

# Genomic Variants and Variations in Malformations of Cortical Development



Saumya S. Jamuar, MD<sup>a,b,c,d,e</sup>,  
Christopher A. Walsh, MD, PhD<sup>b,c,d,f,\*</sup>

## KEYWORDS

- Malformations of cortical development • Genomic variants • Somatic mutation
- Microcephaly • Megalencephaly • Cortical dysplasia • Lissencephaly
- Polymicrogyria

## KEY POINTS

- Development of the cerebral cortex is a tightly regulated process, and disruption in any part of this process can lead to malformations of cortical development (MCDs).
- MCD can primarily be classified into abnormalities of neurogenesis, abnormalities of neuronal migration, and abnormalities of postmigrational development.
- Recent advances in genomic technology have allowed for an unprecedented expansion in the knowledge of these disorders and have elucidated molecular pathways that can serve as targets for therapeutic interventions.

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<sup>a</sup> Department of Paediatrics, KK Women's and Children's Hospital, 100 Bukit Timah Road, Singapore 229899, Singapore; <sup>b</sup> Division of Genetics and Genomics, Manton Center for Orphan Disease Research, Howard Hughes Medical Institute, Boston Children's Hospital, Boston, MA 02115, USA; <sup>c</sup> Department of Pediatrics, Harvard Medical School, Boston, MA 02115, USA; <sup>d</sup> Department of Neurology, Harvard Medical School, Boston, MA 02115, USA; <sup>e</sup> Paediatrics Academic Programme, Duke-NUS Graduate Medical School, 8 College Road, Singapore 169857, Singapore; <sup>f</sup> Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA 02138, USA

\* Corresponding author. Division of Genetics and Genomics, Boston Children's Hospital, Center for Life Sciences 15062.2, 3 Blackfan Circle, Boston, MA 02115.

E-mail address: [christopher.walsh@childrens.harvard.edu](mailto:christopher.walsh@childrens.harvard.edu)

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## CLINICAL BACKGROUND

The development of the human cortex is a complex and tightly regulated process. During development, distinct cell types must proliferate, differentiate, migrate, and integrate to form a highly complex structure, capable of complex cognition, language, and emotion.<sup>1</sup> Disruptions in any of these processes lead to malformations of cortical development (MCD), which are common causes of neurodevelopmental delay and/or epilepsy.<sup>2</sup> Individuals presenting early can show feeding difficulties soon after birth (in some instances, in utero swallowing difficulty may present as polyhydramnios), abnormal head size (microcephaly or macrocephaly), epileptic encephalopathies, or global developmental delay. Some patients with MCD may present early with severe neurologic impairment, whereas others present with epilepsy and mild functional impairment at a later age. Individuals who present later may exhibit focal epilepsy, learning difficulty, and behavioral issues, such as attention deficit hyperactivity disorder.<sup>3</sup> Occasionally, a few individuals may be diagnosed only on screening, as their deficit may not be clinically apparent.

Classification systems for MCDs, first introduced in 1996 and subsequently revised in 2001, 2005, and 2012 to incorporate the improved understanding of cortical development, divide MCDs into 3 major groups, namely, malformations secondary to abnormal neuronal and glial proliferation or apoptosis, malformations due to abnormal neuronal migration, and malformations secondary to abnormal postmigrational development. This system is based on the developmental steps at which the process is first disrupted, the underlying genes and biological pathways affected, and imaging features,<sup>2</sup> although there is surprising overlap in the phenotypes of many genes, reflecting involvement of some genes in more than one stage of development.

Genomic variants are changes in one allele of a gene of an individual compared with a reference genome. Variants may be small (<1 kilobasepair) and include substitutions and small insertions and deletions (indels) or may be large (>1 kilobasepair) and include copy number variants (CNVs) (larger insertions or deletions) and rearrangements, such as translocation and inversion. Lastly, genomic variants also include whole chromosome numerical alterations such as aneuploidy. Although many variants are not associated with disease (and instead are referred to as benign variants), certain deleterious variants that alter the function of a gene may cause disease, representing disease-causing mutations. Human genetic diseases have traditionally been thought to reflect either inherited or de novo (spontaneous) variants. These mutations are present in all the cells of the affected individual and can be detected in any cell of the body, including readily available peripheral blood, and are referred to as germline mutations. Somatic mutation, on the other hand, is a postzygotic mutational event that leads to an individual having 2 or more populations of cells with distinct genotypes, despite developing from a single fertilized egg<sup>4,5</sup>; somatic mutations thus represent a subset of the larger category of de novo mutations.

This review focuses on the recent advances in understanding the genetics of MCDs, including recent updates on the role of somatic mutations in MCDs. Large-scale sequencing projects have led to an exponential increase in the knowledge of the genes associated with MCDs, and the authors address some of these recent discoveries. Although most MCDs are caused by genomic variants, a proportion of MCDs (such as schizencephaly) are associated with nongenomic mechanisms and may be secondary to environmental causes.

## EMBRYOLOGY OF CEREBRAL CORTICAL DEVELOPMENT

The normal human cortex is composed of 6 distinct histologic layers. Its development begins from neuroepithelial progenitors lining the lateral ventricles, which divide to

expand the progenitor pool and then give rise to intermediate progenitors that subsequently divide and give rise to neurons. The neurons migrate from the proliferative ventricular zones toward the pial surface of the brain to form the layered cortex, where the connections between neurons form and mature.<sup>1,6</sup>

The principal excitatory neurons of the cerebral cortex and hippocampus are derived from an embryonic neuroepithelium, with progenitor cells lining the ventricular surface deep in the brain. In animal models, inhibitory neurons that populate the cerebral cortex are formed outside the cortex in a second proliferative zone in the basal forebrain called the ganglionic eminence, which generates the basal ganglia. These neurons migrate large distances in nonradial direction before turning radially to enter the cortex.<sup>7</sup> There is recent evidence that human interneurons are formed by a similar mechanism.<sup>8</sup> Astrocytic glial cells arise from several sources, including progenitors that also generate principal neurons,<sup>9</sup> whereas oligodendrocytes arise in the basal forebrain that generates cells for the entire forebrain.<sup>10</sup>

## RECENT ADVANCES IN GENETICS AND PATHOMECHANISM OF MALFORMATIONS OF CORTICAL DEVELOPMENT

### Overview

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Historically, geneticists have relied on principles of mendelian inheritance to identify genes, which when perturbed, lead to development of specific symptoms. Linkage analysis, homozygosity mapping, positional cloning, and/or candidate gene sequencing have helped identify the genetic causes of many forms of MCDs.<sup>11–14</sup> Studying individuals/families with MCDs allows one to understand the critical components of normal brain development and function.

High-throughput next-generation sequencing (NGS) allows one to interrogate multiple regions of the genome at once to identify tens of thousands of genetic variants in an individual's genome.<sup>15</sup> These variants can then be filtered bioinformatically using certain criteria, such as absence in control population, allele frequency, predicted pathogenicity, and inheritance model, to narrow down the candidate gene list to a few genes. With NGS, causal variants can be identified in a few weeks, and this has led to a surge in the identification of novel genes as well as new alleles in known disease genes. With these recent advances in genetics, certain MCD-related genes, such as *WDR62* and *DYNC1H1*, have been associated with a broad range of malformations, suggesting that some of these genes are implicated in many developmental stages that are functionally and genetically interdependent.<sup>3</sup>

Improved genomic tools have shed light on the role of de novo mutations in intellectual disability.<sup>16</sup> Although traditionally, de novo mutations were considered to have developed in the egg or the sperm of the unaffected parents, there is increasing evidence of the role of postzygotic (or somatic) de novo mutations in neurologic disorders as well. As these mutations may be present in only a small proportion of the cells in the body, traditional methods of testing using leukocyte-derived DNA have been shown to miss most of these somatic mutations.<sup>17</sup> Some of these mutations may be present only in the affected tissue, and testing of nonaffected tissue, such as blood DNA, may not be informative.<sup>17–19</sup>

### Microcephaly

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#### Primary microcephaly

Primary microcephaly (or microcephaly vera) is defined as the clinical finding of a head circumference less than 3 standard deviations (SD) less than the age- and sex-related mean, which is present at birth and is commonly associated with intellectual disability.<sup>20</sup> Genes known to cause primary microcephaly affect pathways involving

neurogenesis, resulting in decreased number of neurons and smaller brain size.<sup>2</sup> These pathways include cell cycle progression and checkpoint regulation (*MCPH1*, *CENPJ*, *CDK5RAP2*<sup>21</sup>), centrosome duplication (*NDE1*<sup>22</sup>), centrosome maturation (*CDK5RAP2*, *CENPJ*<sup>21</sup>), cell proliferation (*STIL*, *ASPM*<sup>23,24</sup>), mitotic spindle formation (*WDR62*, *NDE1*<sup>25,26</sup>), and DNA repair (*PKNP*, *PCNT*<sup>27,28</sup>). Aberrations in these pathways highlight the important role of centrosome in neuronal proliferation. The centrosome is a key microtubule-organizing center that helps maintain the cellular cytoskeleton and coordinate the segregation of duplicated chromosomes during cell division. Mutations in genes encoding centrosomal proteins, or proteins required for proper chromosomal segregation, account for the largest number of genetic causes of microcephaly and may form a common pathway to regulate neuronal progenitor proliferation.<sup>20</sup>

With the exception of *WDR62* (polymicrogyria [PMG], subcortical heterotopia)<sup>25</sup> and *ARFGEF2* (periventricular nodular heterotopia [PVNH]),<sup>29</sup> primary microcephaly genes do not produce obvious brain anomalies except for simplified gyral pattern and hypoplasia of the corpus callosum.<sup>27,30,31</sup> No definable clinicoradiologic characteristics that separate the different types of microcephaly caused by mutations in different stages of the cell cycle have been identified,<sup>2</sup> suggesting that sequencing panels of genes is an efficient diagnostic approach.

### **Postmigrational microcephaly**

In contrast to primary microcephaly, individuals whose head circumference are normal or slightly small (2 SD below mean) at birth, but develop severe microcephaly in the first 1-2 years are referred to as postmigrational microcephaly.<sup>2</sup> In these individuals, brain growth slows during late gestation or early postnatal period after normal early development. Examples of genes involved in this condition include *CASK* (associated with microcephaly with disproportionate cerebellar and brainstem hypoplasia),<sup>32</sup> *MECP2* (Rett syndrome),<sup>33</sup> and *UBE3A* (Angelman syndrome)<sup>34</sup> and genes of proteins related to protein synthesis, including transfer RNA (tRNA) splicing endonuclease subunit genes such as *TSEN54*, *TSEN2*, and *TSEN34*; aminoacyl-tRNA synthetases such as *RARS2*; and *SEPSECS* associated with pontocerebellar hypoplasias.<sup>2,35</sup> Mutations in *QARS*, a cytoplasmic aminoacyl-tRNA synthetase, has been reported in individuals with progressive microcephaly, intractable seizures, diffuse atrophy of the cerebral cortex, and cerebellar vermis and considerably mild atrophy of the cerebellar hemispheres.<sup>36</sup> As the disruption occurs late in cerebral development, these disorders may someday be good candidates for intervention once the molecular causes have been elucidated.

## **Overgrowth-Related Disorders**

### **Megalencephaly and hemimegalencephaly**

Megalencephaly with PMG is a sporadic overgrowth disorder associated with markedly enlarged brain size (head circumference more than 3 SD), sometimes seen with developmental vascular anomalies, distal limb malformations (polydactyly and syndactyly), and mild connective tissue dysplasia, when it is referred to as megalencephaly-capillary malformation-polymicrogyria (MCAP) syndrome.<sup>37</sup> Hemimegalencephaly, on the other hand, refers to asymmetric brain enlargement that is typically isolated, although it has been reported in association with tuberous sclerosis, hypomelanosis of Ito, and Proteus syndrome.<sup>18</sup> Hemimegalencephaly is associated with dysmorphic immature neurons, neuronal heterotopia, and cortical dyslamination.<sup>18,19</sup>

Exome sequencing and targeted deep sequencing have identified de novo germline and postzygotic (or somatic) point mutations in *AKT3*, *PIK3CA*, and *PIK3R2* in

individuals with MCAP syndrome and hemimegalencephaly.<sup>18,19,38</sup> Somatic CNV increases of chromosome 1q involving *AKT3* have also been identified in individuals with hemimegalencephaly.<sup>19,39</sup> Mutations in *PTEN* also lead to megalencephaly, autism, and tumor predisposition.<sup>40</sup> The phosphoinositide 3-kinases (PI3Ks) are a family of signaling enzymes that regulate a wide range of cellular processes, including growth, proliferation, survival, migration, and brain development. *PTEN* is a tumor suppressor gene that antagonizes the PI3K signaling pathway, whereas AKT kinases are downstream effectors of PI3K signaling and have a critical role in growth regulation. Gain of function mutations or increased copy number of *AKT3* hyperactivates the mammalian target of rapamycin (mTOR) pathway, causing increased cell growth, ribosome biogenesis, and messenger RNA translation. The tuberous sclerosis complex (TSC) genes encode negative regulators of mTOR, so that loss of these genes (in tuberous sclerosis) also leads to hyperactivation of mTOR, leading to overgrowth of normal cells and production of abnormal cells in many organs.<sup>38</sup>

### **Focal cortical dysplasia**

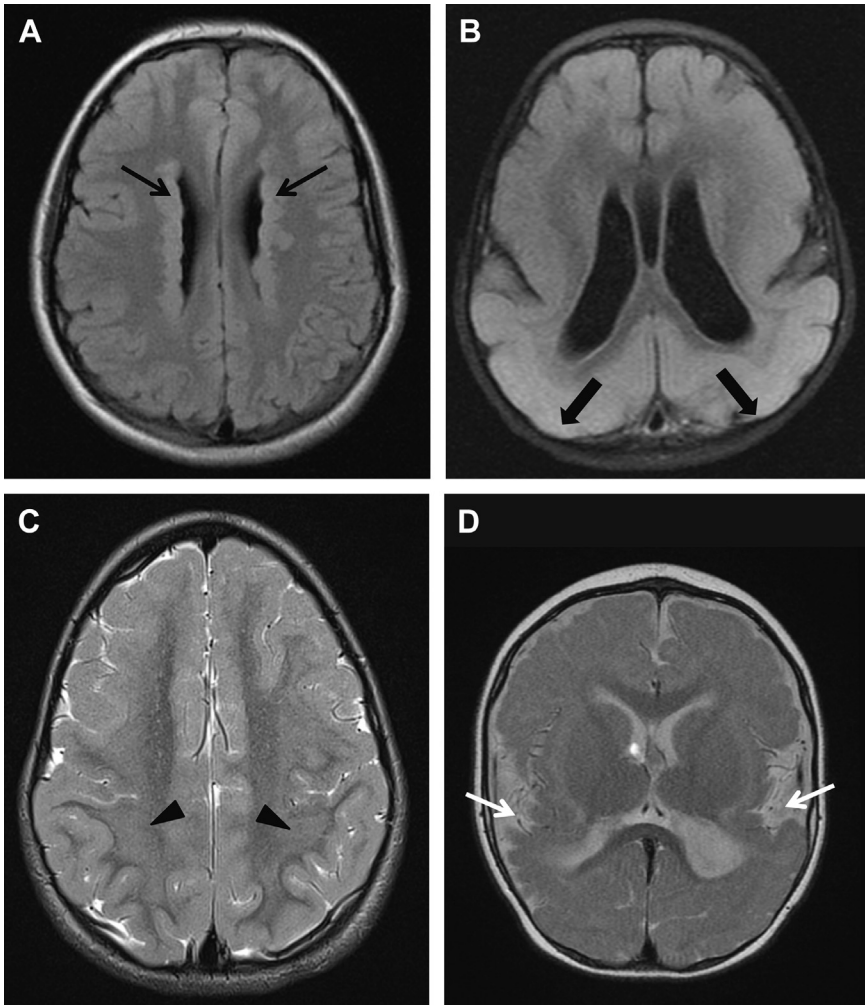
Focal cortical dysplasias (FCDs) are a heterogeneous group of disorders that are characterized by abnormal cortical lamination and defects of neuronal migration, growth, and differentiation involving 1 discrete cortical region, several lobes, or even the entire hemisphere.<sup>41</sup> FCDs are the most common cause of medically refractory epilepsy in the pediatric population. The cause is heterogeneous and can be genetic or environmental. FCDs are classified into 3 groups<sup>42</sup>:

- FCD I: This condition presents with mild symptoms and is often seen in adults, and changes are typically seen in the temporal lobe. Evidence suggests that prenatal and perinatal insults, including extreme prematurity, asphyxia, bleeding, stroke, hydrocephalus, and shaking injury, are commonly observed in patients with FCD I.
- FCD II: Clinical symptoms are more severe with onset typically in childhood. Radiologic changes are seen outside the temporal lobe with predilection for the frontal lobes. Histologic characteristics of FCD II are more homogenous across patients, and evidence points toward genetic mutations that lead to perturbation of the mTOR pathway in the pathogenesis of FCD type IIb.<sup>43</sup> Patients with mutations in *DEPDC5*, which typically cause epilepsy without any imaging abnormality, have been reported with FCD, suggesting a second hit phenomenon, analogous to TSC, with a somatic mutation in the other allele of *DEPDC5* or another gene in the mTOR pathway, causing the malformations in these patients.<sup>44</sup>
- FCD III: This condition is associated with acquired pathology during early development, such as hippocampal sclerosis, vascular malformations, epileptogenic tumor or injury secondary to head trauma, encephalitis, or hypoxic ischemic injury. As seen in FCD IIb, dysregulation of mTOR pathway has been described in certain forms of FCD III.<sup>43</sup>

### **Abnormal Neuronal Migration**

#### **Heterotopia**

Heterotopia can range from PVNH (Fig. 1A) to periventricular linear heterotopia to columnar heterotopia and is secondary to abnormalities of the neuroependyma and failure to initiate migration. Although the exact pathophysiology remains to be elucidated, evidence suggests that injury to the neuroependyma is an important factor in the formation of PVNH. Classic PVNH is associated with mutations in *FLNA*.<sup>13</sup> The phenotype of patients with *FLNA*-related PVNH has been expanded to include a wide spectrum of connective tissue and vascular anomalies, including aortic root dilatation.<sup>45,46</sup> Autosomal recessive forms of PVNH have been described in patients with



**Fig. 1.** Axial magnetic resonance images of the brain showing (A) periventricular nodular heterotopia (black arrows), (B) pachygyria (black arrows), (C) subcortical band heterotopia (arrowheads), and (D) polymicrogyria (white arrows).

mutations in *ARFGEF2*<sup>29</sup> and *C12orf57*.<sup>47,48</sup> Other potential genetic loci for genes associated with PVNH include 7q11.23, 5p15.1, 5p15.33, 5q14.3-q15, and 4p15.<sup>49</sup>

#### **Lissencephaly and subcortical band heterotopia**

Abnormal transmantle migration can lead to agyria (complete absence of gyri), pachygyria (reduced but thickened gyri) (see Fig. 1B), and subcortical band heterotopia or double cortex syndrome (see Fig. 1C). Most cases of lissencephaly are attributable to mutations in *LIS1* (also known as *PFAFH1B1*) and *DCX* and are commonly loss of function alleles. *LIS1* alleles include genic deletions, missense mutations, and nonsense mutations; *DCX* alleles include nonsense mutations, frameshift mutations, genic deletions, and splicing mutations that are distributed across the entire length of the protein, whereas missense mutations cluster predominantly around the 2 functional

microtubule-binding domains and disrupt tubulin binding.<sup>50–53</sup> Germline null mutations in *LIS1* cause classic lissencephaly, whereas germline mutations in *DCX*, which is on the X-chromosome, result in lissencephaly in males and subcortical band heterotopia in females. Somatic mutations in *DCX* and *LIS1*, affecting as few as 10% of leukocytes, have been associated with variable degree of subcortical band heterotopia.<sup>17</sup>

With use of NGS, mutations in additional genes have been identified. These include *TUBA1A* (encoding a neuronal  $\alpha$ -tubulin),<sup>54</sup> *DYNC1H1* (encoding a dynein heavy chain isoform), *KIF2A* (encoding a kinesin heavy chain), *KIF5C* (encoding a member of the kinesin superfamily of proteins), and *TUBG1* (encoding  $\gamma$ -tubulin) and have been reported in patients with milder disease on the lissencephaly spectrum.<sup>17,55</sup> These discoveries highlight the role of cytoskeletal proteins in neuronal migration. In addition, the complex of cytoplasmic dynein with Lis1, Nde1, and Ndel1 has been known to be essential for neuronal migration, and patients with mutations in *NDE1* have been reported in association with microlissencephaly, highlighting the link between microcephaly and lissencephaly.<sup>22</sup>

### **Cobblestone malformations**

Cobblestone malformations are associated with abnormal migration of neurons into the leptomeninges and are a result of deficiencies in the cerebral basement membrane due to defects in O-mannosylation of  $\alpha$ -dystroglycan.<sup>56</sup> This condition leads to abnormal cortical lamination and overmigration of neurons through the incomplete basement membrane into the pial layer.<sup>2</sup> Mutations in multiple genes in the glycosylation pathway have been identified, and mutations in the same gene can cause widely different phenotypes.<sup>57</sup> For example, mutations in *FKRP*, encoding a fukutin-related protein, were initially identified in patients with only severe congenital skeletal muscle defects<sup>58</sup> but since have been identified in patients with milder skeletal system defects<sup>59</sup> and in patients with central nervous system (CNS) malformations and eye involvement.<sup>60</sup> Genes associated with glycosylation within the endoplasmic reticulum (*SRD5A3*)<sup>61</sup> or Golgi apparatus (*ATP6V0A2*)<sup>62</sup> have also been reported in patients with cobblestone malformations.

Dystroglycanopathies can cause a wide range of disorders ranging from isolated brain malformation to intellectual disability with microcephaly and skeletal muscle and eye involvement. These syndromes are commonly referred to as Walker-Warburg syndrome, muscle-eye-brain disease, Fukuyama congenital muscular dystrophy, and congenital muscular dystrophy type 1C and 1D, depending on the extent of tissue involvement.<sup>60</sup> However, elucidation of the genetic underpinnings has prompted a reclassification of the dystroglycanopathies.<sup>57</sup>

Patients with mutations in *GPR56* may also present with cobblestone malformations but do not show a known glycosylation defect. GPR56 is a G protein-coupled receptor that is preferentially expressed in the neuronal progenitor cells of the cerebral cortical ventricular and subventricular zones during periods of neurogenesis but not in the cortical plate or intermediate zone. GPR56 is postulated to regulate cortical patterning, and patients with mutations in *GPR56* have a thin cortex, suggesting a role in cell fate control during neurogenesis.<sup>63</sup>

### **Polymicrogyria**

PMG (see Fig. 1D) refers to a cerebral cortex with many excessively small gyri.<sup>3</sup> The cause of PMG is highly heterogeneous and can be subdivided into two: with schizencephalic clefts likely due to infection or vascular causes and without clefts but with and without associated CNS and non-CNS malformations and with certain types of inborn errors of metabolism (IEM).<sup>2</sup>

### ***Polymicrogyria without clefts***

PMG without clefts can be secondary to a genetic or disruptive process. Isolated PMG is classified by location; however, the genetic cause remains unknown for most of the PMG syndromes.<sup>2</sup> The most common form is bilateral perisylvian PMG, which presents with oromotor dysfunction, intellectual disability, and epilepsy. The clinical presentation of patients with other forms of PMG varies widely and depends on the extent of PMG and presence of other brain malformations, such as cerebellar hypoplasia or microcephaly. PMG can affect cortical areas representing language or primary motor functions, yet these functions can be retained with minimal or no disability.<sup>3</sup>

CNVs, especially deletion of chromosome 1p36.3 and chromosome 22q11.2, have been reported in association with PMG, although the causal genes remain to be identified. Common syndromic associations with PMG include Adams-Oliver syndrome,<sup>64</sup> Joubert syndrome and related disorders,<sup>65</sup> Goldberg-Shprintzen syndrome,<sup>66</sup> Warburg Micro syndrome,<sup>67,68</sup> and Aicardi syndrome.<sup>69</sup> Mutations in genes encoding  $\alpha$ -tubulins, such as *TUBA8*,<sup>70</sup> and  $\beta$ -tubulins, such as *TUBB2B*<sup>71</sup> and *TUBB3*,<sup>72</sup> have been reported in patients with PMG in isolation or in the presence of other brain malformations, including corpus callosum anomalies and optic nerve hypoplasia.

PMG-like cortical malformations have also been reported in patients with IEM, including peroxisomal disorders (such as Zellweger syndrome, neonatal adrenoleukodystrophy), fumaric aciduria, glutaric aciduria type 2, maple syrup urine disease, and mitochondrial diseases.<sup>2,3</sup> However, the pathomechanism of these associations is not well established. Some forms of PMG are also associated with the megalencephalic conditions and cobblestone disorders described earlier, and so those genes should be considered in the differential genetic diagnosis.

### ***Schizencephaly***

In schizencephaly, the cortex edges can be fused (closed lip) or remain at a distance (open lip) and may be unilateral or bilateral. Patients with closed-lip unilateral schizencephaly may present with hemiparesis or motor delay, whereas patients with open-lip schizencephaly present with hydrocephalus or seizures.<sup>3</sup> Histologically, the cortex surrounding the cleft shows loss of laminar architecture, forming irregular heterotopic aggregates of gray matter. Although there was initial evidence of role of mutations in *EMX2* as a cause of schizencephaly,<sup>73</sup> subsequent analysis has not further confirmed this,<sup>74,75</sup> and current understanding supports a nongenetic cause for most cases, likely infection (commonly cytomegalovirus)<sup>76</sup> or vascular event.<sup>77</sup> In addition, young maternal age and monozygotic twin pregnancies have been associated with higher incidence of schizencephaly.<sup>77</sup>

## **DIAGNOSTIC STRATEGY**

### ***Brain Imaging***

In patients presenting with clinical features suggestive of MCD, diagnostic imaging with MRI is recommended to delineate the type of MCD.<sup>3</sup> The key features to look for include distribution and severity of MCD, the cortical surface and border between white and gray matter, cortical thickness, and any other associated brain malformations (such as anomalies of the corpus callosum, brainstem, and cerebellum). Identification of the type of MCD allows the clinician to focus on the malformation-relevant genes.

### ***Tissue Consideration***

Leukocyte-derived DNA from peripheral blood is the most readily accessible tissue for genetic analysis in the clinic and can be used to detect any inherited or de novo germline genomic variants. However, in patients who present with a specific radiologic



phenotype but show negative results on testing for the known malformation-related genes, it is important to consider the role of somatic mutations. In this scenario, DNA derived from buccal swabs has been shown to be more effective in detecting these mutations.<sup>38,78</sup> However, some mutations require direct examination of the affected tissue (in this case, brain), which can be obtained from patients undergoing resection of the affected tissue, for example, for epilepsy surgery.<sup>18,19</sup>

## GENETIC TESTING

### *Single Nucleotide Variants*

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In cases in which 1 or 2 genes are known to be the predominant cause of the MCD phenotype, for example, *LIS1* and *DCX* for lissencephaly and *FLNA* for PVNH, targeted Sanger sequencing of these genes may still be the best approach, although targeted panels are increasingly the first-line test. Given the known genetic heterogeneity of the MCD, such as in pachygyria or PMG, targeted gene panels are useful and cost-effective, by efficiently analyzing multiple genes at once. An alternative strategy is to perform whole exome sequencing (WES), which is the process of sequencing the coding regions of the entire genome in 1 reaction and has been shown to improve diagnostic yield to 25% in undiagnosed cases with mendelian disorder.<sup>79</sup> However, one advantage of targeted gene panel sequencing is that the coverage of the genes of interest is more uniform than in WES. Another advantage is that targeted gene panel obviates the issue related to incidental findings detected on WES (such as mutations in a *BRCA1*, which may place the patient at risk for breast cancer in the future but are not related to the primary phenotype).<sup>80</sup> Lastly, targeted gene panel sequencing also allows for deeper coverage, which in turn is more likely to detect low-frequency somatic mutations.<sup>17</sup>

### *Copy Number Variants*

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CNVs have been associated with certain forms of MCDs, including PMG. Traditionally, these CNVs were detected by cytogenetic analysis with karyotype and fluorescence in situ hybridization (FISH) analysis for specific regions of the genome. However, karyotype analysis has a resolution of approximately 5 megabasepairs, and CNVs smaller than this are not detectable by this method. FISH is specific only for certain regions (eg, 22q11.2) but may be costly and laborious when probing multiple regions across the genome. The advantage of karyotype analysis and FISH is that it provides structural information and can detect translocation. Translocations that disrupt genes of interest have been paramount in mapping of disease-related genes during the past 2 decades.

Chromosome microarray analysis (CMA) allows a clinician to detect submicroscopic CNVs across the genome. The diagnostic yield of CMA in patients with neurologic disorders is about 10% to 15%, and CMA has replaced karyotype analysis as the first-tier test in the evaluation of a child with multiple congenital anomalies, developmental delay, or autism spectrum disorders.<sup>81</sup> Certain forms of CMA using single nucleotide polymorphisms allow for detection of homozygosity in individuals with shared ancestry or consanguinity and can aid in narrowing the list of candidate genes.<sup>82</sup>

## CURRENT MANAGEMENT OF THE DISEASE

### *Investigations*

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Brain imaging with MRI is the first step in managing any patient who presents with signs and symptoms of MCD. If the patient presents with seizures, electroencephalography is prudent to detect any epileptogenic focus, which may be amenable to surgical resection. Other imaging modalities include diffusor tensor imaging, which can be

used to better characterize the perturbation in brain development by evaluating the neuronal tracts,<sup>83</sup> and functional MRI, including magnetoencephalography, which can be used to map brain activity and localize regions affected by pathology.<sup>84</sup>

### **Management**

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The treatment of these individuals is predominantly symptomatic. Developmental delay is managed with neurorehabilitation, including physical and occupational therapy and speech and feeding therapy. Learning disability should be managed based on the severity of learning disability and neurocognitive delay; this could range from additional help in regular school to special education classrooms. Patients with seizures need to be managed with appropriate antiepileptic medications, under the guidance of a neurologist. Occasionally, patients with focal epileptogenic focus may benefit from surgical resection.<sup>85</sup>

Genetic counseling should be provided to individuals in whom a genetic cause is identified and their families and even in those who do not have an identifiable cause but the lesion is known to be genetic, through a referral to a clinical geneticist or genetic counselor. In X-linked disorders, such as *DCX*, *FLNA*, and *ARX*, the carrier mother may be completely asymptomatic and has a 50% risk of having another affected child. Similarly, for disorders inherited in an autosomal recessive manner, the couple has a 25% risk of having another affected child and 50% risk of having an unaffected but carrier child. For disorders with dominant inheritance, both parents should be assessed carefully with detailed physical examination and pertinent investigations, as some of these diseases can have variable expression even within a family. If the parents are affected, albeit mildly, they have a 50% risk of having another affected child. However, if the parents are unaffected, the risk of them being mosaic carriers for the apparent de novo mutations is approximately 4%.<sup>86</sup> In families with known molecular cause, prenatal testing in the form of chorionic villus sampling or amniocentesis can be offered to guide subsequent pregnancies. Preimplantation genetic diagnosis may also be an option for families in which the pathogenic variant has been identified.

### **FUTURE TREATMENT APPROACHES**

The understanding of the genes and pathways associated with MCDs is expanding rapidly. For example, identification of somatic mutations in the *PI3K-AKT-mTOR* pathway in patients with overgrowth-related disorders offers potential opportunity for pharmacologic intervention for these disorders, although this remains untested.<sup>41</sup> *mTOR* encodes the mammalian target for rapamycin and is used commonly as an immunosuppressant. The antiepileptic effects of rapamycin have been evaluated in animal models of cortical dysplasia. For example, in mice with inactivated *TSC1*, rapamycin prevents epilepsy when given early and ameliorates seizure activity when given at a later stage.<sup>87</sup> In patients, administration of rapamycin has been demonstrated to show reduction in the duration and frequency of seizures in a child with TSC<sup>88</sup> and has been associated with reduction in the size of the subependymal giant-cell astrocytomas in patients with TSC.<sup>89</sup>

Similarly, patch clamp recordings from dysplastic neurons from patients with FCD type IIb show excitatory responses of  $\gamma$ -aminobutyric acid type A receptors that are significantly attenuated by the SLC12A2 inhibitor bumetanide,<sup>90</sup> which may justify trials with bumetanide in patients with FCD administered anticonvulsants that increase GABAergic function.<sup>3</sup>

With advances in genomic technology, the understanding of the molecular basis of these MCDs, including the diversity within each MCD and the associated secondary

phenotypes, will continue to improve, which will allow for more rational and targeted treatment options. Identification of pathogenic variant can also allow for prenatal testing to guide future pregnancies in these families.

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