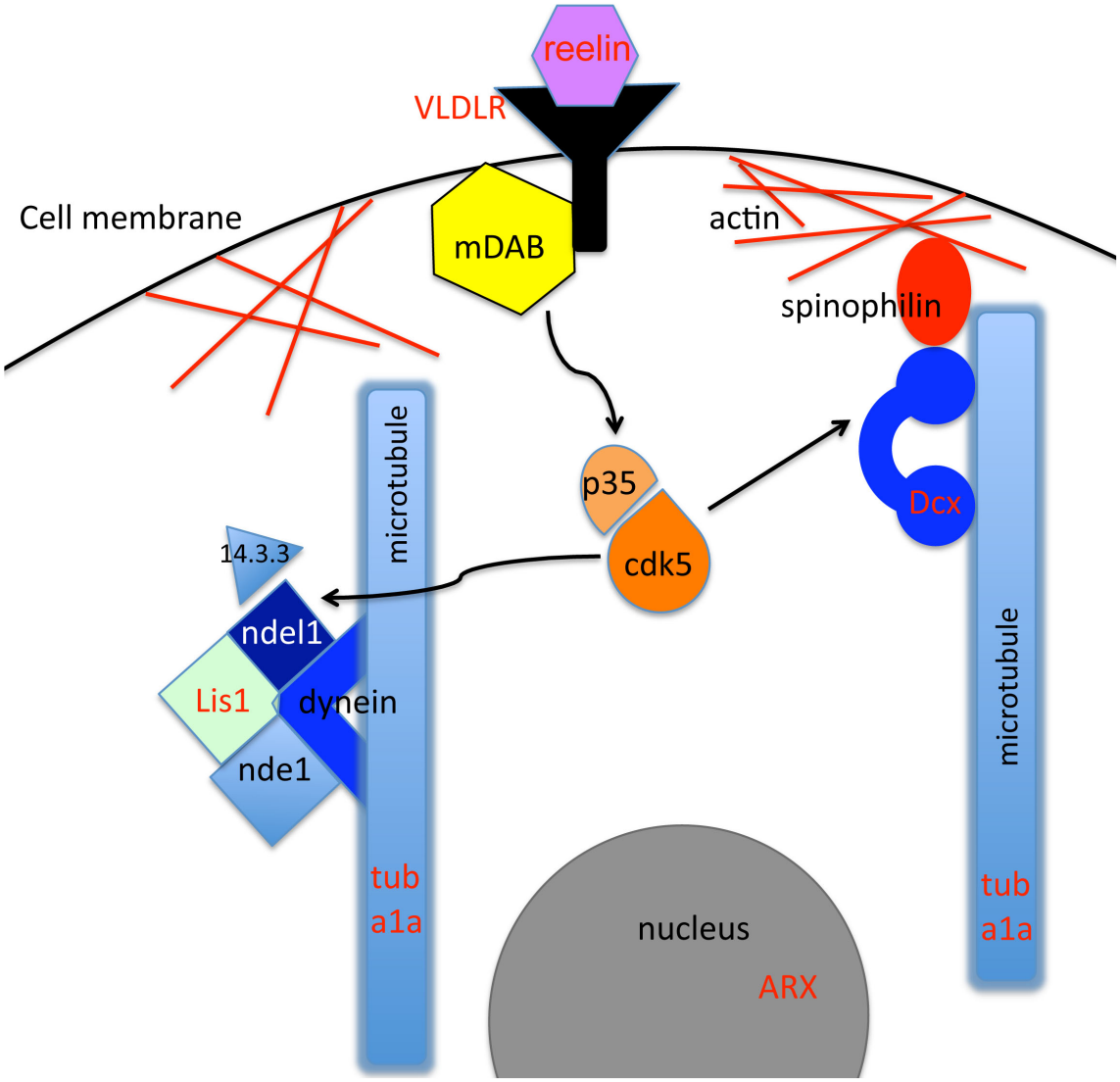

Figure 3. Neuronal migration signaling pathways. A simplified diagram describing the relationship between the known neuronal migration proteins in mouse and human is shown. Protein names encoded by causative genes for human lissencephaly are in red. Reelin is an extracellular ligand, which binds among other receptors, very low-density lipoprotein receptor (VLDLR). Doublecortin (Dcx) is a microtubule binding protein; Lissencephaly1 (Lis1) is an adaptor protein for the minus end motor, dynein and tuba1a is a tubulin isomer. Aristaless (Arx) is a transcription factor. Reelin signaling may activate the cdk5/p35 kinase via mDAB. Cdk5 regulates Dcx microtubule binding and assembly of the Lis1/dynein complex including NDEL1, NDE1, and 14-3-3ε. Dcx interacts with microtubules as well as actin via spinophilin and may be a mediator of actin/tubulin cross talk.

also has similar, but not identical lamination defects including the inverted cortical lamination[17] and cdk5 is thought to be downstream of reelin signaling. In turn, the cdk5 protein is known to interact with both DCX and LIS1.[1, 18] Thus, it has become apparent that the genes identified in mice with neuronal migration defects interact with human

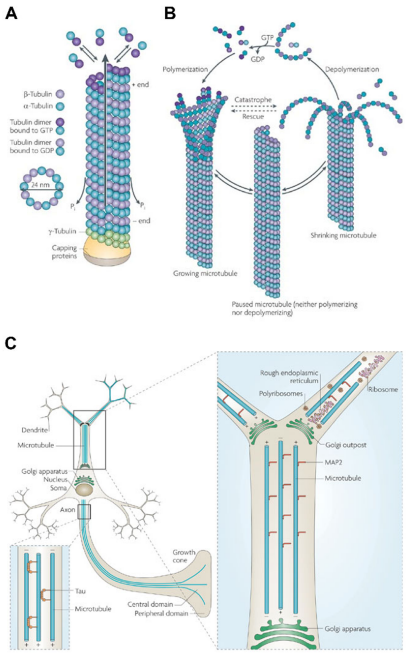

Figure 4. Microtubule regulation in neurons. (A, B) Microtubules are non-covalent cytoskeletal polymers found in all eukaryotic cells that are involved in mitosis, cell motility, intracellular transport, secretion, the maintenance of cell shape and cell polarization. They are polarized structures composed of α- and β-tubulin heterodimer subunits assembled into linear protofilaments. A single microtubule is comprised of 10–15 protofilaments (usually 13 in mammalian cells) that associate laterally to form a 24 nm wide hollow cylinder. The head-to-tail association of the heterodimers makes microtubules polar structures, and they have different polymerization rates at the two ends. In each protofilament, the heterodimers are oriented with their -tubulin monomer pointing towards the faster-growing end (plus end) and their -tubulin monomer exposed at the slower-growing end (minus end). The lateral interaction between subunits of adjacent protofilaments has been described as a B-type lattice with a seam (long arrow, part A in the figure). A third tubulin isoform, γ-tubulin, functions as a template for the correct assembly of microtubules. On addition of a new dimer at the plus end, the catalytic domain of α-tubulin contacts the nucleotide exchangeable site (E site) of the previous β-subunit and becomes ready for hydrolysis; the plus end generally has a minimum GTP cap of one tubulin layer that stabilizes the microtubule structure. When this GTP cap is stochastically lost, the protofilaments splay apart and the microtubule rapidly depolymerizes. During or soon after polymerization, the tubulin subunits hydrolyze their bound GTP and become non-exchangeable. Thus, the microtubule lattice is predominantly composed of GDP–tubulin, with depolymerization being characterized by the rapid loss of GDP–tubulin subunits and oligomers from the microtubule plus end. At the minus end, contact is made between the E site of the new dimer and the catalytic region of the last subunit at the end; therefore, no GTP cap should be present. The properties of microtubules depend on the tubulin isoforms they are made up of — there are three α-tubulins (α 1, α 2 and α4) and five β-tubulins (βI, βII, βIII, βIVa and βIVb) — and on how they have been altered by various forms of post-translational modification, including tyrosination, detyrosination, acetylation, polyglutamylation, polyglycylation, phosphorylation and palmitoylation. Except for tubulin tyrosine ligase, the enzyme that adds a tyrosine to non-assembled -tubulin, most of the modifying enzymes act preferentially on tubulin subunits that are already incorporated into microtubules. Post-translational modifications of tubulin subunits mark subpopulations of microtubules and selectively affect their functions. Although they are not directly involved in determining the dynamic properties of microtubules, post-translational modifications of tubulin, such as the sequential tyrosination-detyrosination-acetylation, correlate well with the half-life and spatial distribution of microtubules. **(C)** Axons have tau-bound microtubules of uniform orientation, whereas dendrites have microtubule-associated protein 2 (MAP2)-bound microtubules of mixed orientation. Dendrites also contain organelles that are not found in axons, such as rough endoplasmic reticulum, polyribosomes and Golgi outposts. Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Neuroscience* 10, 319–332, copyright (2009).

lissencephaly proteins, including the microtubule related proteins, as well to define a neuronal migration signaling pathway (Figure 3).

A key quesion is why the microtubule pathway is important and specific for neuronal migration. During development of pyramidal neurons, newly born neuroblasts undergo changes in morphology that correspond to different stages in neuronal migration (Figure 2B)[19]. The newly born neuroblast in the ventricular zone (VZ) passes into the subventricular zone (SVZ), where it can divide further into additional neurons or alternatively enter into the intermediate zone (IZ). In the IZ, the neuroblast assumes a multipolar morphology, but as it migrates into the cortical plate, the neuron becomes bipolar with a leading process that eventually becomes the dendrite, and a trailing process that will become the axon (Figure 2B). Transport of organelles and polarization of trafficking underlies many of these changes, and since cytoskeletal dynamics and transport regulate cell shape and structure, these pathways are critical for correct neuronal migration. Studies aimed at identifying and characterizing causative genes for neuronal migration disorders have emphasized the importance of microtubule function in human neural development (Table 1). *LIS1, DCX,* and *TUBA1A* encode proteins which are related to microtubule function and microtubule-based transport. The LIS1 protein is a part of the dynein complex, a microtubule motor protein[1]. *DCX* encodes a MAP that has a role for regulating vesicle transport in developing neurites [20]. Finally, *TUBA1A* is a gene that encodes an α tubulin subunit that is enriched during brain development [4]. The other genes, *ARX*, *RELN*, and VLDLR encode for a transcription factor and the ligand and receptor of very low-density lipoprotein receptor system, respectively, that may define upstream or downstream events regulating neuronal migration (Figure 3).

From these studies, it appears evident that the basic biology of microtubules and microtubule-based functions, including transport, is extremely important for proper neuronal development, such as neuronal migration (Figure 4). Microtubules are hollow, tube-shaped polymers that are assembled through polarized polymerization of tubulin heterodimers that are comprised of a variety α- and β-tubulin isoforms. Tubulin subunits α and β are encoded by a family of genes that are structurally similar but have distinctive features important for specific cellular functions. TUBA1A is highly expressed in the nervous system during development and is a causative gene for lissencephaly[13]. Other tubulin genes are causative for different human syndromes including Tubulin β3(TUBB3) which is an axonal-specific tubulin which causes a syndrome- congenital fibrosis of the extraocular muscles (CFEOM3) that is also associated with cognitive defects and a neuropathy[21].

Microtubule assembly is dependent on GTP, which binds to the soluble tubulin heterodimers and, upon hydrolysis, induces a conformational change that favors the polarized polymerization and elongation of the microtubule. Once assembled, however, microtubules are by no means static structures; not only can they be depolymerized, but they are moreover dynamically and developmentally tightly regulated so that their structure, polarization, and function is fine-tuned for the specific requirements of the cell. Some TUBA1A mutations block GTP binding preventing polymerization.[13]

In neurons, as in any other cell type, microtubule polarization results in very defined so-called 'plus' and 'minus' ends, with elongation occurring exclusively at the 'plus' end. In axons, the 'minus' ends are usually oriented towards the cell body, whereas their elongating 'plus' ends project towards the distal regions of the axon. In contrast, in dendrites, the polarity of microtubules is mixed, and the structural differences of microtubules in axons and dendrites likely reflect specific functional differences.[22] Microtubules in axons and dendrites can also be distinguished through their interacting proteins. Microtubule associated proteins are specific for axons or dendrites and are often bound to microtubules in gradients along neural processes.[23] MAPs serve several functions. MAP binding confers structural stability to microtubules and they can also facilitate transport targeting and specificity by either blocking or enhancing motor interaction. Most MAPs compete with motors for binding sites on tubulin and thus are negative regulators of motor transport. DCX, however, does not competitively inhibit motor binding.[24, 25] Moreover, DCX is a MAP that is regulated by phosphorylation by CDK5 and binds microtubules in a signal dependent manner.[18] Our data has shown that DCX facilitates binding of some motors to microtubules. Likewise, post- translational modifications of tubulin including acetylation and polyglutamylation, respectively can also confer stability and enhance motor function on microtubules.[26, 27] And other modifications, such as tyrosination interfere with motor function on microtubules.[27]

Thus polarity, post translational modification, and MAP binding of microtubules is important for transport functions, but the actual transport is mediated by individual microtubule-based motors.[28] Motors are either directed toward the 'plus' or 'minus' ends of microtubules. For example, kinesin motors transport vesicles and organelles towards the 'plus' ends of microtubules, and the single dynein motor transports cargo toward the minus end of the microtubule. In the axon this means that kinesins transport cargoes away from the cell body to distal parts of the axon and dynein mediates transport back toward the cell body. While there is only one 'minus' end motor, dynein, there are 45 kinesins that transport cargo toward the 'plus' end of the microtubule. Thus, achieving cargo identification and coupling for the minus end motor, dynein, is considerably more complex than for the kinesins. The LIS1 protein actually is associated with dynein, the minus end motor and is important as a regulator of dynein force generation.[29]

Thus, mutations in genes that have a major role in microtubule assembly and microtubule-based transport, including *LIS1*, *DCX* and *TUBA1A*, can cause defects in neuronal migration as well as axon and dendrite outgrowth. In this chapter, describing three specific examples, we illustrate how rare genetic causes of lissencephaly may implicate microtubule-based transport in the pathogenesis of cortical dysplasias.

LIS1 IS THE CAUSATIVE GENE ASSOCIATED WITH MILLER-DIEKER LISSENCEPHALY SYNDROME

Haploinsufficiency of *LIS1* (also known as platelet-activating factor acetyl-hydrolase) is known to cause classical lissencephaly, either in isolation (Figure 1A) or as a part of the Miller-Dieker syndrome of 17p13.3 deletion[30] (Figure 1B). Miller-Dieker syndrome also

includes facial dysmorphology, caused by haploinsufficiency of the other genes deleted in the interval. YWHAE encodes the protein 14-3-3ε which is a modifier of Lis1, [31, 32] Patients with *LIS1* mutations alone, have lissencephaly, but are often found to have hypoplasia of the corpus callosum as well as enlarged ventricles, suggesting a role for LIS1 in neuronal migration as well as axon formation[33]. Supporting evidence is provided by the expression pattern of LIS1, which is enriched in neuronal progenitor cells in early development. Moreover, expression of LIS1 in later stages of development supports a wider role of LIS1 function outside of neuronal migration.

Functional characterization of LIS1 has been facilitated by the creation and characterization of an animal model. As predicted by the human genetics, haploinsufficiency of *Lis1* results in structural defects in the brain including dyslamination in the hippocampi.[34] Homozygosity of the mutation is lethal in early embyrogenesis.[35] In addition to having radial migration defects, tangential migration of interneurons is also impaired.[36] However, most striking were the apparent defects in neurogenesis that were not emphasized in the initial clinical description of patients. In the mouse model, Lis1 was found to be critical for expansion of the neuronal progenitor pool through regulating symmetric cell division. Decreased Lis1 is correlated with abnormal spindle pole orientation a marker for the type of cell division- asymmetric to generate a post mitotic neuron or symmetric to generate two progenitors which can divide further.[37]

Mouse genetics and molecular biology has allowed the identification and characterization of proteins interacting with Lis1, most importantly dynein and Ndel1 and Nde1. Lis1 has also been found to interact with dynactin, another member of the dynein complex, and the plus-end microtubule binding protein CLIP-170 that may mediate the assembly of the complex.[38] These proteins have multiple functions during neuronal migration, including nuclear and centrosomal positioning, as well as neurite outgrowth.[39] It is unknown, however, if dynein transport in each of these contexts functions similarly and likely the complexity of the dynein complex including Lis1 facilitates specificity of function. Given its role as the single motor protein responsible for 'minus'-end microtubule transport, it is more likely that defects associated with Lis1-dynein transport are due to specific defects with dynein cargo load. For example, neurite outgrowth problems may be due to defects in retrograde signaling, and neuronal migration and neurogenesis problems may result from disruption of nucleokinesis.[40, 41]

Molecular studies have demonstrated a specific role for LIS1 in the regulation of dynein activity: LIS1 and NDE1 binds to dynein to enhance microtubule binding and prolong force production of the motor protein.[29] As a consequence, LIS1 has been shown to mediate dynein transport of relatively large and heavy cargos, such as the nucleus, while other small vesicular cargoes appear to be unaffected. Unfortunately, other aspects of LIS1 function, including its reported interaction with another lissencephaly gene, *DCX*, as well as its regulation by developmentally important kinases, that may explain aspects of the LIS1 phenotype, including the seizures, remain uncharacterized to date.

DCX MUTATIONS CAUSE BOTH X-LINKED LISSENCEPHALY AND SUBCORTICAL BAND HETEROTOPIA

DCX, or doublecortin, is one of two genes that cause X-linked lissencephaly in affected boys[42, 43](Table 1 and Figure 1E). Women with DCX mutations classically have a migration disorder called subcortical band heterotopia (Figure 1F). X-inactivation to achieve gene dosage compensation in females with *DCX* mutations results in cellular mosaicism: two populations of neurons occur with either the mutant gene or with the normal gene. Affected, mosaic females can have a range of neurodevelopmental phenotypes, including subcortical band heterotopia, a disorder where DCX deficient neurons arrest before reaching the cortical plate to form abnormal islands within the white matter with abnormal axons tracts, rather than frank lissencephaly.[44] In addition, women with *DCX* mutations can exhibit a range of milder phenotypes, including non-syndromic mental retardation or cryptogenic epilepsy without an overt neuronal heterotopia.[45] The degree of dysfunction and severity of phenotype is thought to be due to the skewing of X-inactivation of the X chromosome with the defective DCX.

The genetic mouse model of *Dcx* mutations significantly improved our understanding of the brain phenotype in the human condition. While the mutant mouse with a targeted deletion of *Dcx* does not have an overt cortical migration defect and is more mildly affected[46], it has multiple other defects that have led to a closer examination of the human phenotype. For example, the *Dcx* mutant mouse exhibits disruption of lamination in the hippocampi and white matter defects.[46] These defects were subsequently also described in humans with *DCX* mutations[47]. In addition, defects in migration and morphology of GABAergic interneurons have also been described, which may be a common factor in the pathogenesis of epilepsy in these disorders.

Interestingly, the female *Dcx* mutant mouse with a single undamaged copy of *Dcx* appears to be phenotypically normal.[20] Instead, short hairpin RNA interference (shRNAi) has been used in rats to model the formation and physiology of the subcortical band heterotopia.[48] However, it has to be noted that the same experiment does not produce the heterotopia in the mouse. The shRNAi is introduced by microinjection into the lateral ventricle of an embryonic rat and by applying an electrical pulse, the interference construct is transfected into neuronal progenitors that are adjacent to the ventricle, where the RNAi prevents the expression of Dcx during the migratory phase of neuronal development. Moreover, electrophysiological studies of these rats have shown a decrease of GABA-ergic tone in the cortex overlying the subcortical band, which is populated with not only Dcx deficient excitatory neurons, but also with normal GABA-ergic neurons.[49] These appear to be misdirected and unable to migrate to their normal positions in the cortex. Finally, this model has also been used to show that re-expression of Dcx in the subcortical band neurons during adulthood initiated migration of these neurons into the cortex and further decreased the seizure threshold of the animals.

From animal models, it has become clear that Dcx is a member of family of structurally related functionally redundant proteins that have a role in seizure pathogenesis. In

addition to the single mutant of Dcx, double mutants of Dcx and Dclk1[20, 50] and Dclk2[51] have been published showing much more severe structural effects in the double mutants than single mutants and proving overlapping roles for Dcx family members in development and beyond. All of these animals have been shown to have seizures (Deuel, Walsh, Nobles, unpublished data) and the seizures in the Dcx-/y; Dclk2-/- mutant are thought to emanate from the hippocampus.[51] It is unknown in humans whether seizures are related to the severe temporal lobe dyslamination and how much the disruption of the microcircuitry in the neocortex is contributes to seizure propagation in humans. Epilepsy surgery has not been used on lissencephaly patients, however patients with subcortical band heterotopia have generally not had favorable outcomes after temporal lobectomy.[52]

The molecular role of DCX can be understood by its interaction with other proteins. Mutations in the tandem microtubule binding domains DCX have been shown to abrogate microtubule binding and cause the neuronal migration defects.[53] In addition to microtubules, DCX is known to interact with spinophilin/neurabin II, an actin-binding protein, suggesting a role in actin/microtubule crosstalk, and human mutations disrupting this interaction also cause defects in neuronal migration.[54] Moreover, DCX has been shown to interact with the μ subunit of the clathrin adaptor complex[55], which is involved in vesicle biogenesis from the Golgi complex and in endocytosis as part of clathrin-coated pits. Finally, we have identified a role for Dcx in regulating microtubule-based transport.[20]

MUTATIONS IN αTUBULIN CAUSES LISSENCEPHALY

Heterozygous missense mutations in *TUBA1A*, coding for an α-tubulin isoform that is highly expressed in developing neurons, cause a spectrum of cortical malformations that include lissencephaly and pachygyria. Affected individuals may further be microcephalic and have cortical malformations that range from agyria and posterior pachygyria in severe cases to perisylvian predominant pachygyria in the more common and less severe forms.[56] Findings from autopsies reveal abnormal cortical layering, hypoplastic and disorganized hippocampi, and clusters of heterotopic neurons neurons interspersed within the white matter.

The TUBA1A phenotype (Figure 1C and D) is somewhat distinct from LIS1 and DCX, however. Patients with *TUBA1A* mutations have additional defects that are less commonly associated with *LIS1* and *DCX* mutations, including cerebellar and brainstem hypoplasia, as well as hypoplasia of the anterior limb of the internal capsule. This long tract finding appears to be extremely specific to *TUBA1A* mutations, and is associated with dysmorphic basal ganglia that are lacking a clear separation between the caudate and putamen.[56] As with *LIS1* and *DCX* mutations, hypoplastic and disorganized white matter tracts suggest further disruption in axon growth and guidance beyond simply defects in cell migration. Thus, the patients usually have severe neurological impairment, including mental retardation, spastic diplegia or tetraplegia, facial paralysis, and epilepsy.

In contrast to *DCX* and *LIS1, TUBA1A* mutations have not been extensively modeled in mice. However, a mouse with a mutation in the GTP binding site on Tuba1a in mice has been described with abnormal hippocampal lamination, but no overt migration disorder in the cortex.[13] This mouse appears very similar to the Dcx mutant mouse histopathology and it is likely that on further examination, other abnormalities will be appreciated.

Mutations in genes that encode different α and β-tubulin isoforms, including *TUBB2B, TUBA8,* and *TUBB3*, also cause other congenital neurological syndromes. Mutations in *TUBA8* and *TUBB2B* cause polymicrogyria with and without ocular hypoplasia, respectively.[4, 57] These syndromes are associated with developmental delay and with seizures. In contrast, mutations in *TUBB3* cause a defect in axon guidance; patients present with restrictions in eye movements, mild cognitive impairments, spasticity, and later polyneuropathy. Radiological findings reveal hypoplastic oculomotor nerves, dysmorphic basal ganglia with or without internal capsule hypoplasia, and agenesis or hypoplasia of the corpus callosum and anterior commissure, but no cortical malformations. The TUBB3 syndrome is thus not as severe as the other tubulin mutation syndromes in terms of CNS dysfunction, with the effect that these patients rarely have seizures.[58]

Each of the tubulin syndromes described above can result from mutations in tubulin isoforms that inhibit the formation of microtubules either by interfering with the levels tubulin expression, folding, or function (i.e. GTP binding), yet some pathogenic mutations do not appear to have any discernable effects on these specific tubulin properties. It is thought, however, that these mutations cause the phenotypic defects by disrupting the binding of other proteins to microtubules, including motor proteins such as kinesins and dynein and/or microtubule-associated proteins such as DCX. For example, the R402H mutation in TUBA1A has been shown to lie directly in the groove where DCX is thought to bind to tubulin[13] and DCX is implicated in transport of presynaptic vesicles. In addition, TUBB3 mutations are known to disrupt the function of another kinesin motor protein, KIF21A.[58] These findings strongly suggest that the tubulinopathies may be best understood in terms of a motor defect[59].

MICROTUBULE FUNCTION AND THE PATHOGENESIS OF SEIZURES

Defects in neuronal migration of either excitatory or inhibitory neurons result, broadly speaking, in abnormal neuronal connectivity and circuit formation, as axons are not be able to find their normal post-synaptic targets. Defects in neuronal migration of excitatory neurons may result in the abnormal formation of circuits. The dyslamination of pyramidal neurons may mean the GABAergic interneurons may not be able to form the correct connections. This alone may be enough to tilt the balance in cortical circuitry toward hyperexcitability. In fact, the use of RNAi in the rat model to knockdown Dcx specifically in pyramidal neurons without affecting GABAergic neurons does result in abnormal migration of interneurons to the subcortical band. These animals have been shown to have decreased inhibition in the overlying regions of cortex[49]. However, in

animal models with targeted mutations in Lis1 and Dcx, the interneurons are also affected since these proteins are expressed in every neuron[60, 61]. And while interneuron migration is not totally normal, the inhibitory interneurons still successfully migrate into the cortex. However, it is unknown whether specific subsets of interneurons are preferentially affected in either DCX or LIS1 mediated disorders.

In these migration disorders, however, there might be additional specific molecular defects in the axons themselves, and microtubule dysfunction in such axons may explain the clinical phenotypes observed through disruption of dynein- and kinesin-mediated transport processes and their specific cargoes. For example, the LIS1 syndrome may result from failure of dynein-mediated nuclear translocation during migration. However, the continued absence of retrograde transport in these neurons after the developmental window may affect signaling from distal regions of the axons and dendrites. Although the specific signaling pathways affected are unknown, they may be pathways that respond specifically to neuronal activity such as cdk5, which is both regulated by activity and known to interact with Lis1. In comparison, DCX mutations may cause both a failure of migration and axon outgrowth through defects in anterograde vesicle transport in growing axons and dendrites. In fact, the defect in kinesin-medicated vesicle transport observed in DCX mutant neurons may have far-reaching effects, e.g. impairments of membrane addition, mislocalization of guidance receptors, and ultimately mislocalization of ion channels.

CORTICAL MALFORMATIONS ARE AN IMPORTANT CAUSE OF PEDIATRIC EPILEPSY

Genetic causes of brain malformations have a clearly defined etiology and can be studied with animal models, yet they are relatively rare. In the pediatric population, however, other cortical malformations, especially focal cortical dysplasias (FCD), are the underlying cause of a large percentage of first presentation with seizures {reviewed in [62]}. Epilepsies resulting from cortical malformations are often difficult to control, requiring multiple medications and surgeries, and compared to other epilepsy patients, the societal cost for such cases is disproportionately greater in terms of both health care spending and co-morbidity. However, advances in genetics and neuroimaging have resulted in significant improvements in our understanding of these disorders as well as expansion of our diagnostic capabilities, and we are poised to develop new treatment options for patients suffering from these disorders.

FCD is heterogeneous disorder, which has a wide range of severity by histopathological appearance and thus an unknown (or poorly defined) etiology. The severity of FCD is graded with and correlated with radiological findings. [63,62] Pathological examination of MRI-negative focal epilepsies results in a diagnosis of a mild form of FCD, (Type I FCD) in up to half of the patients who go to surgery for resection of a seizure focus[64]. Type II FCD is more severe and makes up most of the cases, which are diagnosed pre-surgically by MRI. Thus, FCD appears to encompass a wide range of severity that may reflect

multiple etiologies from mild dyslamination to the more severe form with heterotopic neurons, abnormal giant neurons, and the presence of balloon glial cells.[63]

While giant neurons and balloon cells are not found in lissencephaly cases, changes that are associated with mild FCD: heterotopic neurons, and abnormal neuronal polarity, are reminiscent of histopathology seen in human lissencephaly. Studies on mild forms of cortical dysplasias demonstrate cellular defects that are similar to those seen in tissue from animal models of Dcx and Lis1 lissencephaly, including the disruption of lamination, and multipolar heterotopic neurons. Thus, the same pathways that are disrupted in genetic causes of lissencephaly may be important for the pathogenesis of cortical dysplasias, and it is reasonable to hypothesize that microtubule dysfunction may be a lead cause for cortical dysplasia.

In contrast to milder forms of FCD, severe forms, which are more readily diagnosed by imaging, include histopathological features that are also seen in tuberous sclerosis complex (TSC).[65] Both severe FCD (type II) and TSC have in addition to dyslamination, giant neurons and balloon cells (reactive glial cells). Thus, TSC pathway proteins, including mTOR, are other possible candidates for FCD pathogenesis. What remains unclear is whether mild FCD and severe FCD result from the extent of dysfunction of one particular pathway, or if they result from developmental disruption of separate pathways. Finally, it is not inconceivable that FCD is a heterogeneous disorder with several different causes and that subsets of FCD result from molecular pathway dysfunction in terms of severity and other subsets are caused by entirely different pathways. Finally, the pathways that cause FCD and lissencephaly, may be related.

Recent success in manipulation of these candidate pathways in FCD including highlight the importance of understanding pathogenesis of FCD. Manipulation of these pathways have been shown to alter epilepsy in non FCD models: Rapamycin an mTOR inhibitor has been successfully used for treating seizures in animal models of TSC.[66] In addition, a recent study has shown that re-expression of a lissencephaly gene, DCX, in heterotopic neurons of ***adult*** animals resulted in a decrease in the size of the heterotopia and a reduction in seizure threshold.[67] Thus, determining whether these pathways are involved in the pathogenesis of the different types of FCD may result in the development of 1) an appropriate animal model and/or 2) targeting of seizure therapy. With the successful therapeutic intervention in the animal model, the possibility of helping patients with disorders stemming from disruption of the same molecular pathway becomes a real possibility and it is critical to adequately characterize FCD to know whether this can be realized.

This characterization of human FCD will be extremely challenging with obstacles including sample collection, as well as the heterogeneity in patient population in terms of age, gender, treatment and genetic background. In addition, many of the candidate proteins are developmentally expressed, and human samples obtained are typically outside of the window of expression. However, the availability of animal models in all of the stages of development may make detailed phenotypic assessment sand correlation of

molecular pathway defects in human FCD more feasible. As discussed above, mouse models of neuronal migration have been extensively characterized and can be compared with human FCD, as well as the animal model for TSC. Thus, characterization of FCD can be conducted with reference to the animal models of candidate molecular pathways. Furthermore, a detailed characterization of the microcircuitry in both FCD and lissencephaly models may yield meaningful comparisons for understanding the development of hyperexcitability.

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