What disorders of cortical development tell us about the cortex: one plus one does not always make two
M Chiara Manzini and Christopher A Walsh

The unique size and complexity of the human cerebral cortex are achieved via a long and precisely regulated developmental process controlling neurogenesis, neuronal migration and differentiation. Traditionally, disorders of cortical development have been classified on the basis of the most obvious defects in one of these developmental steps. However, the more we learn about the cellular biological roles of genes that are essential for cortical development, the more we realize that these functions map onto molecular processes, but not so cleanly onto anatomical processes. Essential genes might be involved in both proliferation and migration as well as differentiation, reflecting roles for underlying molecular mechanisms in different phases of development and causing a stunning variety of cortical defects.

Introduction

After cortical neurons are generated from radial glial progenitor cells close to the ventricle, they migrate towards the pial surface using radial glial processes as scaffold and form an ordered laminar structure (Figure 1a). Malformations of cortical development have been traditionally classified based on which biological process is likely to be affected and grouped under disorders of proliferation, migration and cortical organization [1,2,3]. In the disorders of proliferation (or of the balance between proliferation and apoptosis), the number of cells is significantly reduced, resulting in an abnormally small head (microcephaly) (reviewed in [4,5,6]). In the disorders of migration, neurons do not reach their correct destination in the cortical plate, either by remaining at the ventricular surface (periventricular heterotopia), arresting in the white matter (subcortical band heterotopia) or forming a disordered, often thickened, cortical plate. This thicker cortex affects the formation of normal gyration, leading to a simplified gyral pattern (pachygyria) or a smooth appearance of the cortical surface (lissencephaly), sometimes with overmigration of neurons to the pial surface (cobblestone lissencephaly) [3,7,8]. The third category, disorders of cortical organization or late migration, comprises mostly the polymicrogyrias, a heterogeneous group of malformations with multiple small gyri and an abnormally thin or thick cortex, sometimes so severely affecting brain structure as to cause clefting between the ventricular and meningeal surface (schizencephaly) [9].

The identification of clear diagnostic criteria to enroll suitable families for genetic analysis has quickened the pace of discovery of genes responsible for many of these diseases as multiple families with the same phenotype could be grouped for linkage studies. The genes associated with each type of disorders often supported the mechanistic hypotheses behind the clinical classification. Almost all microcephaly genes identified to date encode for centrosomal proteins and DNA repair pathway members, whose perturbation is expected to disrupt cell division ([5,10] for review, and [11,12,13,14]). Likewise, genes responsible for lissencephalies and heterotopias are predicted to cause neuronal migration defects, by affecting cytoskeletal stability and trafficking, or by altering responses to extracellular signaling and cellular interactions with the extracellular matrix [7,8]. Only the polymicrogyrias still defy the identification of a common mechanistic cause and may in fact comprise several different disorders.

As new genes are identified and more diverse patient cohorts are used for resequencing, a more complex picture is emerging shaking the one gene/one mechanism/one malformation hypothesis and sometimes identifying all three malformation categories in the same patient. By combining human genetics analyses with functional studies in animal models we are beginning to understand how cortical malformation genes work together to form the normal and diseased cortex. Here, we will discuss recent advances in the study of cortical malformation genes affecting cytoskeletal function to show how phenotypic heterogeneity can be explained by a single gene affecting multiple stages of differentiation or by different genes converging on the same mechanism.
One gene — one malformation? Clinical variability in cortical malformations

α-Dystroglycanopathies, a group of congenital muscular dystrophies associated with brain malformations (also reviewed in this issue), were among the first examples of mutations in the same gene [15] and sometimes the same mutation [16,17] resulting in a varied spectrum of brain phenotypes ranging from cobblestone lissencephaly or pachygyria to polymicrogyria, to no brain defects at all [18,19] and blurring the boundary between disorders of neuronal migration and cortical organization.

In the past year, studies on microcephaly cohorts revealed that the border between disorders of proliferation and migration is also not as sharp as previously thought. The very recent identification of the WDR62 gene as the second most common known genetic cause of microcephaly [11**,12**,13**], 11 years after the mapping of the genetic locus [20], revealed that the malformations associated with mutations in this gene are extremely variable, including pachygyria, lissencephaly, polymicrogyria, schizencephaly, hippocampal and cerebellar abnormalities. Patients with WDR62 mutations can even show considerable hemispheric asymmetry in the findings within a given brain [12**]. Such clinical heterogeneity contributed to the long delay in the identification of WDR62, since many cases would not have been grouped together for linkage studies. However, even ASPM mutations, the...
most common cause of primary microcephaly, have been occasionally associated with variable cortical phenotypes in a large resequencing effort, revealing instances of gyral simplification, polymicrogyria and corpus callosum agenesis [21]. From a clinical genetics standpoint these findings complicate the choice of a specific genetic test for molecular diagnosis, and more resequencing of large patient cohorts with variable malformation patterns may be beneficial to determine the frequency of mutation for each gene and develop better testing guidelines. From the functional standpoint this phenotypic differences support the hypothesis that cortical malformation genes may be involved in multiple developmental steps.

**One gene — one function? The centrosome in proliferation and migration**

Considering the unique morphology of neurons it should not be surprising that the cytoskeleton is emerging as a key element in the regulation of all aspects of neuronal differentiation. Cytoskeletal components or regulators are mutated in all three categories of cortical malformations (Table 1) and understanding the roles of the cytoskeleton in cortical development can help reconcile how different malformations may be caused by the same gene. Six of the seven genes identified for microcephaly vera (or primary microcephaly, defined as a small brain with normal gyral pattern and few or no other associated features) encode proteins localized in or around the microtubule organizing center (MTOC) in the centrosome (ASPM, CDK5RAP2, CENPJ, CEP152, STIL, WDR62) [5*,11*,12*,13*]. Since these proteins are expected to regulate the mitotic spindle, the prevailing hypothesis is that mitotic defects in the neuronal progenitors underlie the reduction in neuronal number in the microcephalic brain [5*]. In fact, recent studies removing Cdk5rap2 [22,23] or Aspm [24,25] in the developing mouse

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Abbreviations: CFEOM — congenital fibrosis of the extraocular muscles, LCH — lissencephaly with cerebellar hypoplasia, PH — periventricular heterotopia, PMG — polymicrogyria, SBH — subcortical band heterotopia. # of alleles were collected from the Human Gene Mutation Database and the following references: [11*,12*,13*,14,54]. % of cases with mutations in the gene were collected from the following references: [3,5*,36].
cortex found early cell cycle exit in the progenitor cells leading to early neuronal differentiation and a likely depletion of the progenitor pool (Figure 1bi). Similar results were observed by knockdown of pericentrin, a centrosomal protein involved in microcephalic osteodysplastic primordial dwarfism [23]. Questions are still open on the exact mechanisms leading to cell cycle exit, whether the mitotic spindle is abnormal as found in some models [22,24] but not in others [23,25], and on the differentiation and survival of the prematurely born neurons. Cortical size reduction in the Aspm mice has been modest compared to the human phenotype [22,25], possibly due to the reduced proliferation potential of murine progenitor responsible for the generation of a much smaller cortex than the human one [26]. The generation of additional mouse models or allelic series involving multiple centrosomal proteins responsible for microcephaly may lead to a more severe phenotype and provide new mechanistic insight into the condition of the mitotic spindle.

The multiple brain malformations identified in microcephaly patients with mutations in WDR62 and to a lesser extent in ASPM argue that the fewer neurons generated may also be impaired in their ability to migrate properly [12**,13**,21], as seen in studies on the lissencephaly gene LIS1, which established the importance of the centrosomal function in both neurogenesis and migration. Lis1 is a microtubule stabilizing protein acting primarily at the centrosome [27,28] and de novo dominant mutations or deletions in the LIS1 gene cause classic lissencephaly [29]. Although mice with heterozygous Lis1 loss of function only show mild lamination defects in the cortex and a delay in neuronal migration [30,31**,32], the combined loss of Lis1 and its binding partners such as the centrosomal proteins Nde1 and Ndel1 and the molecular motor cytoplasmic dynein uncovered how Lis1 acts in multiple developmental processes such as mitotic spindle stability [33,34*], nuclear movement during migration (nucleokininesis) and extension of the leading migratory process [28,31**,35], and the interaction between the microtubules and the actin cytoskeleton [36]. Microcephaly genes, which are associated with the centrosome and cause multiple cortical malformations, may be involved in a similar array of cellular processes. As a result the cells that survive the primary mitotic defects may not be able to complete neuronal migration due to a secondary disruption in the establishment of cell polarity, nucleokininesis and/or leading process extension (Figure 1bii).

**One malformation — one mechanism? The microtubule multi-task**

The discovery of mutations in LIS1 and another microtubule-binding protein DCX in lissencephaly cases and follow-up studies on the function of these proteins firmly established the importance of microtubule stability in the disorders of cortical migrations (for review see [3,7*]). In the past few years, the structural components of the microtubules themselves, α and β tubulin, have been tied to the pathogenesis of cortical malformations with different tubulin isotypes causing different malformations and sometime different syndromes altogether: TUBA1A in classical lissencephaly [37,38], TUBB2B and TUBA8 in two different forms of polymicrogyria [39**,40] and TUBB3 in a wide array of neurological malformations including microlissencephaly, frontal polymicrogyria, gyral simplification or normal cortical appearance all associated with axon guidance defects [41,42]. These findings suggest that different tubulin isotypes have specific roles in cortical development and tubulin disorders will be reviewed in this issue by Tischfield *et al.* We will focus on what these genes can tell us about the etiology of polymicrogyria, which mechanistically is the most elusive of the cortical malformations.

Polymicrogyria is a common cortical malformation and is extremely variable in appearance and topography in both imaging and neuropathology studies [9] occasionally accompanying different cortical disorders. The actual cellular mechanisms of this malformation are unclear and recent comprehensive analyses of hundreds of patients with polymicrogyria suggest that it may in fact comprise several different disorders [43,44]. One possible mechanism for this loss of cortical organization was suggested by studies on the GPR56 gene, which causes bilateral frontoparietal polymicrogyria (BFPP) in humans [45]. In the Gpr56 knockout mouse there are regional cortical organization defects where the radial glia orientation is disrupted and the basal lamina is discontinuous, leading to neuronal overmigration to the pial surface [46*] (Figure 1c). These findings are remarkably similar to those observed in both patient neuropathology and animal models of cobblestone lissencephaly, which is often seen in association with α-dystroglycanopathies, and is also characterized by a disruption of the basal lamina, cortical dysplasia and overmigration, primarily due to defects in the radial glial progenitors [47,48,49*], supporting the hypothesis that BFPP and cobblestone lissencephaly may lie on the same spectrum and belong to a common migration disorder caused by radial glial disruption [9,50].

Unexpectedly, neuropathology studies identified a similar disruption of the radial glial scaffold and neuronal overmigration in a 27-week fetus carrying a dominant missense mutation in TUBB2B, which causes asymmetric polymicrogyria of predominantly frontal distribution [39]. Mechanistically, mutations in these genes appear to lead to radial glial instability via different means, loss of interaction with the basal membrane in the case of Gpr56 [46*] and dystroglycan [47], and cytoskeletal destabilization for TUBB2B [39,51]. An analogous phenotype in the mouse is also observed when the meninges are missing due to mutations in the Foxc1 gene [52,53],
suggested an additional role of structures outside the cortex in regulating its development (also reviewed in this issue).

Together, these findings illustrate how multiple different genetic defects in functionally distinct processes regulating a common structure, the radial glial scaffold, may lead to related anatomical malformations. It will be important to determine whether the radial glia is disrupted in other forms of polymicrogyria, in particular those associated with mutations in *TUBA8* or *TUBB3*, or *WDR62* which also show instances of cobblestone cortex. Other forms of polymicrogyria may have a different origin altogether and only more complete genetic and functional characterizations will help sort through this heterogeneity.

**The future**

The heterogeneity of clinical presentations of cortical malformations and of the genetic causes of these disorders is staggering, but by organizing the disease genes in functional groups and modeling the genetic lesions and functional interactions in the mouse cortex, we can begin to understand how these genes contribute to normal and abnormal cortical development. As these mechanisms are unraveled, the variability of brain phenotypes becomes less daunting as multiple developmental defects can be explained by different roles of the same gene or the concerted function of groups of genes. Owing to the speed in the identification of novel cortical malformations genes in recent years, several mouse models still need to be generated and crossed with the existing ones. However, due to obvious limitations in the mouse cortex, such as the lack of gyration, more detailed neuropathology and better imaging in human brains are also necessary to instruct or corroborate findings in the mouse.

Multiple genetic causes of cortical malformations are still unknown, particularly as it pertains to polymicrogyria and cobblestone lissencephaly, where extreme genetic and clinical heterogeneity makes linkage studies arduous. Next generation sequencing technologies will surpass the heterogeneity via the sequencing of every coding exon in the genome. As these mechanisms are unraveled, the variability of brain phenotypes becomes less daunting as multiple developmental defects can be explained by different roles of the same gene or the concerted function of groups of genes. Owing to the speed in the identification of novel cortical malformations genes in recent years, several mouse models still need to be generated and crossed with the existing ones. However, due to obvious limitations in the mouse cortex, such as the lack of gyration, more detailed neuropathology and better imaging in human brains are also necessary to instruct or corroborate findings in the mouse.

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**References and recommended reading**

Papers of particular interest published within the period of review have been highlighted as:

- of special interest
- of outstanding interest


With [12**,13**] these are the first instance in which next-generation sequencing has been used to identify cortical malformation gene, revealing the extreme phenotypic heterogeneity of WDR62 mutations. Whole-exome sequencing was used in this article (i.e. sequencing of every coding exon in the genome).


With [11**,13**] this article proves the power of next-generation sequencing in identifying previously elusive disease genes. Targeted next-generation sequencing was used here (i.e. sequencing of a large genomic region that could not be tackled using traditional Sanger sequencing).


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Molecular and genetic bases of disease


With reference [34] this study shows the power of allelic series to unravel functional defects in mouse models of cortical malformations where the murine phenotype is less severe than the human one. In this case loss of the lissencephaly gene Lis1 is combined with the loss of interacting centrosomal protein Ndel1.


As in Ref. [32], allelic series of Lis1 with an interacting protein (Ndel1 in this case) allows some insight into the functional versatility of this protein, revealing distinct defects in cortical progenitor proliferation and neuronal migration.


This article describes the initial findings in a mouse model deficient for the polymicrogyria gene Gpr56 and identifies a defect in glial scaffold structure and cortical lamination similar to those observed TUBB2B mutations [39**] and cobblestone lissencephaly models [49].


In a conditional mouse model for cobblestone lissencephaly, the authors dissect the relative roles of dystroglycan in the radial glial scaffold and in neurons, showing how radial glia integrity is necessary for the cortical lamination defects and neuronal overmigration observed in this disorder.


