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MINI-SYMPOSIUM: Etiologies of Focal Epilepsy

Malformations of Cortical Development

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Abstract

Structural abnormalities of the brain are increasingly recognized in patients that suffer from pharmacoresistant focal epilepsies by applying high-resolution imaging techniques. In many of these patients, epilepsy surgery results in control of seizures. Neuropathologically, a broad spectrum of malformations of cortical development (MCD) is observed in respective surgical brain samples. These samples provide a unique basis to further understand underlying pathomechanisms by molecular approaches and develop improved diagnostics and entirely new therapeutic perspectives. Here we provide a comprehensive description of neuropathological findings, available classification systems as well as molecular mechanisms of MCDs. We emphasize the recently published ILEA classification system for focal cortical dysplasias (FCDs), which are now histopathologically distinguished as types I to III. However, this revised classification system represents a major challenge for molecular neuropathologists, as the underlying pathomechanisms in virtually all FCD entities will need to be specified in detail. The fact that only recently, the mammalian target of rapamycin (mTOR)-antagonist Everolimus has been introduced as a treatment of epilepsies in the context of tuberous sclerosis-associated brain lesions is a striking example of a successful translational "bedside to bench and back" approach. Hopefully, the exciting clinicopathological developments in the field of MCDs will in short term foster further therapeutic breakthroughs for the frequently associated medically refractory epilepsies.

INTRODUCTION

Malformations of cortical development (MCD) represent a wide range of cortical lesions resulting from derangements of normal intrauterine developmental processes and involving cells implicated in the formation of the cortical mantle. In the past, these malformations have been defined as "neuronal migration disorders"; however, as not all the cortical abnormalities have been proven to be due to migrational derangements, the general term of MCDs is preferred as a reflection of our improved knowledge on normal and pathological brain development. MCD are now recognized as a heterogeneous group of focal or diffuse anatomical abnormalities, determined by different causes, that develop during crucial periods of cortical ontogenesis and their pathological features depend thus largely on the timing of the defect in the developmental processes, as well as on its causes.

The etiology of malformative disorders often remains uncertain. Recent molecular biologic and genetic studies have, however, greatly expanded our knowledge on brain ontogenesis so that several disorders of cortical development have been recognized and, for some of them, specific causative genetic defects have been identified. Although brain malformations have been recognized by neuropathologists since the 19th century, improved imaging techniques, namely high resolution Magnetic resonance imaging (MRI), have a great diagnostic impact in this field, allowing *in vivo* diagnosis of various MCDs and providing correlations between imaging features, neurological deficits, developmental delays and electro-clinical findings related to epilepsy (126) (Figure 1). Furthermore, new methodological techniques including quantitative image analysis, MR spectroscopy, functional MRI (fMRI), diffusion tensor imaging (DTI) and fiber tract reconstructions are increasingly available and useful in clinical practice, especially for the detection of small malformations. These novel technologies will further improve our understanding about the functional relevance of affected cortical areas.

Due to abnormal rearrangement of neuronal circuitries, MCDs can be intrinsically epileptogenic and this has been addressed by an increasing number of scientific publications during the last decade. The abnormal circuitry may be restricted to the malformed cortical area or may involve adjacent areas anatomically and functionally linked to the disorganized cortex. Due to the intrinsic epileptogenicity of these lesions, most of partial epilepsies previously defined as "cryptogenic" and frequently resistant to pharmacological therapy are now recognized to be associated with malformative cortical lesions and likely susceptible to surgical therapy. As a consequence, an increasing number of epilepsy patients are

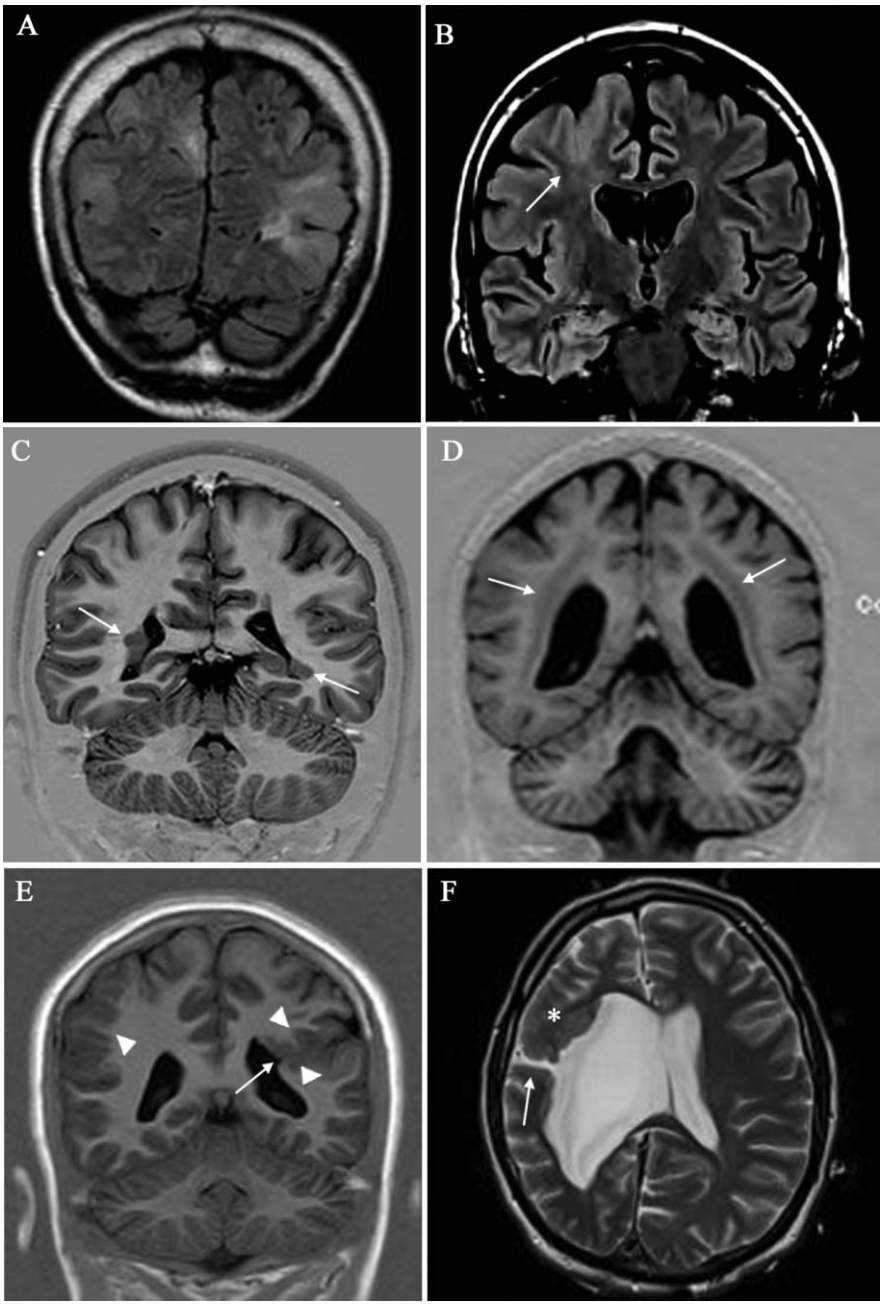


Figure 1. *MRI revealing different types of MCD.* **A.** Multiple bilateral nodules in a patient with TSC. **B.** FCD Type IIb (histologically proven) in the right frontal area; note the tapering from the sulcus toward the ventricle (arrow) considered a landmark for this type of dysplasia. **C.** Bilateral asymmetric periventricular nodular heterotopia (arrows). **D.** Bilateral symmetric band heterotopia (double cortex). **E.** Close-lipped schizencephaly (arrow) associated with polymicrogyria (arrowheads); a polymicrogyric cortex is also evident in the contralateral cortex (arrowhead). **F.** Open-lipped schizencephaly with communication with an enlarged right ventricle (arrow); a malformed cortex is also evident (asterisk) in the frontal cortex of the same side. FCD = focal cortical dysplasia; MCD = malformation of cortical development; MRI = magnetic resonance imaging; TSC = tuberous sclerosis complex.

surgically treated. Increasing availability of surgical specimens also evokes the interest of neuropathologists. Their input not only covers a systematic microscopic workup for diagnosis, but also the possibility to answer basic questions about the pathogenesis of MCDs and related epilepsies. This has been recently made possible by the addition to traditional routine histopathological methods, "new" powerful techniques fruitfully applied to human surgical tissue samples.

A precise incidence of MCDs is not known, but it appears that they are more common, particularly in patients with epilepsy than was recognized in the pre-MRI era. Furthermore, the increasing number of patients admitted to epilepsy surgery has further elucidated the neuropathological incidence of MCD in patients with otherwise unremarkable MRI. In large surgical series of patients with intractable epilepsy, the incidence of postsurgical diagnosis of different types of MCDs progressively increased from the early 1990s to the last few years (77, 132, 199, 219). Although the statistical incidence of MCD in surgically treated patients varies among different centers, biased by different factors such as methodological approaches, patient selection and MRI versus neuropathological diagnosis, it is estimated that 25%–40% of drug-resistant childhood epilepsies are caused by MCD (96) and that

75% of patients with MCD will have epilepsy sometime in their life (133).

Classification systems

An increasing number of malformations are described in the current literature and many types and variants can be encountered in clinical practice. Some malformations have a clear pattern of inheritance, while others are almost exclusively sporadic; the genetic underpinnings of these malformations are increasingly being defined such that by now, more than 30 causative genes have been identified (98, 99). However, environmental causes such as *in utero* infections, trauma and vascular-ischemic events could also be determinants for the generation of cortical malformations.

Abnormal cortical development represents a major cause of epilepsy and severe malformations manifest with profound developmental delay and early onset seizures. Mild malformations may be detected after seizure onset at various ages in otherwise healthy individuals (92, 97). There is no general consensus on these complex structural abnormalities and the current nomenclature is not uniform and, therefore, often misleading. Thus, formulating a classification scheme for MCD is difficult and despite many efforts, it is widely recognized that classifications utilized in the current literature are far from satisfactory. Indeed, a particular defect in corticogenesis may give rise to more than one morphological subcategory of MCD and conversely a morphological subtype of MCD may result from multiple different mechanisms. Due to the complexity of the central nervous system (CNS) and to continuous progress in different fields of neurosciences, a unified and comprehensive classification is far for being formulated and needs continued debate.

Neuropathology has had a great impact in this aspect and careful analysis of neuropathologic material, especially with the aid of new diagnostic methodologies, allowed the distinction and categorization of different forms of MCD. Differences among malformations, previously grouped together, have been revealed by high-resolution MRI, while correlations between morphologically recognized malformations and electro-clinical findings have provided the basis for new classification schemes (12, 148, 157, 166, 200, 204). Recent advances, particularly those derived from genetic analysis, highlight the need for more integrated and complex approaches to clarify MCD.

A combined morphological and genetic scheme referring to malformations of the entire brain has been proposed (75, 179), while Barkovich and coworkers (12) have developed a new classification, specifically addressed to MCD, based on major stages at which cortical development is affected. The categories are based on known developmental steps, pathologic features, genetics (when possible) and neuroimaging features. As focal cortical dysplasias (FCDs) are among the most frequent epileptogenic malformations, particularly susceptible to surgical treatment, a need for a specific classification of these abnormalities was envisaged. Based on combined neuroimaging, electro-clinical and neuropathological findings, different classifications of FCD have been proposed (157, 200). In light of the latest data, including post-surgical outcome, however, a new consensus classification has been proposed by an *ad hoc* task force of the International League Against Epilepsy (ILAE) Diagnostic Methods Commission (23).

FCD

Neuropathology and classification

FCD represents localized malformative brain lesions of unknown cause, frequently encountered in surgical resection specimens from patients with chronic medically intractable epilepsy (21, 190, 193).

Historical background

Taylor and colleagues (204) were first in 1971 to describe neuropathological features of FCD in surgical specimens of 10 epilepsy patients. They reported, as the most evident microscopic feature, the disruption of the normal cortical lamination by the presence of "large aberrant neurones," as well as the presence of "grotesque cells" in both cortex and subcortical white matter (204). The authors suggested that these aberrant ("exotic") populations of nerve cells may underlie the clinical manifestations of certain forms of focal epilepsy and also discuss the similarities with the neuropathological features of cortical lesions in patients with tuberous sclerosis complex (TSC), emphasizing the possibility of a *forme fruste* of TSC (204). Before the comprehensive description provided by Taylor and colleagues, few autopsy cases of focal cerebral gliosis with giant nerve cells in patients with intractable epilepsy had been reported and interpreted as a congenital condition of unknown etiology and pathogenesis (54). Already in 1896, Lombroso and Roncoroni reported focal cytoarchitectural cortical malformations reminiscent of the Taylor-type FCD in patients with epilepsy [reviewed in (44)].

Pathological diagnosis and classification systems

Since the first reports, it has become clear that different histopathological features can be encountered in clinical practice and that the morphological spectrum of FCD is broad, including FCD variants with cortical dyslamination without significant cellular abnormalities. FCD may represent an isolated lesion, although cortical dyslamination can also be detected adjacent to hippocampal sclerosis, glio-neuronal tumors, vascular malformations, as well as adjacent to a large variety of lesions acquired early during brain development (21). During the past 15 years, different FCD classification systems have been proposed (12, 148, 157, 200). The classification published by Palmini *et al* in 2004 was the consensus report of an international workshop and was mainly based on histopathological features, subclassifying the FCD into Type I and Type II categories. Palmini *et al*'s FCD Type I referred to alterations in cortical lamination for Type Ia, with additional cytoarchitectural abnormalities found in Type Ib; Palmini's FCD Type II referred to FCD including both cortical dyslamination and cellular abnormalities (equivalent to the Taylor-type FCD), such as the presence of dysplastic (dysmorphic) neurons (Type IIa) and balloon cells [Type IIb; (157) reviewed in (193)]. During the past 6 years, this classification system has been extensively used as a basis for several scientific reports [reviewed in (190, 193)], but failed to provide consistent associations with clinical and neuroradiological features. Furthermore, the prediction of postsurgical seizure control remained ambiguous, particularly for FCD Type I (21, 121, 132, 193). This variability may possibly reflect inconsistent histological diagnoses,

Table 1. ILAE consensus classification system for FCD. Abbreviations: FCD = focal cortical dysplasia; ILAE = International League Against Epilepsy.

FCD type I *(isolated)*	Ia: abnormal microcolumnar arrangement of neocortex	Ib: abnormal tangential cortical lamination	Ic: abnormal radial and tangential cortical lamination	
FCD type II *(isolated)*	IIa: FCD with dysmorphic neurons		IIb: FCD with dysmorphic neurons and balloon cells	
FCD type III *(associated with principal lesion)*	IIIa: FCD in the temporal lobe associated with hippocampal sclerosis	IIIb: FCD adjacent to a glial or glioneuronal tumour	IIIc: FCD adjacent to a vascular malformation	IIId: FCD adjacent to any other lesion acquired during early life

Three-tiered ILAE classification system for FCDs. Modified from (23).

especially for Type I FCDs, which in the Palmini *et al* classification included different entities (ie, isolated and associated FCD variants). Accordingly, a recent study reported excellent interobserver concordance for the diagnosis of FCD Type II, but relatively poor neuropathologic inter- and intraobserver agreement for the Type I subtypes (41). These findings suggested the need to refine neuropathological diagnostic criteria of FCD subtypes to achieve improved reproducibility and enhanced insight into the clinicopathological correlations of FCD subtypes. A task force of the ILAE has recently generated a new consensus classification of FCD subtypes based on histopathological features and representing the basis for prospective clinical studies addressing the epileptogenicity of different FCD variants (23)

The ILAE classification system of FCD consists of a three-tiered system, including both isolated and associated FCD variants [Table 1 (23)]. FCD Type I (Table 1 and Figure 2B,C) refers to isolated forms of FCD, characterized by an abnormal cortical layering, which may affect one or multiple lobes and is often observed in young patients with severe epilepsy and psychomotor retardation (22, 23, 122, 202). The new ILAE classification includes three FCD Type I subtypes, according to the pattern of cortical dyslamination (Table 1). FCD Type Ia is characterized by abnormal radial migration, as well as neuronal maturation with immature small diameter neurons organized in microcolumns, resembling the microcolumnar organization pattern described during the early stages of cortical development [(165) Table 1; Figure 2B]. However, the diagnosis of this FCD subtype requires particular attention when studying specimens of temporal polar cortex, including areas that tend to maintain a columnar appearance (64). In addition to the microcolumnar organization, increased numbers of heterotopic neurons are often observed and hypertrophic neurons can also be encountered outside layer 5 (19, 23). FCD Type Ib (Table 1; Figure 2C) is characterized by abnormal cortical layering affecting the 6-layered tangential organization of the neocortex and including a large spectrum of histopathological features ranging from alterations of the entire neocortical architecture to a more subtle abnormal layering involving specific layers, such as layer 2 or layer 4 or both (23). Also in this FCD subtypes, heterotopic neurons in white matter and hypertrophic neurons (outside layer 5) can be encountered, as well as normal neurons with disoriented dendrites (19, 23). FCD Type Ic refers to isolated lesions characterized by abnormal cortical layering affecting both radial and tangential cortical organization [Table 1; (23)]. Immunocytochemical analysis using antibodies directed against neuronal nuclear antigen (NeuN; Figure 2) can improve the visualization of the cortical layer abnormalities characteristic of the FCD Type I subtypes; heterotopic neurons within the white matter can be visualized using NeuN or microtubule-associated protein 2 (MAP2) immunohistochemistry (19).

FCD Type II refers to an isolated malformation characterized by disrupted cortical lamination and cytological abnormalities and [as in the previous classification system (157)] includes two subtypes, FCD Type IIa (with dysmorphic neurons, but without balloon cells) and FCD Type IIb (with dysmorphic neurons and balloon cells) (Table 1; Figure 2D–L; (23)). The cortical dyslamination in FCD Type II is often pronounced, without any recognizable layers (except for Layer I). In addition, blurring of gray/white matter junction is often observed, because of increased heterotopic neurons in white matter. Dysmorphic neurons in FCD Type II can be identified in routine hematoxylin and eosin (H&E) and cresyl violet (Nissl-staining) staining. However, because they accumulate neurofilament proteins (phosphorylated and nonphosphorylated) in their cell bodies, antibodies directed against these proteins (2F11 or SMI32) may be useful to detect dysmorphic neurons, showing their abnormal shape, size and orientation (Figure 2E,G,I). Balloon cells (histologically identical to giant cells in tubers of TSC patients) can be detected in routine H&E; however, vimentin and the splice variant of GFAP, GFAP-δ, may represent helpful markers for the demonstration of balloon cells in FCD specimens (30, 129, 142). In addition, some balloon cells also express the CD34 epitope (70) and the adhesion molecule on glia [AMOG; (31)]. Reduction of myelin staining, particularly evident in Type IIb, can be visualized using Luxol fast blue or Klüver–Barrera staining (19).

A major advance of the 2011 classification is represented by the FCD Type III category where FCD is combined with other potentially epileptogenic pathologies (20, 187). FCD Type III consists of four different subtypes: IIIa associated with hippocampal sclerosis (HS); IIIb associated with tumors; IIIc associated with vascular malformations; IIId associated with any other lesion acquired during early life [Table 1; Figure 2O–Q; (22)]. The histopathological features of FCD Type III subtypes may be similar to those detected in FCD Type I, with alterations in architectural (cortical dyslamination) and/or cytoarchitectural composition of the neocortex (hypertrophic neurons); however, distinct patterns can be identified in specific variants, such as FCD Type IIIa. In approximately 10% of surgical specimens of patients with HS, the temporal cortex shows an abnormal band of small and clustered "granular" neurons in the outer part of Layer II [(81, 206); Figure 2M]. In addition, small "lentiform" nodular heterotopias have been reported in a subset of patients with HS (23). As discussed previously, immunocytochemical analysis using antibodies directed against NeuN, MAP2 and neurofilament proteins can improve the visualization of architectural and/or cytoarchitectural alterations of the cortex. Additional antibodies directed against CD34, p53, mutant IDH1

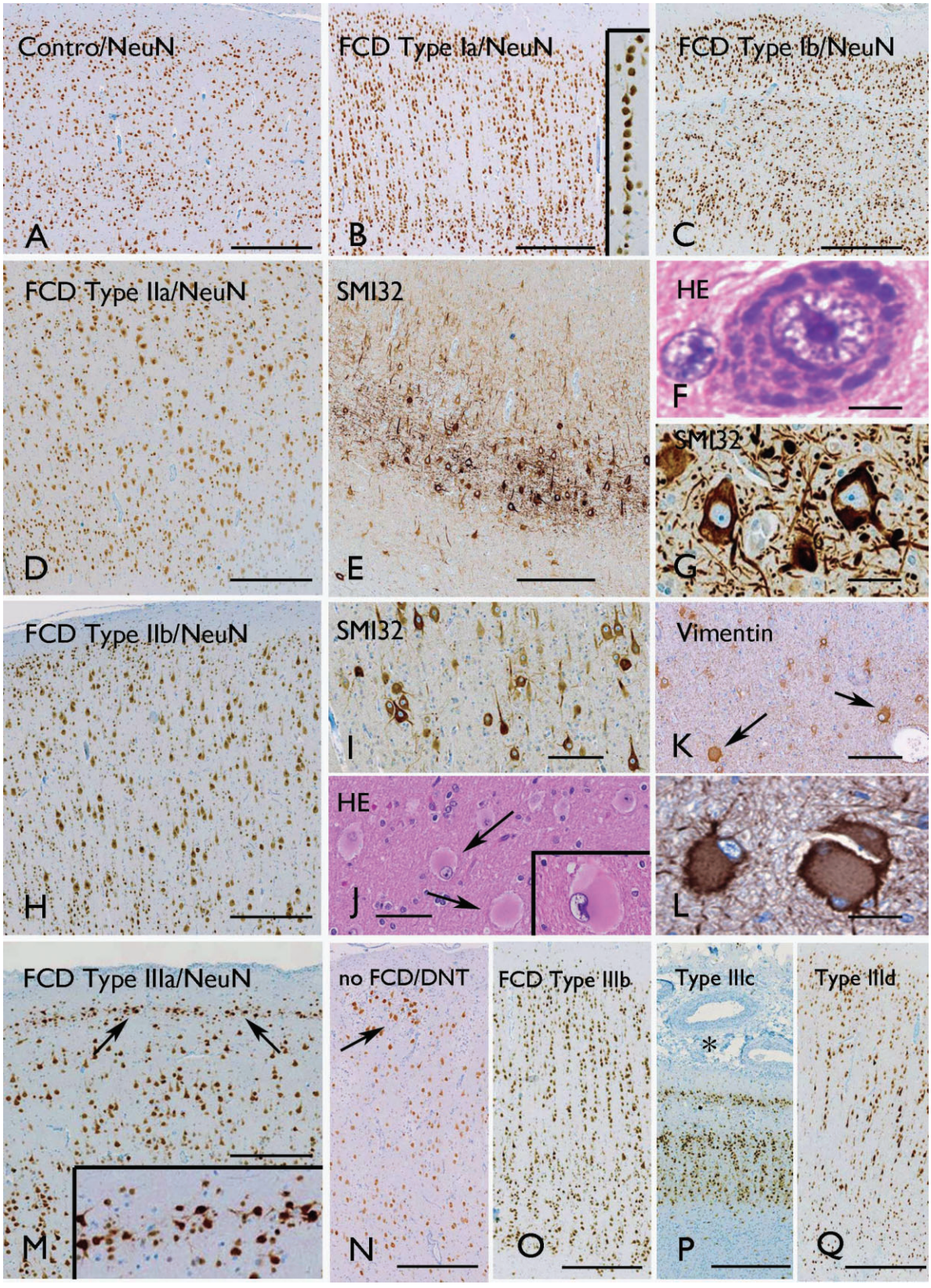


Figure 2. *FCD variants*. ILAE classification system (19, 23): **A.** Normal appearing neocortex (NeuN). **B.** Microcolumnar arrangements of small diameter neurons in FCD type Ia (NeuN); microcolumns are composed of more than eight neurons (insert in B). **C.** Abnormal cortical layering affecting the 6-layered tangential organization of the neocortex in FCD Type Ib (NeuN). **D–G.** FCD Type IIA with cortical dyslamination (D, NeuN) and dysmorphic neurons with enlarged nuclei and aggregates of Nissl substance (F, H&E), as well as accumulation of nonphosphorylated neurofilaments (E, G; antibody SMI32). **H–L.** FCD Type IIb with cortical dyslamination (H, NeuN), dysmorphic neurons with accumulation of nonphosphorylated neurofilaments (I, antibody SMI32) and balloon cells (J, H&E, arrows and insert; K, L, vimentin, arrows in K). **M.** FCD Type IIIa with the characteristic abnormal Layer II (NeuN, arrows and insert in M). **N.** Neocortex adjacent to a dysembryoplastic neuroepithelial tumor (DNT) with clear cell tumor infiltrates that disrupt the cortical architecture (NeuN, no FCD); **O.** Neocortex adjacent to a DNT with microcolumnar arrangements, but without tumor infiltration (FCD Type IIIb, NeuN). **P.** Abnormal cortical architecture in a patient with meningioangiomatosis (FCD Type IIIc, NeuN). **Q.** Abnormal cortical architecture adjacent to a glial scar (NeuN). Scale bar in A: A–E, H, M–Q: 500 μm; I, K: 160 μm; J: 80 μm; G, L: 40 μm; F: 12 μm. FCD = focal cortical dysplasia; H&E = hematoxylin and eosin; ILAE = International League Against Epilepsy; NeuN = neuronal nuclear antigen.

and Ki-67 can also be helpful in the evaluation of FCD Type IIIb, to exclude neocortex infiltrated by neoplastic cells, which should not be diagnosed as FCD (19, 23). The new FCD classification system is based on histopathological criteria and its major aim is to improve the identification and homogeneity of different entities (ie, isolated and associated FCD subtypes). The next challenge is represented by the re-evaluation of this new classification, involving different international centers and addressing the clinical, electrophysiological and imaging features, and the postsurgical seizure control. A collaboration between different neuropathology departments has already been established to examine both the interobserver and intraobserver variability associated with application of the ILAE classification system. In addition, we should now look forward to collaboration between clinicians and basic scientists to improve our still limited understanding of the underlying molecular mechanisms of FCD subtype formation (see below).

FCD—Molecular basis

With respect to molecular alterations in cortical malformations, major objectives aim at improving our understanding of gene defects and dysregulated signaling cascades, as well as molecular and cellular mechanisms mediating hyperexcitability. Given the revised classification of FCD, our current knowledge of the molecular pathology for distinct entities, particularly the FCDs Type I, is still limited. In contrast, molecular alterations of FCD type II are better understood. However, the delineation of cellular or molecular differences between these lesion types that refer to histological differences has the potential to become highly relevant with respect to the development of new diagnostic and therapeutic perspectives. Recent data suggest differential expression of several stem cell proteins and aberrant phosphorylation of mammalian target of rapamycin (mTOR) substrates, that is, S6 and S6 kinase 1 proteins, to distinguish Type II from Type I FCDs, that is, biomarkers for diagnostic pathology in resected epilepsy specimens (156). Epilepsy-associated focal lesions and brain malformations in patients suffering from familial syndromes have served as an argument for overlapping molecular pathogenesis. Several clinicopathological studies have pointed to a role of specific genes and pathways in epilepsy-associated glioneuronal lesions (15, 17, 150), generally underlying rare familial disorders such as tuberous sclerosis complex (TSC) (211, 212). TSC, as discussed in detail next, is caused by germline mutations of the *TSC1* (hamartin) and *TSC2* (tuberin) genes (67, 163). Generally, sporadic epilepsy patients with FCDs as well as benign glioneuronal tumors do not present with additional TSC-associated stigmata (171). Nonetheless, FCD Type IIb brain specimens typically have sequence alterations in *TSC1* (139). A base transition in exon 17 (2415C > T; His732Tyr) is increased in FCD Type IIb compared with controls. This base exchange is located in the interaction domain of hamartin with tuberin (212). Intriguingly, in FCD Type IIa, abundant genomic polymorphisms were detected in intron 4 of TSC2, but no allelic variants in exon 17 of *TSC1* were observed (139). Are there potential functional consequences for allelic variants increased in FCD Type IIb? Co-immunoprecipitation assays with tuberin revealed reduced tuberin binding of hamartin compared with wild-type hamartin, similar to two TSC1 stop mutants. Furthermore, aberrant nuclear distribution of hamartin *in vitro* was observed, suggesting a fundamental functional impairment of hamartin (136). Several transgenic mouse studies have pointed to *TSC1* with a major role for cellular abnormalities reflecting those in cortical malformations (144). Only recently, tuber/FCD Type IIb mimicking lesions in mice have been generated by using a sophisticated transgenic strategy (71). Hamartin and tuberin constitute a tumor suppressor mechanism, which plays a central role in the insulin/PI3K-signaling pathway (107, 128). The PI3K pathway is critical for cell size- and growth-control and cortical development (198). Binding of insulin to its membrane receptor activates the cascade components PI3K, Akt, TSC1/TSC2, mTOR (mammalian target of rapamycin) and the transcription factors p70S6kinase (S6K), as well as ribosomal S6 protein (S6) (68, 80). Inactivation of the TSC1/TSC2 complex by phosphorylation through Akt results in phosphorylation of mTOR and subsequent activation of the respective transcription factors interfering with cell size control; differential activation of distinct upstream elements have been demonstrated in FCD IIb (106, 143, 182, 183). Activation of individual PI3K-pathway downstream components has also been reported in FCD Type IIb, that is, the eukaryotic translation initiation factor (eIF) 4G and *phospho*-S6 (15, 150). FCD Type II, but not FCD Type I cases exhibit activation of the mTOR cascade with strong neuronal expression of the phosphorylated isoform of S6 protein (108). Interfering with this cascade provides intriguing therapeutic perspectives in animal models of TSC-associated lesions as well as in human TSC-related malformations (5, 123, 145). Intriguingly, Everolimus has been established recently as the first mTOR inhibitor for the treatment of TSC patients with subependymal giant-cell astrocytomas. Evidence supports reduction in tumor volume and improvement in epilepsy (123).

Recent data have pointed to a role of doublecortin-like (DCL), a protein critically involved in neuronal division and radial migration

Figure 3. *Tuberous sclerosis*. Panel **A,B.** Coronal sections of the brain (32-year-old patient with a germline mutation in the *TSC2* gene; (26)), showing several regions with blurring of the cortex/white matter junction (arrows) and subependymal nodules (SENs; arrowheads and insert in A). Panel **C.** Post-mortem MRI; 3D-FLAIR image, showing the hyperintense tuber (arrow), as well as SEN with calcifications (arrowhead). Panels **D,E.** Histologic stains [D: hematoxylin and eosin (H&E) and E: Luxol fast blue-PAS-staining]. Note the cortical tuber (arrows in D and E) with decreased density of myelinated fibers (E) and the calcified SEN (arrowhead in D). Panel **F.** Low magnification view showing strong GFAP immunoreactivity within cortical tubers (arrows). Panel **G.** H&E stain showing a low magnification image of a SEN appearing as an oval-shaped compact and calcified lesion, with overlying ependyma, projecting into the lateral ventricle; insert a in G: calcification; inserts b–c: high-magnification microphotographs of H&E stain showing the cellular components of a SEN, including relatively small, plump glial cells with eosinophilic cytoplasm (b) and giant aberrant cells with large nuclei displaying different size and shape (c). Panel **H.** H&E stain of cortical tubers showing the disorganized cortical cytoarchitecture with multiple punctate calcifications (arrows). Panel **I.** NeuN stain showing the disorganization of the neuronal component within the cortical tuber. Panel **J.** Dysmorphic neurons. Panel **K.** Giant cell with pale eosinophilic cytoplasm and balloon-like appearance. Panels **L,M.** Microphotographs showing strong phospho-S6 ribosomal protein (pS6) immunoreactivity in a giant cell within the subcortical white matter (L), and in dysmorphic neurons within the dysplastic cortex (M). Scale bars: F: 0.8 cm; G: 400 μm; H–I: 300 μm; J–M: 40 μm. MRI = magnetic resonance imaging; NeuN = neuronal nuclear antigen.

during early corticogenesis in FCD as well as TSC. Balloon cells in FCD and giant cells in TSC, as well as dysmorphic neurons in both entities were observed to express strongly DCL. The prominent postnatal expression of DCL by cell elements of FCD and TSC supports an important role for this microtubule-associated protein (28). Furthermore, in FCD Ia taken from patients up to 4 years of age, prominent DCX-positive [DCX(+)] immature cells have been observed along the junction of layers I and II, with processes extending into the molecular layer. Such expression patterns in layer II of FCD Ia, but not in FCD II, may indicate delayed or abnormal cortical maturation rather than ongoing cytogenesis (194). Understanding mechanisms mediating increased excitability of respective lesions is for obvious reasons highly important. Recent analyses in experimental models of seizures and in temporal lobe epilepsy have pointed to a critical role of high-mobility group box 1 and toll-like receptor 4 signaling in mediating hyperexcitability and seizures (230). Although microglia reactivity is increased in FCD I compared with controls, the number of HLA-DR-positive cells is substantially denser in FCD II. There, a striking presence of perivascular and parenchymal CD3(+) T lymphocytes,a predominance of CD8(+) T-cytotoxic/suppressor lymphocytes, a few dendritic cells, andexpression of components of the complement cascade, IL-1β and MCP1 suggested the activity of both innate and adaptive immunities (108).

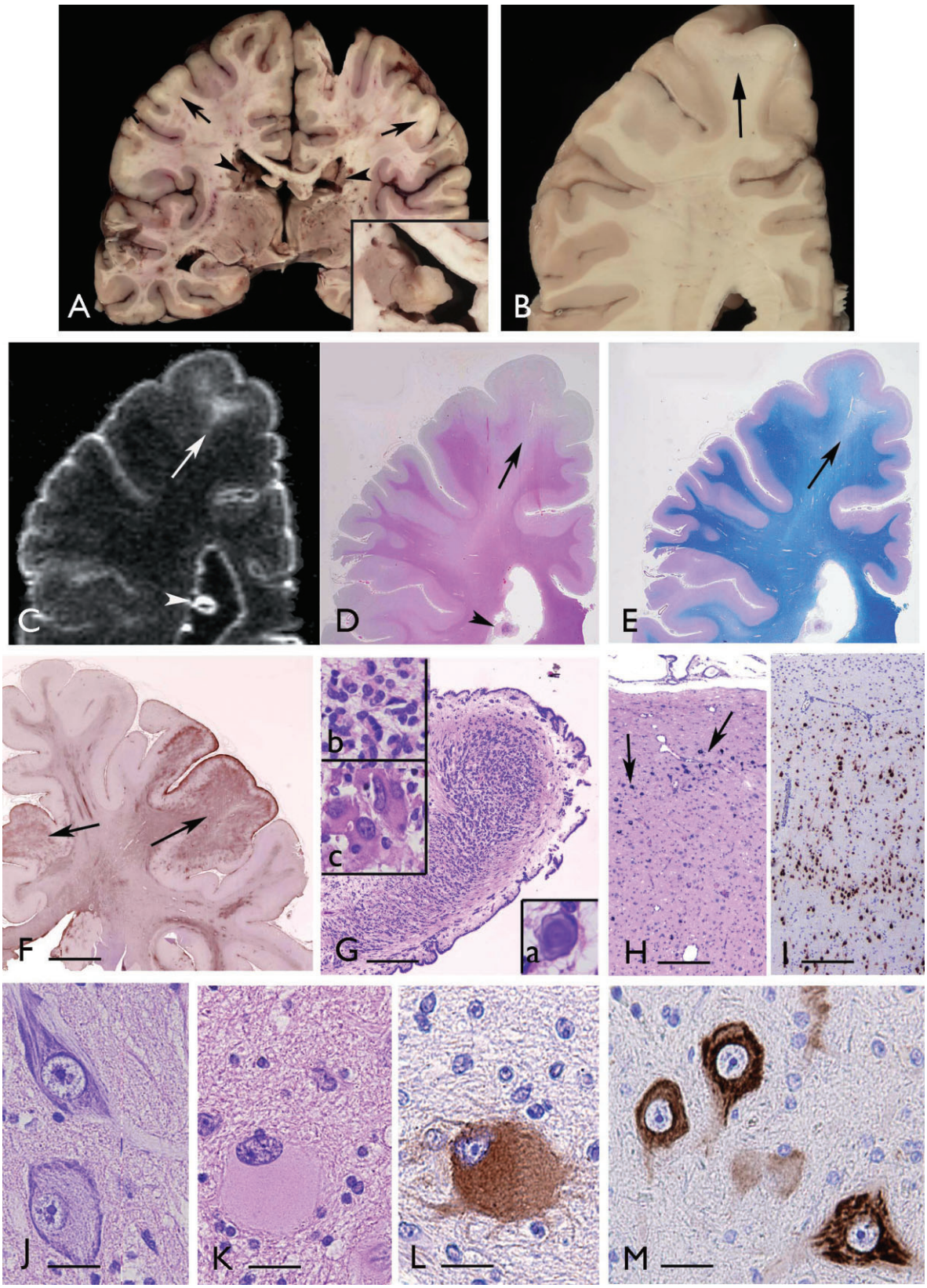
TUBEROUS SCLEROSIS

TSC is an autosomal dominant, multisystem disorder that results from mutations in the *TSC1* or *TSC2* genes (67, 211). CNS involvement is common in TSC and the neurological manifestations of TSC are the most disabling, including: developmental delay, neurobehavioral abnormalities such as autism, and severe epilepsy (33, 55). Although a clear genotype–phenotype correlation has not been established, the majority of studies suggest that patients with mutations in *TSC2* have a more severe neurologic phenotype (9, 57, 154). Complex structural brain abnormalities are believed to underlie the neurological symptoms of TSC patients. Neuropathological examination of post-mortem and surgical TSC brain specimens reveals three major lesions: subependymal nodules, subependymal giant cell tumors and cortical tubers (63, 151). However, recent advances in neuroimaging allow the detection of more subtle structural brain abnormalities present throughout the brain, which are believed to contribute to the complex neurological features of TSC (63, 89, 135).

Subependymal nodules (SENs) represent small (usually multiple) benign proliferative lesions lining the ventricular system that are believed to develop in fetal life and to be asymptomatic; microscopically, SENs often contain calcifications and are mainly composed of glial cells with variable morphology (plump or elongated glial cells), sometimes including small clusters of giant cells [(26, 87, 151); Figure 3]. SENs may grow and develop into subependymal giant cell astrocytomas (SEGAs), which are low-grade, slow-growing tumors that arise from the periventricular region and can cause obstructive hydrocephalus (1, 87, 151).

Cortical tubers are focal developmental malformations detected, as single or multiple lesions (Figure 3), in more than 80% of patients with TSC [for reviews, see (52, 155)]. Several cases have been reported with prenatal detection of CNS lesions (43, 86, 222) and with histopathological confirmation of tubers as early as 20 weeks gestation (158), indicating that tubers form during embryonic brain development, probably between weeks 9 and 20 of human gestation. Cortical tubers display cortical dyslamination with different cell types, including dysmorphic neurons, reactive astrocytes and giant cells [(26, 87, 151, 230); Figure 3]. There is no obvious cytological difference between the dysmorphic neurons observed in tubers of TSC patients and those detected in specimens of patients with FCD Type IIa/b. Similarly, the giant cells in TSC are histologically identical to balloon cells detected in FCD Type IIb [large cell body and opalescent glassy eosinophilic cytoplasm; (23)]. Such cells have been shown to express both neuronal and immature glial markers, suggesting a failure to differentiate prior to migration into the cortex [(28, 51, 131, 197); for review, see (155)].

A growing body of evidence suggests that cortical tubers are not static lesions, but are instead highly dynamic, exhibiting evolving features over time. Recently, the cystic evolution of tubers has gained interest because of its strong association, in approximately 50% of patients, with a TSC2 gene mutation and with a more aggressive seizure phenotype (45, 46). Cyst-like tubers possibly represent the end stage of a large spectrum of degenerative changes, affecting the white matter. An additional study suggests that astrogliosis in tubers is a dynamic process as well, with progression of astrocytes from "reactive" to "gliotic," supporting the hypothesis that tubers are dynamic lesions (192).



The identification of the TSC1 and TSC2 genes has facilitated our understanding of the molecular mechanisms involved in the development of malformative and neoplastic lesions in TSC brain. Loss of function mutations results in a constitutive activation of the mTOR cascade, which plays a critical role in the regulation of cell growth and proliferation (42, 128, 156). Several studies have provided evidence of cell-specific activation of the mTOR pathway in tubers, showing that dysmorphic neurons and giant cells are strongly immunoreactive for the phosphorylated isoform of p70S6 kinase1 and ribosomal protein S6 [pS6 (15, 28, 150, 155, 197); Figure 3]. Expression of activated (phosphorylated) components of the mTOR pathway, such as pS6, now permits the analysis of the cellular components of tubers not simply on the basis of their morphology, but on the basis of the specific pathogenetic defect underlying the development of these lesions. Interestingly, recent observations in fetal brain indicate that mTOR hyperactivation represents an early event in the pathogenesis of tuber formation and is strictly linked to cytoarchitectural features characteristic of tubers (Crino and Aronica, unpub. obs.).

Many TSC-associated tumors show loss of heterozygosity (LOH) at either the TSC1 or TSC2 locus (4, 42, 88). There is still debate about whether this "two-hit" hypothesis explains tuber formation (115, 164). A recent study, by sequencing *TSC1* and *TSC2* in microdissected pS6–immunolabeled giant cells, identified in 5 out of 6 tuber specimens a somatic mutation in single giant cells that was not detected in whole tuber sections or leukocyte DNA, suggesting that a single somatic inactivating mutations may represent a mechanism underlying the formation of tubers adjacent to morphologically normal cortex (53).

Epilepsy is reported in over 80% of patients diagnosed with TSC and significantly affects psychomotor development and quality of life. Failure to respond to anticonvulsant drug treatment is particularly common in TSC patients (47, 55). Cortical tubers are believed to represent the neuropathological substrate of epilepsy in TSC patients. Recent advances in structural and functional imaging techniques have resulted in more accurate preoperative evaluation and improvements in surgical outcome (32, 217). However, the relationship between the tuber and the epileptogenic zone remains a challenge (32). Increasing evidence support the importance of the perituberal cortex in TSC, as well as in other malformations of cortical development [for review, see (220)]. Although epilepsy surgery, targeting tubers, often results in seizure freedom (120, 217), some patients continue to have seizures after target tuber resection and a few cases of TSC patients becoming seizure free after resection of nontuberal cortex have been reported [(216); for review, see (220)].

The cellular mechanism(s) underlying the epileptogenicity of cortical tubers remain largely unknown and possibly involved multiple mechanisms. Recent studies support the role of developmental alterations affecting the balance between excitation and inhibition in the epileptogenicity of cortical tubers [for review, see (102, 220)]. Abnormal glutamate homeostasis, increased AMPA-receptor-mediated currents, impaired astrocytic gap junction coupling and potassium buffering, which would support seizure generation, have been described in mouse models of TSC (203, 216, 223, 228). Large-scale gene expression studies indicate alterations in glutamatergic and GABAergic synaptic transmission within tubers in TSC patients, involving reduced expression of the glial glutamate transporter (GLT-1 or EAAT2), reduced expression levels of GABAA receptor subunits and reduced expression of potassium channels (29). Selective alterations of ionotropic glutamate receptor subunit, as well as of metabotropic glutamate receptor (mGluR) subtypes, have been observed in the different cellular components of cortical tubers (27, 197, 218). Another recent development is represented by the demonstration in cortical tuber of a complex activation of pro-inflammatory signaling pathways, including chemokines, complement, IL-1β and Toll-like receptor (TLR) mediated pathways (25, 29, 230), as well as evidence of activation of the plasminogen system (109); high expression of several factors involved in the regulation of angiogenesis has also been detected [(29); for review, see (7)]. Accordingly, epidermal growth factor (EGF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), which regulate angiogenesis and cell growth in the developing brain, have recently been shown to be up-regulated in human SEGAs and tubers as well as in a mouse model of TSC, providing potential new target for therapy development in TSC (159).

HEMIMEGALENCEPHALY

Hemimegalencephaly (HMEG) is a rare malformation of cortical development characterized by enlargement and cytoarchitectural abnormalities of one cerebral hemisphere. In 1835, Sims in his article on "hypertrophy and atrophy of the brain" (186) discusses several autopsy cases of hypertrophy which have fallen under his observation, including one case of hypertrophy of one hemisphere. Since then, small series reports have been published, indicating that HMEG may occur as an isolated malformation or in syndromic form and that is associated with developmental delay, motor deficits and severe epilepsy with onset typically within the first few months of life (112, 176, 180, 207, 210). Epilepsy is often poorly controlled by antiepileptic drugs, requiring surgical therapy to remove or functionally disconnect the epileptogenic area within the affected hemisphere. However, epilepsy surgery in HMEG patients yields modest postsurgical seizure and cognitive outcomes (114). Radiologically, HMEG, in both isolated and syndromic forms, is typically characterized by an enlarged cerebral hemisphere with abnormal gyral pattern, thickened cortex and white matter abnormalities, with loss of gray-white matter differentiation, on the enlarged side (74, 117, 153, 224). Structural changes have been also reported in structures outside the involved hemisphere, including ipsilateral olfactory nerve enlargement, cerebral vascular dilations, cerebellar enlargement with abnormal architecture of the cerebellar folia (181). The spectrum of CNS structural abnormalities includes cases variously defined as "hemi-megalencephaly," "focal megalencephaly" or "localized megalencephaly," in which the abnormalities are detected only over a partial area of one hemisphere (58, 127, 153). In these cases, the detection of associated extracerebral abnormalities has been suggested to provide a clue for differentiating localized megalencephaly from multilobar cortical dysplasia (153).

HMEG has been reported in patients with various syndromes, such as Proteus syndrome (62), neurofibromatosis (56), hypomelanosis of Ito (195), Klippel–Weber–Trenauany syndrome (208), TSC (39) and linear sebaceous nevus syndromes (74, 174, 191). In both isolated and syndromic forms, a large spectrum of morphological alterations MCD can be observed [for review, see (74, 76, 141)]. Macroscopic examination of the brain often reveals an abnormal gyral pattern (pachygyria, polygyria, polymicrogyria)

and increased thickness of the cortex of the enlarged hemisphere (with blurring of the cortex-white matter junction; Figure 4); microscopically, in the large majority of cases a severe dyslamination is observed throughout the cortex together with the presence of hypertrophic and dysmorphic neurons, as well as heterotopic neurons in the white matter and leptomeningeal glioneuronal heterotopia [(6, 24, 61, 76, 172, 175, 226); for review, see (74, 76, 141); Figure 4]. Balloon cells have also been detected in HMEG specimens and have been considered a typical component of HMEG (11, 76). However, in a large series of surgery patients with HMEG, balloon cells were identified in less than 50% of the surgical specimens (175), with no detection in fetal examples (24). The observation that complex cytoarchitectural abnormalities are observed prenatally, also at a fetal age of 22 weeks (141), supports the hypothesis of an underlying primary genetic defect which affects the early stages of cortical development (between 10 and 20 weeks) involving the proliferation, migration and differentiation of precursor cells.

One unresolved question is whether syndromic HMEG is distinct from the sporadic forms or reflect a spectrum of hemispheric brain malformations, sharing common mechanisms of pathogenesis. The histopathology of HMEG with complex cytoarchitectural abnormalities, including in some cases the presence of dysmorphic neurons and balloon cells a [as in FCD Type IIb; (23)], has suggested a primary disorder of proliferation (196) or a disturbance of cellular lineage and cellular growth (74, 179). Barkovich *et al* (11) include HMEG among the non-neoplastic disorders of proliferation (with abnormal cell types) together with tuberous sclerosis and cortical dysplasia with balloon cells (FCD Type IIb). Salamon *et al* (175) suggested that pathogenesis of HMEG probably involves an increased proliferation of the progenitor cells together with partial failure of postneurogenesis apoptosis in the molecular layer and subplate. Other authors (141) suggested the possibility of an accelerated neuronal differentiation as underlying pathogenetic mechanism, also emphasizing the importance of migration abnormalities. Excessive production and/or function of neuronal growth factors have been suggested to contribute to enhanced neuronal growth and/or differentiation. Attention has been focused on the potential role of EGF (119), nerve growth factor (NGF) and its high-affinity receptor (6), as well as on the role of VEGF (24, 227).

Recent work from Crino and coworkers points to the role of new converging cell signaling pathways (Wnt/β-catenin- and mammalian target of rapamycin, mTOR-pathway) in the pathogenesis of HMEG [(8, 24, 49, 227); Figure 2]. The identification of aberrant mTOR signaling in malformations of cortical development (such as FCD Type IIb, TSC and HMEG), characterized by cortical dyslamination, cytomegaly and intractable epilepsy, suggests a pathogenic link between these malformations, possibly representing a spectrum of disorders of mTOR signaling [so-called "TORopathies"; (50, 221)]. It has been suggested that HMEG could result from somatic mutations in genes encoding for proteins that regulate mTOR signaling pathway, occurring in progenitor cells in one hemisphere during the early stages of corticogenesis (50). A hemizygous deletion within chromosome 15q11.2–15q13.1 has recently been detected in the affected hemisphere in a single case of isolated HMEG (16).

Little is known about the epileptogenesis of HMEG. Using target cDNA array analysis and immunocytochemistry, the expression of multiple neurotransmitter receptors in surgery and autopsy HMEG specimens has been investigated (14, 24). These studies indicate that changes in expression and/or composition of ionotropic and metabotropic glutamate receptors could play a role in epileptogenesis in HMEG, as suggested for other types of focal malformations of cortical development [for review, see (220)]. In addition, the presence of activated glial cells in HMEG (24) may also be related to the epileptogenicity of this disorder through different mechanisms, including the production of inflammatory cytokines, such as IL-1β; (184, 213). Accordingly, accumulating clinical evidence strongly supports the relevance of inflammation in the pathophysiology of human epilepsy (7, 214).

LISSENCEPHALY

Impaired cortical development can be caused by aberrant proliferation, differentiation or migration of neural precursor cells and neurons. Obviously, the latter defect leads to aberrant neuronal localization and lack of physiological cortical lamination (157). Migration of virtually all cortical neurons is severely affected in so-called lissencephaly, that is, an abnormally smooth cerebral surface (215). Classic lissencephaly has a prevalence of 11.7 per million births (60). However, the frequency of milder forms of lissencephaly is not known. Hallmarks of the disorder are severe epilepsy and mental retardation, which in many patients go along with death at young ages. In normal brain development, the agyric cerebral surface, which is present until the 11th week of gestation, is replaced by gyri, which develop from the area of the sylvian fissure within the following weeks. Gyration is physiologically accomplished by approximately the 32nd fetal week. The critical time window for lissencephalic aberrations to initiate is between fetal weeks 11–13 (113). Lissencephaly/agyria and pachygyria, that is, cortical architecture somewhat more advanced, represent a spectrum of cortical aberrations.

Lissencephaly represents a genetic disorder with mutations particularly in two genes, that is, doublecortin (*DCX*) on the X chromosome (84) and *LIS1* on chromosome 17 (161, 169, 170). Mutations in *DCX* cause lissencephaly (XLIS) in hemizygous males and subcortical band heterotopia (SBH) in heterozygous females (65, 162). DCX is a microtubule-associated phosphoprotein functionally important for cytoskeletal plasticity during axonal outgrowth, neuronal maturation and cell migration (104). Aberrant DCX function impairs the cellular shape and cytoskeletal function as well as migration of cells (84, 85, 178). Furthermore, *DCX* represents a candidate gene for women with cryptogenic epilepsy and retardation in the absence of male relatives or a known relevant family history (97). Intriguingly, in a rat model of subcortical band heterotopia (SBH) generated by *in utero* RNA interference of the Dcx gene, it was shown that aberrantly positioned neurons can be stimulated to migrate by re-expressing Dcx after birth. Restarting migration in this way both reduced neocortical malformations and restored neuronal patterning. Furthermore, the capacity to reduce SBH continued into early postnatal development. Intervention after birth reduces the convulsant-induced seizure threshold to a level similar to that in malformation-free controls. For disorders of neuronal migration, these data provide an entirely novel perspective to reactivate developmental programs and, both, reducing the size of cortical malformations and seizure risk (140).

Heterozygous deletions of the chromosomal region 17p13 comprising Lissencephaly 1 (*LIS1*) causes the so-called Miller–Dieker

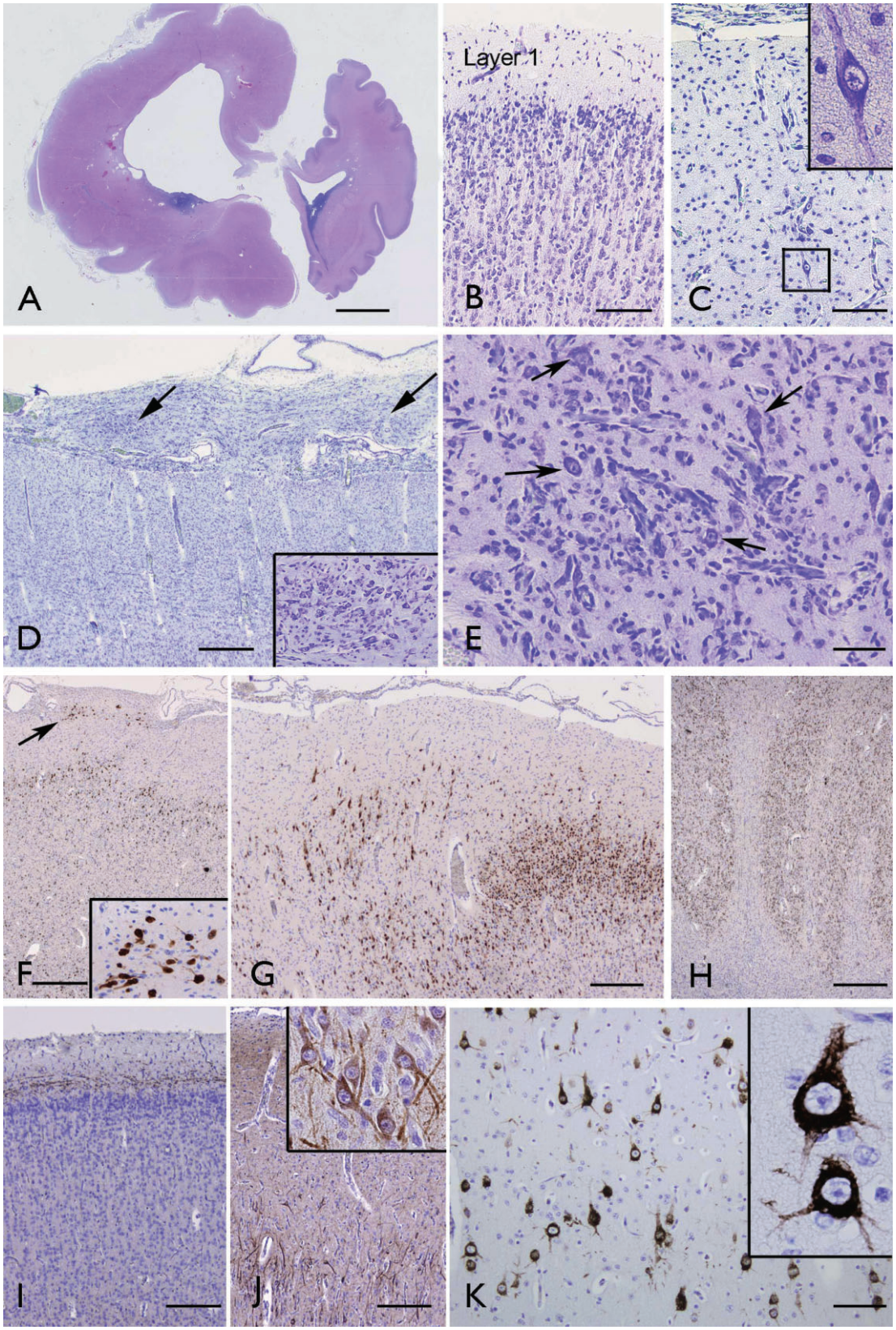


Figure 4. *Hemimegalencephaly (HMEG).* **A.** Coronal section showing diffuse and severe hyperthrophy of the left hemisphere with lateral ventricular dilatation, periventricular cysts, pachygyria and thickened cortex (32 weeks gestation; HMEG plus epidermal nevus (24). Panels **B–D.** Clear difference in the architecture of the cortex between the right side ("normal" side; B, cresyl violet) and the affected left side (HMEG; C, D, cresyl violet). HMEG side (C and D) shows severe cortical disorganization with loss of lamination and presence of cytomegalic neurons (insert in C) and thickened leptomeninges with clusters of heterotopic cells (arrows and insert in D). Panel **E** shows (arrows) a cluster of dysmorphic neurons within the affected cortex (cresyl violet). Panels **F,G.** (NeuN in HMEG cortex): loss of lamination with irregular distribution of neuronal cells and clusters of NeuN-positive elements in Layer I (arrow and insert in F). **H.** NeuN staining in the occipital HMEG cortex with polymicrogyria. **I,J.** Neurofilament immunoreactivity in the unaffected (I) and affected (J) cortex, showing intense cytoplasmic staining in dysmorphic neurons (J). **K.** Phospho-S6 ribosomal protein (pS6) staining; strong positivity is observed in a population of dysmorphic neurons within the affected cortex (K and insert). Scale bars: A: 1028 mm; B–C: 250 μm; D: 400 μm; E: 125 μm; F–H: 400 μm; I–J 250 μm; K: 200 μm. NeuN = neuronal nuclear antigen.

syndrome, which includes lissencephaly, craniofacial defects and substantial EEG abnormalities and is generally associated with a reduced lifespan. *LIS1* encodes a subunit of the enzyme acetylhydroxylase and is particularly expressed by Cajal–Retzius cells (167, 185). Other genes located on chromosome 17p are likely to be involved in the phenotype of the Miller–Dieker syndrome such as the gene YWHAE encoding a protein termed 14-3-3e (209). It has been pointed out that the pachy- or agyral alterations associated with *LIS1* are stronger in the posterior occipital area, whereas the structural abnormalities in XLIS particularly affect the anterior occipital brain (65).

In animal models, *LIS1* is differentially activated by seizure activity (185). Nuclear distribution factor E-homolog 1 (NDE1), LIS1 and NDE-like 1 (NDEL1) together participate in essential neurodevelopmental processes, including neuronal precursor proliferation and differentiation, neuronal migration and neurite outgrowth (229). NDE1/LIS1/NDEL1 interacts with Disrupted in Schizophrenia 1 (DISC1) and the cAMP-hydrolyzing enzyme phosphodiesterase 4 (PDE4). DISC1, PDE4, NDE1 and NDEL1 have each been raised as genetic risk factors for major mental disorders. Recently, it was shown that DISC1 and PDE4 modulate NDE1 phosphorylation by cAMP-dependent protein kinase A (PKA). PKA-dependent phosphorylation of the NDE1/LIS1/NDEL1 complex is DISC1-PDE4 modulated and was suggested to regulate its neural functions (35). Interestingly, experimental approaches such as *in utero* knockdown of calpain by short hairpin RNA has shown potential to rescue defective cortical layering in Lis1(+/–) mice. Therefore, calpain inhibition can represent a potential therapeutic intervention for lissencephaly (225). *DCX* is expressed mainly in the developing brain. Epileptic seizures are generally regarded as a result of complex functional changes based on disruption of the normal development of the cortex and white matter and an impaired neuronal network structure.

A further variant of lissencephaly with autosomal-recessive transmission is because of deletions in the reelin (*RELN*) gene (103). Generally, moderately severe pachygyria and extensive cerebellar hypoplasia coincide. In addition to seizures, severe hypotonia and developmental delay constitute clinical hallmarks of this syndrome. Type II lissencephaly's structural correlate is generally referred to as "pachygyric micropolygyria" (3). The cerebral surface is agyric but not entirely smooth. Histological assessment reveals abundant and confluent microgyri. In the cerebellum, cortical heterotopias occur in a multiple fashion. Type II lissencephaly is frequently associated with oculocerebellomuscular anomalies such as Walker–Warburg syndrome (177) as well as Fukuyama-type congenital muscular dystrophy (79, 173). Macroscopic inspection of lissencephalic brains reveals absence (agyric) or decreased presence (pachygyric) of convolutions. In pachygyria, the gyri are plain and smooth. Microscopically, there is blurring of gray and white and impaired cortical structure. Two types of lissencephaly are distinguished (59): *Type I* is represented by a thick, rarely differentiated cortex under a smooth, largely agyric cerebral surface. There are only limited portions of white matter. The cortex generally comprises four layers:

- a marginal (molecular) layer;
- an outer neuronal layer;
- a cell-depleted layer with tangentially orientated myelinated fibers; and
- an inner neuronal layer.

The respective architecture is frequently observed in the Miller–Dieker syndrome. The neurons with impaired migration can generate an additional cortical layer in the white matter, which underlies the double cortex formation. In contrast, *Type II* lissencephaly shows morphological CNS alterations with pachygyria, insufficient gyration of the cerebral surface and widened gyri (Figure 5). The claustrum is frequently absent and respective differences can be distinguished frequently by MRI (118). In the medulla and cerebellum, aberrant cerebellar cortex architecture and heterotopias of the nucleus olivaris can be observed (113). At the transition zone of the pachygyric to normal cortical areas, the outer neural layer continues into the normal cortex, whereas the inner neural layer does not continue in the normal cortex. This element can differentiate pachy- from polymicrogyria.

Only recently, tubulin alpha1A (TUBA1A), encoding a critical structural subunit of microtubules, has been implicated in lissencephaly. Novel and recurrent TUBA1A mutations are present in approximately 1% of children with classic lissencephaly and approximately 30% of children with lissencephaly with cerebellar hypoplasia (125). Mutations in the so-called TUBA1A gene are found in patients with lissencephaly, pachygyria and polymicrogyria. Mutations in TUBA1A occur *de novo* and in the form of familial recurrence because of parental somatic mosaics in a parent (111). However, further genes such as the recently described GPR56, an orphan G protein-coupled receptor (GPCR) from the family of adhesion GPCRs (137), may be linked to lissencephaly like maldevelopmental patterns in the future.

HETEROTOPIAS

Heterotopia comprises a group of MCD characterized by the presence of excessive neurons, either isolated or clustered, in the subcortical white matter (Figure 6). According with the location and

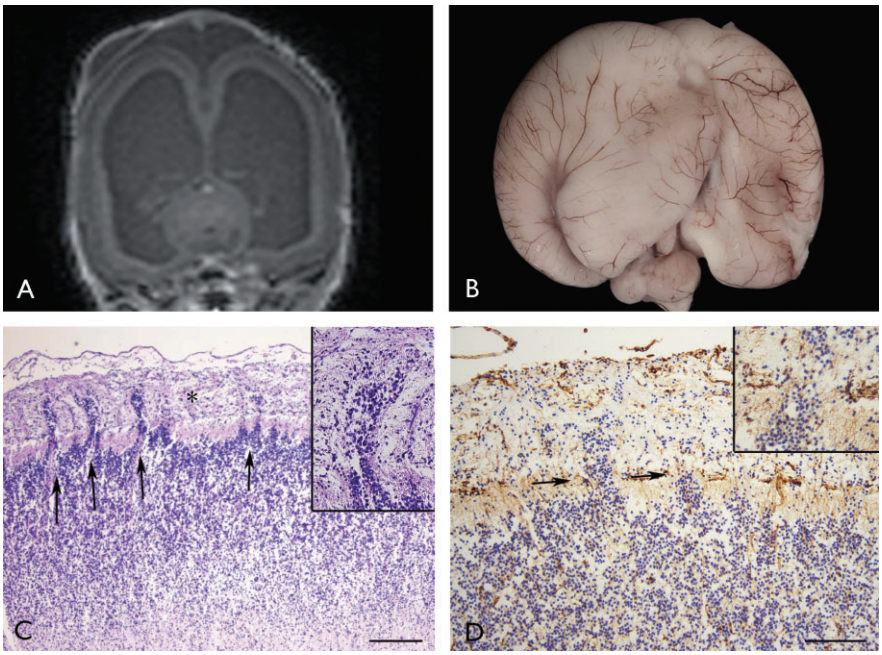


Figure 5. *Type II lissencephaly*. Fetal brain at 22 weeks of gestation with Walker–Warburg syndrome (POMT1 mutation). **A.** Post-mortem MRI (hydrocephalus); **B.** External view of the brain. **C.** (H&E) cortical plate showing focal disruption of the pia-glial limitans (arrows) with overmigration of cells into the leptomeninges (asterisk; high magnification in insert). **D.** Vimentin staining showing the multiple breaches of the pia-glial limitans (arrows; high magnification in insert). Scale bars: A: 240 μM; B: 120 μM. H&E = hematoxylin and eosin; MRI = magnetic resonance imaging.

distribution of heterotopic neurons, three groups are currently described:

- neuronal heterotopia characterized by individual misplaced neurons in the white matter;
- nodular heterotopia with nodules of gray matter within the white matter; and
- band (or laminar) heterotopia (also defined as double cortex).

Although originally only subependymal nodular and band (laminar) forms were considered in the recent classification by Barkovich *et al* (12), the presence of abundant neurons in the white matter, frequently associated with different forms of MCD, is also considered. Gray matter heterotopia can be diffuse or localized. Diffuse forms include subcortical laminar heterotopia and extensive forms of bilateral periventricular nodular heterotopia. Localized forms can be subependymal, unilateral or bilateral, subcortical (nodular, laminar), or may extend from the subependymal region to the cortex unilaterally (transamantle). Among the different types of nodular heterotopia (NH), the number of bilateral and unilateral cases is approximately equal and bilateral symmetrical NH account for about one-third of bilateral cases.

NH

The prevalence of NH has not been ascertained in the general population or patients with epilepsy (2, 66), but diagnoses of NH by MRI have been reported in 13%–20% of large series of epileptic patients with MCD [Figure 1C; (13, 66, 134, 166)]. Two main groups of NH are currently recognized: (i) subependymal heterotopia (SHE) subsuming periventricular nodular heterotopias (PNH) and (ii) subcortical heterotopia (SCH), further subdivided on the basis of imaging and clinical data (10, 12, 13, 66, 201). Some PNH can be causally related to mutations of specific genes and chromosomal rearrangements [see (160) for references]. An X-linked dominant inheritance has been established for familial bilateral periventricular nodular heterotopia (classical bilateral PNH) in females, a condition also characterized by a high incidence of spontaneous miscarriages especially in male fetuses and a 50% recurrence risk in the female offspring of affected women. Linkage analysis mapped this disorder to Xq28 and the gene responsible, filamin 1 (FLN1), has been identified. Sporadic cases of bilateral PNH both in females and males have also been described [see (94) for references.]. However, a genetic etiology has not always been found in other forms presenting single or multiple unilateral or bilateral nodules, sometimes associated with other brain malformations and thus suggesting that NH may represent a heterogeneous disorder (13, 152, 201).

Available surgical specimens were rare in the past as it was generally considered that epileptic patients with PNH did not respond to surgical treatment. However, recent reports indicate that in patients with unilateral heterotopia, surgery can be highly beneficial when epileptogenic zone is carefully identified (201). The surgical success also contributed to the neuropathological studies of these malformations providing new data on the intrinsic organization of the nodule and also suggesting possible etiological mechanisms

In all cases, the nodules consist of masses of neurons, with well-defined boundaries, surrounded by white matter fibers, some of which infiltrate the nodules, suggesting a functional connection between the nodules and the overlying cortex (Figure 5). Neuropathologic investigations (147) reported that all of the nodules, regardless of their size and number (single or multiple), lobe and depth of location (subependymal and/or subcortical), have similar morphological and immunocytochemical characteristics. Similarly organized nodules have also been reported in patients with the filamin 1 gene mutation (116, 205). The reported data showed that the nodules contain pyramidal cells as well as all of the subtypes of inhibitory interneurons with apparent normal morphology (205). Interestingly, the presence

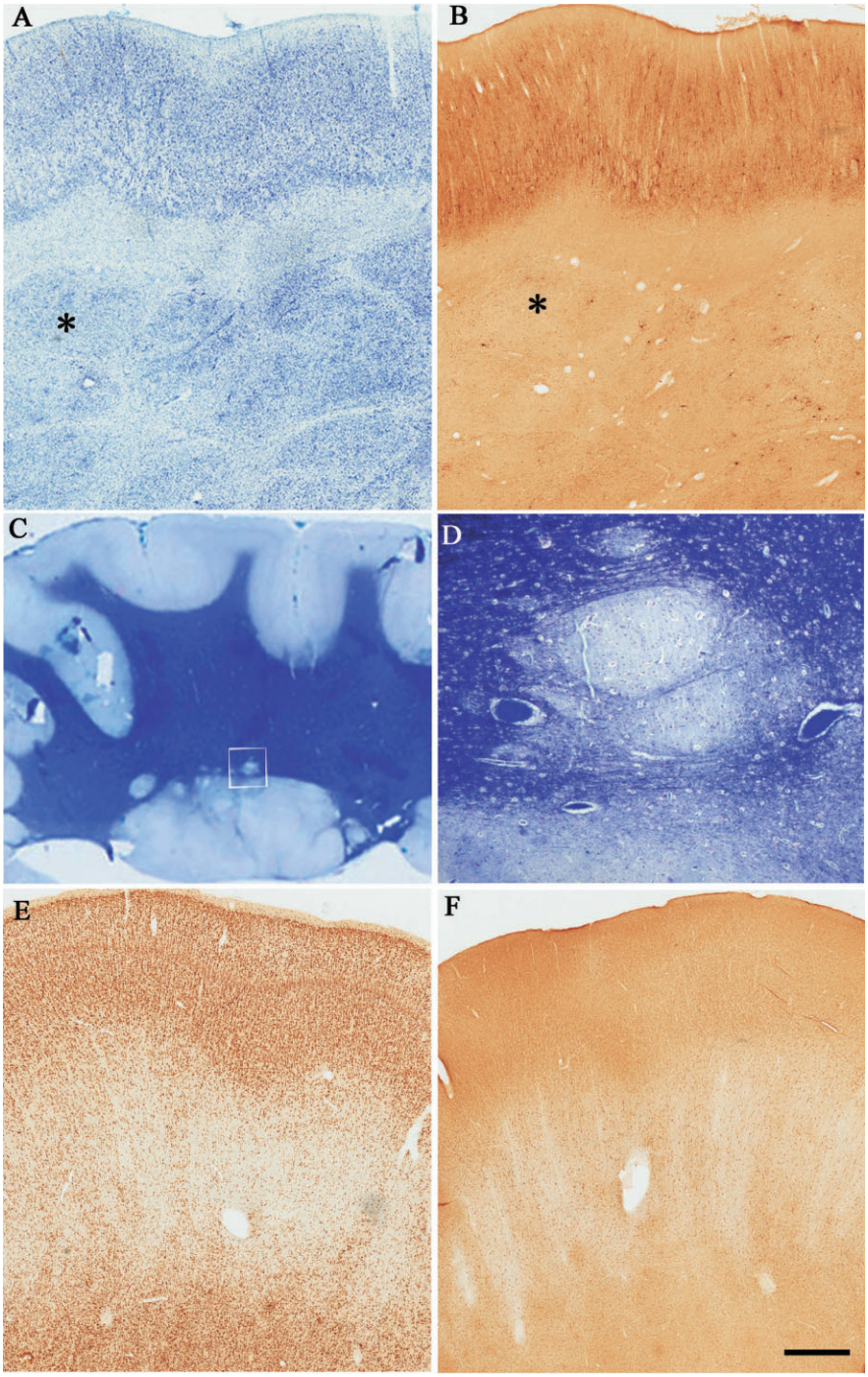


Figure 6. *Heterotopia*. **A,B.** Thionin stained and neurofilament immunostained coronal serial sections (asterisk is indicating the same nodule) from a surgical specimen showing multiple subcortical nodules. Note that the overlying cortex appears disorganized and no lamination can be detected. **C.** Low-power photomicrograph of a Luxol fast blue-stained section of occipital cortex, white matter, subependymal region with nodular formations around the ventricle and overlying cortex. **D.** High-magnification detail of one of the nodules in C (square). **E,F** show two serial coronal sections immunostained with NeuN and MAP2 respectively from a patient with "double cortex." The heterotopic band consists of unlayered cortex with haphazardly organized neurons. The white matter between the "outer" and "inner" cortex contains abundant heterotopic neurons arranged in columns. MAP2 = microtubule-associated protein 2; NeuN = neuronal nuclear antigen.

of cell-free zones with small vessels, reminiscent of the molecular layer, and glial cells, with radiating fibers similar to radial pattern of superficial gliosis, was described, suggesting a rudimentary laminar pattern within the nodules. In addition, small Reelin-immunoreactive neurons within the cell-free zone were identified, mirroring their normal location in the molecular layer of the overlying cortex (205). This peculiar organization was further confirmed by studies performed by analyzing the expression patterns of three layer-specific genes (Ror β, ER81 and Nurr1) in tissues from patients with subcortical NH and demonstrating the prevalent expression of neurons belonging to layer VI and layer V in the external border of the nodules, with those belonging to layer IV in a more internal region (82).

The hypothesis that in subcortical NH, some of the Cajal–Retzius, Reelin-secreting, cells remain displaced within the subplate during early stages of cortical development and that these cells, wrongly located in this transient layer, attract migrating neurons into a heterotopic position leading to the formation of a

heterotopic malformation with a rudimentary laminar organization, was postulated (82). This hypothesis was indirectly confirmed by Kubo *et al* (124), demonstrating that ectopically expressed Reelin in developing mouse cortex is able to determine subcortical nodular aggregates with laminar organization similar to those observed in human pathology. Although MRI frequently suggests a polymicrogyric cortex overlying the NH, recent neuropathologic data reported abnormal gyration but without histological abnormalities consistent with the typical four-layered or unlayered polymicrogyria. However, various degrees of cortical laminar disorganization similar to those observed in FCD has been described with reduced expression of calcium binding proteins, suggesting an impairment of the GABAergic system. In other cases, a normal cortical organization can be found although cortical disruption can be noted in areas where nodules invade the cortex (147, 201, 205). When NH involves the temporal lobe, hippocampal sclerosis may also be present.

Band heterotopia

This type of malformation, also defined as laminar heterotopia or double cortex, is included by Barkovich and coworkers (12) among those due to abnormal neuronal migration and incorporated in the subgroup defined as lissencephalies/subcortical band heterotopia spectrum. However, from the neuropathological aspect, this malformation is frequently considered within the broad group of "heterotopia." Being a diffuse cortical malformation, frequently genetically determined, only a few neuropathological reports are available (138). Due to its frequent association with intractable epilepsy, focal surgical resections have been proposed in selected patients. However, despite the fact that in some surgically treated patients, satisfactory postsurgical outcome has been achieved, in general, surgery is considered to yield inadequate results in these cases. Despite the small number of surgical specimens, careful anatomical descriptions are available, thanks to the high-resolution imaging also providing genotype/phenotype correlations of this malformation (93). The gross anatomical aspect observed on both MRI (Figure 1D) and available specimens shows a continuous bilateral, roughly symmetrical, band of gray matter located between the cortical mantle and the ventricles. The thickness of the band varies among patients and seems to correlate with the degree of associated mental retardation. A slight reduction in number of gyri and pachygyria can be observed in the "outer" cortex, but in general, it is of normal thickness and formed by the regular six layers with a normal distribution of neuronal elements with no cytological alterations. A zone of white matter of variable thickness containing abundant heterotopic neurons frequently arranged in columns is present between the "outer" and "inner" cortex (Figure 5E,F). The heterotopic band consists of unlayered cortex with small and medium-sized rounded or pyramidal neurons. Although macroscopically the heterotopic band appears as a continuous aggregate of neurons, at the microscopic level it appears discontinuous and interrupted by radially oriented fibers. Within the white matter and in the heterotopic band, the pyramidal neurons are haphazardly oriented and nonpyramidal neurons express different calcium-binding proteins, suggesting the presence of different subpopulations of GABAergic interneurons.

POLYMICROGYRIA

Polymicrogyria (PMG) is a cortical malformation characterized by an excessive number of small irregular gyri separated by shallow sulci leading to an irregular cortical surface with a lumpy aspect. PMG can be localized to a single gyrus or can involve a portion of one hemisphere; it can be bilateral and asymmetric, bilateral and symmetric, or diffuse. PMG may escape or be confused with "thickened cortex" at imaging investigations when CT scan or low-field MRI are performed but, if appropriate age-related protocols are used with high-resolution MRI, it can be reliably differentiated from other forms of MCD and identified by the presence of numerous cortical gyration even in the absence of irregular gray/white matter junctions. The extent and location of PMG in different brain regions can determine a variety of specific syndromes with a wide range of clinical manifestations, from severe encephalopathy with intractable epilepsy to only selective impairment of cognitive function. Several malformation syndromes have been described such as bilateral perisylvian, bilateral parasagittal parieto-occipital, bilateral frontal, and unilateral perisylvian or multilobar types (90, 95). However, the most common form of PMG is represented by the bilateral perisylvian PMG characterized by distinct clinico-pathological features and consistent familial recurrence (93).

Various patterns of inheritance have been described for the different subtypes of polymicrogyria (90). No individual genes have been linked to any of the bilateral forms with isolated polymicrogyria, although a mutation in *MECP2* was observed in a male patient with bilateral perisylvian disorder and severe neonatal encephalopathy (83). A recent hybrid genetic-MRI approach led to the identification of the homeobox gene *PAX6* as a factor, in which mutations can result in unilateral polymicrogyria (149). As observed frequently for other types of cortical malformations, polymicrogyria may be part of multiple congenital anomaly syndromes and is frequently observed in the vicinity of encephaloclastic lesions, such as schizencephaly [Figure 1E,F (38)]. PMG can also be secondary to exogenic causes such as infections including cytomegaly, toxoplasmosis, rubeola, as well as to impaired hemodynamic disturbances, particularly referred to the perfusion area of the middle cerebral artery.

Although PMG is frequently associated with epileptic syndromes, the mechanisms of epileptogenesis related to PMG are not entirely understood. In an experimental model in which microgyri are generated by a freezing insult, it has been shown that functional abnormalities extend beyond the anatomic malformation (110, 168). These data corroborate the observations in humans revealing that the cortex surrounding the PMG is also involved in epileptogenesis and suggest that surgical resection restricted to the polymicrogyric area is not sufficient to substantially attenuate or abolish recurrent epileptic seizures (188, 189). Polymicrogyria is, however, not inevitably associated with epilepsy, and it may present as developmental delay or congenital hemiparesis.

In PMG, the gyri are atypically organized with abnormalities of the physiological laminar cytoarchitectural structure. Although a variety of intermediate neuropathological phenotypes exist, two main histological types are observed, the unlayered and the four-layered types. In the unlayered PMG, the molecular layer is continuous and does not follow the convolution profiles. The neurons have a radial distribution without any laminar organization (72).

This aspect suggests an early disruption of normal neuronal migration with subsequent cortical disorganization. By contrast, the four-layered PMG shows a four-layered laminar structure composed by a molecular layer, outer neuronal layer, nerve fiber layer and inner neuronal layer. Apparently, the third layer (nerve fiber) is a result of cellular necrosis. Occasionally, in the neuronal cell layers, granular as well as pyramidal neurons can be observed, resembling residuals of the normal six-layer cortical architecture. This subtype of malformation is thought to be the result of perfusion failure, occurring between the 20th and 24th weeks of gestation, leading to intracortical laminar necrosis with consequent late migration disorder and postmigratory overturning of cortical organization (73, 78). These two histopathological subtypes do not necessarily have a distinct origin, as both may coexist in contiguous cortical regions (18, 93).

SCHIZENCEPHALY

Por-/schizencephaly represent generally cystic lesions of cortical brain areas. Anatomical consequences are given by aberrant, immediate connections between the lateral ventricles and the subarachnoidal space. Schizencephaly is currently grouped with polymicrogyria among the malformations of cortical development (MCD; group IIIA 1,2) (11). The immediate pathological substrate of schizencephaly is given by a defect presenting with a transcortical cleft with open and partially closed parenchymal portions decorated by cortical tissue portions (Figure 1E,F).

For schizencephaly, several pathogenetic factors and etiological aspects exist, encountering regional lack of proliferating neurons and glial cells, which impairs cortical architecture. Furthermore, local impairment of neuronal migration or circumscribed ischemic necrosis comprising destruction of the radial glial fibers during early gestation has been claimed (91). Congenital cytomegaly virus infections have been linked with severe brain malformations including schizencephaly (34). Epidemiological data suggest that schizencephaly occurs more frequently in the fetuses of younger mothers and is associated with septo-optic dysplasia, suggesting that the two conditions may be of common origin (105). The majority of schizencephaly patients are sporadic, but familial schizencephaly has been described. Recent data suggest molecular alterations in schizencephaly, encountering germline mutations in the EMX_2 homeobox gene in approximately 70% of the cases (36, 37, 69). However, these data could not be confirmed by other studies (101). Sequencing analyses did not reveal genomic aberrations of *LHX2*, a gene with an important cortical patterning role; or *HESX1* or *SOX2*, genes that have been associated with septo-optic dysplasia in schizencephaly (146). As schizencephaly frequently occurs with additional cerebral malformations, such as hypoplasia or aplasia of the septum pellucidum or optic nerve, the involvement of genes important for the establishment of midline forebrain structures has been hypothesized and analyzed. These experiments resulted in observing heterozygous mutations in the *SIX3* and *SHH* genes in a total of three unrelated patients and one fetus with schizencephaly; one of them without obvious associated malformations of midline forebrain structures. Three of these mutations have previously been reported in independent patients with holoprosencephaly. SIX3 operates immediately upstream of SHH, and the SHH pathway represents a key regulator of ventral forebrain patterning. These data suggest that schizencephaly may represent a pathogenetic aspect of a more complex aberration of ventral forebrain development in some patients, that is, belonging to the pathological pattern of holoprosencephaly related to mutations in SHH pathway components (101).

Schizencephaly frequently affects central brain regions as well as sites perfused by the middle cerebral artery. Hypoxic damage is of major importance during fetal development until the end of cortical maturation. Transitions of schizencephaly with polymicrogyria (72) (*type 1*) and co-occurrence of schizencephaly with radial orientation of the gyri adjacent to the cortical defect (*type 2*) have been described (18, 40, 130). Schizencephaly often occurs bilaterally. However, the severity of the pathological manifestations frequently varies, that is, a porus is often present only on one side of the brain, whereas only abnormal gyration is present on the contralateral side. The inner glio-ependymal surface can project to the external cortical surface with the structural correlate of a membranous, pale membrane. Clefts may be associated with a range of other malformations of septum, optic nerve, callosum or hippocampus (100). Epilepsy surgery represents a therapeutic option only in a minor fraction of schizencephalic patients with pharmacorefractory epilepsies (188). A major differential diagnosis of schizencephaly is given by cystic necrosis due to inappropriate perfusion at peri- and postnatal stages of development. However, these cystic lesions do not generally show communication between the inner and outer cerebrospinal fluid spaces.

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