Neurodevelopmental Disorders as a Cause of Seizures: Neuropathologic, Genetic, and Mechanistic Considerations

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This review will consider patterns of developmental neuropathologic abnormalities—malformations of cortical development (MCD)—encountered in infants (often with infantile spasms), children, and adults with intractable epilepsy. Treatment of epilepsy associated with some MCD, such as focal cortical dysplasia and tubers of tuberous sclerosis, may include cortical resection performed to remove the "dysplastic" region of cortex. In extreme situations (eg, hemimegalencephaly), hemispherectomy may be carried out on selected patients. Neuropathologic (including immunohistochemical) findings within these lesions will be considered. Other conditions that cause intractable epilepsy and often mental retardation, yet are not necessarily amenable to surgical treatment (eg, lissencephaly, periventricular nodular heterotopia, double cortex syndrome) will be discussed. Over the past 10 years there has been an explosion of information on the genetics of MCD. The genes responsible for many MCD (eg, *TSC1*, *TSC2*, *LIS-1*, *DCX*, *FLN1*) have been cloned and permit important mechanistic studies to be carried out with the purpose of understanding how mutations within these genes result in abnormal cortical cytoarchitecture and anomalous neuroglial differentiation. Finally, novel techniques allowing for analysis of patterns of gene expression within single cells, including neurons, is likely to provide answers to the most vexing and important question about these lesions: *Why are they epileptogenic?*

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Introduction

Neocortical malformations are now a recognized cause of intractable epilepsy, often occurring in infants (to produce infantile spasms, ISS) and children, occasionally presenting in adolescents and even adults. Surgical therapy for some of these lesions is now acceptable, indeed often *indicated*, in carefully selected patients (31, 76, 88). The specimens that result from such complex operations continue to provide neuropathologists with novel brain tissues that may yield clues to the pathogenesis of seizures in individuals at all ages. The most commonly observed morphologic substrate of ISS—cortical dysplasia (CD), a type of malformation of cortical development (MCD)—appears to represent a profound maldevelopment of the cerebral cortical mantle (83, 108). It seems logical or intuitive that such a malformation could be associated with anomalous electrical discharges resulting in seizures (39). Precisely how this occurs, however, is not known although inroads to understanding pathogenetic mechanisms will be the focus of this article. The challenges to neuropathologists who examine material from patients with MCDs (whether at autopsy or, increasingly in the surgical pathology cutting room) are *a)* to try to explain the origin of the often bizarre structural findings in the resected tissue, including what represents primary or secondary abnormalities, and *b)* to attempt to comprehend *precisely* how and why these aberrations of the cerebral cortex produce intractable seizures, including ISS (112).

Surgery for the treatment of pediatric epilepsy is usually performed at tertiary medical centers, primarily because this is where the ancillary pre-operative investigations necessary to determine an optimal operative strategy and outcome are readily available. The resulting corticectomy specimen needs to be handled in such a way as to optimize its value for research, as well as providing the most appropriate neuropathologic diagnosis (32, 83, 115). For example, at UCLA Medical Center, a fragment of cortex is usually provided to the neurophysiology laboratory directly from the operating room (75). The remaining tissue, depending upon its size, is in most cases aliquoted such that representative fragments are snap frozen for subsequent neurochemical/molecular studies, if indicated or of interest, and others fixed in

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paraformaldehyde and cryopreserved for immunohistochemistry or embedded in plastic (lowicryl or LR White medium), to be used in subsequent immuno-ultrastructural investigations or high resolution light microscopic immunocytochemistry. Recent developments in molecular biological technologies such as cDNA array analysis and tissue microdissection permit analysis of gene expression in immunohistochemically labeled tissue (see below). Small tissue fragments are fixed in glutaraldehyde for electron microscopy, which is not necessarily carried out on every specimen but may be helpful in some cases. The remaining formalin-fixed tissue is routinely processed in paraffin blocks and stained with hematoxylin and eosin, silver stains (eg, modified Bielschowsky technique to show cytoskeletal anomalies) and the Klüver-Barrera technique, which highlights neuronal morphology and often subcellular architectural features, as well as integrity of the myelinated white matter. Using this technique, any "blurring" of the cortex-white matter junction that may represent evidence of suboptimal or deranged neuronal migration to the neocortex can be easily visualized, even without placing the section under the microscope. Immunohistochemical studies are carried out on selected tissue blocks after initial review of the case, and will be discussed and illustrated in greater detail below.

Studies from most major pediatric epilepsy surgery centers report that CD is the most common neuropathologic abnormality encountered when cortical resection is performed to treat ISS and related intractable seizure disorders of infancy or childhood (91, 113, 114, 115). However, other types of lesion are occasionally encountered, including uni- or multifocal cystic/gliotic encephalopathy, possibly representing the result of intrauterine or perinatal anoxic-ischemic injury or infarcts. When encountered in a corticectomy specimen, these manifest as regions of grossly apparent encephalomalacia with cystic cavitation, surrounded by dense astrocytic gliosis and often regions of dystrophic punctate calcification, making it nearly impossible to make precise judgements about their etiology or pathogenesis (114, 115). Rarely, Sturge-Weber-Dimitri syndrome (encephalotrigeminal angiomatosis) presents as a surgically treatable lesion during the first year of life, though more commonly this lesion is operated on in older children (32, 41). Rasmussen encephalitis (RE) is a comparatively rare, though important, cause of epilepsia partialis continua (EPC) in older children; it is often treated surgically (32, 33, 114). Primary CNS neoplasms, rare in the first year of life but more common in older children, usually manifest (in infancy) with

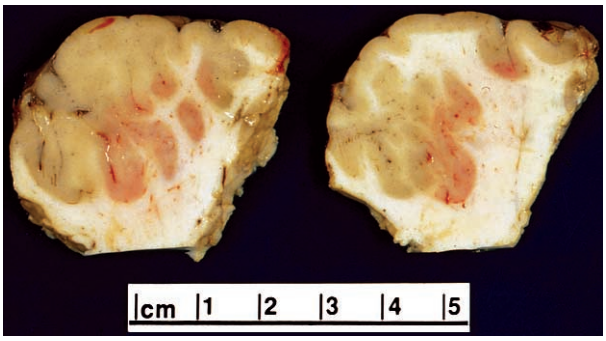

Figure 1. Gross appearance of surgically resected cerebral cortex and underlying white matter from a 4-year-old boy with intractable epilepsy. Note marked irregular thickening of cerebral cortex with blurring of the cortex-white matter junction (left), and abundant heterotopic gray matter (right).

enlargement of the head and a tense fontanel; about 20% of infants with such a tumor present with a neurologic syndrome that may include seizures (95).

An MCD (including focal cortical dysplasia, FCD) may be suspected upon examination of slices of the fixed corticectomy specimen, which sometimes show a widened cortical ribbon, blurring of the cortex-white matter junction, or irregular nodules of gray matter within subcortical white matter (Figure 1). Klüver-Barrera-stained sections highlight these abnormalities—FCD is often identified by holding such a section against the light. Hemimegalencephaly, a profound MCD that affects an entire cerebral hemisphere, may demonstrate especially severe abnormalities in cut slabs of the gross specimen (27, 59). Microscopic features of FCD (83) include cortical dyslamination of variable severity, often focally accentuated adjacent to regions of entirely normal appearing cortex, the presence of mushroom-like disorganized excrescences of neuroglial tissue protruding into the subarachnoid space, persistence of neurons in cortical layer I, excessive (single) neurons in the subcortical white matter and nodular heterotopias of disorganized neuroglial tissue in the deep subcortical white matter. Histopathologic features of severe FCD include bizarre, enlarged neurons with cytoskeletal disorganization, often best demonstrated using a silver stain, and balloon cells that resemble gemistocytic astrocytes, with oval pale glassy eosinophilic cytoplasm (see below). These 2 cell types appear randomly scattered among each other in a patternless arrangement accentuated by the generalized cortical disorganization. Many of the cells in such foci show histologic features suggesting the existence of an indeterminate or undifferentiated phenotype, with both neuronal and glial features, eg, a cell may possess a characteristic nucleolat-

ed nucleus (typical of neurons) but show glassy, hyaline eosinophilic cytoplasm, a feature more suggestive of astrocytic differentiation. Immunohistochemical studies (see discussion below) confirm that many cells of indeterminate or "uncommitted" phenotype are present in such lesions (32, 115). Some specimens show focal neuronophagia with surrounding microglia, a histologic feature which suggests ongoing death of neurons (113).

Malformations of Cortical Development

Malformations of cortical development (MCD) include a heterogeneous group of disorders also referred to as cortical dysplasias (CD) and neuronal migration disorders (NMDs), in which the normal hexalaminar structure of the cerebral cortex is disrupted and individual neural morphologies may be aberrant (for review, see 2). MCD can uniformly affect broad regions of the cerebral cortex as in classical lissencephaly and hemimegalencephaly, or may be restricted to focal areas such as tubers in the tuberous sclerosis complex (TSC) or Taylor type focal cortical dysplasia (FCD). MCD may also exhibit large collections of heterotopic neurons, as in syndromes of subcortical band heterotopia and periventricular nodular heterotopia. In some examples of MCD, the normal 6-layered organization of the cerebral cortex is replaced by a more primitive 4-layered arrangement, as in lissencephaly and polymicrogyria. In other MCD, eg, FCD or tubers of TSC, there is a virtual loss of all lamination. The morphology of individual neurons in most MCD is abnormal, suggesting a pervasive disruption of many steps important in neuronal development. In the past, MCD have been categorized on the basis of structural pathological features such as gyral patterning (agyria, pachygyria, polymicrogyria). However, positional cloning efforts in well-defined familial pedigrees over the past decade have permitted identification of single gene mutations in select forms of MCD that have led to more precise molecular diagnostic classification of these disorders.

What are the Key Clinical Issues in Patients with MCD?

The single unifying feature of all MCD is a strong clinical association with epilepsy (2, 19). For example, it is estimated that MCD may account for 20% of all epilepsies (10, 53) and in select extensive MCD such as lissencephaly, hemimegalencephaly, and TSC, seizures may occur in 70 to 90% of affected patients. Even smaller malformations such as focal heterotopia or focal dysplasias are strongly associated with often intractable seizures. Estimates are that nearly 30% of cortical specimens resected as treatment for focal neocortical epilepsy contain some type of cortical developmental malformation. Recent advances in neuroimaging have demonstrated that many cases of "cryptogenic" epilepsy are actually associated with small regions of cortex containing subtle cytoarchitectural abnormalities (microdysgenesis). Finally, a subgroup of adult patients with temporal lobe epilepsy exhibit radiographic and histopathologic evidence of MCD either alone or in combination with hippocampal sclerosis ("dual pathology") suggesting that MCD is clinically relevant in both pediatric and adult epilepsy patients (55). From a clinical perspective, virtually all seizure subtypes, eg, generalized tonic-clonic, complex partial, atonic, myoclonic, atypical absence seizures and infantile spasms, have been described when the broad family of MCD are considered in toto. Satisfactory seizure control in patients with MCD may be particularly difficult to attain despite antiepileptic drug polytherapy, and surgical resection is often necessary (76). Unfortunately, even with extensive attempts at localization of the seizure focus and skilled neurosurgical technique, seizure cure following surgical resection of focal CDs is successful in less than 50% of patients. Even worse, certain patients are not surgical candidates at all.

Developmental Contextual Background for MCD

Development of the human cerebral cortex is initiated at gestational week 7 and continues through week 24 (for review, see 49, 79, 96). The cortex is formed in four broad stages: *1)* mitosis and proliferation of neural progenitor cells in the embryonic ventricular zone (VZ), *2)* commitment to a neural lineage and exiting mitotic phases of the cell cycle, *3)* dynamic migration of postmitotic neurons out of the VZ, and *4)* the establishment of cortical laminae in the evolving cortical plate through an "inside-out gradient." Neurons destined to reside in deeper cortical laminae, eg, layer VI, arrive first in the nascent cortical plate, and subsequent waves of neurons destined for more superficial layers, eg, V, IV, III, must migrate through each preceding and established layer.

The histopathologic features of MCD syndromes provide a visual anatomic map that reflects the molecular mechanism causing the malformation. Indeed, as we endeavor to understand the pathogenesis of each MCD subtype it is important to place the putative molecular events responsible for the malformation into an appropriate developmental context, since the genesis of each malformation is inexorably linked to cortical develop-

MCD Subtype	Locus	Gene	Protein
Miller-Dieker lissencephaly	17p13	*LIS-1*	PAFAH1B1
Subcortical band heterotopia (SBH)	Xq22	*DCX*	DCX or doublecortin
X-linked lissencephaly (XLIS)	Xq22	*DCX*	DCX or doublecortin
Periventricular nodular heterotopia (PH)	Xq28	*FLN1*	filamin1
Tuberous sclerosis complex (TSC)	9q34	*TSC1*	hamartin
	16p13	*TSC2*	tuberin
Fukuyama congenital muscular dystrophy (FCMD)	9q31	*FCMD*	fukutin
Muscle-eye-brain disease (MEB)	1p32	*POMGnT1*	POMGnT1

DCX, doublecortin; FLN1, filamin1; PAFAH1B1, platelet activating factor acetylhydrolase β subunit; POMGnT1, protein *O*-mannose β1,2-*N*-acetylglucosaminyltransferase

Table 1. Genetics of MCD syndromes.

mental processes. The genes responsible for MCD have important functions in one or more stages of normal cortical development. Thus, the histopathologic features of each MCD render a fascinating view of the role that responsible gene plays in cortical development by demonstrating the sequelae of loss of encoded protein function. In addition, the effect of each gene mutation also highlights the developmental epoch in which that gene contributes to corticogenesis; these critical time points provide a framework to understand the interface between gene mutations and abnormal neural development. For example, gene mutations that alter mitosis will result in an effect confined to the proliferative stages of cortical development but may have little effect on post-mitotic neurons. Similarly, a gene mutation that alters cytoskeletal assembly during dynamic phases of neuronal migration will have distinct effects in an actively migrating neuron versus a neuron that has already achieved its laminar destination. In sum, a defined molecular event occurring within a specific developmental context results in a characteristic malformation.

Molecular Neurobiology of MCD

Only in the past decade have we begun to understand the molecular pathogenesis of select MCD. While developmental malformations may be observed in a variety of neurological disorders without a definitive molecular correlate, such as dyslexia, autism, and schizophrenia, MCD may be observed in the setting of large chromosomal rearrangements such as trisomy syndromes and as a consequence of single gene mutations. Indeed, positional cloning strategies in human pedigrees with inherited (autosomal and sex-linked) forms of MCD have led to the identification of at least 8 genes (Table 1) directly responsible for human MCD that are associated with epilepsy (for review, see 117) and most of these genes are being studied in transgenic or knockout mouse strains. As we learn more about the function of these genes, we can begin to classify MCD with a more mechanistic nomenclature, that is, as disorders of neural proliferation or migration. Several MCD occur by a yet to be defined mechanism during an unknown epoch. Furthermore, although MCD are initiated at specific timepoints, effects on later developmental processes may also be sequelae of the initial inciting event. For example, disorders of cell proliferation such as TSC also likely include disorganized neural migration, while migrational disorders such as lissencephaly are also likely to show defects in cell process outgrowth and synaptogenesis.

Disorders of cellular proliferation. The tuberous sclerosis complex is an autosomal dominant disorder characterized by cerebral cortical tubers that are regions of focal CD strongly associated with epilepsy. Tubers are the likely source of seizure initiation in TSC patients (50, 67). Microscopically, the normal hexalaminar structure of cortex is lost within a tuber. Neurons exhibiting aberrant somatodendritic morphologies and cytomegaly (dysplastic neurons) are abundant within them. Giant cells, a unique cell type not seen in any other neurologic disorder other than severe FCD, are a defining feature of tubers (116). These bizarre cell types exhibit extreme cytomegaly, shortened processes of unknown identity (axons or dendrites), and are often bi- or multinucleated. Extensive astrocytosis is a variable feature of tubers (for review, see 22). TSC results from mutations in one of 2 non-homologous genes, *TSC1* (111) and *TSC2* (110). The *TSC1* gene encodes a protein, hamartin, and has virtually no homology to known vertebrate genes. The *TSC2* gene encodes a 200 kD protein tuberin that is structurally distinct from hamartin. Both hamartin and

tuberin mRNA and protein are widely expressed in normal tissues including brain, liver, adrenal cortex, cardiac muscle, skin, and kidney (51, 61, 65, 80). Identification of an encoded coiled-coil domain in the carboxy region of hamartin (111) raised the possibility of a functional protein-protein interaction with tuberin (and other proteins) that has recently been demonstrated in *Drosophila* (89). Indeed, hamartin interacts with the ezrin-radixin-moesin (ERM) family of actin-binding proteins and may contribute to cell-cell interactions, cell adhesion, and cell migration (70). Tuberin contains a hydrophobic N-terminal domain and a conserved 163 amino acid carboxy terminal region that exhibits sequence homology to the catalytic domain of a GTPase activating protein (GAP) for Rap1. As a member of the superfamily of Ras-related proteins, Rap1 likely functions in regulation of DNA synthesis and cell cycle transition. Tuberin displays GAP activity for Rap1 and co-localizes with Rap1 in the Golgi apparatus in several cell lines (120, 121). The GAP activity of functional tuberin may modulate the effects of Rap1 on G- to S-phase transition during cell division. Mutations in *TSC2* might result in constitutive activation of Rap1 leading to enhanced cell proliferation or incomplete cellular differentiation. Recent studies in *Drosophila* suggest that hamartin and tuberin form a functional heteromeric complex that is an important component of a pathway that modulates insulin receptor or insulin-like growth factor mediated signaling (43, 89). This pathway functions downstream of the cell signaling molecule Akt to regulate cell growth and potentially cell size. Thus, loss of hamartin or tuberin function following *TSC1* or *TSC2* mutations may result in enhanced proliferation of neural and astrocytic precursor cells and increased cell size characteristic of dysplastic neurons and giant cells commonly found in tubers of TSC. Of course, enhanced cell size may compromise neuronal migration and account for the loss of lamination within tubers. Alternatively, loss of hamartin or tuberin function may independently compromise neural migration via an interaction with ERM or actin binding proteins. Recently it has also been shown that hamartin and tuberin interact with the G2/M cyclin-dependent kinase CDK1 and its regulatory cyclins A and B (14). Abnormalities of radial glia have also been implicated in the pathogenesis of CNS lesions of TSC (87).

An interesting issue in TSC is whether the cellular constituents of tubers collectively reflect the cellular manifestations of TSC gene haploinsufficiency (heterozygosity) versus complete loss of gene function in tubers (loss of heterozygosity). For example, all somatic cells in TSC patients (including neurons), contain a single mutated copy of either TSC gene and are thus haploinsufficient or heterozygous. With the interesting exception of tubers, it has been shown that a "second hit" somatic mutation occurs in the second unaffected TSC allele in a variety of TSC lesions resulting in loss of function of either hamartin or tuberin. These lesions exhibit loss of heterozygosity. A "second-hit" mutation has not been conclusively identified in tubers, and recent studies suggest that haploinsufficiency effects alone may lead to tuber formation. Indeed, several studies have demonstrated tuberin expression in tubers despite a known *TSC2* mutation genotype (60). Alternatively, only a select population of cell types in tubers, eg, giant cells, may actually have sustained 2 mutational hits and thus these cells represent the effects of loss of function in maturing developing neurons. This important concept implies that only a defined population of cell types in tubers, eg, giant cells, may actually have sustained 2 mutational hits and reflect loss of gene function during brain development. Other adjacent cell types in tubers may be mere innocent (and haploinsufficient) bystanders whose migration into cortex has been interrupted. This conundrum has not been resolved, yet it remains a point of critical relevance in understanding the pathogenesis of TSC.

Disorders of neuronal migration. Lissencephaly is a severe developmental brain malformation characterized by loss of the normal gyral patterns in the cerebral hemispheres, marked disorganization of the cerebral cortical cytoarchitecture, and a high association with profound neurologic deficits and epilepsy. The lissencephalies comprise a group of disorders sharing similar pathologic features that result from mutations in distinct genes. Recent evidence has shown that the identified genes in each syndrome play important roles in normal neural migration and thus, these MCD result from defects in neural migration. There are 2 pathological subtypes of lissencephaly, type I (classical) and type II (cobblestone). In type I lissencephaly, the cortex is thickened and without gyri. The normal hexalaminar structure is reduced to a 4-layered pattern including a marginal, superficial cellular, sparsely cellular, and deep cellular laminae. Cells in the deeper layers are dysmorphic and exhibit features of pyramidal, fusiform, or rounded neurons without clear radial orientation. One of the first MCD genes discovered was *LIS-1* on chromosome 17p13.3 in patients with the autosomal recessive Miller-Dieker lissencephaly syndrome (98). This syndrome is an autosomal recessive disorder characterized

by classical lissencephaly, profound mental retardation, epilepsy, and craniofacial dysmorphism. Hemizygous deletions within the *LIS-1* gene are associated with Miller-Dieker lissencephaly in over 90% of patients. The encoded *LIS-1* protein is the beta subunit of platelet activating factor acetylhydrolase (PAFAH1B1). Platelet activating factor (PAF) is a potent phospholipid messenger molecule whose intra- and extracellular levels are controlled by PAFAH, which functions as a degradative enzyme for PAF. LIS-1 is a beta subunit component of a trimeric $\alpha 1/\alpha 1/\beta$ complex. PAFAH1B1 contains 7 repetitive stereotyped tryptophan and aspartate repeats (WD40 repeats) that likely function in protein-protein interactions. Stimulation of the platelet activating factor receptor disrupts neuronal migration in vitro (7) and mutations in the *LIS-1* gene may lead to defective nucleokinesis (movement of the neuronal nucleus during dynamic phases of neural migration). LIS-1 is expressed in Cajal-Retzius cells, the ventricular neuroepithelium, a subset of thalamic neurons, and in the subplate in fetal brain specimens (16, 97). The LIS-1 protein interacts with microtubules (101) and may function in destabilization of these cytoskeletal elements during neuronal migration (for review, see 35). A fungal homolog of LIS-1, *NudF*, has been identified in *Aspergillus nidulans* and interacts with a downstream protein NUDE (56). These proteins interact with cytoplasmic dynein/dynactin subunits and may contribute to nuclear transport. Two LIS-1-interacting proteins, Nudel and a mammalian homolog NudE, identified by a yeast 2-hybrid screen, are components of the dynein motor complex and microtubule organizing centers (34) that are critical for neuronal migration. In addition, LIS-1-dynein interactions may also regulate cell division. In mutant *LIS-1* mouse strains, there is a range of disorganization of cortical cytoarchitecture including abnormal hippocampal and cortical lamination (54) and electrophysiologic studies have demonstrated hyperexcitability in this tissue (38)

X-linked lissencephaly (XLIS) is also, by histologic criteria, a classic lissencephaly although a few abnormally large gyri (pachygyri) may be noted. While the neuropathologic features of XLIS are virtually indistinguishable from Miller-Dieker lissencephaly and mental retardation and epilepsy are invariably present in affected patients, there are no associated craniofacial abnormalities in XLIS. Mutations in the doublecortin gene (*DCX*) on chromosome Xq22 in hemizygous males result in lissencephaly (29, 46), whereas *DCX* gene mutations in females result in the subcortical band heterotopia syndrome (see below). The mutational spectrum includes deletion, nonsense, missense, and splice donor site mutations (77), many of which are clustered in two regions of the open reading frame (48). The 40 kDa DCX protein is normally expressed in post-mitotic neurons during the limited time window surrounding neuronal migration (human gestational weeks 12-20; [93]), and thus, mutational effects will be exerted only during this dynamic phase of cortical development (48). A small proportion of mature neurons also express DCX (84). DCX is a microtubule associated protein that specifically interacts with, stabilizes, and stimulates tubulin polymerization via a binding domain ("beta-grasp" superfold motif) that that has been shown to be disrupted in patients with DCX mutations (109). DCX co-precipitates with LIS-1 protein (12) suggesting that these 2 molecules may reflect a pivotal common pathway in assembly of the neuronal cytoskeleton during dynamic phases of neuronal migration (47). A recent study has also shown that DCX interacts directly with the AP-1 and AP-2 adaptor complexes involved in clathrin-dependent protein sorting and potentially vesicular trafficking (42). Thus, in addition to effects on neuronal migration, mutations in DCX might compromise movement of select proteins or vesicle bound molecules such as neurotransmitters that may enhance excitability and foster seizures (see below). A related molecule, doublecortin-like kinase (DCLK) shares sequence similarity to DCX in its N-terminal region and is also co-localized with microtubules (11). DCLK is also a substrate for the cysteine protease calpain (11). DCX contains a consensus substrate site for c-Abl, a non-receptor tyrosine kinase that also modulates cytoskeletal assembly (46). The association with c-Abl may herald an important mechanistic link to specific cell pathways since a mutation in the mouse *disabled1* gene, a c-Abl binding protein, also results in abnormal neuronal migration.

Although *LIS-1* and doublecortin mutations account for approximately 70 to 80% of classical lissencephaly syndromes, other lissencephalies exist that do not result from mutations at either of these loci. A recent study reported 2 consanguineous pedigrees, in which an autosomal recessive lissencephaly syndrome associated with cerebellar hypoplasia, was mapped to chromosome 7q22 by linkage analysis (57). The investigators postulated that the mutational locus for this syndrome would be at or near 7q22 since this chromosomal region contains the *reelin* (*RELN*) gene and mutations in this gene in mice result in neocortical migration abnormalities and cerebellar hypoplasia, similar to those seen in the patient cohort. Reelin is a secreted protein that modulates neuronal migration by binding to several cell sur-

face molecules including the very low density lipoprotein receptor, the apoprotein E receptor 2, $\alpha 3\beta 1$ integrin, and protocadherins. *RELN* is encoded by 65 exons and spans more than 400 kilobase pairs of genomic DNA. A precise 85 base pair deletion corresponding to exon 36 was identified in one pedigree that resulted in abnormal splicing of exon 35 to exon 37. In the second pedigree, a second distinct mutation was identified in which 148 base pairs corresponding to exon 42 were deleted. Both mutations produced a translational frameshift followed by a premature termination codon and resembled naturally occurring mouse *reelin* alleles. Western blot analysis of serum from affected patients demonstrated reduced or absent reelin protein expression. Thus, other lissencephaly candidate genes may exist.

Periventricular nodular heterotopia (PH) and subcortical band heterotopia (SBH) are X-linked disorders characterized by differential phenotypes in males and females (for review, see 71). Nodules of abnormal neurons and astrocytes separated by layers of myelinated fibers are identified along the lateral ventricles beneath the cortex in female PH patients (52, 90). PH in females results from mutations in the *filamin1* (*FLN1*) gene, which is located on chromosome Xq28 (40). Hemizygous males die in utero although rare male cases occur (see below). The encoded protein filamin1 is an actin-cross-linking phosphoprotein that modulates actin reorganization necessary for cellular locomotion. In *Drosophila*, filamin is required for ring canal assembly and actin organization during oogenesis (72). The precise mechanisms by which loss of filamin1 function in PH leads to nodular accumulations of neurons remains to be fully defined. A likely scenario is that neurons within the nodules are unable to migrate successfully out of the embryonic ventricular zone as a consequence of actin cytoskeletal dysfunction and remain trapped within the nodules. While *DCX* gene mutations in males cause lissencephaly, *DCX* gene mutations in females are associated with the SBH ("double cortex") syndrome in which there is a bilaterally symmetric band of cortical neurons extending through the underlying white matter of the centrum semiovale (4). The subcortical bands contain heterotopic neurons which are of small pyramidal shape without clear radial orientation. Neurons may be arrayed into clusters, sheets, or wide bands. Interestingly, however, the overlying cortex exhibits normal cytoarchitecture. The SBH is separated from the overlying cortex and underlying ventricles by normal white matter. A recent study has suggested that PH may result from radial glial fiber disruption (100).

The sexual dimorphism of the PH and SBH/XLIS syndromes likely reflects differential expression of the mutant or normal (*FLN1*) or *DCX* gene alleles within select populations of neurons during brain development. Females with PH or SBH carry one normal and one mutant allele for either the *filamin1* or *DCX* genes. As a consequence of X-chromosome inactivation (Lyonization), one of these alleles is no longer used for gene transcription. It has been speculated that neuronal migration will be compromised in neurons in which the normal allele is inactivated and the mutant gene is expressed. Neurons expressing the mutant allele will become the cellular constituents of either the nodules in PH or the band heterotopia in SBH. In contrast, those cells that inactivate the mutant allele and express the normal allele will migrate to the appropriate destination and come to comprise the overlying "normal" cortex. In males, only a single X-chromosome is present, and thus, if the mutant gene is inherited, the effect is either lissencephaly (XLIS) or a lethal state in PH. Sporadic male cases of PH that result from truncating mutations in *FLN1* have been reported (103).

Disorders of unspecified mechanism or developmental context. The Fukuyama muscular dystrophy syndrome (FCMD) and muscle-eye-brain disease (MEB) are rare, autosomal recessive disorders (17, 18) that exhibit "cobblestone" (Type II) lissencephaly in which there is a complete loss of regional and laminar organization that is distinct from classical (type I) lissencephalies such as the Miller-Dieker syndrome. FCMD is seen primarily in Japan and is associated with a debilitating muscular dystrophy as well as seizures. The FCMD gene encodes the protein fukutin, which maps to chromosome 9q31(66) and may function as a secreted protein. MEB is associated with retinal dysplasia, congenital myopathy, and lissencephaly. The MEB gene, POMGnT1 (124) encodes an acetylglucosaminyltransferase (POMGnT1), which participates in *O*-mannosyl glycan synthesis, is located on chromosome1p32-p34 (17).

Polymicrogyria (PMG), hemimegalencephaly (HME), and schizencephaly (SCHZ) are fascinating disorders that remain poorly understood. All 3 malformations have a high association with epilepsy although HME has perhaps the most devastating clinical manifestations. In PMG, the cortex exhibits multiple small microgyri which reveal a 4-layered lamination pattern. It is not clear that PMG is a malformation of development per se and may instead reflect a destructive process occurring during later stages of corticogenesis. PMG

	Age	Sex	Clinical manifestation	Location of lesion	Pathological diagnosis
1.	10 weeks	M	infantile spasms	rt. frontal, temporal, parietal	Cortical dysplasia, severe
2.	10 weeks	F	infantile spasms	frontal, temporal	Cortical dysplasia, severe
3.	1 y.o.	F	intractable epilepsy	rt. parietal	Cortical dysplasia, severe
4.	1 y.o.	M	intractable epilepsy, HME	rt. frontal, insular	Cortical dysplasia, severe
5.	1 y.o.	M	intractable epilepsy	lt. parietal	Cortical dysplasia, severe
6.	2 y.o.	M	intractable epilepsy	occipital	Cortical dysplasia, severe
7.	27 y.o.	M	complex partial seizure	rt. parietal	Cortical dysplasia, severe
8.	42 y.o.	F	intractable epilepsy	orbitofrontal	Cortical dysplasia, severe
9.	44 y.o.	M	intractable epilepsy	lt. frontal	Cortical dysplasia, severe
10.	39 y.o.	M	temporal lobe epilepsy	mesial temporal lobe	Chaslin's gliosis
11.	29 y.o.	F	temporal lobe epilepsy	mesial temporal lobe	Ammon's horn sclerosis
12.	44 y.o.	M	temporal lobe epilepsy	mesial temporal lobe	Ammon's horn sclerosis
13.	3 y.o.	F	intractable epilepsy	parietal	Chaslin's gliosis
14.	4 y.o.	F	epilepsia partialis continua	parietal	Rasmussen encephalitis
15.	15 y.o.	M	epilepsia partialis continua	parietal	Rasmussen encephalitis

Abbreviation: HME; hemimegalencephaly

Table 2. Characteristics of the patients with FCD from whom corticectomy tissue was studied.

has been reported in association with a variety of neurological syndromes but a specific molecular pathogenetic mechanism has not been identified. HME is characterized by massive enlargement of one cerebral hemisphere with abnormal genesis of both cortical and subcortical structures. The opposite hemisphere may be histologically normal but subtle malformations or even focal dysplasias within it have been reported radiographically or in rare autopsy specimens (59). The cortex shows a complete loss of laminar organization and the presence of dysplastic, heterotopic, and large "balloon" neurons. The cellular phenotype of these cells has not been defined although they express many distinct intermediate filament proteins (25, 27). HME was at one time believed to be associated with TSC; however, this has been shown not to be the case. Early studies suggested that HME was a proliferative disorder and that DNA polyploidy could be demonstrated within it. However, more recent studies have not supported this hypothesis and the mechanism of the malformation remains to be defined. The most curious aspect of HME is the clear asymmetry of the malformation, which suggests a very early and pervasive abnormality in cortical development selectively affecting one cerebral hemisphere.

Focal cortical dysplasias (FCD) such as the Taylor type, small subcortical heterotopias, and microdysgenesis are identified frequently in resected surgical specimens from patients with neocortical epilepsy. The precise developmental epoch in which FCD are generated is unknown and there is little data on whether FCD reflects an abnormality in cell proliferation, migration or laminar destination (37). Correlative electrophysiologic, neuroimaging and pathologic studies have been carried out in patients with FCD (107). The search for candidate genes responsible for FCD is an area of intense research. There are 2 competing hypotheses regarding the formation of FCD. The first states that these lesions result from an abnormality affecting a single neural precursor cell which in turn undergoes successive rounds of division to yield a clonal progeny that comprise the cellular constituents of FCD. This scenario might develop if a somatic mutation were to occur in a single progenitor cell. One intriguing possibility is that a mutation in one of the known MCD genes occurs as a somatic event in a neural precursor, that then gives rise to a clonal population of neurons within the FCD. The alternative hypothesis is that an external event affects the development of multiple precursor cells that yield multiple nonclonal cell types as progeny. Histologically, multiple cell types are present within FCD including neurons with dysmorphic features (dysplastic neurons), neurons within the subcortical white matter (heterotopic neurons), and neurons with excessively large cell bodies (neuronal cytomegaly). Thus, a pivotal question is whether these cell types all reflect a central pathogenetic process affecting their laminar distribution and morphology or alternatively whether there is a select cell type that is actually the "dysplastic" cell type. Indeed, we are not sure whether cortical dysplasia reflects a regional or cellular abnormality or if in fact, FCD reflects an abnormality in radial glial cells. These considerations are relevant

Antibody	Type (clone)	Specificity	Dilution	Source
Neurofilament	monoclonal (2F11)*	NF-H (200kDa), NF-M (160kDa), NF-L (70kDa)	1:400	DAKO
Neurofilament	monoclonal (N52)**	NF-H (200 kDa)	1:50	Chemicon
Ezrin	monoclonal (18)	ezrin (80kDa)	1:70	Transduction Laboratories
Moesin	monoclonal (38)	moesin (78kDa)	1:150	Transduction Laboratories
Moesin Ab-1	monoclonal (38/87)	moesin (78kDa), radixin (80kDa)	1:500	NeoMarkers
Tuberin	polyclonal	ap1 tuberin	1:300	Johnson MW et al (60, 61)
Hamartin	polyclonal	C-term hamartin	1:800	Johnson MW et al (60, 61)
GFAP	polyclonal	glial fibrillary acidic protein	1:300	DAKO
Ki-67	monoclonal (MIB-1)	Ki-67 nuclear antigen	1:80	DAKO

* reacts with the phosphorylated form of the 70kDa component of the three major polypeptide subunits of neurofilament
** reacts with the phosphorylated and dephosphorylated H-chain of neurofilament

Table 3. Primary antibodies of potential value in study of FCD/MCD.

since the precise phenotype of cells within FCD has not been clearly defined. While MCDs rarely, if ever, have a familial inheritance pattern, the histologic features of FCD suggest a consistent or uniform etiology (21, 83). It has been speculated that FCD results from early somatic mutations in one of the known MCD genes, or in a yet to be defined, or even novel gene. Alternatively, some suggest that FCD is a late occurring event, possibly even a post-natal event (73) resulting from external injury such as trauma or hypoxia-ischemia. An interesting recent study demonstrates discordant incidence of select FCD in monozygotic twins, suggesting that these lesions result from acquired factors, such as prenatal insults, or postfertilization genetic abnormalities (9). Furthermore, it is not clear at what point in cortical development FCD occurs.

An interesting feature of many dysplastic, heterotopic, and "balloon" or enlarged neurons is the expression of a variety of cytoskeletal genes and proteins, many of which are expressed under normal circumstances only in neural precursor cells. These proteins are normally expressed on a regulated developmental schedule during corticogenesis and are necessary for appropriate neuronal differentiation, migration, and process outgrowth. For example, expression of select intermediate filament (IF) proteins such as nestin, α-internexin, and vimentin, proteins typically found in immature neurons, has been reported in subpopulations of dysplastic and heterotopic neurons within FCD (25, 122). Thus, detection of select IFs in FCD that are normally expressed at either early or late stages of cortical development may yield clues to the maturational phenotype of dysplastic neurons and supports the hypothesis that these cells may retain components of an immature developmental phenotype. However, dysplastic neurons contain abnormal accumulations of highly- and non-phosphorylated neurofilament (NF) protein isoforms which are normally expressed in more differentiated neurons (30).

Immunohistochemical Characterization of FCD (MCD).

Cortical dysplasia (CD), as noted above, is one of the major neuropathologic abnormalities in patients with medically intractable epilepsy who undergo cortical resection or hemispherectomy (88, 114). Histological characteristics of CD, especially focal cortical dysplasia (FCD) of Taylor type (108), include neuronal dyslamination and cytologic features of neuronal dysmorphism with concomitant cytoskeletal abnormalities as well as proliferation of large and/or giant "balloon cells." Many of these balloon cells have been reported to show immunohistochemical evidence of both neuronal and astrocytic differentiation. Given the essentially identical morphologic features of FCD among patients of different ages, however, it is enigmatic that they give rise to both infantile spasms and, in others, intractable epilepsy with onset in adolescence or in adulthood.

We have performed a qualitative evaluation of abnormal neuroglial cells in FCD by means of immunohistochemical detection of the developmentally regulated proteins, hamartin and tuberin, gene products of *TSC1* and *TSC2*, respectively (see above), 2 of the ERM (ezrin, radixin, moesin) proteins, ezrin and moesin, as well as intermediate filaments, GFAP and neurofilament, and quantitative analysis of immunostained sections to evaluate differences in expression of these proteins between patients with early-onset infantile spasms and late-onset epilepsy. Nine archival cases of pathologically confirmed FCD were compared with 6 surgical cases of epilepsy with other neuropathologic abnor-

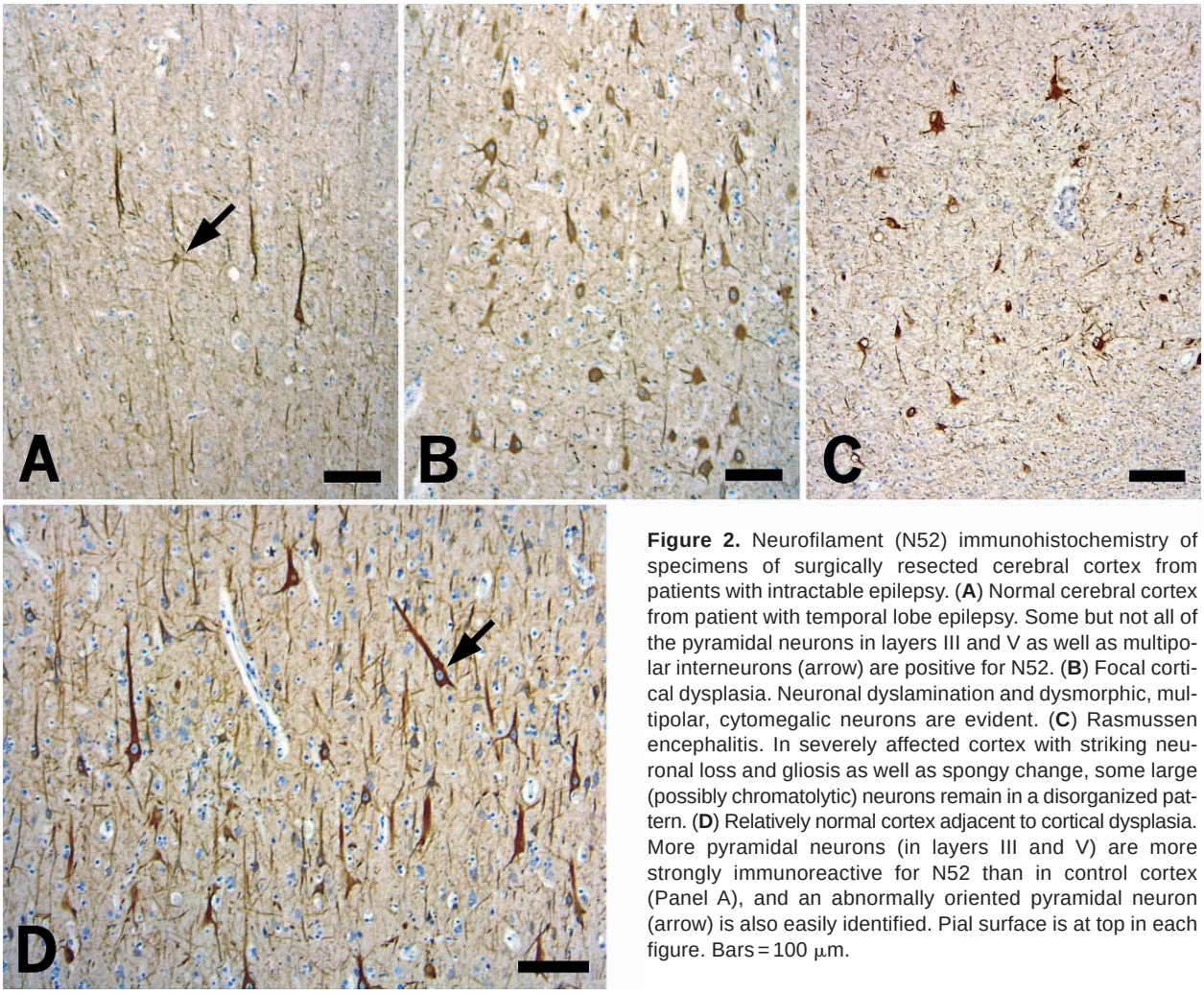

Figure 2. Neurofilament (N52) immunohistochemistry of specimens of surgically resected cerebral cortex from patients with intractable epilepsy. (**A**) Normal cerebral cortex from patient with temporal lobe epilepsy. Some but not all of the pyramidal neurons in layers III and V as well as multipolar interneurons (arrow) are positive for N52. (**B**) Focal cortical dysplasia. Neuronal dyslamination and dysmorphic, multipolar, cytomegalic neurons are evident. (**C**) Rasmussen encephalitis. In severely affected cortex with striking neuronal loss and gliosis as well as spongy change, some large (possibly chromatolytic) neurons remain in a disorganized pattern. (**D**) Relatively normal cortex adjacent to cortical dysplasia. More pyramidal neurons (in layers III and V) are more strongly immunoreactive for N52 than in control cortex (Panel A), and an abnormally oriented pyramidal neuron (arrow) is also easily identified. Pial surface is at top in each figure. Bars = 100 μm.

malities. The clinical characteristics of patients from whom tissue was obtained are briefly summarized in Table 2. These included 9 cases of pathologically-confirmed focal cortical dysplasia (FCD) of Taylor type (108), 2 cases of Ammon's horn sclerosis, 2 cases of Rasmussen encephalitis, and 2 cases showing no diagnostic abnormalities. All cortical dysplasia cases were pathologically diagnosed as "severe" (83), showing disruption of cortical laminar structure, presence of large/giant dysmorphic neurons as well as "balloon cell" change. Primary antibodies used are summarized in Table 3. Immunohistochemistry was performed according to commercial product specification and using previously published protocols (60, 61). Sections were counterstained with hematoxylin.

Neurofilament Immunohistochemistry

The antibody 2F11 helps to clearly detect abnormal neurons in terms of cytoplasmic expression of phosphorylated neurofilament. Phosphorylated neurofilament (p-NF) exists within axons, whereas non-phosphorylated neurofilament (NF) exists in cell bodies of normal neurons. 2F11 immunohistochemistry detected small numbers of dysmorphic and cytomegalic neurons and neurites in FCD cases as well as axons passing through the white matter, indicating abnormal p-NF expression within cell bodies of those dysplastic cells. Balloon cells were rarely stained with this antibody. The antibody N52 recognizes both p-NF and NF (102), and as expected detects more cells of neuronal origin and/or neuronal phenotype than does the antibody 2F11. In fact N52 detected more neuronal cells including most of the dysmorphic and cytomegalic neurons, many balloon cells, neurites and axons as well as rela-

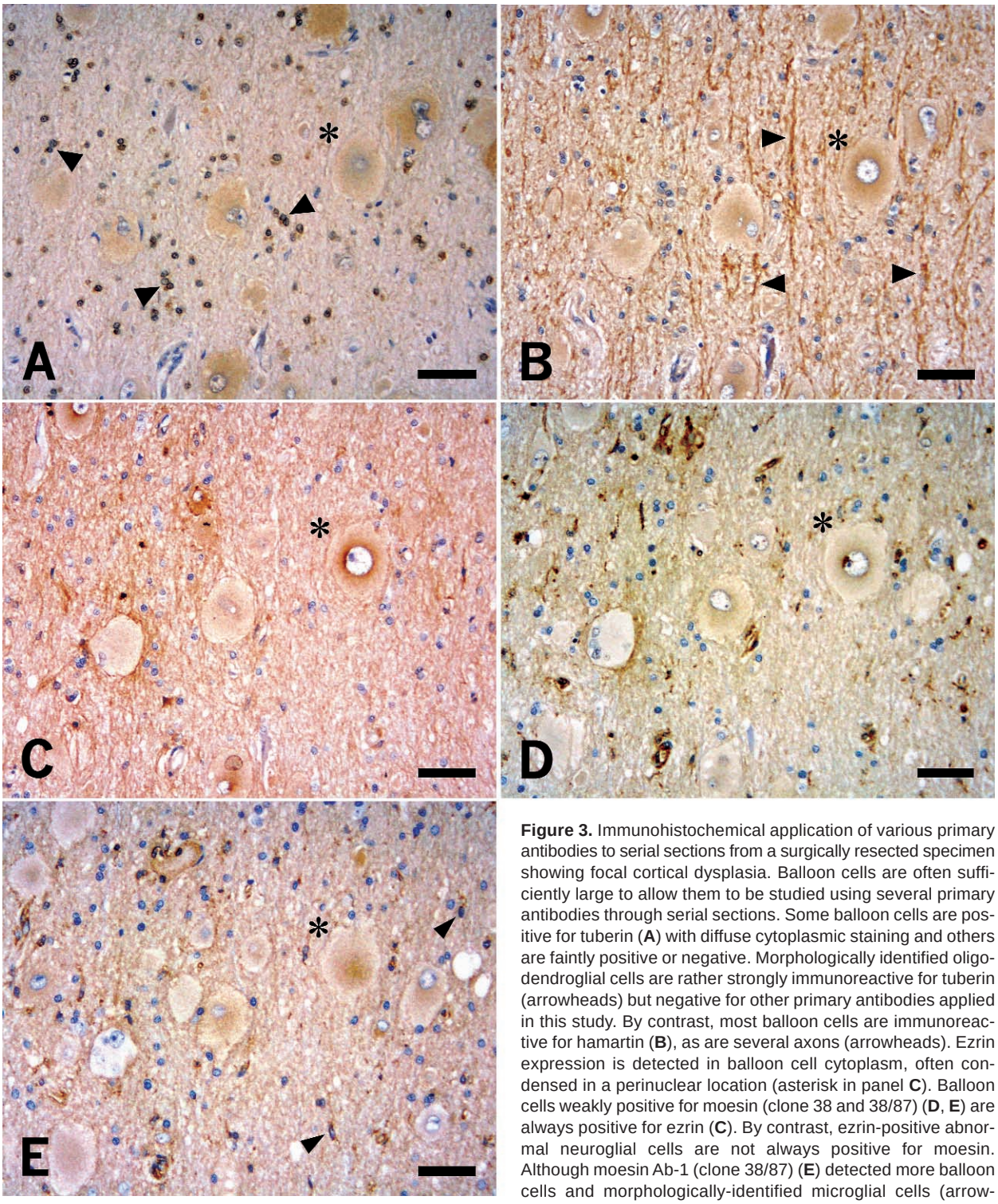

Figure 3. Immunohistochemical application of various primary antibodies to serial sections from a surgically resected specimen showing focal cortical dysplasia. Balloon cells are often sufficiently large to allow them to be studied using several primary antibodies through serial sections. Some balloon cells are positive for tuberin (**A**) with diffuse cytoplasmic staining and others are faintly positive or negative. Morphologically identified oligodendroglial cells are rather strongly immunoreactive for tuberin (arrowheads) but negative for other primary antibodies applied in this study. By contrast, most balloon cells are immunoreactive for hamartin (**B**), as are several axons (arrowheads). Ezrin expression is detected in balloon cell cytoplasm, often condensed in a perinuclear location (asterisk in panel **C**). Balloon cells weakly positive for moesin (clone 38 and 38/87) (**D**, **E**) are always positive for ezrin (**C**). By contrast, ezrin-positive abnormal neuroglial cells are not always positive for moesin. Although moesin Ab-1 (clone 38/87) (**E**) detected more balloon cells and morphologically-identified microglial cells (arrowheads) with stronger intensities than did moesin (clone 38), there was no significant difference in the cellular component labeled by both primary antibodies. Asterisks in all panels indicate the same cell. Bars = 50 μm.

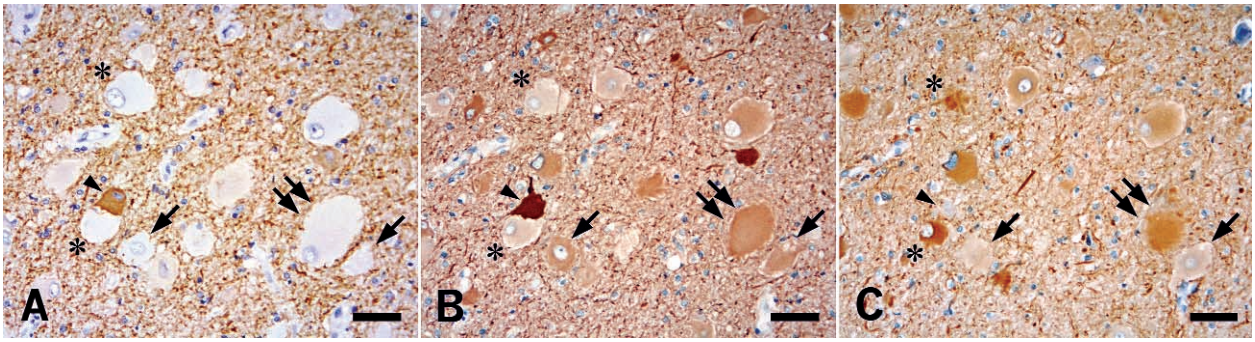

Figure 4. Balloon cells show various patterns of differentiation. Some show only glial (GFAP) or neuronal (neurofilament) differentiation, while others show both GFAP and neurofilament immunoreactivity. Only a few balloon cells are immunoreactive for phosphorylated neurofilament (arrowhead in panel **A**), however, most are immunoreactive to non-phosphorylated neurofilament (**B**) with varying intensities. Some are immunoreactive for both GFAP and non-phosphorylated neurofilament (double arrows in panels **B** and **C**). Symbols and arrows in all panels indicate the same cell. Arrowhead: A balloon cell that is p-NF-positive, NF-positive, and GFAP-negative. Arrows: Balloon cells that are p-NF-negative, NF-positive, and GFAP-negative. Asterisks: Balloon cells that are p-NF-negative, NF-negative, and GFAP-positive. Double arrow: A balloon cell that is p-NF-negative, NF-positive, and GFAP-positive. Bars = 50 μm. (Panel A: Section immunostained with 2F11; Panel B: Section immunostained with N52; Panel C: Section immunostained with GFAP)

tively normal pyramidal neurons in cortical layers 3 and 5 and a small number of (what appeared to be) multipolar interneurons adjacent to regions of FCD (Figures 2A, B, 4A, B). Presence of subpial neurons, increased neurons in layers 1 and 2 of the cortex, and single heterotopic neurons in the white matter were also clearly demonstrated with the antibody N52. In relatively normal cortex, such as neocortex remote from the apparent dysplastic foci, and the middle and/or inferior temporal neocortices from specimens with Ammon's horn sclerosis, some of the pyramidal neurons in layers 3 and 5, and (what appeared to be) interneurons were labeled with N52. In cortex adjacent to dysplastic lesions, entorhinal cortex from Ammon's horn sclerosis, and relatively preserved cortex of Rasmussen encephalitis cases, more pyramidal neurons were more strongly labeled by the antibody N52 than in normal neocortex remote from the lesions. N52 immunohistochemistry was helpful in detecting occasional neuronal cells of abnormal polarity and abnormal location in otherwise relatively normal cerebral cortex (Figure 2D), obviously suggesting the usefulness of applying this probe for detecting some components of microdysgenesis. These findings suggest the upregulation of NF in dysplastic cells and also in pyramidal neurons within relatively normal cortex adjacent to FCD, indicating the relevance to physiologic and functional alteration of those cells associated with epileptic activity. In advanced cases of Rasmussen encephalitis, with severe neuron loss, gliosis, and chronic inflammatory changes manifested by patchy perivascular lymphocytic infiltration as well as spongy state with capillary proliferation, some large chromatolytic neurons remained and were strongly positive for N52 (Figure 2C).

TSC Gene Products and ERM Protein Expression in FCD

Recently hamartin, the gene product of *TSC1*, has been reported to bind to and interact with ezrin (70), one of the ERM (ezrin, radixin, and moesin) proteins which belong to the band-4.1 superfamily of membrane-cytoskeleton linking proteins (106). ERM proteins are involved in the formation of microvilli, cell-cell adhesion, maintenance of cell shape, cell motility and membrane trafficking (74). It has been shown that ezrin and radixin are specifically expressed in rat cultured astrocytes at their filopodia and microvilli, suggesting that ERM proteins may be involved in astrocytic pathology (28). In fact, in vitro study has shown that ezrin mediates motility and invasiveness of glioma cells (119), and ezrin expression in human astrocytomas has also been reported (45). We have investigated the expression of 2 ERM proteins, ezrin and moesin, in tubers of tuberous sclerosis, and demonstrated that expression of both proteins colocalized with the expression of hamartin and tuberin in a population of abnormal neuroglial cells (62). Expression of ERM proteins, tuberin and hamartin, may be of interest in studying both FCD and tubers of TSC, given their similar neuropathologic features.

In FCD, diffuse cytoplasmic staining with tuberin and hamartin was observed in most neurons within comparatively normal cortex. In dysplastic lesions,

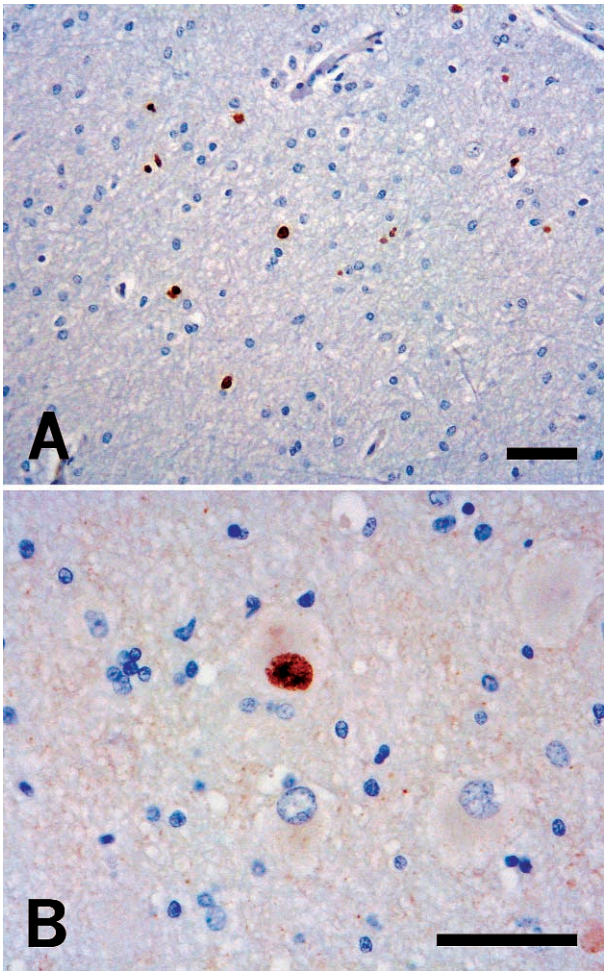

Figure 5. Ki-67 (MIB-1) immunohistochemistry on a surgically resected specimen showing focal cortical dysplasia. (**A**) Focally prominent MIB-1 labeling in relatively normal white matter. (**B**) Abnormal neuroglial cells including dysmorphic, cytomegalic neurons and balloon cells are rarely labeled with MIB-1, suggesting their low proliferative activity. Bars = 50 μm.

tuberin and hamartin expression was robust in dysmorphic and cytomegalic neurons with a granular appearance in their cytoplasm, often in the vicinity of the nucleus, suggesting colocalization with Nissl substance and/or Golgi apparatus as previously described (120, 121). Tuberin and hamartin expression in balloon cells varied: Some balloon cells showed weak immunoreactivity more marked in the center of their cytoplasm; others showed positive staining more marked in the periphery of their cytoplasm. In many balloon cells, both proteins were seen to be colocalized although a few balloon cells were immunoreactive only for hamartin or negative for both proteins. This suggests that hamartin immunostaining detected more abnormal neuroglial cells than did tuberin immunostaining. Patterns of ezrin expression in FCD were almost identical to GFAP expression except for the immunoreactivity in balloon cells. Astrocytic cells with eosinophilic cytoplasm and well-developed processes, morphologically identical to reactive astrocytes, were strongly positive for both GFAP and ezrin. Ezrin expression was observed in more balloon cells than those positive for GFAP. Vascular endothelial cells and a population of CD68-positive microglia (data not shown) were positive for moesin. Moesin expression was also detected in some of the balloon cells in FCD which were positive for ezrin, and was observed in a smaller population than ezrin-positive balloon cells. Conversely moesin-positive balloon cells were always positive for ezrin, and also positive for tuberin and hamartin. Some balloon cells with nuclear atypia showed strong ezrin- and moesin-immunoreactivity in the vicinity of the nucleus. Ezrin and moesin expression was not detected in any dysmorphic or cytomegalic neurons having apparent Nissl substance, and positivity for N52. Examples of immunohistochemical studies with various primary antibodies on serial sections are shown in Figures 3 and 4.

Quantitative Analysis of Immunostained Specimens

The numbers of immunoreactive cells (using different antibodies) in cortical dysplasia were randomly counted using a calibrated ocular grid with ×20 objective lens. Total number of immunoreactive cells (without regard to their phenotype) per mm^2 and (%) positive cells were calculated. This represents a preliminary and admittedly crude quantitative analysis on immunostained sections of FCD to evaluate differences in protein expression between early-onset and late-onset epilepsy groups by counting immunoreactive cells. No significant difference in protein expression (in terms of immunoreactive cell numbers) between those two groups was noted. This suggests that these proteins are not expressed in an age-specific pattern.

MIB-1 Labeling Index in FCD

Although FCDs are thought to be a consequence of abnormal neuronal migration and are classified as a type of malformation of cortical development (MCD, see above), and thus are thought to exist at birth, there is debate as to whether abnormal neuroglial cells in FCD have significant proliferative potential. MIB-1 labeling indices were extremely low within definite focal cortical dysplasia; ie, almost no MIB-1 labeling of balloon cell nuclei was noted and only rare nuclei of glial cells with-

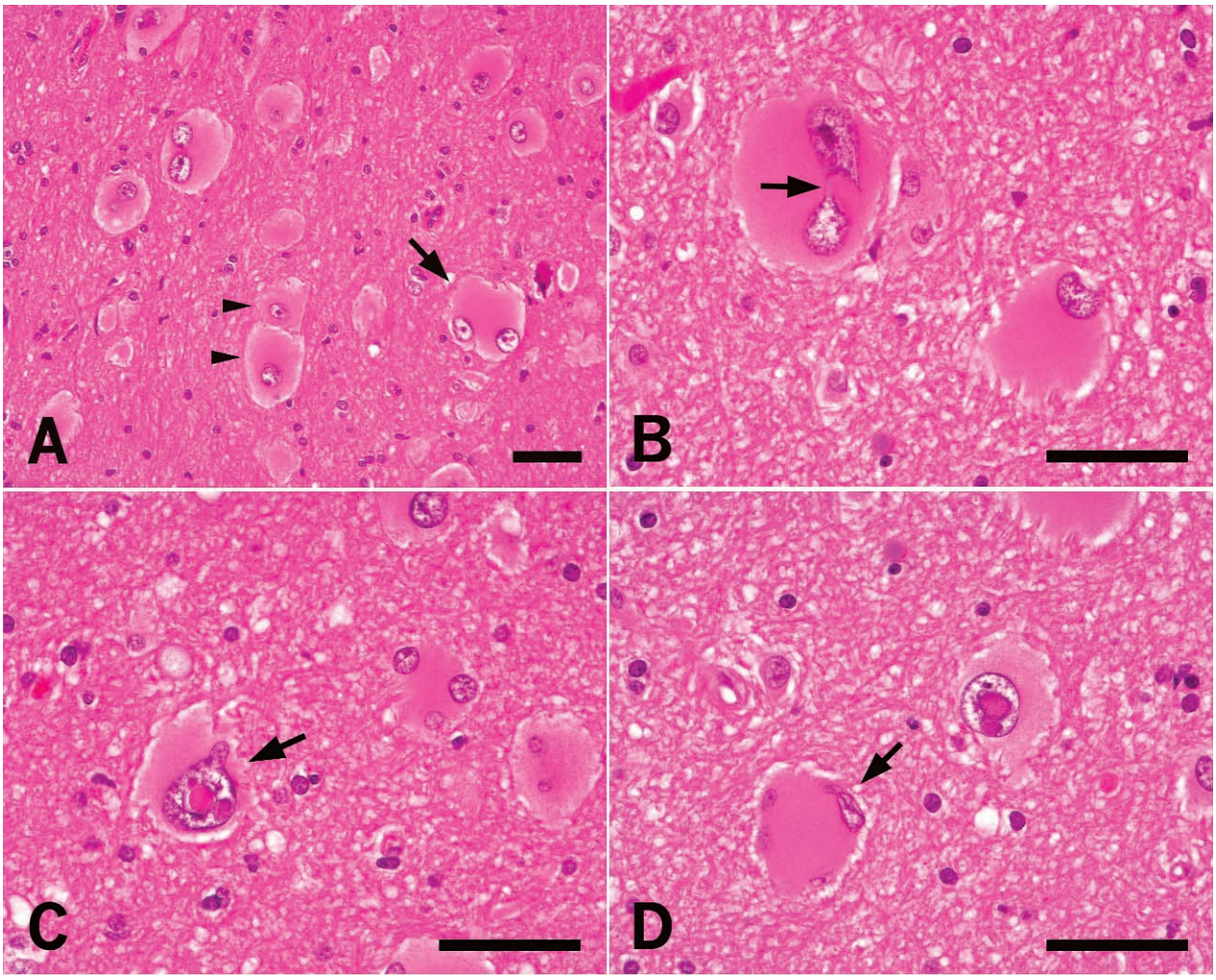

Figure 6. Balloon cells with marked nuclear atypia. Hematoxylin and eosin staining. (**A**) Balloon cells often show bi-nucleation (arrow). Paired balloon cells are also seen (arrowheads). (**B**) A balloon cell with a nuclear bridge connecting two nuclei (arrow). (**C**) A balloon cell with marked nuclear atypia and/or nuclear invagination with nuclear budding (arrow). (**D**) A balloon cell with multinucleation and/or micronucleation (arrow). Bars = 50 μm

in a given field were seen in each case. Relatively high MIB-1 labeling indices of up to 6%, however, were focally encountered within normal-appearing white matter adjacent to dysplastic cortex (Figure 5A). Careful observation revealed some balloon cells showing severe nuclear atypia with nuclear budding and cleavage. In bi- and/or multi-nucleated balloon cells, bridges and/or "strings" connecting the 2 nuclei were observed (Figure 6). Given these observations together with extremely low MIB-1 labeling index of balloon cells, these findings suggest the existence of amitotic nuclear division of balloon cells, which may be related to the proliferation of abnormal neuroglial cells within FCD foci. Multinucleated and micronucleated cells are thought to be destined to die but physically they may continue to divide a few times before dying (6). Further studies (including analysis of DNA content) in abnormal cells may elucidate the biological significance or the mechanism of formation of abnormal neuroglial cells in FCD, which may also contribute to understanding the mechanism of seizure onset in FCD.

Epileptogenesis and MCD: How Does Cortical Maldevelopment Lead to Epilepsy?

A pivotal question directly relevant to both clinical and laboratory investigations of MCD is why cortical malformations are so highly associated with epilepsy? Indeed, the majority of patients with extensive MCD suffer from medically intractable seizures and require antiepileptic drug polytherapy. An overriding hypothesis

that has been supported by clinical studies is that in patients with focal types of dysplasia, the seizures emanate from the malformation rather than surrounding cortex. For example, in Taylor type FCD, tubers, and polymicrogyria, intracranial grid recordings have clearly demonstrated that the ictal onset zone is within the malformation (86, 92). An important recent study using human depth electrode recording showed that seizures in patients with PH may emanate directly from the heterotopia (68). Interestingly, in a rat model of SBH, seizures were shown to emanate from the overlying cortex (15) and not the heterotopia, although this finding has not been confirmed in humans. Surgical resection in SBH patients does not seem to be as effective as more focal resections in other MCD (5).

Several theories of epileptogenesis have been proposed based on studies in human MCD and in animal models of MCD that include altered synaptic connectivity, aberrant expression of molecules that mediate synaptic transmission, and an imbalance between excitatory and inhibitory impulses associated with the dysplasia. Solid evidence has been provided in favor of each of these hypotheses and it is likely that, broadly speaking, epilepsy in MCD reflects contributions from multiple contingent and convolved factors. However, it is unclear at this time whether the mechanisms of epileptogenesis in MCD will be similar or distinct across multiple MCD subtypes. In other words, epilepsy in MCD may be more akin to a final common electrophysiologic or clinical pathway for a variety of malformations. There may be important differences in epileptogenesis between individual MCD syndromes. As a corollary to this question, within each MCD why are their distinct seizure phenotypes? For example, how can we mechanistically explain or investigate the occurrence of infantile spasms and complex partial epilepsy in tuberous sclerosis or in 2 distinct MCD subtypes such as tuberous sclerosis and lissencephaly? Implicit in this question is whether clinical epilepsy syndromes or semiologic subtypes are specific for each MCD subtype or whether they reflect maturational contextual differences in cortical development. This point is critical since the clear failures in antiepileptic drug treatment for many patients with MCD may imply an essential flaw in drug utility as a direct consequence of the pathoanatomic differences among these different brain lesions. Indeed, an inherent question in this idea is whether future anti-epileptic drug design may be predicated on individual cellular and molecular differences in epileptogenesis among different MCD subtypes. A final point is that it is often a tacit assumption that aberrant cortical cytoarchitecture alone is responsible for seizures in MCD. This is an especially important consideration since MCD have been well-documented in patients with no history of epilepsy. Certainly, disorganized synaptic connectivity within MCD and between MCD and the surrounding cortex may enhance the likelihood of recurrent and propagated synchronous, paroxysmal discharges. However, an alternative hypothesis is that some of the genes responsible for individual forms of MCD may in fact also serve as epilepsy susceptibility loci that confer hyperexcitability to neurons regardless of their laminar positioning.

Mechanisms of epileptogenesis in MCD have been investigated in human tissue and animal models of MCD. Data addressing the physiological properties of neurons within MCD remains sparse largely as a consequence of technical limitations of studying resected tissue in vitro, including limited viability of the tissue slice, the paucity of connections between the slice and surrounding cortex, and lack of true control tissue. Surprisingly little is known about mechanisms controlling excitatory and inhibitory tone in several important MCD syndromes including lissencephaly, band heterotopia, and nodular heterotopia. However, one MCD subtype that has been studied using cellular, electrophysiologic and recently, molecular strategies, is Taylor type FCD since this MCD subtype is frequently identified in neocortical epilepsy surgical specimens as well as in temporal lobe resections. The cellular constituents of FCD may include a variable population of dysplastic neurons, heterotopic neurons, and large "balloon" neurons intermingled with neuronal subtypes of indeterminate laminar destiny. It is important to remember that it has yet to be defined whether all neurons within foci of FCD are in fact themselves abnormal, or within an incorrect laminar position, and thus how we view the physiologic responses of these cells may depend on how we weight the contributions of each cell type. One hypothesis that has received experimental support is that there is decreased inhibitory and enhanced excitatory tone in FCD (3, 105). The few studies that have addressed this hypothesis directly with field potential and intracellular recording within human FCD specimens have shown that dysplastic cortex is hyperexcitable (78, 75) and that a subset of dysplastic neurons generate repetitive ictal discharges in response to the K^+ channel blocker 4AP that can be blocked by NMDA receptor antagonists (3). These electrophysiological responses may result from several possible causes. First, several reports have demonstrated a reduction in the number of parvalbumin, somatostatin, and GAD65 immunolabeled inhibitory interneurons in FCD (36, 104). In cor-

tical tubers, there are few GABAergic neurons identified by GAD65 immunolabeling and the expression of the vesicular GABA transporter (VGAT) mRNA, a marker for GABAergic neurons, is also reduced (118). Interestingly, using proton magnetic resonance spectroscopy to assay tuber samples, an increase in GABA was defined (1) suggesting a possible compensatory response. Neurons within nodular subcortical heterotopias are largely calbindin immunoreactive, suggesting a GABAergic phenotype that has failed to migrate into cortex (52). Taken together these studies suggest that there is a paucity of GABAergic interneurons in MCD that represents reduction in the genesis of GABAergic cells, a selective failure of GABAergic cell migration from the median eminence during development, or enhanced death of GABAergic neurons. A second contributory factor to hyperexcitability in MCD is a selective reduction in $GABA_A$ receptor subunits in FCD which argues that the synaptic machinery to modulate neural inhibition within FCD may be diminished. Reduced expression of $GABA_A$ $\alpha 1$, $\alpha 2$, $\beta 1$, and $\beta 2$ receptor subunit mRNAs is observed in human FCD (20) and GABAergic terminals identified in close proximity to balloon neurons in FCD do not appear to make functional synapses (44). The reduction in GABAergic neurons and loss of GABAergic receptor subunits would alter critical inhibitory synaptic control and render neurons in FCD more susceptible to sudden and prolonged excitability. A third factor related to hyperexcitability is that recent reports have demonstrated the expression of several glutamate receptor subunits to be enhanced in FCD (20, 64, 123). For example, expression of the NR2B site has been shown to be increased in FCD (20, 81). This site has been shown to modulate sustained calcium mediated depolarization and is an ideal candidate protein to account for recurrent hyperexcitability in FCD. The expression of the NR2B site was correlated with more widespread epileptiform abnormalities detected by surface grid electrodes (85). Increased NR2B mRNA and protein expression has been defined in tubers of the tuberous sclerosis complex by cDNA array and receptor ligand pharmacology (118). Increased expression of several other glutamate receptor subunits including GluR1, GluR2 have been defined in FCD (20, 64). Diminished coupling of NR1 with calmodulin, reported in 3 FCD specimens, is an interaction that is essential for inactivation of the NR1 complex (82). Two recent studies suggest that the number of excitatory neurons, as evidenced by expression of the neuronal glutamate transporter EAAT3/EAAC1, is increased in tubers (118) and in FCD (23) suggesting that regional hyperexcitability in select MCD may result from enhanced numbers of excitatory neurons as well.

Corroborative studies in several animal models of MCD support the idea of an imbalance between excitatory and inhibitory tone in MCD (58). These models include the freeze induced microgyrus, lesions caused by methylazoxymethanol (MAM), and in utero radiation, and several spontaneous and engineered rodent strains such as the flathead, OTX-1, p35, TISH, and NZB strains. The histologic features of these individual strains are quite distinct and only very few of them, eg, the flathead strain, exhibit spontaneous seizures. Yet differential expression of select glutamate and GABA receptor subunits have nonetheless been demonstrated within them. For example, in the freeze microgyrus lesion model, selective upregulation of the NR2B site has been shown using patch clamp recordings (26) and there is a reduction in parvalbumin immunolabeled neurons within the microgyrus (58). Interestingly, in a rodent model of cortical dysplasia generated by in utero irradiation, there is a reduction in the numbers of cortical parvalbumin and calbindin immunoreactive neurons (99). In rats treated with methylazoxymethanol (MAM), altered expression of GluR2, NR2A, and NR2B was reported within the heterotopic cell islands in the hippocampus and neocortex (94). In the mouse Lis1 mutant strain, hyperexcitability in the CA3 hippocampal sector reflected the cytoarchitectural disruption observed, including displaced somatostatin and parvalbumin immunoreactive neurons (38). Disruption of ion channel function may also contribute to epileptogenesis of MCD although little data in expression of these channels in human brain tissue is available. In the freeze lesion model, loss of an inwardly rectifying potassium current as well as a reduction in gap junction coupling have been demonstrated (8) although similar findings in humans are unknown. In the MAM model, selective reduction in Kv4.2 potassium channel was observed in heterotopia (13). These findings suggest that animal models of cortical malformations can provide important insights into epileptogenesis associated with MCD.

Experimental Strategies for Studying Epilepsy in MCD

The studies needed to assay the molecular pathogenesis of MCD will require implementation of novel strategies including the generation of animal models that closely recapitulate human pathologic abnormalities, direct electrophysiological recording from human specimens, identification of candidate loci in family pedigrees, and the application of gene array technologies to both ani-

mal and human specimens. For some disorders such as FCD, positional cloning approaches will be limited by the lack of family pedigrees and thus, gene array platforms offer a rapid strategy to screen numerous gene families. Quantification of relative gene expression will provide clues to the mechanisms by which mutations can alter cortical cytoarchitecture. However, a significant limiting factor common to most MCD is that there are neurons of diverse phenotype within a given malformation that potentially make distinct contributions to epileptogenesis and neurological dysfunction in these patients. Thus, the implementation of single cell molecular biology to the study of cortical malformations provides an attractive strategy to assay gene expression in single microdissected and phenotypically characterized neurons. For example, this approach has provided the first studies using cDNA array analysis to assay MCD associated with epilepsy including FCD (20), TSC (24, 69, 118), and hemimegalencephaly (25) that have yielded clues to the cellular and molecular heterogeneity of neurons in these malformations.

The experimental protocol to amplify cellular poly (A) mRNA from fixed tissue using an oligo-dT primer was first described using resected surgical epilepsy specimens (24) and has subsequently been outlined in detail (63). The approach uses an oligo-dT primer coupled to a T7 RNA polymerase promoter to convert poly (A) mRNA into cDNA directly on the fixed tissue section by in situ (reverse) transcription (IST). In general, tissue sections are immunolabeled prior to this so that cells can be characterized experimentally by phenotypic characteristics. Following immunolabeling, tissue sections are treated with proteinase K to reverse excessive fixation and then washed in diethyl pyrocarbonate (DEPC)-treated water. To initiate IST, an oligo-dT(24) primer-T7 RNA polymerase promoter is annealed to cellular poly (A) mRNA directly on the tissue section at room temperature. cDNA is synthesized on the section with avian myeloblastosis reverse transcriptase (AMVRT). At this point, cDNA can be extracted from cellular poly (A) mRNA and then processed for mRNA amplification and synthesis of a radiolabeled RNA probe. This pool of aRNA will reflect to be total population of genes present in the whole section. Alternatively, single immunolabeled cells may be microdissected so that the mRNA can be amplified and used as a probe of cDNA arrays to define gene expression in that specific cell. Single cells are microdissected from sections under light microscopy using a glass electrode or a stainless steel microscalpel guided by a joystick micromanipulator. Fixed cells are then aspirated into a second glass microelectrode and transferred to a microfuge tube reaction buffer and incubated at 40°C for 90 minutes to insure cDNA synthesis in the single dissected cell.

cDNA extracted from whole sections or generated from single cells served as a template for synthesis of double stranded template cDNA with T4 DNA polymerase I. mRNA was amplified (aRNA) from the double stranded cDNA template with T7 RNA polymerase. aRNA served as a template for a second round of cDNA synthesis with AMVRT, dNTPs, and N(6) random hexamers. cDNA generated from aRNA was made double stranded and served as template for a second aRNA amplification incorporating ^{32}PCTP. Radiolabeled aRNA was used to probe candidate cDNA arrays.

The ability to measure gene expression provides a unique strategy to assay the coordinate expression of multiple mRNAs in phenotypically defined cell types, since interpretation of gene expression assays of whole brain regions is complicated by contributions from multiple distinct cell types within a tissue sample. Indeed, analysis of gene transcription in single disease affected cells versus adjacent unaffected cells provides a unique opportunity to understand specifically which genes may be responsible for the pathologic features of the disease in question within select populations of cells. Furthermore, alterations in mRNA levels have been correlated with and thus can be used to predict, changes in expression of proteins in single cells.

Summary and New Directions: Targeted Therapy for Epilepsy in MCD

Prior to the 1990s, the molecular pathogenesis of MCD was largely the source of speculation. However, with the discovery of several genes responsible for MCD including *LIS1*, *DCX*, *FLN1*, *TSC1* and *TSC2*, it is clear that single gene mutations may account for numerous subtypes of MCD. The identification of MCD genes now permits in depth analysis of the proteins encoded by these genes and may aid in designing new therapies targeted at these molecules. Thus, in the future, we may hope to modulate pathways in select MCD syndromes so that specific agents can be used to treat seizures in, for example, PH or TSC. Perhaps even more exciting is the potential to design therapeutic strategies to actually abolish or prevent the development of these malformations in utero.

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