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SCIENTIFIC COMMENTARIES
Complication begets clarification in classification

As recently as 12 years ago, breakthroughs aided by laboratory neuroscience and modern neuroimaging had made differentiation and classification of malformations of cortical development seem straightforward (Barkovich *et al.*, 2001). Some resulted from abnormal neuronal proliferation (microcephalies, megalencephalies), others from abnormal neuronal migration (heterotopia and lissencephaly), and still others from abnormal cortical organization (mostly polymicrogyrias). The protein products of the few genes known to be associated with these disorders (*LIS1* and *DCX* with lissencephaly; *FLNA* with heterotopia) support these mechanistic concepts, as the lissencephaly genes are important in the migration of neurons (specifically in the extension of leading processes and nucleokinesis, the process by which the nucleus is able to follow the leading process); and the assumption that mutations of *FLNA* (which produces an actin cross-linking phosphoprotein important for cell locomotion) in neurons impair their migration is logical. Although details needed clarification, everything seemed to make sense.

The discovery that tubulin mutations cause brain malformations was easily incorporated into existing malformation classifications. That some infants with lissencephaly had mutations of *TUBA1A* (Poirier *et al.*, 2007) did not dislodge existing concepts, as both LIS1 and DCX were known to be microtubule-associated proteins (MAPs), which complex with microtubules and/or microtubule motor proteins during cell migration. Reports that mutations of *TUBB2B* are associated with polymicrogyria (Jaglin *et al.*, 2009) posed more difficulties, but caused no great surprise, as polymicrogyria is hard to explain. This malformation of cortical development is associated with many different conditions, including prenatal injury (infection or ischaemia) and many mutations. The causative mechanisms are unknown, and the appearance on imaging varies greatly. Still, it was easy to accept that disruption of the complex (and poorly understood) processes of terminal neuronal migration and cortical organization could result from *TUBB2B* mutations. Similarly, the report that *TUBB3* mutations are associated with congenital fibrosis of the optic nerves (Poirier *et al.*, 2010) was interesting but not altogether surprising. Microtubules play a large part in axonal navigation through the developing brain, particularly at growth cones—the axonal leading process. The attractive signal received by actin-rich filopodia in the tip (peripheral zone) of the growth cone is transduced to the cytoskeleton, causing polymerization of actin and extension of microtubules moving the growth cone in the direction of attraction;

simultaneously, actin is depolymerized and filopodia retract from areas expressing neutral or repulsion signals. This process allows growth cones to navigate through the developing brain to their targets. Abnormalities of microtubules thus impair axon navigation and result in diminished size of nerves and white matter bundles, manifesting in patients with tubulin mutations as abnormalities of the corpus callosum, cranial nerves and internal capsule.

The past year has qualified these simple formulations. We now know that mutations of *TUBA1A* cause not only lissencephaly but also a polymicrogyria-like malformation of cortical development (Poirier *et al.*, 2012), and that mutations of *TUBB2B* cause congenital fibrosis of the extraocular muscles, axon dysinnervation, polymicrogyria-like malformations of cortical development, pachygyria, as well as more complex developmental disorders (Cederquist *et al.*, 2012; Guerrini *et al.*, 2012; Romaniello *et al.*, 2012). Two reports published in this issue of *Brain* now reveal that *TUBB2B* mutations can cause lissencephaly; that both *TUBA1A* and *TUBB2B* mutations can cause a spectrum of cortical malformations associated with mutations that affect specific portions of the tubulin heterodimer (Cushion *et al.*, 2013: see pages 534–546); and that specific mutations of TUBB3 (the E410K phenotype) cause a fascinating new phenotype that can largely be interpreted as resulting from an axonal path-finding defect (Chew *et al.*, 2013: see pages 520–533). This new information builds upon earlier work (Poirier *et al.*, 2010; Tischfield *et al.*, 2010) in advancing our understanding of how microtubules function in brain development and how effects of specific mutations of these genes alter the structure and, consequently, the function of their protein products.

Early neuronal development is complex: the neural tube rapidly expands from proliferation of progenitor cells, which differentiate and migrate before organizing into discrete components. While *en route* to their destination, neurons extend both large leading processes that aid their migration and small processes that develop into neurites (axons and dendrites) that elongate and form synapses, allowing communication with other neurons and the formation of networks. The dramatic changes in the structure of neurons as they migrate and connect with other neurons in distant parts of the nervous system can only be accomplished by active changes in the cytoskeleton, in particular those occurring in and adjacent to cilia, centrosomes and microtubules (Kuijpers and Hoogenraad, 2011). Therefore, it is not surprising that mutations in genes involved in the formation of these structures result in multiple and varied malformations. In addition to knowledge of

when the gene is expressed and the degree of gene redundancy, an understanding of these processes underlying malformations requires knowledge of how each gene mutation affects the structure of its protein product; more specifically, how altered structure of the protein product affects its interaction with other molecules in specific molecular pathways has to be determined. By mapping the sites of amino acid substitutions resulting from *TUBB2B* and *TUBA1A* mutations onto a predicted 3D model of an α/β-tubulin heterodimer within microtubule polymer structures, Cushion *et al.* (2013) have correlated the location of several mutation-altered proteins to the sites of the α/β-tubulin interface and the binding site of DCX to tubulin. Sites near the α/β-tubulin interface should be important in formation of the heterodimer and its stability, both of which are crucial for rapid changes in the cytoskeleton (e.g. axonal pathfinding) of the developing neuron. It has been reported that some TUBB3 mutations (p.E205K, p.A302V, p.M388V and p.M323V) impair tubulin heterodimerization (Poirier *et al.*, 2010; Tischfield *et al.*, 2010), while others increase microtubule stability (Poirier *et al.*, 2010); either of these effects could impair axonal pathfinding in which actin in filopodia rapidly polymerizes and microtubules are simultaneously built (through heterodimerization) or deconstructed upon encountering attractive or repulsive signals, respectively. Mutations that predominantly interfere with heterodimerization may not affect cortical development; indeed, E410 and other proteins that cause congenital fibrosis of the extraocular muscles are located on the opposite face of the heterodimer from those affected by malformations of cortical development resulting from *TUBB3* mutations (Poirier *et al.*, 2010). In addition, E410 is located at a kinesin motor protein-binding site of β-tubulin, near the α-tubulin interface, and is likely to be involved in heterodimerization (Fig. 1). The exact mechanisms by which the process is altered remain to be determined. Cushion *et al.* (2013) also note that the TUBB2B R380 site is located at the DCX-binding site of β-tubulin, close to the α-tubulin interface, but on the opposite side from proteins affected in congenital fibrosis of the extraocular muscles. As mutations of DCX are known to cause pachygyria and lissencephaly, it seems likely that these impact neuronal migration, and it is to be expected that mutations affecting R380 cause malformations of cortical development, in this case polymicrogyria-like cortical malformations. It will be interesting to see, in future studies, whether other tubulin-related causes of malformations of cortical development are associated with mutations near binding sites of other microtubule-associated proteins.

Finally, use of the term 'PMG-like MCD' (polymicrogyria-like malformations of cortical development) in the article by Cushion *et al.* (2013) brings to the front an issue that has been simmering for a decade. Physicians who deal frequently with malformations of cortical development have known for a long time that the term 'polymicrogyria' includes several, perhaps many, different malformations that have superficial similarities. The so-called 'cobblestone malformations', in which neurons migrate into the subarachnoid space through gaps in the pial limiting membrane are often considered to be polymicrogyrias. Other conditions with deep undulations of thin cortex, and those with tiny undulations and rather thick cortex are also put in this group, as are many other disorders with distinct patterns (discussed in
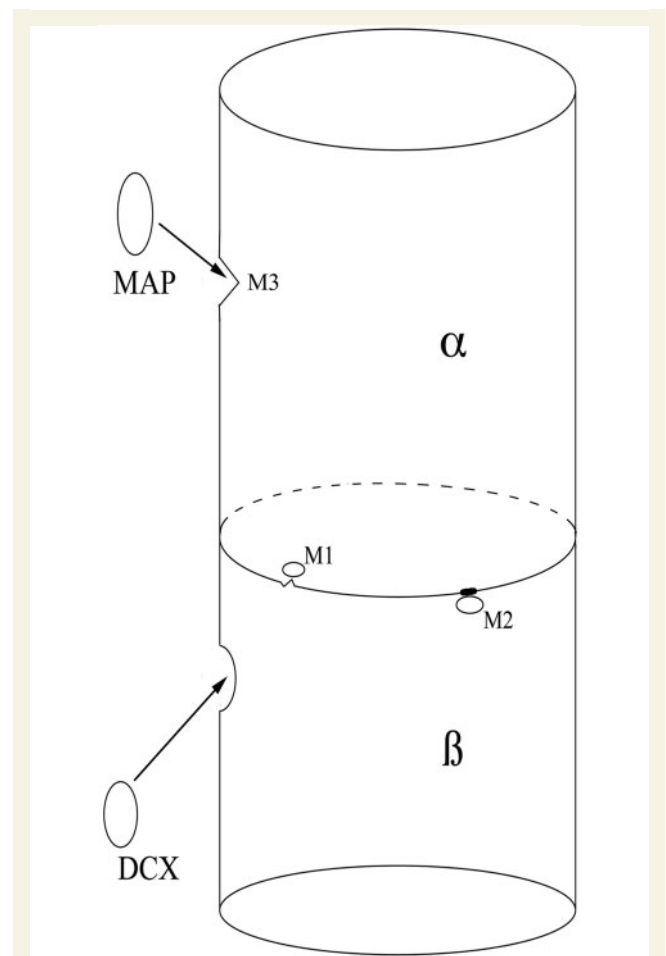

Figure 1 Schematic showing the effects of mutations on the structure and stability of microtubules. This depicts a microtubule heterodimer, composed of alternating α- and β-subunits. Mutations near the interface of the subunits can interfere both with the dimerization process (owing to unstable regions of the interface that break down, M1) and rapid breakdown of the dimers (owing to impaired breakdown of abnormal regions of interface, M2) that are essential for neuronal migration and axonal pathfinding. This likely leads to hypoplastic axonal structures such as the cortical spinal tracts, corpus callosum and cranial nerves. Interaction with microtubule-associated proteins (MAPs) such as DCX (in β-subunit) is essential for other microtubule functions, such as mitosis and neuronal migration. Mutations near binding sites of the microtubule-associated proteins (M3 in α-subunit) can interfere with microtubule-associated protein binding, disturbing these critical functions in a manner similar to mutations of the microtubule-associated proteins themselves. These mutations are more likely to result in a malformation of cortical development.

Barkovich, 2010). 'Polymicrogyria' is also described with infection, ischaemia and many different genetic mutations, not all of which are known directly to affect the brain. The same can be said of lissencephalies and heterotopia and focal cortical dysplasias. Such classifications were acceptable and useful in the past, when the molecular mechanisms could not be determined. Knowledge has now advanced to the point where these fascinating disorders

should be classified by the effects of specific mutations on molecular structure and their consequent ramifications upon molecular pathways. It is expected that this will create realistic opportunities for the development of effective therapies.

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Oligodendroglia: new players in amyotrophic lateral sclerosis

The contribution of glial cells to the neuropathology and phenotype of amyotrophic lateral sclerosis (ALS) has become a focus for basic and translational research. Whereas astrocytes and microglia are studied in great detail, the contribution of oligodendrocytes has been relatively neglected. Oligodendrocytes, one of the most important glial cells of ectodermal origin, serve to endow axons with myelin sheaths that are important for the propagation of signals in the CNS. Very recently, it has been demonstrated that they also contribute to axonal energy supply and metabolism (Lee *et al.*, 2012). Oligodendrocytes have been shown to contribute to the neuropathology of ALS (Neumann *et al.*, 2007; Seilhean *et al.*, 2009; MacKenzie *et al.*, 2011). In some subforms of the disease, such as that seen in SOD1^{D90A} mutation carriers, there is evidence that the pyramidal tracts are demyelinated, but research on their functional contribution to the disease process is in its early stages.

Now, in work that is complementary to Lee *et al.* (2012), Wim Robberechts and colleagues report on oligodendrocytic dysfunction both in human patients suffering from ALS and a mouse model created by overexpressing the SOD1^{G93A} mutation (Philips *et al.*, 2013 in this issue). In human autopsies the authors find TDP-43^{+} inclusions not only in spinal cord neurons, but also in oligodendrocytes; longitudinal studies in SOD1^{G93A} mice reveal morphological pre-apoptotic changes of oligodendrocytes, even before the onset of neuronal loss. In contrast, the absolute number of oligodendrocytes is maintained during the course of the disease; this apparent paradox being explained by an increased number and turnover of oligodendrocyte precursor cells (Ng2+ cells). The resulting oligodendrocytes are dysfunctional in the SOD1^{G93A} mouse model since they express lower levels of myelin basic protein and the lactate transporter MCT1 (monocarboxylate transporter 1). The latter