Genetic Basis of Brain Malformations

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Abstract

Malformations of cortical development (MCD) represent a major cause of developmental disabilities, severe epilepsy, and reproductive disadvantage. Genes that have been associated to MCD are mainly involved in cell proliferation and specification, neuronal migration, and late cortical organization. Lissencephaly-pachygyria-severe band heterotopia are diffuse neuronal migration disorders causing severe global neurological impairment. Abnormalities of the *LIS1, DCX, ARX, RELN, VLDLR, ACTB, ACTG1, TUBG1, KIF5C, KIF2A,* and *CDK5* genes have been associated with these malformations. More recent studies have also established a relationship between lissencephaly, with or without associated microcephaly, corpus callosum dysgenesis as well as cerebellar hypoplasia, and at times, a morphological pattern consistent with polymicrogyria with mutations of several genes *(TUBA1A, TUBA8, TUBB, TUBB2B, TUBB3,* and *DYNC1H1)*, regulating the synthesis and function of microtubule and centrosome key components and hence defined as tubulinopathies. MCD only affecting subsets of neurons, such as mild subcortical band heterotopia and periventricular heterotopia, have been associated with abnormalities of the *DCX, FLN1A,* and *ARFGEF2* genes and cause neurological and cognitive impairment that vary from severe to mild deficits. Polymicrogyria results from abnormal late cortical organization and is inconstantly associated with abnormal neuronal migration. Localized polymicrogyria has been associated with anatomo-specific deficits, including disorders of language and higher cognition. Polymicrogyria is genetically heterogeneous, and only in a small minority of patients, a definite genetic cause has been identified. Megalencephaly with normal cortex or polymicrogyria by MRI imaging, hemimegalencephaly and focal cortical dysplasia can all result from mutations in genes of the PI3K-AKT-mTOR pathway. Postzygotic mutations have been described for most MCD and can be limited to the dysplastic tissue in the less diffuse forms.

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The development of the human cerebral cortex is a complex dynamic process that occurs during several gestational weeks [Gleeson and Walsh, 2000]. During the first stage, stem cells proliferate and differentiate into young neurons or glial cells deep in the forebrain, in the ventricular and subventricular zones lining the cerebral cavity. During the second stage, cortical neurons migrate away from their place of origin: most cells migrate along the radial glial fibers from the periventricular region towards the pial surface, where each successive generation passes one another and settles in an inside-out pattern

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within the cortical plate. When neurons reach their final destination, they stop migrating and order themselves into specific 'architectonic' patterns guiding cells to the correct location in the cerebral cortex. This third phase involves the final organization within the typical 6 layers of the cortex, associated with synaptogenesis and apoptosis [Barkovich et al., 2012].

Abnormal cortical development is increasingly recognized as a cause of developmental disabilities and epilepsy. This recognition is largely due to improved magnetic resonance imaging (MRI) resolution, which makes it possible to assess the distribution and depth of cortical sulci, cortical thickness, the boundaries between grey and white matter, and variations in signal intensity. Abnormalities of any or all of these features may be observed in different malformations of cortical development (MCD), which may be restricted to discrete cortical areas or may, alternatively, be diffuse [Guerrini et al., 2008; Guerrini and Dobyns, 2014].

A classification scheme has been developed categorizing MCD into 3 major groups that recapitulate the main developmental steps as malformations of cell proliferation, neuronal migration, or postmigrational cortical organization and connectivity. Although the classification of MCD has advanced substantially during the past decade, in practice only a few categories are used, including lissencephaly (LIS), polymicrogyria, schizencephaly, focal cortical dysplasia (FCD), and periventricular nodular heterotopia (PNH). However, emerging evidence suggests that MCD are far more heterogeneous than this classification suggests [Guerrini and Dobyns, 2014].

So far, more than 100 genes have been associated with one or more types of MCD. The biological pathways include cell-cycle regulation at many steps (especially mitosis and cell division), apoptosis, cell-fate specification, cytoskeletal structure and function, neuronal migration and basement-membrane function, and many inborn errors of metabolism. A subset of MCD genes – especially those associated with megalencephaly – are associated with postzygotic (i.e., mosaic) mutations [Lee et al., 2012].

Genetic testing needs accurate assessment of imaging features and familial distribution, if any, and can be straightforward in some disorders but requires a complex diagnostic algorithm in others. Because of substantial genotypic and phenotypic heterogeneity for most of these genes, a comprehensive analysis of clinical, imaging, and genetic data is needed to properly define these disorders. Exome sequencing and ultra-high field MRI are rapidly modifying the classification of these disorders [Guerrini and Dobyns, 2014].

In this themed issue dedicated to the genetic of epilepsy, we limited our contribution to those malformations that have more frequently been associated with epilepsy.

Lissencephaly and Subcortical Band Heterotopia

LIS or smooth brain and the associated malformation known as subcortical band heterotopia (SBH) are the classic malformations associated with deficient neuronal migration [Dobyns et al., 2012].

LIS (from the Greek words 'lissos' meaning smooth and 'enkephalos' meaning brain) is a neuronal migration disorder characterized by absent (agyria) or decreased (pachygyria) convolutions, cortical thickening, and a smooth cerebral surface (fig. 1A) [Barkovich et al., 2012; Guerrini and Dobyns, 2014]. Different subtypes of LIS are readily distinguished based on the number of cortical layers affected and include 2-layered, 3-layered, and 4-layered forms [Forman et al., 2005]. The most common, classical LIS (4-layered form), features a very thick cortex (10–20 mm vs. the normal 4 mm) and no other major brain malformations. The cytoarchitecture consists of 4 primitive layers, including an outer marginal layer, which contains Cajal-Retzius neurons (layer 1); a superficial cellular layer, which contains numerous large and disorganized pyramidal neurons (layer 2) corresponding to the true cortex, a variable cell-sparse layer (layer 3), and a deep cellular layer (composed of medium and small neurons), which extends more than half the width of the mantle (layer 4) [Golden and Harding, 2004]. SBH is a related disorder in which bands of grey matter are interposed in the white matter between the cortex and the lateral ventricles (fig. 1B) [Guerrini and Parrini, 2010]. Histopathology demonstrates that heterotopic neurons settle close to the cortex in a pattern suggestive of laminar organization.

Brain Imaging
The severity of LIS and SBH varies from complete or nearly complete agyria (grades 1 and 2), to mixed agyria-pachygyria (grade 3), to pachygyria only (grade 4), to mixed pachygyria-SBH (grade 5), and finally to SBH only (grade 6) [Dobyns et al., 2012]. Based on genetic findings, the full range of LIS now extends from severe LIS with cerebellar hypoplasia to classic LIS to SBH, and also includes a polymicrogyria-like cortical malformation that can be distinguished from both LIS and typical polymicrogyria by high-resolution brain imaging.
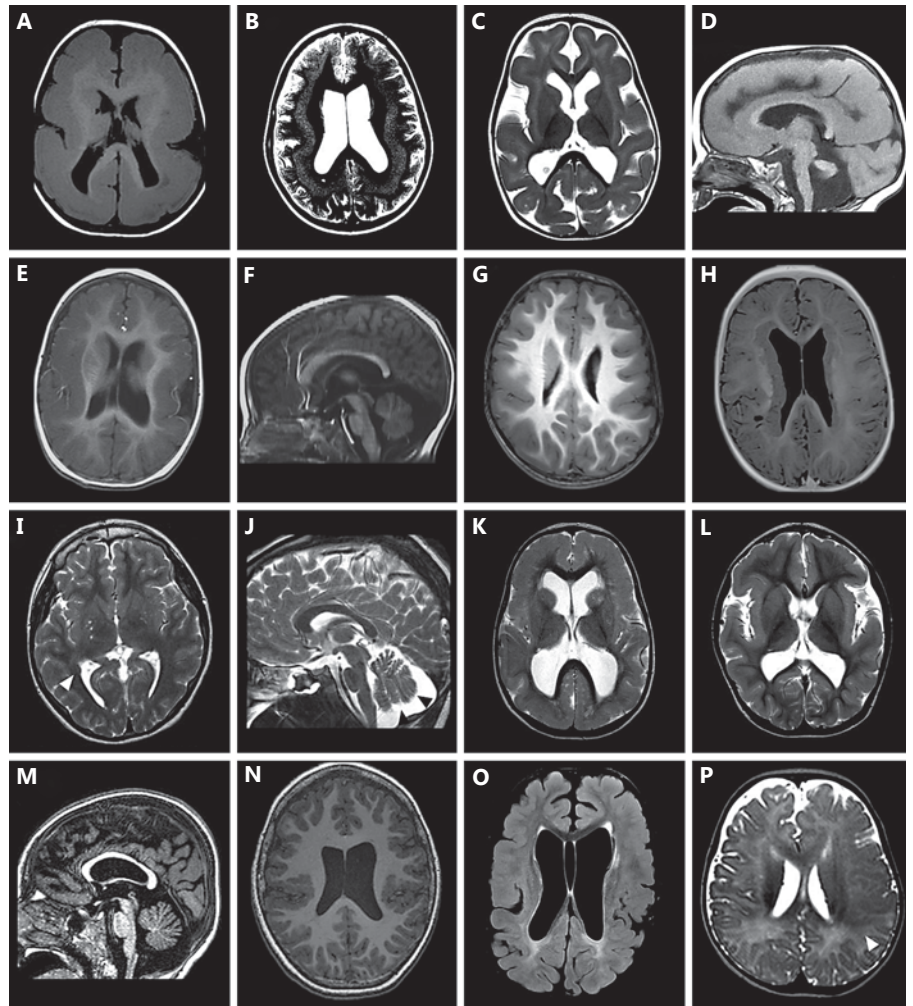
Fig. 1. Brain MRI of patients with different malformations of the cerebral cortex. **A** T1-weighted axial section. Posterior to anterior pachygyria in a boy with *LIS1* mutation. **B** T2-weighted axial section. Diffuse SBH in a girl with *DCX* mutation. **C, D** T2-weighted axial section and T1-weighted sagittal section. Lissencephaly and cerebellar hypoplasia in a girl with *RELN* mutation. **E, F** T1-weighted axial section and T1-weighted sagittal section. Thickened cortex with simplified gyral pattern and cerebellar hypoplasia in a girl with *TUBA1A* mutation. **G** T1-weighted axial section. Diffuse simplified gyral pattern with prominent thickening and infolding of the sylvian fissures in a boy with *TUBB2B* mutation. **H** T1-weighted axial section. Typical, classical bilateral PNH in a girl with an *FLNA* mutation. Bilateral nodules of subependymal heterotopia are contiguous and rather symmetric, extensively lining the ventricular walls. **I, J** T2-weighted axial section showing mild colpocephaly with uni- lateral PNH (white arrowhead) and T2-weighted sagittal section through the midline, showing cerebellar vermis hypoplasia (black arrowhead) with mega cisterna magna in a patient carrying a deletion in the 6q27 chromosomal region. **K** T2-weighted axial section. Bilateral frontoparietal polymicrogyria in a boy with *GPR56* mutation. **L, M** T2-weighted axial section and T1-weighted coronal section. Pachygyria and perisylvian polymicrogyria in a girl with *DYNC1H1* mutation. **N** Axial T1-weighted section in a patient with a mosaic *PIK3R2* mutation. **O, P** T1-weighted and T2-weighted axial images form patients carrying mosaic mutations in the *MTOR* gene with different percentages of mosaicism [**O**: p.Thr1977Ile, 20% of mosaicism in blood, **P**: p.Ser2215Phe, 5.5% of mosaicism in dysplastic brain tissue) showing bilateral cortical dysgyria (**O**) and focal cortical dysplasia (**P**, white arrowhead)].

Clinical Features

Children with the most common types of LIS (or SBH) typically appear normal as newborns. Most affected children come to medical attention during the first year of life due to neurological deficits in the first weeks or months [Barkovich et al., 2012]. The major medical problems encountered are ongoing feeding problems and epilepsy of many different types that are often intractable.

Classical LIS is rare, with a prevalence of about 12 per million births. Patients with severe LIS have early devel-

opmental delay, early diffuse hypotonia, later spastic quadriplegia, and eventual severe or profound mental retardation. Seizures occur in over 90% of LIS children, with onset before 6 months in about 75% of the cases [Guerrini and Filippi, 2005]. Between 35 and 85% of children with classic LIS develop infantile spasms, often without classic hypsarrhythmia. Most LIS children will subsequently continue to have epileptic spasms in association with other seizure types. The EEGs show diffuse, high-amplitude, fast rhythms, which are considered to be highly specific for this malformation.

The main clinical manifestations of SBH are mental retardation and epilepsy [Barkovich et al., 1994]. Epilepsy is present in almost all patients and is intractable in about 65% of the cases. About 50% of these epilepsy patients have focal seizures, and the remaining 50% have generalized epilepsy, often within the spectrum of Lennox-Gastaut syndrome. Those with more severe MRI abnormalities have significantly earlier seizure onset and are more likely to develop Lennox-Gastaut syndrome [Guerrini and Filippi, 2005].

Children with some LIS syndromes (especially Miller-Dieker syndrome, and severe forms of LIS with cerebellar hypoplasia or the X-linked syndrome of LIS with abnormal genitalia) have a severe course, and the most severe forms have high mortality rates. However, these data do not apply to children with less severe forms of LIS, SBH, or LIS with cerebellar hypoplasia, as all of these disorders are associated with better motor and cognitive function and longer survival [Dobyns et al., 2012].

Genetic Basis and Diagnosis

LIS, SBH, and LIS with cerebellar hypoplasia always have a genetic cause. Studies to date have identified 14 *LIS* genes (table 1), which account for roughly 90% of patients. However, 2 major genes have been associated with classic LIS and SBH. The *LIS1* gene causes the autosomal form of LIS [Reiner et al., 1993], while the *DCX* gene is X linked [des Portes et al., 1998; Gleeson et al., 1998]. Although each gene can result in either LIS or SBH, most cases of classic LIS are due to deletions or mutations in the *LIS1* gene [Mei et al., 2008], whereas most cases of SBH are due to mutations in the *DCX* gene [Matsumoto et al., 2001]. *LIS1* encodes a 45-kDa protein (PAFAH1B1), which functions as a regulatory subunit of platelet-activating factor acetylhydrolase (PAF-AH) [Hirotsune et al., 1998]. PAFAH1B1 heterozygous mutant mice show a dose-dependent histopathological disorganization of cortical lamination as well as hippocampal and cerebellar cortical defects [Hirotsune et al., 1998].

DCX encodes a 40-kDa microtubule-associated protein (DCX) that is expressed in migrating neuroblasts [Gleeson et al., 2000a]. The DCX protein contains 2 tandem-conserved repeats. Each repeat binds to tubulin, and both repeats are necessary for microtubule polymerization and stabilization.

LIS1-related LIS is more severe in the posterior brain regions (posterior-to-anterior gradient), whereas *DCX*-related LIS is more severe in the anterior brain (anterior-to-posterior gradient). About 60% of patients with posterior-to-anterior isolated LIS carry genomic alterations or mutations involving *LIS1* [Mei et al., 2008]. A simplified gyral pattern in the posterior brain, with underlying SBH, has been associated with mosaic mutations of *LIS1* [Sicca et al., 2003]. Miller-Dieker syndrome is characterized by severe 4-layered LIS with diffuse agyria and no clear gradient, a typical facial appearance (prominent forehead, bitemporal hollowing, short nose with upturned nares, protuberant upper lip and a small jaw), and other birth defects (e.g., heart malformations) [Cardoso et al., 2003]. Miller-Dieker syndrome is caused by a deletion in *LIS1* and contiguous genes in the 17p13.3 region. Deletion of 2 additional genes, *CRK* and *YWHAE*, telomeric to *LIS1*, may contribute to the most severe LIS grade and dysmorphic features [Cardoso et al., 2003].

Most *DCX* mutations cause anterior-to-posterior SBH/pachygyria. Mutations in *DCX* have been found in all reported pedigrees and in 80% of sporadic females and 25% of sporadic males with SBH [Matsumoto et al., 2001]. Genomic deletions in the *DCX* gene have been identified in females with sporadic SBH and in males with X-linked LIS [Mei et al., 2007]. Maternal germline or mosaic *DCX* mutations may occur in about 10% of the cases of either SBH or X-linked LIS [Gleeson et al., 2000b]. Hemizygous males with *DCX* mutations have classical LIS, but rare boys with missense mutations and anteriorly predominant SBH and rare females with *DCX* mutations and normal brain MRI have been described [Guerrini et al., 2003]. Rarer forms of LIS have been identified.

X-linked LIS with absent corpus callosum and ambiguous genitalia (XLAG) results from mutations in the *ARX* gene [Kato et al., 2004]. Affected patients show LIS with a posterior-to-anterior gradient, absent corpus callosum, a moderate increase of cortical thickness (only 6–7 mm), atrophic striatal and thalamic nuclei, postnatal microcephaly, neonatal-onset epilepsy, hypothalamic dysfunction including deficient temperature regulation, chronic diarrhea, and ambiguous genitalia with micropenis and cryptorchidism. Most XLAG patients die within one year after birth [Okazaki et al., 2008].

Table 1. Genes and chromosomal loci associated with MCD

Cortical malformation	Pattern of inheritance	Gene	Locus	OMIM
Lissencephaly				
MDS	AD	*LIS1*	17p13	601545
ILS or SBH	AD	*LIS1*	17p13.3	601545
ILS or SBH	X-linked	*DCX*	Xq22.3q23	300121
Pachygyria and Baraitser-Winter syndrome	AD	*ACTB*	7p22.1	102630
Pachygyria and Baraitser-Winter syndrome	AD	*ACTG1*	17q25.3	102560
ILS or SBH	AD	*TUBA1A*	12q12q14	602529
XLAG	X-linked	*ARX*	Xp22.13	300382
LIS cerebellar hypoplasia	AR	*RELN*	7q22	600514
LIS cerebellar hypoplasia	AR	*VLDR*	9p24.2	192977
LIS cerebellar hypoplasia	AR	*CDK5*	7q36.1	123831
Posterior predominant pachygyria	AD	*DYNC1H1*	14q32.31	600112
Posterior predominant agyria/pachygyria	AD	*KIF2A*	5q12.1	602591
ILS	AD	*TUBA1A*	17q13.12	602529
ILS	AD	*TUBB2B*	6p25.2	612850
Posterior predominant pachygyria	AD	*TUBG1*	17q21.2	191135
Classic LIS with variable gradient[a]	AD	*KIF5C*	2q23.1	604593
Periventricular nodular heterotopia				
Classical bilateral PNH	X-linked	*FLNA*	Xq28	300017
Ehlers-Danlos syndrome and PNH	X-linked	*FLNA*	Xq28	300017
Facial dysmorphisms, severe constipation and PNH	X-linked	*FLNA*	Xq28	300017
Fragile-X syndrome and PNH	X-linked	*FMR1*	Xq27.3	309550
Microcephaly and PNH	AR	*ARFGEF2*	20q13.13	605371
ACC, PMG and PNH	AD	*ERMARD*	6q27	615532
PNH and Van Maldergem syndrome type 2	AR	*FAT4*	4q28.1	612411
PNH and Van Maldergem syndrome type 1	AR	*DCHS1*	11p15.4	603057
Donnai-Barrow syndrome and PNH	AR	*LRP2*	2q24q31	600073
PNH with limb abnormalities (limb reduction abnormality or syndactyly)	X-linked	–	Xq28	
Williams syndrome and PH	AD	–	7q11.23	
PH	AD	–	5p15.1	
PH	AD	–	5p15.33	
PH	AD	–	6p25	
PH	AD	–	4p15	
PH	AD	–	5q14.3q15	
PH	AD	–	22q11	
PH and steroid sulfatase deficiency	AD	–	Xp22.3	
PH	AD	–	Xp22.11	
PH and Smith-Magenis syndrome	AD	–	17p11.2	
ACC and PNH	AD	–	1p36.22pter	
Polymicrogyria				
Bilateral frontoparietal PMG	AR	*GPR56*	16q13	604110
Asymmetric PMG	AD	*TUBB2B*	6p25.2	612850
PMG and rolandic seizures, oromotor dyspraxia	X-linked	*SRPX2*	Xq21.33q23	300642
PMG and ACC, microcephaly	AD	*TBR2*	3p21.3p21.2	604615
PMG and aniridia	AD	*PAX6*	11p13	607108
PMG and microcephaly	AR	*NDE1*	16p13.11	609449
PMG and microcephaly	AR	*WDR62*	19q13.12	613583
PMG and fumaric aciduria	AR	*FH*	1q43	136850
PMG and 'band-like calcfication'	AR	*OCLN*	5q13.2	602876
Perysilvian PMG and CHARGE syndrome	AD	*CHD7*	8q12.1q12.2	608892
PMG and Warburg Micro syndrome	AR	*RAB3GAP1*	2q21.3	602536
PMG and Warburg Micro syndrome	AR	*RAB3GAP2*	1q41	609275
PMG and Warburg Micro syndrome	AR	*RAB18*	10p12.1	602207

Table 1 (continued)

Cortical malformation	Pattern of inheritance	Gene	Locus	OMIM
PMG-like, microcephaly, ACC	AD	*DYNC1H1*	14q32.31	600112
PMG-like, microcephaly, ACC, CBLH	AD	*TUBA1A*	17q13.12	602529
PMG-like, microcephaly, ACC, CBLH	AR	*TUBA8*	22q11.21	605742
PMG-like, microcephaly, ACC, CBLH	AD	*TUBB3*	16q24.3	602661
PMG-like, microcephaly, ACC, CBLH	AD	*TUBB*	6p21.33	191130
PMG-like, microcephaly, ACC	AR	*EOMES*	3p24.1	604615
PMG and Goldberg-Shprintzen syndrome	AR	*KIAA1279*	10q21.3	609367
PMG and CK syndrome	X-linked	*NSDHL*	Xq28	300275
PMG and CEDNIK syndrome	AR	*SNAP29*	22q11.21	604202
PMG and Knobloch syndrome	AR	*COL18A1*	21q22.3	120328
PMG	AD	–	1p36.3pter	
PMG and microcephaly	AD	–	1q44qter	
PMG and facial dysmorphisms	AD	–	2p16.1p23	
PMG and microcephaly, hydrocephalus	AD	–	4q21q22	
PMG	AD	–	21q2	
PMG	AD	–	6q26q27	
PMG	AD	–	13q3	
PMG	AD	–	18p11	
PMG and Di George syndrome	AD	–	22q11.2	
Bilateral perysilvian PMG	AD	*PIK3R2*	19p13.11	603157
Bilateral temporooccipital PMG	AR	*FIG4*	6q21	609390
PMG and microcephaly	AR	*RTTN*	18q22.2	610436
Megalencephaly-polymicrogyria and dysplastic megalencephaly				
MPPH, DMEG	AD	*AKT3*	1q43q44	611223
Weaver syndrome	AD	*EZH2*	7q36.1	601573
MCAP	AD	*PIK3CA*	3q26.32	171834
MPPH	AD	*PIK3R2*	19p13.11	603157
FCD	Somatic	*MTOR*	1p36.22	
FCD	AD	*DEPDC5*	22q12.2q12.3	604364
FCD	Somatic	*AKT3*	1q43q44	
FCD	AD	*NPRL3*	16p13.3	

AD = Autosomal dominant; AR = autosomal recessive; ACC = agenesis of the corpus callosum; CBLH = diffuse cerebellar hypoplasia; DMEG = dysplastic megalencephaly; ILS = isolated LIS; MCAP = megalencephaly-capillary malformation syndrome; MDS = Miller-Dieker syndrome; MPPH = megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome; PH = periventricular heterotopia; PMG = polymicrogyria; – = not known.
ª Posterior to anterior or anterior to posterior.

Autosomal recessive LIS with cerebellar hypoplasia consists of mild frontal predominant LIS, plus severe hippocampal and cerebellar hypoplasia and dysplasia. This pattern has been associated with mutations in *RELN* [Hong et al., 2000] or *VLDLR* (an essential cell-surface receptor for reelin) (fig. 1C, D) [Boycott et al., 2005].

Mutations in the *ACTB* and *ACTG1* genes are associated with Baraitser-Winter cerebrofrontofacial syndrome which is characterized by the combination of hypertelorism, a broad nose with a large tip and prominent root, congenital nonmyopathic ptosis, ridged metopic suture, arched eyebrows, iris or retinal coloboma, sensorineural deafness, shoulder girdle muscle bulk, and progressive joint stiffness [Rivière et al., 2012a; Di Donato et al., 2014; Verloes et al., 2015]. Many patients with *ACTB* and *ACTG1* mutations have pachygyria with an anterior-to-posterior severity gradient, similar to that observed in males with *DCX* mutations [Rivière et al., 2012a; Verloes et al., 2015].

A mutation in the *CDK5* gene has been identified in 4 individuals with LIS and cerebellar hypoplasia born from a highly consanguineous family [Magen et al., 2015].

Laboratory Investigations and Genetic Counseling

In patients with classic LIS, cytogenetic and molecular investigations are part of the diagnostic process. When Miller-Dieker syndrome is suspected, array-CGH analysis is indicated. When isolated LIS is diagnosed, careful assessment of the antero-posterior gradient of the abnormal cortical pattern will suggest whether to investigate *LIS1* or *DCX*. When LIS is more severe posteriorly, it is worth performing MLPA first to rule out *LIS1* deletions/duplications. If a deletion/duplication is not found, *LIS1* sequencing should then be performed. In boys whose MRI shows more severe pachygyria in the frontal lobes, sequencing of the *DCX* gene is indicated. In patients with SBH, direct sequencing of *DCX* should be performed. If a *DCX* mutation is not found, MLPA analysis then should be performed. Direct sequencing is also indicated in the mothers of patients harboring a *DCX* mutation or other female relatives. Analysis of the *ACTB* and *ACTG1* genes should be performed in patients with frontal predominant pachygyria who are not harboring *DCX* mutations.

All reported *LIS1* alterations are de novo. Given the theoretical risk of germline mosaicism in either parent (which has never been demonstrated for *LIS1*), a couple with a child with LIS is usually given a 1% recurrence risk. When a *DCX* mutation is found in a boy with LIS, mutation analysis of *DCX* should be extended to the proband's mother, even if her brain MRI is normal. If the mother is a mutation carrier, the mutation will be transmitted according to Mendelian inheritance. If the mother is not a carrier, there still is a risk of germline mosaicism, which is roughly estimated ~5%.

Tubulinopathies and Related Disorders

Mutations of tubulin genes were first reported as causing LIS (for *TUBA1A*) [Keays et al., 2007] or polymicrogyria (for *TUBB2B*) [Jaglin et al., 2009]. However, the cortical malformations seen in most individuals with mutations of tubulin or tubulin motor genes comprise a wide spectrum of morphological abnormalities whose characteristics are at times distinctive but can also overlap with those of LIS and polymicrogyria by brain imaging and neuropathology [Cushion et al., 2013; Poirier et al., 2013]. The full range of these malformations vary from extreme LIS with completely absent gyri, total agenesis of the corpus callosum, and severe cerebellar hypoplasia; to less severe LIS with moderate-to-severe cerebellar hypoplasia; to classic LIS, and to an atypical polymicrogyria-like cortical malformation with cerebellar hypoplasia [Cushion

et al., 2013; Poirier et al., 2013]. Cortical thickness varies and can be mildly thin, normal, or mildly thick. Most children with mutations of tubulin genes have severe intellectual disability and intractable seizures.

Genetic Basis and Diagnosis

Nine genes (*KIF2A*, *KIF5C*, *TUBA1A*, *TUBA8*, *TUBB*, *TUBB2B*, *TUBB3*, *TUBG1*, and *DYNC1H1*; table 1) associated with tubulinopathies have been identified so far [Bahi-Buisson et al., 2014]. Findings from functional studies suggest that abnormal brain development in tubulinopathies results from a dominant negative effect of heterozygous missense mutations (in the absence of loss-of-function mutations) on the regulation of microtubule-dependent mitotic processes in progenitor cells, and on the trafficking activities of the microtubule-dependent molecular motors KIF2A, KIF5C, and DYNC1H1 in postmitotic neuronal cells [Poirier et al., 2013].

The most severe phenotype of tubulinopathies consists of LIS with cerebellar and brainstem hypoplasia. These children all have profound deficits, intractable epilepsy and short survival. Heterozygous missense mutations in the *TUBA1A* gene have been found in this syndrome, with *TUBA1A* accounting for ~30% of the patients (fig. 1E–G) [Kumar et al., 2010; Cushion et al., 2013]. A less severe phenotype consists of moderate LIS with cerebellar hypoplasia with pachygyria, with callosal defects and moderate hypoplasia of the brainstem and cerebellum [Morris-Rosendahl et al., 2008; Kumar et al., 2010]. A third phenotype has isolated LIS sequence with posterior predominant LIS matching the *LIS1* pattern [Kumar et al., 2010]. Finally, some patients exhibit polymicrogyria plus variable agenesis of the corpus callosum as well as constant hypoplasia of the brainstem and cerebellum [Kumar et al., 2010].

Laboratory Investigation and Genetic Counseling

Testing is available for all of the tubulin and tubulin motor genes so far identified. For all but *TUBA8*, only de novo heterozygous missense mutations have been found. Several families with 2 affected sibs have been reported, explained by low-level gonadal mosaicism in one parent [Zillhardt et al., 2016]. The phenotype (if any) associated with truncation or deletion mutations of most of the tubulin genes is unknown. Probably, these genes are intolerant to loss-of-function mutations, as also suggested by the observation in the ExAC Database (http://exac.broadinstitute.org/); the tubulin genes, with the exception of *TUBA8*, have a probability of loss-of-function intolerance near to 1.00, a value consistent with an almost

complete intolerance. A single family with a tubulinopathy phenotype has been reported with a homozygous mutation of *TUBA8* [Abdollahi et al., 2009].

Neuronal Heterotopia

There are 3 main groups of heterotopia: periventricular (usually nodular: PNH), subcortical and leptomeningeal (glioneuronal heterotopia found over the surface of the brain), of which only the first 2 can be detected by imaging. PNH is by far the most frequent. SBH is a mild form of LIS and has been dealt with in a previous section.

Periventricular Nodular Heterotopia
PNH consists of nodules of grey matter located along the lateral ventricles with a total failure of migration of some neurons [Barkovich et al., 2012; Guerrini and Dobyns, 2014]; it ranges from isolated, single, to confluent bilateral nodules (fig. 1H). The overlying cortex may show an abnormal organization. When the nodules are bilateral and numerous, a genetic basis is probable, and associated brain malformations are often reported [Parrini et al., 2006].

Brain Imaging
Patients with the classic X-linked PNH typically have bilateral contiguous nodules that spare the temporal horns and mild cerebellar vermis hypoplasia with megacisterna magna [Parrini et al., 2006]. Patients with rare autosomal recessive bilateral PNH can have severe congenital microcephaly and thin overlying cortex with abnormal gyri [Sheen et al., 2004]. In the fairly common posterior predominant syndromes, PNH is limited to the trigones, temporal and occipital horns, and can be associated with overlying polymicrogyria, hippocampal and cerebellar hypoplasia, or hydrocephalus [Pisano et al., 2012].

Clinical Features
Although most patients with PNH come to medical attention because they have focal epilepsy of variable severity, there is a wide spectrum of clinical presentations, including several syndromes with intellectual disability and dysmorphic facial features. There is some correlation between the size of PNH and the likelihood of concomitant structural abnormality of the cortex and clinical severity [Parrini et al., 2006], but there seems to be no correlation between the size and number of heterotopic nodules and cognitive outcome or epilepsy severity. Most females with

PNH due to *FLNA* mutations have epilepsy, with normal or borderline cognitive level. Age at seizure onset is variable. Most patients have focal seizures, which can be easily controlled or refractory [Parrini et al., 2006]. There is no clear relationship between epilepsy severity and the extent of nodular heterotopia.

Genetic Basis and Diagnosis
The most common syndromic form of PNH (X-linked PNH) consists of bilateral contiguous or near contiguous nodules and is most often caused by *FLNA* mutations. All other PNH syndromes are rare.

X-linked PNH is a clinically and genetically heterogeneous disorder occurring most frequently in women, associated with high rates of prenatal lethality in male foetuses, and a 50% recurrence risk in the female offspring. Almost 100% of the families and 26% of sporadic patients harbor mutations in the *FLNA* gene [Parrini et al., 2006], which also causes cardiovascular abnormalities in some patients of both sexes and gut malformations in boys. Only a few male patients with PNH owing to *FLNA* mutations have been reported [Guerrini et al., 2004]. *FLNA* encodes a large actin-binding phosphoprotein that stabilizes the cytoskeleton and contributes to the formation of focal adhesions along the ventricular epithelium [Fox et al., 1998]. *FLNA* may be required for the initial attachment of neurons onto the radial glial scaffolding before migration from the ventricular zone [Lu et al., 2006]. Failure of migrating neurons to attach to radial glia is one likely mechanism leading to the formation of heterotopia.

A rare recessive form of PNH owing to mutations of the *ARFGEF2* gene was described in 2 consanguineous pedigrees [Sheen et al., 2004] in which affected children had microcephaly, severe cognitive delay, and early-onset seizures. The *ARFGEF2* gene encodes a protein designated BIG2 that converts guanine diphosphate to guanine triphosphate. This activates ADP-ribosylation factors, which regulate vesicle trafficking and transport of molecules from the cell interior to the cell surface, where they can bind to other molecules or be secreted by the cell [Sheen et al., 2004]. Thus, BIG2 may assist in the transport of FLNA to the cell surface.

Other genetic forms of PNH have been associated with chromosomal rearrangements, in particular 6q27 deletion (fig. 1I, J) including *C6orf70* (also known as *ERMARD*) mapping in 6q27, and a few additional putative causal genes (*EML1, FAT4* and *DCHS1*; table 1) [Cappello et al., 2013; Conti et al., 2013; Kielar et al., 2014].

Laboratory Investigations and Genetic Counseling

FLNA mutation analysis should be performed in patients with 'classical' bilateral PNH. When PNH is associated with microcephaly, the autosomal recessive form caused by *ARFGEF2* mutations should be ruled out. In patients with PNH associated with other brain malformations or extraneurological defects, array CGH should be considered.

Classical PNH is much more frequent in women and likely to be caused by *FLNA* mutations. Among carrier women, about half have de novo *FLNA* mutations, whereas the remaining have inherited mutations. Although maternal transmission is much more likely, father-to-daughter transmission is possible. Given that germline mosaicism of *FLNA* has never been reported, the recurrence risk (for other children) seems to be very low when a mutation is found in the proband but neither parent is a carrier. Counseling is very difficult when PNH is not related to either *FLNA* or *ARFGEF2,* and array-CGH study is advised. Familial PNH unrelated to these genes is exceptionally low.

Polymicrogyria

The term polymicrogyria defines an excessive number of abnormally small gyri that produce an irregular cortical surface with a lumpy aspect [Bielschowsky, 1916]. Polymicrogyria can be limited to a single gyrus, involving a portion of one hemisphere, be bilateral and asymmetrical, bilateral and symmetrical, or diffuse. Sometimes, it is associated with deep clefts that may extend through the entire cerebral mantle to communicate with the lateral ventricle (schizencephaly) [Barkovich and Kjos, 1992]. Macroscopically, polymicrogyria appears as an irregular or pebbled cortical surface. The perisylvian cortex is the most frequently affected. The cortex often appears thickened to 8–12 mm, but when viewed microscopically, it is overfolded and not necessarily thick. While polymicrogyria most often occurs as an isolated malformation, it can co-occur with several other brain malformations, including microcephaly, megalencephaly, grey matter heterotopia, ventriculomegaly as well as abnormalities of the septum pellucidum, corpus callosum, brainstem and cerebellum.

When schizencephaly is present, the cortex edges can seem to fuse (closed lips) or stay at distance (open lips). The clefts of schizencephaly can be unilateral or bilateral. An area of polymicrogyria can occur in the cortex contralateral to a unilateral cleft [Yakovlev and Wadsworth, 1946].

Brain Imaging

Using CT and low-field strength MRI, polymicrogyria is difficult to discern and may only appear as a mildly thickened, irregular cortex. For this reason, polymicrogyria is frequently misdiagnosed as pachygyria or LIS. With use of high-field or ultra-high-field strength MRI with appropriate age-specific protocols, polymicrogyria can be reliably differentiated – especially the classic form of polymicrogyria with perisylvian location, schizencephaly, and several other forms – from other cortical malformations and its extent fully recognized [De Ciantis et al., 2015]. The imaging appearance of polymicrogyria varies with the patient's age. In newborns and young infants, the malformed cortex is very thin with multiple, very small undulations. After myelination, polymicrogyria appears as thickened cortex (usually 6–10 mm) with irregular cortex-white matter junction. In schizencephaly, the grey matter lining the cleft has the imaging appearance of polymicrogyria with an irregular surface, deep infolding (the cleft), mildly thick cortex, and stippling of the interface between grey and white matter. Schizencephaly is often bilateral but frequently asymmetrical; the contralateral hemisphere should be closely assessed for milder clefts or polymicrogyria without cleft [Barkovich and Kjos, 1992].

Polymicrogyria has been described in a number of topographic patterns. Most of these are bilateral and symmetrical, the most common of which is bilateral perisylvian polymicrogyria, although the perisylvian form may be asymmetric or unilateral. Other bilateral symmetric forms are generalized, bilateral frontal, and parasagittal parieto-occipital polymicrogyria [Guerrini et al., 1997, 2000; Barkovich et al., 1999; Leventer et al., 2010], although little or no neuropathologic data is available to support the classification.

Clinical Features

The clinical manifestations of polymicrogyria vary widely and depend on several factors. The most severe outcomes occur in children with severe microcephaly (−3 SD or smaller), abnormal neurological examination, widespread anatomic abnormality, and additional brain malformations such as heterotopia or cerebellar hypoplasia. The best outcomes are in individuals who have localized unilateral polymicrogyria without other malformations. Polymicrogyria can affect eloquent cortical areas representing language or primary motor functions, yet these functions can be retained with little or no disability [Guerrini and Barba, 2010].

Bilateral perisylvian polymicrogyria is associated with mild to moderate intellectual disability, epilepsy, and im-

paired oromotor skills. More severely affected patients have minimal or no expressive speech. The frequency of epilepsy in these patients is 60–85%, although seizure onset may not occur until the second decade, usually between 4 and 12 years of age [Kuzniecky et al., 1993; Barkovich et al., 1999]. Seizure types include atypical absences (62%), atonic and tonic drop attacks (73%), generalized tonic-clonic (35%), and focal seizures (26%).

Patients with closed-lip schizencephaly typically present with hemiparesis or motor delay, whereas patients with open-lip schizencephaly typically present with hydrocephalus, seizures and intellectual disability, which can be severe [Packard et al., 1997]. Seizure types include infantile spasms, complex partial seizures as well as tonic, atonic, and tonic-clonic seizures, although these are less common.

Genetic Basis and Diagnosis

Numerous causes, both genetic and nongenetic, have been associated with polymicrogyria. Nongenetic causes other than hypoxia or hypoperfusion relate mainly to congenital infections, primarily cytomegalovirus [Evrard et al., 1989; Barkovich and Lindan, 1994]. Although the number of cases of polymicrogyria secondary to cytomegalovirus is not known, the real magnitude of the problem is probably underestimated, and an analysis for cytomegalovirus infections of dry blood spot should be performed in newborns with this malformation.

Polymicrogyria is associated with a wide number of patterns and syndromes as well as mutations in several genes (table 1). Various polymicrogyria syndromes have been described, which have been designated according to their lobar topography [Barkovich et al., 2012].

Various types of single-gene inheritance have been hypothesized for polymicrogyria, based on observations of families with X-linked [Guerreiro et al., 2000; Villard et al., 2002; Santos et al., 2008], autosomal dominant [Guerreiro et al., 2000; Chang et al., 2006], and autosomal recessive forms [Hilburger et al., 1993; Guerreiro et al., 2000]. Bilateral frontoparietal polymicrogyria, (fig. 1K) has been associated with mutations in the *GPR56* gene in families with recessive pedigrees [Piao et al., 2004]. However, the imaging characteristics of bilateral frontoparietal polymicrogyria resemble those of the cobblestone malformative spectrum (muscle-eye-brain disease and Fukuyama congenital muscular dystrophy) [Guerrini and Dobyns, 2014].

Polymicrogyria occurs with a few types of severe congenital microcephaly, such as autosomal recessive syndromes associated with mutations in the *WDR62* [Bilgüvar et al., 2010], *NDE1* [Alkuraya et al., 2011], or *KATNB1* [Mishra-Gorur et al., 2014] genes. Polymicrogyria with microcephaly or normal head size has been reported with several tubulin and tubulin motor genes, especially *TUBB2B* (fig. 1G) [Jaglin et al., 2009; Guerrini et al., 2012] and *DYNC1H1* (fig. 1L, M) [Poirier et al., 2013]. Autosomal recessive forms of polymicrogyria have been linked to mutations of *RTTN* [Kheradmand Kia et al., 2012]. Also, polymicrogyria has now been reported in several megalencephaly syndromes. Recently, it has been demonstrated that mutations in *PIK3R2*, a pivotal gene of the PI3K-AKT-mTOR pathway, may account for up to 15% of all patients with bilateral perisylvian polymicrogyria with or without megalencephaly (fig. 1N) [Mirzaa et al., 2015].

Some copy-number variants have been associated with polymicrogyria (table 1), but only deletions in 1p36.3 and 22q11.2 are common [Robin et al., 2006; Dobyns et al., 2008]. Indeed, when these 2 loci are excluded, copy number variants seem to be rare. A causal gene has not been identified for any of these loci.

Megalencephaly, Hemimegalencephaly and Focal Cortical Dysplasia

The term megalencephaly refers to an abnormally large brain that exceeds the mean for age and gender by 2 SD [DeMyer, 1986]. Megalencephaly has most often been classified simply as a disorder of brain size, but recent studies have shown that megalencephaly with normal cortex by imaging, megalencephaly with polymicrogyria, and dysplastic megalencephaly (including classic hemimegalencephaly) as well as FCD can all result from mutations in genes in the PI3K-AKT-mTOR pathway [Lee et al., 2012; Poduri et al., 2012; Rivière et al., 2012b].

Hemimegalencephaly and FCD constitute a spectrum of malformations of cortical development with shared neuropathological features. The former is primarily defined by macroscopic enlargement of (more or less) one hemisphere, while FCD is primarily defined by histopathology. As currently classified [Blümcke et al., 2011], FCD encompasses a wide spectrum of cortical malformations with variable features, including microscopic neuronal heterotopia, dyslamination, and abnormal cell types. FCD has been divided into 3 major types and 9 subtypes based on histopathological features [Blümcke and Spreafico, 2011; Blümcke et al., 2011]. Type 1 FCD is characterized by abnormal cortical lamination, type 2 FCD includes cortical dyslamination with dysmorphic

neurons (2a) and balloon cells (2b), type 3 FCD occurs in combination with other brain lesions (e.g., tumors) [Blümcke et al., 2011].

The histological changes in hemimegalencephaly, which can be considered as an extreme hemispheric form of FCD, are similar, if not identical, to FCD type 2, with cortical dyslamination and dysmorphic neurons, without (type 2a) or with (type 2b) balloon cells, blurred junctions between grey and white matter, and increased heterotopic neurons in white matter [De Rosa et al., 1992; Blümcke et al., 2011; Guerrini et al., 2015].

Brain Imaging

The most common cortical malformation in megalencephaly is perisylvian polymicrogyria that looks very similar to perisylvian polymicrogyria in patients with normal or small head size. The cortical changes in hemimegalencephaly are severe and consist of an enlargement of part or a complete hemisphere (or less often bilateral asymmetrical involvement) with no consistent preference for which lobes of the brain are enlarged [Salamon et al., 2006]. Patients with FCD usually have enlarged gyri with either smooth or irregular cortical surface and increased subcortical signal intensity. Some individuals with FCD type 2b have migration tracts underlined by hyperintense radially oriented white matter bands that extend from the dysplastic cortex to the periventricular region, an imaging feature that has been termed transmantle dysplasia [Barkovich et al., 1997].

Clinical Features

The developmental and health complications of megalencephaly differ widely. The most common problems include developmental delay, intellectual disability, and seizures that can start early in life and be intractable. Children with diffuse symmetrical or mildly asymmetrical megalencephaly, with or without associated polymicrogyria, have a large head size at birth that soon exceeds +3 SD [Mirzaa et al., 2012a]. Their early development is delayed, and later cognitive development varies from normal to severe intellectual disability. Seizures can begin at any time in childhood.

Individuals with hemimegalencephaly have early developmental delay and more severe intellectual disability than those with diffuse symmetrical or mildly asymmetrical megalencephaly. Epilepsy usually begins in the first weeks or months of life and can include infantile spasms and other epileptic encephalopathies.

The most common clinical sequelae of FCD are seizures. Developmental delay, cognitive disability, and focal neurological deficits are only observed with extensive dysplasias. Early seizure onset has been associated with infantile spasms with asymmetrical or focal features [Guerrini and Filippi, 2005]. FCD type 2 is a frequent cause of focal status epilepticus and, together with FCD type 1, is the most common pathological substrate in surgical series of epilepsy.

Genetic Basis and Diagnosis

Megalencephaly without cortical malformations occurs in benign autosomal dominant macrocephaly, a poorly defined disorder. Several syndromes have been associated with megalencephaly and include neurofibromatosis type 1 due to *NF1* microdeletions that also involve the *RNF135* gene and Sotos syndrome (with NSD1 mutations), Weaver syndrome (with *EZH2* mutations), in addition to Bannayan-Riley-Ruvalcaba syndrome, Cowden syndrome, and severe megalencephaly with autism (all 3 with *PTEN* mutations) [Mirzaa et al., 2012b]. Megalencephaly with polymicrogyria occurs in megalencephaly-capillary malformation syndrome (with mutations of *PIK3CA*) and megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (with mutations of *PIK3R2* or *AKT3*) [Mirzaa et al., 2012b, 2015]. Hemimegalencephaly most often occurs without syndromic features, and has recently been associated with mosaic mutations of *PIK3CA*, *AKT3*, and *MTOR* (fig. 1O, P) [Lee et al., 2012]. Hemimegalencephaly has also been associated with the linear nevus sebaceous syndrome (also known as Schimmelpenning syndrome) and, rarely, with CLOVES syndrome (congenital lipomatous overgrowth with vascular, epidermal, and skeletal anomalies), tuberous sclerosis, hemihypertrophy, and hypomelanosis of Ito [D'Agostino et al., 2004; Tinkle et al., 2005].

The highly focal and variable nature of FCD type 2b, and the pathological resemblance to tubers in tuberous sclerosis, led to the hypothesis that somatic mosaic mutations of genes that encode proteins in the PI3K-AKT-mTOR pathway, which includes the tuberous sclerosis associated genes *TSC1* and *TSC2*, were implicated in FCD [Crino, 2009]. This hypothesis has been in part confirmed by studies documenting pathogenic germline and mosaic mutations in the *mTOR* gene or in other genes belonging to the PI3K-AKT-mTOR pathway (i.e., *AKT3*, *DEPDC5*, and *NPRL3*) in the dysplasic tissue of FCD type 2a and 2b [D'Gama et al., 2015; Lim et al., 2015; Sim et al., 2015]. In addition, somatic duplications in the 1q chromosomal region encompassing the *AKT3* gene have been associated with megalencephaly, hemimegalencephaly, and FCD type 1b [Poduri et al., 2012; Wang et al., 2013; Conti et al., 2015].

When performing genetic testing for disorders possibly caused by mosaic mutations, the screening of multiple tissues (e.g., blood, saliva, and skin fibroblasts) is advisable. Indeed, although testing DNA extracted from blood is the gold standard for identifying de novo constitutive mutations, the analysis of different tissues may help in identifying mutations that are only present in a subset of somatic cells [Nellist et al., 2015].

Conclusions

The clinical spectrum of epilepsy associated with MCD is broad. Understanding the genetic and molecular basis of these malformations is advancing rapidly with new genes being reported frequently, providing evidence that different cortical malformations are probably secondary to abnormalities disrupting different developmental stages. The recent advances in next-generation sequencing have opened new perspectives in the study of the genetic causes of MCD. In particular, massive parallel sequencing of panels targeting key genes involved in cortical development represents a fast and cost-effective tool for the genetic diagnosis of these malformations. The screening of multiple genes in the same experiment also has the invaluable advantage of boosting new genotype-phenotype correlations and possibly identifying genetic causes of MCD for which molecular diagnosis is still missing. However, depending on the number of genes screened in each experiment, next-generation sequencing analysis may provide large numbers of variants whose interpretation is at times difficult. To overcome misleading interpretations of variants, it is always necessary to evaluate their potential pathogenic effect in the context of each patient's phenotype. As a consequence, a tight interaction between referring physicians and molecular biologists is mandatory.

Disclosure Statement

The authors declare to have no conflict of interest related to the contents of this article.

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