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© 2014 John Wiley & Sons Ltd. Cortical dysplasia (CD) is a neurodevelopmental disorder due to aberrant cell proliferation and differentiation. Advances in neuroimaging have proven effective in early identification of the more severe lesions and timely surgical removal to treat epilepsy. However, the exact mechanisms of epileptogenesis are not well understood. This review examines possible mechanisms based on anatomical and electrophysiological studies. CD can be classified as CD type I consisting of architectural abnormalities, CD type II with the presence of dysmorphic cytomegalic neurons and balloon cells, and CD type III which occurs in association with other pathologies. Use of freshly resected brain tissue has allowed a better understanding of basic
mechanisms of epileptogenesis and has delineated the role of abnormal cells and synaptic activity. In CD type II, it was demonstrated that balloon cells do not initiate epileptic activity, whereas dysmorphic cytomegalic and immature neurons play an important role in generation and propagation of epileptic discharges. An unexpected finding in pediatric CD was that GABA synaptic activity is not reduced, and in fact, it may facilitate the occurrence of epileptic activity. This could be because neuronal circuits display morphological and functional signs of dysmaturity. In consequence, drugs that increase GABA function may prove ineffective in pediatric CD. In contrast, drugs that counteract depolarizing actions of GABA or drugs that inhibit the mammalian target of rapamycin (mTOR) pathway could be more effective.

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Basic Mechanisms of Epileptogenesis in Pediatric Cortical Dysplasia

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SUMMARY

Cortical dysplasia (CD) is a neurodevelopmental disorder due to aberrant cell proliferation and differentiation. Advances in neuroimaging have proven effective in early identification of the more severe lesions and timely surgical removal to treat epilepsy. However, the exact mechanisms of epileptogenesis are not well understood. This review examines possible mechanisms based on anatomical and electrophysiological studies. CD can be classified as CD type I consisting of architectural abnormalities, CD type II with the presence of dysmorphic cytomegalic neurons and balloon cells, and CD type III which occurs in association with other pathologies. Use of freshly resected brain tissue has allowed a better understanding of basic mechanisms of epileptogenesis and has delineated the role of abnormal cells and synaptic activity. In CD type II, it was demonstrated that balloon cells do not initiate epileptic activity, whereas dysmorphic cytomegalic and immature neurons play an important role in generation and propagation of epileptic discharges. An unexpected finding in pediatric CD was that GABA synaptic activity is not reduced, and in fact, it may facilitate the occurrence of epileptic activity. This could be because neuronal circuits display morphological and functional signs of dysmaturity. In consequence, drugs that increase GABA function may prove ineffective in pediatric CD. In contrast, drugs that counteract depolarizing actions of GABA or drugs that inhibit the mammalian target of rapamycin (mTOR) pathway could be more effective.

Introduction

Cortical neurodevelopment in mammals is a complex, dynamic process that involves the birth of neurons, their growth and differentiation, migration to their final destination, and synaptic formation. This process is longer and more complex in humans, therefore increasing susceptibility to developmental errors [1,2]. Both genetic and environmental factors cumulatively dictate and influence the fate of neuronal development [3]. Aberrant cell migration and differentiation is responsible for cortical dysplasia (CD), the most common histopathological substrate in surgical cases of pediatric epilepsy [4,5].

Focal CD associated with epilepsy, first described by Taylor and associates in 1971, is characterized by dyslamination, ectopic neurons in the white matter, and the presence of abnormal cytomegalic neurons and balloon cells that exist only in one other etiology, tuberous sclerosis complex (TSC) [6]. Erroneous cortical development can occur at the level of neuronal-glial proliferation and differentiation during migration of neurons to the cortical plate and during intracortical organization [7,8]. Faulty differentiation leads to the occurrence of abnormal cells in CD, in particular dysmorphic cytomegalic neurons, balloon cells, and immature pyramidal neurons. There is still debate about the proper definition of these cells, and a general consensus has not been reached. In this review, we will adhere, as much as possible, to the most recent nomenclature [9], but we also take into account previous definitions. Dysmorphic cytomegalic pyramidal neurons, sometimes called dysplastic, are misshapen, enlarged cells with abnormal orientation, cytoskeletal structure, atypical dendritic processes, and rich in neurofilaments [6,9–12]. Balloon cells have thin membrane, pale, glassy and eosinophilic cytoplasm with an eccentric nucleus, express neuronal and glial markers [13], and are morphologically similar to, but usually larger than, gemistocytic astrocytes and without proliferative potential [9,12,14]. Immature neurons derive from neuroblasts, have a small somatic size, and do not accumulate neurofilaments [9]. However, not all cases of CD present these aberrant cells and the need for a classification of different types of CD based on degree of severity soon became evident as more pathological specimens from epilepsy surgery cases became available.

Recent advances in identification of different CD types, histopathological substrates, imaging, and in vitro studies using resected tissue have allowed a better understanding of the diversity of pathologies and epileptogenic mechanisms. In this review, we examine possible mechanisms of epileptogenesis in different CD
types based on histopathological substrates and aberrant membrane and synaptic properties.

**Classifications of CD**

Several classifications of the complex structural abnormalities of CD have been proposed that account for the degree of severity depending on either the pathological characteristics or the origin of the pathology (see Figure 1). In 1995, a grading system for CD was introduced based on histopathology of resected human brain specimens. Timing of the developmental insult in early, mid- and late gestational stages resulted in severe, moderate and mild CD, respectively. Mild CD was characterized by pathological features such as cortical disorganization, heterotopic white matter and molecular layer neurons, persistent subpial granular cell layer, and marginal gli-o-neuronal heterotopia. In moderate CD, in addition to those features, polymicrogyri and neuronal heterotopia were present. Finally, in the severe form of CD, in addition to features present in mild and moderate CD, there were balloon cells and neuronal cytomegalgy [11]. This classification, although not generally used today, is the only one that explicitly suggests an inverse correlation between degree of CD severity and timing of initial insult during embryogenesis.

The Palmini classification separated CD into two main types based on histopathological findings [12]. Intracortical laminar and columnar disorganization was reported as the major feature of CD. This classification separated CD type I based on cortical dyslamination as well as misorientation of cells secondary to excess neurons in the subcortical white matter and CD type II based on dyslamination and the presence of abnormal cellular elements such as dysmorphic pyramidal neurons and balloon cells. CD type II was further divided into type IIa based on the presence of dysmorphic neurons and type IIb where abnormal elements also included balloon cells.

The most recent CD classification was proposed by the International League Against Epilepsy (ILAE). It is based on a three-tiered system that distinguishes whether pathological findings of CD are isolated or associated with other epileptogenic lesions [9]. CD types I and II are considered isolated findings, while CD type III is a variant as there exists a principal lesion associated with CD (dual pathology). Similar to the Palmini classification, CD type I was defined by cytoarchitectural abnormalities. Further subdivisions were proposed with CD type Ia characterized by radial dyslamination, CD type Ib characterized by tangential dyslamination, and CD type Ic where dyslamination is both radial and tangential. In CD type II, in addition to dyslamination, there are abnormal dysmorphic neurons, type IIa, or dysmorphic neurons and balloon cells, type IIb. Lastly, in CD type III, in addition to CD, there is another lesion present, likely associated with epileptogenicity. CD type III is further divided into subtypes. CD type IIIa refers to CD associated with hippocampal sclerosis in the temporal lobe, CD type IIIb is associated with a CNS tumor, CD type IIic is associated with a vascular malformation, and CD type IIId is associated with another lesion acquired early in life either secondary to a trauma or encephalitis. It is important to classify precisely different types of CD as it can lead to the appropriate tailoring of treatment modalities based on differential epileptogenic mechanisms [9,13].

**Clinical Manifestations**

The cerebral cortex plays a vital role in a number of functions including memory, attention, perceptual awareness, thought, language, and consciousness. Accordingly, individuals affected with CD exhibit a wide array of symptoms depending on the extent and region of the brain involved. Epilepsy is the main symptom of CD, and it is often refractory to antiepileptic drugs (AEDs) [16]. Symptoms may appear at any age including in adulthood, although seizures mostly start in childhood. Individuals with CD may also present with behavioral disturbances, psychomotor retardation, and learning disabilities [9,17,18]. Other manifestations that have been reported include homonymous hemianopsia [19,20] and sleep-related epilepsy [21].

Children with CD type I are more likely to be mentally retarded and present maladaptive behaviors more frequently compared with children with CD type II [17]. In addition, surgical outcome is significantly worse in patients with CD type I [17,22], probably because it is more difficult to delimit the structural lesion and achieve a complete surgical resection [4,23,24]. Patients with CD type II manifest symptoms earlier than those with CD type I. Furthermore, those with larger lesions tend to present symptoms earlier than patients with smaller lesions. Evidence suggests that patients with CD type II usually have multilobar lesions with extratemporal involvement, mainly within the frontal lobe. Hence, seizures that occur early during the neonatal period or childhood are more likely to be due to CD type II with more extensive, multilobar involvement [9]. With central or parietal lesions, neurological findings include motor and sensory limb deficits and facial paresis, as well as poor bilateral hand coordination. Nystagmus and hemianopsia are observed in patients with posterior lesions. The motor deficits can become permanent if seizures are medically refractory, as is the case in status epilepticus, which poses a grave prognostic factor [20]. The cognitive impairment tends to be broad, ranging from mild impairment in those with limited CD to severe cognitive disorder and autism spectrum disorder in those with extensive lesions as well as earlier onset of epilepsy. The CD cases that present with psychiatric symptoms also tend to have earlier onset of epilepsy, and their lesions are located more posteriorly [20].

**MRI and EEG Findings**

Magnetic resonance imaging (MRI) is the most common method used to locate the lesion(s) in CD. In children with positive MRI findings, seizures tend to be earlier in onset and intractable [25–27]. The MRI in CD type I includes segmental or lobar atrophy that is usually associated with reduced volume of the subcortical white matter, some blurring of the gray–white matter junction, and abnormal patterns of gyri and sulci. This type often involves the temporal lobe [4]. The MRI findings in CD type II include cortical thickening, blurring of the gray–white matter junction on T1- and T2-weighted images, increase in signaling of white matter on T2-weighted images and fluid attenuated inversion recovery (FLAIR) T2-weighted images, and decrease in the white matter signal in T1-weighted image. The change in white matter signaling tapers toward the ventricle, resulting in the
## CLASSIFICATIONS OF CORTICAL DYSPLASIA & PRINCIPAL HISTOPATHOLOGICAL & CLINICAL FEATURES

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<td>Severe (early gestation)</td>
<td>Dys, Mis Hetero Mol. Layer Neurons Polymicrogyri Neuronal Cytomegalgy &amp; Balloon Cells</td>
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<th>ILAE (2011)</th>
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<th>CD Ib Dys (tang), Mis Hypertrophic &amp; Immature Neurons</th>
<th>CD Ic Dys (radial &amp; tang), Mis Hypertrophic &amp; Immature Neurons</th>
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<td>CD IIa Dys, Mis Dysmorphic Cytomegalgy &amp; Immature Neurons + Balloon Cells</td>
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<td>CD IIIa Dys + Hippocampal Sclerosis</td>
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#### Clinical Features
- Older Age at Seizure Onset
- Seizures (less frequent)
- Maladaptive Behaviors & Mental Retardation (frequent)
- Worse Surgical Outcome

- Earlier Age at Seizure Onset
- Seizures (more frequent)
- Maladaptive Behaviors & Mental Retardation (less frequent)
- Better Surgical Outcome

#### MRI
- Segmental/Lobar Atrophy
- Reduced White Matter Volume
- Some Blurring of Grey/White Matter Junction
- Abnormal Pattern of Gyri & Sulci

- Cortical Thickening
- Hyperintense White Matter on T2
- Hypointense on T1
- Blurring of Grey/White Matter Junction
- Transmantle Sign

#### PET
- Hypometabolism of Dysplastic Region

#### EEG/ECOG
- CEDs or REDs
- Reduced Background Activity
- Repetitive, High Amplitude Fast-spikes Followed by Slow Waves Interspersed with Flat Periods
- High Frequency Oscillations (80-450 Hz) & Fast Ripples (250-500 Hz)

#### Potential Generators of Epileptic Activity
- Immature & Hypertrophic Pyramidal Neurons
- PGA (regular spiking interneurons)

- Immature & Dysmorphic Cytomegalgy Pyramidal Neurons
- Cytomegalgy Interneurons
- PGA (regular spiking interneurons)

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**Figure 1** Different classification systems used to define cortical dysplasia (CD) types based on the presence of architectural and cellular abnormalities. In the original description of focal cortical dysplasia (FCD) by Taylor et al. [6], different types were not defined but the authors noticed that not all cases presented with balloon cells. The ILAE classification of CD is currently the more widely accepted. We also include the relationship of different types of structural lesions with clinical manifestations, as well as potential cellular mechanisms that could participate in the generation of epileptic discharges.
“transmantle sign” [28]. The subcortical white matter may also appear hyperintense on T2-weighted imaging and hypointense on T1-weighted imaging [29,30].

In CD types I and II, if the lesions are very small, the MRI study may be negative regardless of the severity of the symptoms posing a challenge for surgical treatment [31–33]. For example, CD type II associated with intractable partial epilepsy with early onset produces neurological deficits as well as cognitive impairment and is missed on MRI studies in one-third of affected subjects [34]. Nevertheless, detection of the “transmantle” sign in CD type II can be greatly improved using 3 Tesla MRI, which has better spatial resolution [35].

Precise localization of epileptic foci and improved outcome can benefit from the use of multimodality imaging [36]. In the case where MRI is negative in individuals with intractable epilepsy, fluoro-deoxyglucose positron emission tomography (FDG-PET) coregistration and ictal single photon emission computed tomography (SPECT) may help to identify structural lesions [37–40]. In this process, FDG-PET images are superimposed on the MRI while color-coding the area as well as degree of hypometabolism to identify subtle CD foci. Use of FDG-PET/MRI coregistration may be superior to MRI studies alone, as borders of the lesions are identified more clearly, thus promoting success in complete resection of these lesions [38,41].

Electroencephalography (EEG) and intraoperative electrocorticography (ECoG) are other important tools in the localization of the epileptogenic substrate. The intrinsic epileptogenicity of CD tissue, manifested by continuous or rhythmic epileptogenic discharges (CEDs or REDs) recorded directly from the structural lesion, has been demonstrated using ECoG recordings [42]. These types of discharges could be used as a diagnostic tool for CD [43]. Stereo EEG confirmed a high correlation between REDs and an existing dysplastic lesion [20]. Other EEG findings in CD include absence of background activity and repetitive, high-amplitude fast spikes followed by high-amplitude slow waves interspersed with flat periods [9]. Abnormal EEG background is a common finding in CD that is multilobar or affects the posterior rather than the anterior brain in both children and adults [20]. The predominant seizures observed during chronic EEG monitoring in CD are nocturnal as well as sleep-related seizures [34]. More recently, EEG high-frequency oscillations (80–450 Hz) and fast ripples (250–500 Hz) have been used as localizing tools for CD [44–46] and also can be used as biomarkers for epileptogenic areas.

Architectural and Cellular Abnormalities in CD

Neuronal cortical growth takes place in a central to peripheral direction from the subventricular zone, and new neurons are sequentially added as new layers [47–49]. This pattern continues until the formation of the six layers is completed, with each layer containing neurons of various shapes, sizes and density with different nerve fiber organization [1,50,51]. The Reelin signaling pathway plays a crucial role in the laminar organization and orientation of radially migrating neurons [52]. Reelin is a large extracellular matrix (ECM) protein [53,54] produced by the transient Cajal–Retzius (C-R) cells localized in the marginal zone of the developing cortex [50] and also later by some interneurons [55]. Deficient Reelin signaling is the main cause of architectural malformation and polarity defects of cortical neurons [52]. Interestingly, in CD types I and II, the density of C-R cells is increased and can be used as a marker of these types of dysplasia [56]. Furthermore, any disturbance in the ECM of the developing cortex can potentially interfere with the appropriate distribution of migrating neurons leading to supernumerary, misplaced neurons (Kostovic et al., 2014, this issue). Indeed, it has been demonstrated that the composition of the ECM of CD differs from that of nonmalformed tissue, mainly by the altered expression of certain ECM molecules and by the presence of an increased number of astrocytic processes [57].

Architectural Abnormalities

In pediatric CD, increased number of neurons in the upper cortical layers, specifically in the molecular layer, as well as in the white matter has been observed [58], which could indicate overproduction of neurons later during corticogenesis. Furthermore, the post-neurogenesis programmed cell death (apoptosis) appears to be disrupted in CD, leading to further increase in neuronal densities [59,60]. In contrast, stereological studies in CD (types I and II) tissue indicated that overall neuronal densities are decreased compared with unaffected cortex [61]. However, this study included pediatric and adult cases (age range 1–81 years) and secondary neuronal loss or simply cortical volume expansion commonly observed in CD could not be ruled out as an explanation for reduced neuronal density. Obviously, more studies are warranted to determine more precisely changes in neuronal densities based on the new CD classification system and using a more homogeneous population in terms of age, histopathology, and CD type.

Microscopic examination of dysplastic tissue at low power shows localized disruption of normal cortical lamination. For example, layer IV is discontinuous and layers V and VI are barely distinguishable [62]. In CD type Ia, there is abundance of neurons and persistence of microcolumns (>8 neurons aligned vertically) [9]. In CD type Ib, the six-layered tangential composition of cortex fails to get established. In type Ic, both abundance of microcolumns and abnormal tangential layer composition are observed [9]. There is blurring of the border between gray and white matter, and the white matter shows reduced myelin staining [9]. Cortical lamination, except for layer I, is also disrupted in CD type Ila/b. CD type Ila contains small displaced neurons with blurring of boundaries of gray–white matter [9]. In CD type IIIa, there is cortical dyslamination as well as cortical hypoplasia near the tumor site without cortical infiltration [9]. Whether dysplasia adjacent to the tumor truly reflects dual pathology or a single developmental lesion has been questioned [63]. CD type IIa is characterized by cortical dyslamination or cortical hypoplasia near the vascular malformation [9]. Finally, CD type IIIb has cortical dyslamination and hypoplasia in the presence of other lesions acquired early in life [9].

Using layer-specific markers, studies found abnormal laminar expression patterns in CD [64] and also that disruption of cortical lamination is brain region dependent, most prominent in frontal CD and in layers III and VI [65]. In addition, layer-specific gene
expression in CD type II cases revealed that normal-appearing pyramidal neurons are better organized in different laminae than dysmorphic neurons and balloon cells, which show more altered migratory patterns [66].

Cellular Abnormalities

The following description of morphological cellular abnormalities is largely based on our own studies using biocytin and immunocytochemical staining in tissue samples from children (ages 2 months to 14 years) with CD [10,67–69]. CD type I contains misoriented pyramidal neurons, pyramidal neurons with tortuous processes, and hypertrophic pyramidal neurons with otherwise normal morphology. Furthermore, one may encounter clusters of pyramidal neurons with immature characteristics, noted for their smaller round or pyramidal somata, thin, varicose processes and immature spines [10]. Some of the microcolumns described in CD type Ia/c may in fact be formed by these immature-looking pyramidal neurons (Figure 2).

In CD type Ia/b, abnormal cells include primarily dysmorphic cytomegic neurons and balloon cells. Immature pyramidal neurons can also be encountered. Dysmorphic cytomegic neuronal neurons are characterized by large soma, abnormal thickness of the initial portion of apical dendrite and axon [10]. Unusually dense Nissl substance with wild appearance and abnormal distribution of neurofilament proteins can be observed [9,70,71]. Cytomegic interneurons are another abnormal cell type found in CD type II (in particular associated with hemimegalencephaly, HME) [67]. They are characterized by their large size, 2–3 times larger than normal interneurons. Cytomegic interneurons are similar to basket cells and also have increased number of dendrites. They label for calbindin and parvalbumin but not for calretinin, suggesting they may originate in the medial ganglionic eminence [67]. Balloon cells are the hallmark of CD type IIb. The cytoplasm in balloon cells is filled with hypertrophic endoplasmic reticulum and intermediate filaments in addition to at least one eccentric nucleus [8]. Balloon cells do not have detectable Nissl substance, and they are large and pale with multiple nuclei and present in all layers but tend to concentrate in the upper layers and white matter. They are reminiscent of gemistocytic or fibrillary astrocytes, without dendritic spines or visible axons, and their processes are tortuous [10,14,69]. They also express glial fibrillary acidic protein (GFAP), as well as immature neuronal and stem cell markers [13,72]. Finally, in some CD type II cases, cells with combined aberrant morphological characteristics have been observed. These are called “intermediate cells” due to their neuronal and glial appearance [73].

Tuberous sclerosis complex shares histopathological similarities with CD type IIb. TSC is an autosomal dominant disorder with genetic mutations of TSC1 and TSC2 coding for hamartin and tuberin, respectively. The mutation leads to excessive activation of the mammalian target of rapamycin (mTOR) pathway that is normally inhibited by TSC1 and TSC2 proteins [74]. As a result, benign tumors (hamartomas) are produced in multiple organs. In the brain, hamartomas can lead to epileptic activity and disturbance of cognition [75]. Balloon cells found in CD type IIb are similar to the “giant” cells reported in TSC [76]. In addition, similar to balloon cells in CD, “giant” cells in TSC variably express both neuronal and glial markers [77].

In Vitro Electrophysiology of CD tissue

Pioneer work using slices in vitro demonstrated that CD tissue is highly sensitive to some proconvulsant drugs [78,79]. Treatment with 4-aminopyridine (4-AP), a K⁺ channel blocker that augments neurotransmitter release, leads to the generation of N-methyl-D-aspartate (NMDA) receptor-mediated ictal discharges as well as GABA and glutamate receptor-mediated interictal discharges [80]. GABA can increase the K⁺ concentration leading to initiation of seizure activity [81]. In nondysplastic tissue treated with 4-AP, ictal activity is not observed, and there is only periodic generation of interictal-like events that are GABA mediated.

Studies have been carried out to evaluate the electrophysiological properties of normal and abnormal cells in CD. Visualization of individual brain cells is made possible by the use of infrared video microscopy in combination with differential interference contrast optics [10,82]. Use of this technology in conjunction with whole-cell patch clamp recordings assists in more accurate characterization of different cell types and correlation with physiological function. Tissue from younger patients (<5 years of age) is optimal as there is less myelination and therefore better visualization. Biocytin is used to further characterize morphological features after electrophysiological studies.

Dysmorphic cytomegic pyramidal neurons have abnormal passive membrane properties marked by larger cell capacitance, consistent with increased membrane area, longer time constant, and lower input resistance compared with normal-appearing pyramidal neurons. The amplitude of macroscopic Ca²⁺ currents and Ca²⁺ influx also are larger in this type of neuron, leading to hyperexcitability [10,73]. They also have reduced sensitivity to Mg²⁺, suggesting NMDA receptors can be activated at more hyperpolarized membrane potentials [83]. Changes in GABA_A receptor subunit composition have been demonstrated based on slower GABA current kinetics [15]. However, the sensitivity of these cells to GABA is not changed in CD tissue compared to nondysplastic tissue [84]. Similar to cytomegic pyramidal neurons, cytomegic GABAergic interneurons are hyperexcitable and display spontaneous membrane depolarizations and bursting activity [67].

The balloon cells, found in CD type IIb, are characterized by round cell bodies. These cells do not have voltage-gated Na⁺ or Ca²⁺ currents. The input resistance is usually high, and there are no spontaneous synaptic currents [10,73]. These cells are not sensitive to application of excitatory amino acids, which points to lack of ability of these cells to be epileptogenic [10,13,85]. Likewise, the “intermediate” cells observed in CD type IIb and TSC do not display active inward currents [73].

The electrophysiological properties of the misoriented pyramidal neurons and normal-appearing pyramidal neurons with tortuous processes are normal in terms of capacitance, input resistance, and time constant [10]. The neurons with immature characteristics have low membrane capacitance, high input resistance, short time constant, normal Na⁺ current but small Ca²⁺ currents, and abundance of GABA inputs [10].
Figure 2 Simplified diagram of architectural and cellular abnormalities in cortical dysplasia (CD) compared with normal cortex. In CD type I, laminar organization becomes more tenuous and pyramidal neuron misorientation, with or without tortuous processes, is profuse. Immature pyramidal neurons also are observed, sometimes organized as microcolumns or as clusters. In CD type II, lamination is almost lost except for a distinct layer I. Balloon cells can be seen mainly in the upper layers and in white matter. Dysmorphic cytomegalic pyramidal neurons and cytomegalic interneurons are scattered throughout the cortical plate. In addition, clusters of immature pyramidal neurons can be seen. The role of granular and polymorphic neurons in dysplastic cortex remains unknown. This diagram was inspired by a figure in reference [72].
Proposed Mechanisms of Epileptogenesis in CD

The exact mechanisms of epileptogenesis in pediatric CD are not yet fully understood, but they involve complex interactions among different cell types and are not simply due to an imbalance in glutamatergic/GABAergic neurotransmission [84]. In addition, while some mechanisms of hyperexcitability are similar among CD types, there must be differences based on dissimilar histopathology. In CD type I, there are no dysmorphic cytomegalic neurons that could account for epileptogenic activity. Thus, other cellular types and mechanisms have to be invoked. In particular, dyslamination and the presence of immature, misoriented, and hypertrophic pyramidal neurons with or without tortuous processes could certainly lead to aberrant connectivity. In CD type II, the dysplastic neurons are postulated as essential pathogenetic elements [86] and the source of ictal discharges [20]. Of the different dysplastic cells, cytomegalic pyramidal neurons are more likely to be epileptogenic, while balloon cells are not epileptogenic [85]. On the other hand, the cytomegalic interneurons have been shown to be hyperexcitable and display spontaneous membrane depolarizations similar to paroxysmal depolarizing shifts, making them potential generators of epileptic activity [67].

Role of Glutamate Receptors

The pattern of glutamate receptor expression in CD is consistent with increased excitability [77]. The concept of abnormal NMDA receptor distribution was first introduced by Speрафico et al. in 1998 [19] and later confirmed by numerous studies [83,87]. There is altered expression and co-assembly of GluN2A- and GluN2B-type NMDA receptor subunits, as well as upregulation of the GluN1 subunit in CD [88–92]. In support, the functional role of NMDA receptors in CD became evident by abolition of 4-AP-induced ictal discharges by NMDA receptor antagonists [80]. Cytomegalic pyramidal neurons in CD have abnormal composition of NMDA receptor subunits and reduced Mg2+ sensitivity [83]. Thus, glutamate can activate neurons with decreased Mg2+ sensitivity at hyperpolarized membrane potentials. It is important to note that the abnormality in NMDA receptor subunit composition, in addition to decreased Mg2+ sensitivity, is a feature of developing neurons [93,94]. As neurons with reduced sensitivity to Mg2+ can fire more readily, a role of these neurons in the induction of seizures can be suggested [83]. Of the non-NMDA receptor subunits involved in epileptic activity, GluA2/3 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits are highly expressed in dysplastic neurons [95,96]. Metabotropic glutamate receptors, particularly mGluR1 and mGluR5, also are highly expressed in dysplastic cortical regions [97].

Role of GABA Receptors

In CD tissue, there is reduced density of GABAergic interneurons as well as reduced expression of GABA transporters which leads to altered inhibition [98]. Expression of parvalbumin and calbindin is decreased in CD. The populations of calbindin- and parvalbumin-positive cells are scattered abnormally in all layers with small clusters in some regions and scarce presentation in others [15,19,98]. When comparing morphological studies of CD types I and II, it is evident that glutamic acid decarboxylase (GAD)-labeled interneurons are decreased in number in CD type II as compared to CD type I [15,62]. Intriguingly, the dysplastic cortex also is noted for increased expression of GABAergic terminals surrounding the excitatory cytomegalic pyramidal neurons [8,62]. The source of these terminals has not been determined, but the cytomegalic interneurons are one possible source.

The functional role of GABA is different in immature compared to adult brain tissue. In immature pyramidal neurons, GABAergic synaptic inputs are depolarizing and potentially excitatory [99,100]. In CD, the cytomegalic and immature pyramidal neurons retain features of immature cortex with a predominance of GABA synaptic activity and GABA acting as an excitatory rather than inhibitory neurotransmitter [68,101]. This can provide an explanation for the relative insensitivity of CD tissue to benzodiazepine treatment [102]. GABA receptors in CD also display poor response to zolpidem and low reactivity to Zn2+, indicating changes in subunit composition [15,84]. In nondisplastic tissue, zolpidem binds to GABA receptors with z1 subunits. Zn2+ blocks GABA receptors that lack z2 subunits. In CD type II, there are fewer GABA receptors with the mature z1 subunit and more with the immature z2 subunit [15,84]. The GABA1 receptor subtype also plays a role in icetogenesis of CD tissue. For example, it has been shown that ical activity is initiated by GABA1 receptor activation causing increases in [K+]o facilitated by a reduced ability of GABA1 receptors to control GABA release from interneuron terminals [81].

Pacemaker GABA Synaptic Activity (PGA)

In a number of pediatric cases with CD types I, II, and III, rhythmic clockwise GABA receptor-mediated synaptic events (usually 5–10 Hz) are observed in immature, cytomegalic, and a subpopulation of normal-appearing pyramidal neurons [101]. These events are dependent on action potentials and are unaffected by glutamate receptor blockade. Regular spiking (double-bouquet or bitufted) GABAergic interneurons are a possible source of PGA. PGA is an abnormal signal, but probably normal in emergent cortical networks, that could play a role in neuronal synchronization contributing to epileptogenesis in CD. It can lead to membrane depolarizations in immature, normal-appearing, and cytomegalic pyramidal neurons, making these cells more excitable and capable of widespread synchronization. PGA could be the source of CEDs and REDs observed on the EEG of CD cases [20]. In fact, an early ECoG study hypothesized that this type of epileptic discharges indicated the presence of an intradysplastic pacemaker operating in a self-sustained, unstoppable fashion [42]. The discovery of synaptic PGA in CD tissue lends support to this idea.

The mTOR Pathway

The mTOR signaling pathway malfunctions in several disorders in which there is cortical malformation such as TSC and HME. Recently, epileptogenicity in CD was postulated to be due to the interplay between the mTOR pathway and structural and electrophysiological neuronal changes [103]. Thus, there have been
reports of enhanced mTOR signaling in CD type II. Abnormalities in the mTOR pathway can lead to increase in cell growth, which can account for the presence of balloon cells and cytomegalic neurons in CD [103–106]. In HME, another form of severe CD, de novo somatic mutations in the PIK3CA, AKT3, and MTOR genes were demonstrated in a high percentage of affected individuals [107]. This indicates aberrant activation of mTOR signaling, leading to a gain of function and cytomegaly. Indeed, mutational analysis of the TSC1 gene indicates that this gene may contribute to the development of CD type IIb [108].

The Dysmaturation Hypothesis of CD

Failure of maturation and migration during corticogenesis can lead to persistence of neurons that are immature and/or have mal-developed synaptic circuits. In CD tissue, many signs of dysmaturation have been demonstrated. For example, from the onset of migration (~5 weeks) until midgestation, the fetal neocortical plate exhibits a radial microcolumnar architecture [109]. Persistence of the fetal cortical architecture can be observed in CD type Ia/c and is suggestive of maturational arrest [109]. In addition, persistent and increased expression of embryonic cortical layer-specific markers (SATB2, FOXP1, and TBR1) in CD and HME brains indicates disrupted neuronal migration and dysmaturity [110]. Dysmaturity is more evident in CD type II than CD type I [111], and expression of early progenitor cell markers can distinguish CD type II from CD type I [112].

At the cellular level, both dysmorphic and normal-looking pyramidal neurons from CD tissue display immature markers [113,114]. Balloon cells, believed to originate from glyo-neuronal precursors such as radial glial cells, are enriched with stem cell markers including nestin, vimentin, Pax6, CD133, CD34, GFAP, and MAP2 [115–117]. Intriguingly, they also express the vesicular glutamate transporter VGLUT2 [116], as well as the primarily astrocytic markers EAAT2/GLT1 [118]. Expression of undifferentiated cell markers suggests that balloon cells suffered an arrest in development and differentiation [119,120]. Further, the chloride transporters NKCC1 and KCC2 also are differentially expressed. In control brains, NKCC1 expression, which is high during early development, occurs at very low levels. In contrast, expression is very high in age-matched patients with CD type IIb or HME and is observed in large dysplastic neurons and balloon cells [121]. In contrast, in CD and HME patients, KCC2 immunoreactivity is characterized by less neuropil staining compared with controls [121,122].

Based on electrophysiological, morphological, and immunocytochemical studies, a unified hypothesis to explain mechanisms of epileptogenesis in CD, known as the dysmaturation hypothesis, was postulated [94]. Accordingly, in CD, there is incomplete cortical development as a result of events failing to take place at different times during corticogenesis. Developmental alterations during the late second or early third trimester would account for severe and more diffuse CD (type II and HME), while events occurring closer to birth (after the subplate has nearly disappeared) would explain more focal and milder forms of CD (type I). However, it is important to acknowledge that some elements of CD pathogenesis, such as the presence of balloon cells (CD type IIb), might begin much earlier during corticoneurogenesis [11]. Developmental arrest or delay leads to preservation and mal-positioning of a substantial number of subplate and radial glia-like cells. Common to CD types I and II is the dysmaturity of neuronal networks and GABA synaptic activity with depolarizing actions similar to immature, developing networks which are highly epileptogenic [123,124]. The interaction of dysmature and retained subplate neurons with mature cells and networks not working in harmony can lead to chaotic electrophysiological behavior and epileptic activity [94,125] (Figure 3). Indeed, spontaneous plateau depolarizations and bursts of action potentials, similar to those observed in cytomegalic interneurons, occur in subplate neurons as early as 20 gestational weeks in the relative absence of external stimulation [126], demonstrating that subplate neurons display “mature” firing properties capable of initiating synchronous discharges. However, this is only part of the equation as even in normal brains, there is preservation of a substantial number of subplate neurons which remain as interstitial neurons in the white matter [1,2] (see also Kostovic et al., present issue). It is possible that interstitial neurons are more confined and do not interact actively with cortical circuits as may occur with retained subplate neurons in CD. Additionally, spontaneous depolarizations and bursting are observed in cytomegalic interneurons not in cytomegalic pyramidal neurons, whose function appears to be more like an amplification device instead of a generator of epileptiform activity [67,85]. Obviously, other factors are involved in the epileptogenic circuitry.

An important question is whether or not the dysmaturity hypothesis of epileptogenesis for pediatric CD cases can be applied to adults. We can expect that the structural lesion and aberrant circuitry that generate epileptic activity are constantly evolving. While immature membrane and synaptic electrophysiological properties can be observed in cells recorded in older children, up to 14 years of age [10,101], we do not know whether they also occur in adults. The presence of immature morphological markers in adult cases would suggest this is probably the case. However, we need to await further experimentation to support this idea. At least in one of the few reports of electrophysiological single-cell recordings from adult CD patients, the relative abundance of GABA synaptic activity observed in children does not seem to occur, at least in normal-appearing pyramidal neurons [98].

Other Potential Mechanisms of Epileptogenesis in CD

While substantial strides have been made to understand the mechanisms of epileptogenesis in CD, many other potential factors remain underexplored. For example, upregulation of the voltage-gated sodium channel Nav1.3 in CD type IIb could play a role in increased excitability of cytomegalic pyramidal neurons and interneurons [127]. Electrotropic propagation via gap junctions also could contribute to epileptogenesis in CD tissue [128]. Gap junctions play an essential role in migration, synchronization, and 4-AP oscillatory activity [129,130]. Studies in pediatric CD tissue demonstrated a positive correlation between age and the presence of dye-coupling [131]. In addition, immature pyramidal neurons filled with biocytin frequently show coupling (personal observation).
The exact role of balloon cells in epileptogenesis in CD type IIb also remains obscure. Several studies have found evidence for increase in mechanisms leading to clearance of glutamate in areas that contain balloon cells, hence decreasing the spread of epileptogenic activity [118,132]. In that sense, balloon cells could play a protective or antiepileptic role. As mentioned earlier, balloon cells express EAAT2/GLT1 suggesting a role in glutamate buffering [118]. In addition, subsets of balloon cells and astrocytes from CD type IIb tissue also display high expression of gap junction-forming connexin 43, allowing spatial buffering of extracellular ions and neurotransmitters [133]. However, if balloon cells, which also express VGLUT2, are able to release glutamate, they could in fact contribute to epileptogenesis [134]. Finally, the enlargement and tortuosity of the ECS in CD type II may alter extrasynaptic volume transmission and thus might represent another factor contributing to CD epileptogenicity [57].

Conclusions and Future Perspectives
Cortical dysplasia offers a privileged glimpse into the immature albeit pathological brain. A better definition and classification of the different CD types has helped understand basic mechanisms. But despite advances in imaging, cellular electrophysiology, and molecular biology, the exact etiological and pathophysiological mechanisms of this debilitating disorder remain unclear.
Future studies are indicated to further investigate the underlying etiology of the different types of CD. Recently, it was reported that specimens from CD type IIb cases express the human papillomavirus type 16, primarily in balloon cells, suggesting an infectious etiology, but this finding requires confirmation [135]. This observation is important as this virus is a potent activator of the mTOR pathway [136]. Other studies have found the presence of cytomegalovirus infection in fetal brains [137], and interestingly, the virus showed higher tropism for stem cells/radial glial cells. On the other hand, a genetic predisposition to CD also is the focus of current research [138].

Identifying different types of CD lesions on MRI tends to be a challenge as some are not detectable due to their subtle nature. Improvement in imaging modalities to detect CD type I and type II lesions can help achieve better outcomes [38]. In addition, although there are effective neuroimaging and surgical techniques to identify and control seizures in CD [24], the diagnosis and treatment of this disorder continues to pose a challenge and many cases remain underdiagnosed and not effectively treated. The poor response to AEDs could be accounted for by a combination of mechanisms. There is altered GABA function in pediatric CD which can cause depolarization, hyperexcitability, and alterations in intracellular chloride concentration, requiring other means to normalize the cell electrophysiology rather than the use of AEDs that increase GABA function [15]. There also is altered composition of GABAA receptor subunits as well as levels of NKCCI and KCC2 in CD type II and TSC with increases in NKCCI and decrease in KCC2. Agents such as bumetanide that inhibit NKCCI may prove effective when added to the AED therapy and are the subject of ongoing studies [102,139,140]. Animal studies have found promising results with combination therapy with barbiturates and bumetanide in neonatal rat brain with increased efficacy of GAB-Aergic inhibition [141,142] (see also studies by Khazipov et al. and Dzhala and Staley, this issue). Research is currently ongoing to evaluate the safety and efficacy of this combination therapy in human neonates. The result can certainly help tailor the treatment approach for an effective seizure control in patients suffering from CD and TSC and are nonresponsive to AEDs [102].

Use of rapamycin, an inhibitor of the mTOR pathway, has proved positive in decreasing epilepsy in mice [103,143]. Although effective, this medication is accompanied by a series of adverse effects that seriously limit its use [143]. It will be important to evaluate the role of this class of medications for the treatment of CD, specifically those with more desirable side effect profile than rapamycin. Electrical stimulation of thalamic nuclei also showed promise in the modulation of paroxysmal activity in CD [144]. Finally, the FDA approved recently a new treatment for epilepsy using a neurostimulator implant that serves to detect the onset of seizures and deliver electrical stimulation to terminate ictal discharges [145]. This can lead to future research involving more precise stimulation techniques such as the use of optogenetics [146,147].

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Conflict of interest
The authors declare no conflict of interest.

References

Basic Mechanisms of CD Epileptogenesis

S. Abjadjad et al.
Basic Mechanisms of CD Epileptogenesis


