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The clinico-pathological spectrum of Focal Cortical Dysplasias: a consensus classification proposed by an *ad hoc* Task Force of the ILAE Diagnostic Methods Commission

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There is considerable debate regarding the terminology used for abnormal cell types, which has been inconsistently used in previous classification systems. The following definitions were based on microscopic inspection of 4-7μm thin sectioned, formalin-fixed and paraffin embedded surgical specimens. Representative examples were given in Figure 10.

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Abstract

Focal cortical dysplasias (FCDs) are localized regions of malformed cerebral cortex and are very frequently associated with epilepsy in both children and adults. A broad spectrum of histopathology has been included in the diagnosis of FCD. Characteristic findings include aberrant radial or tangential lamination of the neocortex (FCD Type I) and cytological abnormalities (FCD Type II). An ILAE task force has re-evaluated available data and proposes a clinico-pathologic classification system of FCDs. The major change since a prior classification represents the introduction of FCD Type III, which occurs in combination with Hippocampal Sclerosis (FCD Type IIIa), or with epilepsy-associated tumors (FCD Type IIIb). FCD Type IIIc is found adjacent to vascular malformations, whereas FCD Type IIId can be diagnosed in association with epileptogenic lesions acquired in early life (i.e., traumatic injury, ischemic injury or encephalitis). Hence, FCD Type I will now refer to isolated lesions, which present either as radial (FCD Type Ia) or tangential (FCD Type Ib) dyslamination of the neocortex, microscopically identified in one or multiple lobes. FCD Type II is an isolated lesion characterized by cortical dyslamination and dysmorphic neurons without (Type IIa) or with balloon cells (Type IIb). Architectural abnormalities adjacent to or within gross malformations of cortical development are frequently observed and not distinguished as a specific FCD variant. This three-tiered classification system will help to better characterize specific clinico-pathological entities and is an important basis to further explore imaging, electro-clinical features, and postsurgical seizure control as well as underlying molecular pathomechanisms.

Keywords

Epilepsy; Seizures; Hippocampal Sclerosis; Cortical Dysplasia; Neuropathology

Introduction

Focal cortical dysplasias (FCDs) were first described in detail by David Taylor et al. in 1971. They reported on 10 patients with drug-resistant epilepsy who underwent surgical resection (Taylor et al. 1971). Microscopic examination revealed a peculiar histopathology including cortical disorganization, large bizarre neurons and, in half of the patients, balloon cells. Since then, the term “FCD” has been widely used for a large spectrum of lesions comprising cortical dyslamination, cytoarchitectural lesions and underlying abnormalities of white matter (Palmini et al. 2004). With ongoing advances in presurgical neuroimaging techniques, more subtle cortical abnormalities can be identified as potential epileptogenic foci. Following surgery, there is often the expectation that the reporting pathologist will identify a corresponding distinct abnormality rather than give a negative report such as ‘non-specific minor changes or within normal limits’. The pathologist should be able to provide robust and consistent objective criteria for any cortical abnormality with findings that are reproducible and reliable between laboratories. An *ad hoc* ILAE Task Force (created under

the Commissions of Therapeutic Strategies and Pediatrics with follow up in the Commission of Diagnostic Methods) has made an attempt, therefore, to review available literature on clinical presentation, imaging findings and histopathological features of distinct clinico-pathological FCD variants and propose a refined clinico-pathological classification system. It is the sincere expectation of our group, that this first international consensus classification will be helpful for clinical practise as well as motivating further research strategies to improve our clinico – imaging – histological and genetic understanding of FCDs.

Previous Classification Systems of FCDs

During the past 15 years, different FCD classifications have been introduced. A neuropathological grading system was proposed (Mischel et al. 1995), which described the spectrum of histopathological abnormalities in a series of 77 surgical specimens, i.e. balloon cells, neuronal cytomegaly, neuronal heterotopia, polymicrogyria, marginal heterotopia, neurons in the molecular layer, heterotopic white matter neurons and cortical disorganization. In many epilepsy centers, the epileptogenic lesion is diagnosed only by MRI analysis (Barkovich et al. 2005), but yet, there are no highly sensitive imaging parameters available which can reliably differentiate among FCD subtypes. The classification system of a previous working group report is now widely used (Palmini et al. 2004). By this scheme, FCDs can be histopathologically distinguished into Type I and II. FCD Type IA referred to architectural disturbances of cortical lamination, and FCD Type IB included also cytoarchitectural abnormalities, i.e. hypertrophic (not dysmorphic, see terminology issues below) pyramidal neurons outside Layer 5. Dysmorphic neurons are the histopathological hallmark of FCD Type IIA. Microscopic identification of dysmorphic neurons and eosinophilic balloon cells specifies FCD Type IIB.

Clinico-radiological and pathological presentation of FCDs

Focal cortical dysplasias can be located in any part of the cortex. They have variable size and location, and may also affect multiple lobes. FCD Type II is more frequently encountered in extra temporal areas, particularly in the frontal lobe. Unless the area of FCD is large, patients do not suffer from severe neurological deficits and the main clinical manifestation is epilepsy. Seizures can start at any age (but usually during early childhood) and are very often drug resistant. Seizure semiology depends on the location of the lesion, and patients with both Type I and Type II dysplasias generally present high seizure frequency (Tassi et al. 2002, Tassi et al. 2010). They can also exhibit behavioural disturbances, especially those with early onset epilepsy, and whether this occurs more frequently for FCD involving the temporal lobe remains an important issue. The presence of focal, rhythmic epileptiform discharges is the most characteristic feature of the scalp EEG in patients with FCD, frequently showing spatial correlation with the lesion (Gambardella et al. 1996). First, by means of electrocorticography (ECoG) and then with intracerebral recordings, intrinsic epileptogenicity of dysplastic tissue has been demonstrated, particularly in FCD Type II with evidence of a peculiar interictal activity never observed in other forms of MCD (Chassoux et al. 2000, Palmini et al. 1995). In contrast, inconsistencies in the clinical presentation of patients with FCD Type I most likely result from the difficulty to classify them accurately by microscopic inspection (Chamberlain et al. 2009).

The neuroimaging characteristics of FCDs are a very important component of the clinical assessment (Barkovich et al. 2005, Colombo et al. 2009, Lerner et al. 2009). Among reported findings are increased cortical thickness, blurring of the cortical-white matter junction, increased signal on T2 weighted images, a radially-oriented linear or conical transmantle stripe of T2 hyperintensity, cortical thinning, and localized brain atrophy. Unfortunately, none of these signs are consistent or completely reliable. For instance, in the immature and unmyelinated brain, increased T2 signal, localized or transmantle, is difficult

to identify, as is the cortical-white matter junction blurring: cortical-white matter junction MR blurring is a normal finding during the postnatal stage of brain maturation (Barkovich et al. 1988). Incomplete myelination can also give the appearance of cortical thickening on T2 FLAIR and T1 weighted images, as partially myelinated white matter becomes transiently isointense to cortex. As recently discussed (Colombo et al. 2009), it seems essential to study the brain with true T2 weighted images as well as T1 weighted and T2 FLAIR images. Beyond these limitations of imaging, published data suggest that patients histopathologically diagnosed with the same FCD subtype (according to Palmini's classification system) have different imaging characteristics (Krsek et al. 2008, Lerner et al. 2009). This makes no physical sense, as entities with identical histology should have identical imaging characteristics. In view of the recently described difficulty in reliably diagnosing FCD pathology, especially when mild (Chamberlain et al. 2009), it seems that imaging variability reflects inconsistent histological diagnoses, likely combined with the fact that different entities have been included together in a single histological category in the past. We hope that this new classification will allow more consistent histological-MRI correlations and that better MRI interpretations will guide better management.

The new “FCD classification system” also takes into account insights from experimental neurodevelopmental studies (Battaglia et al., 2009), i.e., sustained plasticity and neurogenesis in the postnatal brain, which is compromised by various pathogenic conditions. Similarily, the “dysmature cerebral developmental hypothesis” suggested that there is partial failure in later phases of cortical development that might explain the distinctive histopathology of CD and that local interactions of dysmature cells with normal postnatal neurons promote seizures (Cepeda et al. 2006). We propose a three-tiered classification system (Table 1) distinguishing isolated FCDs (FCD Type I and II) from variants associated with other (potentially) epileptogenic lesions (FCD Type III). We propose in addition that mild forms of cortical malformations (mMCD) should be included in the classification, although their clinical impact will need further clarification (see below). Notwithstanding, any classification system using histopathological examination will rely on sufficient and representative surgical tissue as well as standardized laboratory protocols (see supplementary material).

1. Focal Cortical Dysplasia Type I

Definition: Focal Cortical Dysplasia Type I is a malformation presenting with abnormal cortical layering, either compromising radial migration and maturation of neurons (FCD Type Ia) or the 6-layered tangential composition of the neocortex (FCD Type Ib). The combination of both variants will be classified as FCD Type Ic.

1.1. Focal Cortical Dysplasia with abnormal radial cortical lamination (FCD Type Ia)

1.1.1. Histopathological findings—This variant is characterized by abundant microcolumnar organization (most prominent within Layer 3). A “microcolumn” is defined by more than 8 neurons aligned in vertical direction (Figure 1), if **i.)** the section is cut perpendicular to the pial surface; **ii.)** a 4μm thin paraffin embedded section is used, **iii.)** NeuN immunohistochemistry is applied, and **iv.)** aligned neurons present with a small diameter and cell size of < 250 μm^2 (Hildebrandt et al. 2005). Microcolumns resemble ontogenetic columns described during normal cortical development (Rakic 1988). They can be also seen at lower frequency and with fewer neurons in non-epileptic brain samples as well as in the vicinity of other principal lesions (see below). The border towards white matter is usually less sharply demarcated due to increased numbers of heterotopic neurons. Cellular abnormalities can be encountered in this variant, and include (1) immature small diameter neurons or (2) hypertrophic pyramidal neurons outside Layer 5. The diagnosis of

FCD Type I variants will need particular attention, however, when studying agranular or dysgranular areas of the temporo-polar lobe (Ding et al. 2009).

1.2. Focal Cortical Dysplasia with abnormal tangential cortical lamination (FCD Type Ib)

1.2.1. Histopathological findings—Failure to establish a 6-layered tangential composition of the isocortex is a hallmark of this variant (and should, therefore, always be used with caution in non-six layered allo- or proisocortical areas). The entire neocortical architecture may be affected (Figure 2A) without any recognizable layering (with the exception of Layer 1). Other subtypes are restricted to abnormal layering of Layer 2, Layer 4 or both. Layer 2 can be either missing or is significantly depleted of the characteristic population of small pyramidal neurons. This pattern results in a blurred demarcation between Layers 1 and 2, as well as between Layers 2 and 3, whose boundaries are very well defined in non-epileptic controls. Layer 4 can also be missing (Figure 2C) or is obscured and less distinguishable from Layers 3 and 5. The border with white matter is usually less sharply demarcated due to increased neuronal cells. Cellular abnormalities can be encountered in this variant, and include (1) immature neurons with a small diameter or (2) hypertrophic pyramidal neurons outside Layer 5 or (3) normal neurons with disoriented dendrites. These observations and other cellular alterations requiring sophisticated neuroanatomical techniques are not mandatory, however, to establish the diagnosis of FCD Type I variants.

1.3. Focal Cortical Dysplasia with abnormal radial and tangential cortical lamination (FCD Type Ic)

This variant refers to those isolated lesions, in which histopathological inspection reveals both, abnormal radial and tangential cortical lamination. Histopathological hallmarks are identical to those specified in Sections 1.1.1 and 1.2.1. This FCD variant is diagnosed only as an isolated lesion and not in combination with any other pathology. It has to be clarified in the future, however, whether such lesions occur within patients with more widespread abnormalities linked to mental retardation and/or multiple congenital abnormality syndromes.

1.4. Electro-clinical and imaging findings

A recent series studied 215 consecutive patients with proven histopathological diagnosis of Type I FCD (according to Palmini's classification system) and specifically compared electro-clinical and imaging findings as well as postsurgical outcome when FCDs occurred isolated or associated with hippocampal sclerosis and tumors (Tassi et al. 2010). Significant differences were found between both FCD cohorts. Isolated FCDs were observed in 31% of this series and characterized by more frequent seizures, negative MRI, multilobar involvement as well as worse post-surgical seizure control (46% Engel Class I). In contrast, associated FCD Type I patients presented with a similar clinical phenotype than those epilepsy patients suffering from HS or tumors alone (most frequently with temporal lobe involvement). Further studies using cohorts of isolated FCD Type I variants are required to characterize reliable presurgical MRI signal changes. A recent series examined 18 children (mean age at surgery was 7.6 years) with multilobar FCD Type I and severe drug-resistant seizures (Blumcke et al. 2010). Significantly reduced ‘hypoplastic’ volumes of affected compared to the non-affected hemispheres were correlated with the occurrence of microcolumns suggesting severe developmental disturbances (and/or retardation) in isolated FCD variants (FCD Type Ia according to the new classification system). Using diffusion imaging, voxel-based analysis and measures of blurring of gray-white matter transition may also identify significant abnormalities. A more routine use of high-field MRI scanners and new MRI contrasts as well as FDG-PET/MRI coregistration will enhance sensitivity and

specificity (Salamon et al. 2008). Techniques such as MR spectroscopy, FDG-PET or new PET tracers, e.g. ^{11}C-alphamethyl tryptophan may infer the presence of abnormality but specificity needs to be established in this particular cohort of patients.

1.5. Perspectives

Our approach to classify three isolated FCD Type I variants will need re-evaluation for its feasibility in clinical practise. However, there is still no clue for any underlying pathomechanism. The Task Force is confident, however, that scientific studies addressing more homogeneous groups FCD variants will improve our understanding of this disease entity.

One limitation of this clinico-pathological classification system has to be also mentioned. If only small, fragmented or non-representative surgical tissue specimens were submitted for histopathological diagnosis, the distinction between isolated and associated FCD subgroups and variants will be difficult to obtain. If no specific diagnosis can be achieved, a descriptive formulation of microscopic features should be given (Supplementary Figure 11). We do not recommend to use “probable or suspect FCD” as diagnostic terms.

2. Focal Cortical Dysplasia Type II

Definition: Focal Cortical Dysplasia Type II is a malformation presenting with disrupted cortical lamination and specific cytological abnormalities, which differentiates FCD Type IIa (dysmorphic neurons without balloon cells) from FCD Type IIb (dysmorphic neurons and balloon cells).

2.1. Focal Cortical Dysplasia with dysmorphic neurons (FCD Type IIa)

2.1.1. Histopathological Findings—The hallmark of this FCD variant is the presence of dysmorphic neurons, which present with a significantly enlarged cell body and nucleus, malorientation, abnormally distributed intracellular Nissl substance and cytoplasmic accumulation of neurofilament proteins. There are no balloon cells present (to be confirmed by immunohistochemistry). Discrimination of individual cortical layers is almost impossible (with the exception of Layer 1). Other cortical layer abnormalities are frequently encountered and should not be separately classified, including abnormal isocortical layer organization adjacent to the main lesion, as well as heterotopic neurons in Layer 1 or white matter.

2.1.1.1. Dysmorphic neurons (Figure 3E/F) were first described by Crome (Crome 1957) and Taylor (Taylor et al. 1971). Dysmorphic neurons are exclusively characterized by the following set of severe cytological abnormalities: (1) Neuronal cell diameters are significantly enlarged, ranging from 16-43μm compared to 12-25μm in normal appearing pyramidal neurons in Layer 3; (2) The cell nucleus diameter is also significantly enlarged, ranging from 15-28μm compared to 10-18 μm in normal pyramidal cells in Layer 3; (3) Nissl substance is aggregated and displaced towards the cell membrane; (4) Phosphorylated (antibody 2F11) and nonphosphorylated neurofilament isoforms (SMI-32) accumulate in their cytoplasm (Figure 3E). Cell shape is not a defining hallmark of this peculiar cell type, as they can present with pyramidal or interneuronal phenotpyes. Dysmorphic neurons can be distributed throughout the entire cortical thickness or locate within the white matter. The demarcation from FCD Type IIa towards adjacent normal appearing neocortex is variably ranging from a “sharp” to “smooth” transition. In the latter examples, isolated dysmorphic neurons can be identified distant from the core of the main lesion. In addition, multiple FCD Type II lesions have been recognized individually contributing to seizure generation (Fauser et al. 2009).

2.1.1.2. Cortical dyslamination (Figure 3D) is always present. It differs from that described for FCD Type I, in which individual cortical layers are obscured or cortical thickness may be decreased. In FCD Type II, there is no identifiable cortical layering except Layer 1. Whether cortical thickness is normal or increased remains to be clarified but likely not be changed significantly (Andres et al. 2005, Chandra et al. 2007). One obvious difficulty is, however, to delineate the precise border between cortex and white matter. In addition, thickness measurements need always to be performed at the "center of lesion" rather than being randomly selected.

2.1.1.3. Junction at gray/white matter is usually blurred with increased heterotopic neurons in white matter. These neurons may also be dysmorphic. The precise border between cortex and white matter is usually difficult to delineate.

2.2. Focal Cortical Dysplasia with dysmorphic neurons and balloon cells (FCD Type IIb)

2.2.1. Histopathological Findings—The hallmark of this FCD variant is the presence of dysmorphic neurons (significantly enlarged with accumulation of neurofilament proteins) and balloon cells (Sisodiya et al. 2009). Cortical lamination is frequently disrupted with the exception of Layer 1 (Figure 4E). The myelin content may also be altered in underlying subcortical white matter. Other cortical layer abnormalities are frequently encountered and should not be separately classified, including abnormal isocortical layer organization adjacent to the main lesion, as well as heterotopic neurons in Layer 1 or white matter. Histopathologically similar lesions are observed in cortical tubers and other gross MCDs, i.e. hemimegalencephaly or schizencephaly.

2.2.1.1. Balloon Cells present with a large cell body and opalescent glassy eosinophilic cytoplasm (using H&E stain), which lacks Nissl substance (Figure 4H). Multiple nuclei are often present and small nuclei may be joined by nuclear "bridges". Balloon cells can occur at any cortical location (including Layer 1) and are often found in the underlying white matter. Balloon cells may gather in small aggregates but can also be found displaced within adjacent "normal" brain tissue. Balloon cells commonly accumulate intermediate filaments vimentin and nestin (Garbelli et al. 1999, Urbach et al. 2002). They have variable GFAP and neurofilament staining patterns. In rare examples, co-expression of both markers was reported suggesting glial and neuronal lineage determination, i.e. intermediate cells (Talos et al. 2008). Balloon cells may also express the GFAP-delta variant (Martinian et al. 2009), or other stem cell markers, i.e., SOX2, CD133, beta-1 integrins or the onco-fetal antigen CD34 (Fauser et al. 2004, Yasin et al. 2010). Balloon cells have gross histomorphological similarities with giant cells [according to NIH Consensus Meeting in 2000; (Hyman and Whittemore 2000)], and which can be observed in cortical tubers from patients with Tuberous Sclerosis Complex. Despite the similarities between both cell types, which may not be distinguishable by routine histomorphological work-up, we will refer to the term "balloon cell" in our classification to specify this cell population in FCD Type IIb.

2.2.1.2. Dysmorphic neurons. There is no obvious cytological difference between dysmorphic neurons observed in FCD Type IIa or IIb (Figures 3F vs. 4F).

2.2.1.3 Intermediate cells. *In vitro* electrophysiological recordings as well as immunohistochemical analysis showed a broad spectrum of abnormal membrane properties and phenotypic specifications in cells obtained from surgical FCD Type IIb lesions. Whereas balloon cells mostly presented with glial-like features, dysmorphic neurons (pyramidal-like or interneuronal-like variants) revealed atypical hyperexcitable intrinsic membrane properties (Andre et al. 2007, Cepeda et al. 2006). Hence, there is a rare cell type, which shares glial and neuronal features and which may be defined as "intermediate-like" cells, as already shown in TSC (Talos et al. 2008).

2.2.1.4. Cortical dyslamination. Like in Type IIa, cortical dyslamination is a hallmark of FCD Type IIb and the border towards Layer 1 often remains visible (Figure 4E).

2.2.1.5. Borders between gray and white matter. The boundary between gray and white matter is always blurred in FCD Type IIb.

2.2.1.6. Altered myelin content in white matter. There is usually a reduction of myelin staining in the underlying white matter, which can be histochemically verified using Luxol-Fast-Blue or similar appropriate staining protocols during routine neuropathological work-up of surgical specimens (Figure 4D). However, there is yet no published data available clarifying the origin of reduced myelin content nor suggesting significant differences between FCD subgroups.

2.3. Imaging

FCD Type IIb is often characterized by hypo-, de-, or dysmyelination in the subcortical white matter. On T1WI, such changes cause blurring of the gray-white matter junction and mimic increased cortical thickness (Colombo et al. 2009). Increased subcortical white matter signal is visible on T2WI and T2 FLAIR images (Figure 4A). However, the cortex can be seen to have normal thickness on T2WI. The white matter signal alterations frequently taper from the crown of a gyrus or bottom of a sulcus towards the ventricle, reflecting the involvement of radial glialneuronal units. This “transmantle sign”, first described by Barkovich in 1997, is almost exclusively found in FCD Type IIb, but its detection largely depends on an optimized angle and thin MRI sectioning (Barkovich et al. 1997). Blurring between cortex and white matter on T1WI and T2 FLAIR images is often more pronounced than in FCD Type I. Frequently, the border appears sharp on T2WI. Abnormal cortical gyration and sulcation, better evaluated on 3D surface rendering, are frequent findings in FCD Type IIb and sometimes focal enlargement of the subarachnoid spaces seems to point to the dysplastic lesion, assisting in the diagnosis. In contrast, FCD Type IIa is not always detected on in vivo MRI and is harder to identify than FCD Type IIb.

2.4. Clinical and Electrophysiological findings

Data suggest that individuals with FCD Type II coming to surgery have a younger age of seizure onset, shorter epilepsy duration and increased seizure frequency compared to FCD Type I (Palmini et al 2004, Fauser et al 2006, Lerner et al 2009), although not consistently (Kresk et al 2009); these factors will also be influenced by the extent of the lesion. Seizure presentation itself will be age and location related. There is a characteristic interictal intralesional electrical activity detectable in FCD Type IIb. Intracerebral recordings (Stereo-EEG) are usually characterized by total absence of background activity and a distinctive pattern of repetitive, high amplitude, fast spikes, followed by high amplitude slow waves, interspersed with relatively flat periods. Repetitive bursts of low-amplitude high frequency oscillations interspersed with flat periods can also be seen. Similar patterns can be obtained from subdural and epidural (sometimes also by surface) EEG recordings. During drowsiness and slow sleep, fast spikes become more prominent, increase in frequency and tend to spread into contiguous non-lesional areas (Nobili et al. 2009). During REM sleep, there is a marked decrease in electrical abnormalities.

2.5. Perspectives

A yet unresolved issue addresses the clinical differentiation between FCD Type IIa and IIb, either with respect to history, seizure presentation, electrophysiological findings, MRI features, or surgical procedures and postsurgical seizure control after complete lesionectomy. If no such differences can be identified in the future, the distinction between both variants will need careful reconsideration. Yet, the histopathological distinction

between FCD Type IIa and IIb may be problematic, if non-representative or small surgical specimens were submitted for microscopic inspection. New biomarkers including imaging, immunohistochemical stainings, or genetic profiling may be helpful to resolve this obstacle.

Abnormal cortical lamination will be detectable in the vicinity of both FCD Type II variants. We are presently considering this association as a part of FCD Type II and not as a separate FCD Type III subtype, although we cannot exclude a specific role in epileptogenesis. In rare cases, FCD Type IIa or IIb will occur with other principle lesions, i.e., hippocampal sclerosis, cavernomas or tumors. We want to explicitly state, that this association is classified as "Dual" or "Double" pathology (see supplementary material on terminology issues) but not as FCD Type III variant.

3. Focal Cortical Dysplasia Type III

Definition: Focal Cortical Dysplasia Type III refers to cortical lamination abnormalities associated with a principal lesion, usually adjacent to or affecting the same cortical area/lobe. Four variants should be distinguished: FCD Type IIIa associated with hippocampal sclerosis; FCD Type IIIb associated with tumors; FCD Type IIIc associated with vascular malformations and FCD Type IIId associated with any other principal lesion acquired during early life.

3.1 Focal Cortical Dysplasia associated with hippocampal sclerosis (FCD Type IIIa)

3.1.1. Histopathological Findings—In this variant the temporal cortex shows alterations in architectural organisation (cortical dyslamination) or cytoarchitectural composition (hypertrophic neurons outside Layer 5) in patients with hippocampal sclerosis (HS, syn. Ammon's horn sclerosis). The etiology and pathogenesis of FCD Type IIIa remains to be determined, but is likely related to the pathogenesis or effect of HS. Note that we do not consider HS with FCD Type IIIa as "Dual Pathology" (see supplement).

The following patterns should be recognized as FCD Type IIIa variants:

3.1.1.1. HS with architectural abnormalities in the temporal lobe, i.e. loss of Layers 2 or 4. This category also includes the occurrence of hypertrophic neurons outside Layer 5, which still share a pyramidal morphology and accumulate phosphorylated neurofilaments. This histopathological variant may not be very different from isolated FCD Type I

3.1.1.2. HS with temporal lobe sclerosis (TLS) (Thom et al. 2009)

3.1.1.3. HS with TLS **AND** heterotopic neurons in subcortical white matter

3.1.1.4. HS with TLS **AND** small "lentiform" heterotopias in subcortical white matter

3.1.1.5. HS with small "lentiform" heterotopias in subcortical white matter

The following patterns should not be included as FCD Type IIIa variants:

- **A.** Neuronal cell loss confined to hippocampus, amygdala or entorhinal cortex, i.e. mesial temporal sclerosis (MTS)
- **B.** HS with heterotopic neurons in the deep white matter of temporal lobe, but no other architectural alteration. Neuronal heterotopia includes also blurring of gray/white matter junction. The pathogenic and epileptogenic significance of this frequent finding has yet to be clarified.

C. HS and any other principal lesion in the temporal lobe, i.e. tumors, FCD Type IIa/IIb, vascular malformations, glial scars or MCDs (other than FCD Type IIIa) should be classified as "Dual Pathology".

3.1.2. Temporal Lobe Sclerosis—In HS patients, an abnormal band of small and clustered "granular" neurons can be observed in the outer part of Layer 2 in approx. 10% of temporal lobe surgical specimens, designated as Temporal Lobe Sclerosis (Garbelli et al. 2006, Thom et al. 2009). TLS is likely to present severe neuronal cell loss in Layers 2 and 3 with associated laminar gliosis (GFAP-positive astrogliosis; Figure 5C) and cortical re-organisation. Horizontal bundles of myelinated axons can be observed to a variable degree in these cases using H&E-Luxol-Fast-Blue stainings. In 40% of HS/TLS cases more severe involvement of the temporal pole is seen, whereas extensive involvement throughout the temporal lobe occurs in 20%. There is no correlation between this FCD variant and MRI findings in these patients.

3.1.3. Small "lentiform" heterotopias or heterotopic neurons in white matter—In HS patients, small "lentiform" nodular heterotopias can be identified within the temporal lobe (Figure 6E/F). They usually remain undetected by MRI (Meroni et al. 2009). Radial orientation along the gray/white matter junction is characteristic and cellular composition is usually formed by projecting neurons. These small "lentiform" heterotopias are distinct from the larger nodular heterotopias, which are readily identified by MRI, may be present in any location of the white matter and are histologically characterized by projecting and local circuit neurons (Meroni et al. 2009). A diagnostic pitfall results from a similar normal anatomical structure located within the depth of the temporal lobe close to the claustrum.

Another frequent alteration presents with isolated heterotopic neurons either at **(1)** the gray/white matter junction (Figure 6 C) or **(2)** in deep subcortical white matter location (Figure 6 D). Both findings are very often encountered in surgical specimens obtained from epilepsy patients, although its pathogenic or epileptogenic significance remains undetermined.

3.1.4. Hypertrophic neurons—In temporal lobe specimens obtained from HS patients, hypertrophic neurons accumulating phosphorylated or non-phosphorylated neurofilament proteins can be observed in Layers 2, 3 or 4 (see Appendix and Figure 10). In normal "control" human cortex, these pyramidal neurons are usually allocated to Layer 5. Please note, that staining intensities with non-phosphorpylated NFP antibodies (i.e. SMI 32) increase with age and that neuronal hypertrophy can be observed also in non-epilepsy related pathologies.

3.2 Focal Cortical Dysplasia associated with tumors (FCD Type IIIb)

3.2.1 Histopathological Findings—The histopathological hallmark of this new FCD variant is an altered architectural (cortical dyslamination, hypoplasia without six-layered structure) and/or cytoarchitectural composition (hypertrophic neurons) of the neocortex, which occur adjacent to tumors (Ganglioglioma, Dysembryoplastic Neuroepithelial Tumor (DNT, syn. DNET) or other epilepsy-associated neoplasms [for review see (Blumcke 2009)]. It is important to exclude tumor infiltration in areas of cortical abnormalities before establishing the diagnosis of FCD. The etiology and pathogenesis of FCD Type IIIb remains to be determined, but is likely an acquired process. It should not be considered, therefore, as "Double Pathology" (see supplement).

Histopathologically, cortical architecture may be severely disturbed (infiltration by tumor cells need to be excluded) with a small cortical ribbon (hypoplasia) and effacement of six-layered organization (Figure 7B). We cannot exclude, that the compromised cortical

architecture results from an acquired dysplasia secondary to the development of the principal lesion. Notwithstanding, seizure activity may arise from altered networks in this affected cortical area.

3.3 Focal Cortical Dysplasia associated with vascular malformations (FCD Type IIIc)

3.3.1. Histopathological findings—Alterations in architectural (cortical dyslamination, hypoplasia) or cytoarchitectural composition of the neocortex (hypertrophic neurons), which occur adjacent to vascular malformations (cavernomas, arteriovenous malformations, leptomeningeal vascular malformations, telangiectasias, meningioangiomatosis). The etiology and pathogenesis of FCD Type IIIc remains to be determined, but is likely an acquired process. It should not be considered, therefore, as "Double Pathology".

The histopathologic pattern is similar to that described for other FCD Type III variants, and can be identified adjacent to the principal lesion. Cortical architecture may be severely disturbed (Figure 8B). However, we cannot exclude the possibility, that the compromised cortical architecture is acquired secondary to the development of the principal lesion, but seizure activity may arise from altered networks in this affected cortical area (Ferrier et al. 2007).

FCDs may be associated with abnormal sulcation and are drained by a single, large vein. This might be interpreted as a venous angioma on MRI scans. If the suspected angioma cannot be verified by histopathological examination, the FCD likely occurs as "isolated" lesion and should be classified as FCD Type I or Type II variant, respectively.

3.4 Focal Cortical Dysplasia associated with other lesions acquired during early life (FCD Type IIId)

3.4.1 Histopathological findings—The histopathological hallmark of this new FCD variant is an altered architectural (cortical dyslamination, hypoplasia without six-layered structure) or cytoarchitectural composition (hypertrophic neurons) of the neocortex, which occur adjacent to other lesions acquired during early life (not included into FCD Type IIIa-c). These lesions comprise a large spectrum including traumatic brain injury (Lombroso 2000, Marin-Padilla et al. 2002), glial scarring after prenatal or perinatal ischemic injury or bleeding (Figure 9), and inflammatory or infectious diseases, i.e. Rasmussen encephalitis, limbic encephalitis, bacterial or viral infections.

3.5 Focal Cortical Dysplasia associated with clinically suspected principal lesion, but lesion not available for histopathological examination (FCD Type III, not otherwise specified, NOS)

If FCD Type I patterns are histopathologically detected in a patient with a clinically suspected principal lesion, but **(1)** the principal lesion is not available for microscopic inspection (entire sample should be embedded and sectioned for microscopic inspection), or **(2)** tissue may not be available for microscopic analysis after the surgical procedure, the neuropathological diagnosis of FCD Type III (NOS) should be considered (supplementary Figure 11).

3.6. Clinical and electrophysiological presentation in FCD Type III

The cohort of HS patients with FCD Type IIIa has not yet been characterized with respect to clinical presentations and electroclinical findings. Previous studies have described some of these patients, with respect to the generation of ictal and interictal activity (Chabardes et al. 2005, Maillard et al. 2004). Another study (Fauser and Schulze-Bonhage 2006) specifically correlated ictal onset patterns in temporal lobe epilepsy patients with respective

histopathology, i.e. HS and FCD Type I (according to Palmini's classification system). Approx. 40% of seizures arise from the amygdala/hippocampus complex, 35% from the temporal neocortex (including the cortical dysplasia), 22% were simultaneously recorded from both sites, and 2% from other regions. The interictal patterns obtained from FCDs in the temporal regions were similar to those seen over extratemporal areas. This study showed, that dysplastic tissue in the temporal neocortex is often epileptogenic (Fauser and Schulze-Bonhage 2006).

3.7. Perspectives

Meta-analysis of studies addressing the clinical presentation and histopathological alterations in patients with FCD Type I (according to previous Palmini classification) demonstrate a very large variability (Blumcke et al. 2009). Postsurgical outcome was also less comparable between studies, with a broad range of Engel 1 seizure control in 21 to 67% of operated patients. One obstacle is that different cohorts were included, i.e., children vs. adults, isolated vs. HS-associated FCD variants. The data suggest, therefore, that different clinico-pathological entities were encountered within the Palmini classification of FCD Type I. The major objective of the proposed new FCD classification system is to separate these different entities (FCD Type I vs. Type III). The most reliable strategy to classify these subtypes is a histopathological distinction between isolated and associated FCD subtypes (Spreafico and Blumcke 2010). Yet, the histopathological distinction between isolated and associated FCD variants remain problematic, if non-representative or small surgical specimens were submitted for microscopic inspection. The development of new and reliable biomarkers will be helpful to resolve this obstacle.

We also need to address the issue of whether FCD Type IIIa is an acquired pathology with accompanying reorganizational dysplasia resulting from hippocampal sclerosis, rather than being a distinct pathological entity. The latter would favor the hypothesis that HS is the consequence of chronic epileptogenicity of the temporal lobe due to the dysplasia. Several aspects argue for a common etiology between HS and FCD Type IIIa. Patients from both groups have a similar age at onset and a similar history of febrile seizures as an initial precipitating injury (Marusic et al. 2007); no other clinical differences have yet been identified between isolated HS and HS/FCD Type IIIa cases (Thom et al. 2009). Accordingly, postsurgical outcome is similar in patients with HS only and with FCD Type IIIa (Tassi et al. 2010). Notwithstanding, a standardized histopathological evaluation of HS patterns also needs to be established by an international consensus. Atypical HS variants should be histopathologically identified, i.e. predominant pyramidal cell loss in only CA4 or CA1 regions, as they may associate with a less favorable postsurgical outcome (Blumcke et al. 2007) or may account for the different types of temporal lobe seizures in TLE patients (Kahane and Bartolomei 2010).

Re-organisation of the cortical cytoarchitecture can be observed adjacent to a destructive cortical pathology including an infarct, chronic encephalitis, traumatic brain injury or vascular malformation (Hart et al. 1998, Kremer et al. 2002, Marin-Padilla et al. 2002). It is likely to be a reflection of the ongoing plasticity and response to injury of the maturing as well as adult cortex. If acquired in the early years there are likely to be additional abnormalities of the myeloarchitecture. Such acquired dysplasias should be distinguished from primary dysplasias. We believe that the term “progressive cortical dysplasia” (Marin-Padilla et al. 2002) may be misleading and should be replaced by FCD Type IIId.

Architectural or cytoarchitectural disorganization can always be identified in the vicinity of gross malformations of cortical development, i.e. with polymicrogyria, hemimegalencephaly, schizencephaly, nodular heterotopia, or cortical tubers. We, therefore, suggest not including these abundant architectural and/or cytoarchitectural disorganization

patterns as specific FCD Type III variants until studies show that the presence of such dysplasias relate to divergent clinical outcome.

In rare cases, FCD Type IIa or IIb will occur with other principle lesions, i.e., cavernomas or tumors. We want to explicitly state, that this association is classified as “Double” pathology (see supplementary material on terminology issues) but not FCD Type III variant, as both lesions have most likely an independent pathogenesis. The same applies for the rare association between FCD Type IIa/IIb with hippocampal sclerosis which should be classified as “Dual Pathology”, although this important issue will need further scientific elaboration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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8. Appendix

8.1 Glossary of abnormal cell types

Dysmorphic neurons: essential component of FCD Types IIa and IIb. Their soma and nuclei are abnormally large. They are disoriented in the cortex with abnormal aggregates of Nissl substance and phosphorylated or non-phosphorylated neurofilament accumulation in cytoplasm. They mostly represent altered pyramidal neurons but can also show features consistent with those of interneurons.

Hypertrophic neurons: resemble large pyramidal cells of Layer 5 abnormally located in Layers 1, 2, 3 or 4. Dendrites’ orientation and arborisation may be altered, but there is no obvious intracellular pathology affecting the nucleus or Nissl substance.

***Immature neu*ons:** develop from neuroblasts and have a small diameter and cell size (< 250 mm²). They do not accumulate non-phosphorylated neurofilaments. They are observed in large numbers in vertically oriented microcolumns (FCD Type Ia).

Dysplastic neurons are the neuronal components of glio-neuronal tumors, i.e. Gangliogliomas and Dysembryoplastic Neuroepithelial Tumor.

Balloon cells have a large cell body with opaque eosinophilic cytoplasm which lacks Nissl substance on Haematoxylin & Eosin stains. They rarely express cytoplasmic/ immunohistochemical differentiation with glial (GFAP) or neuronal markers (NFP). Multiple nuclei can be seen.

8.2 Glossary of Terminology

In order to avoid the confusion that has been created by various usages of descriptive and diagnostic terms pertaining to malformations of cortical development, we utilize in this revised classification system the following definitions:

Dysplasia (synonymous with Dysgenesis and Malformation): this is a general term referring to any tissue that is imperfectly developed in embryonic or fetal life. However, Dysplasia is a diagnostic term used here to identify specific malformations of the cortex, the so-called Focal Cortical Dysplasias (FCDs) irrespective of their diverse histological appearances which are addressed by this classification system.

Heterotopia: misplaced tissue or cells within their normal organ of origin.

Hamartoma: is a tumor-like non-neoplastic mass (> 1mm) of malformed tissue (Wolf and Wiestler 1993), composed of normal cells in their normal site which exhibit disorganized architecture. A **hamartia** is a small glio-neuronal lesion which is not grossly visible (< 1mm).

Ectopia: is a normally formed organ or tissue in an abnormal site within the body. We do not refer to this definition in our classification system.

Dyslamination is a compromized tangential or radial organization of cortical architecture. It may be observed in any of the proposed FCD subtypes.

Dual Pathology is not yet comprehensively defined (Cendes et al. 1995), and is still ambiguously used in clinical and histopathological practice. We propose the following definition: Dual Pathology refers only to patients with hippocampal sclerosis, who have a second principal lesion affecting the brain (which may be located also outside the ipsilateral temporal lobe), i.e. tumor, vascular malformation, glial scar, limbic/Rasmussen encephalitis, or MCD (including FCD Type IIa/IIb). Ipsilateral temporo-polar atrophy with increased T2 signal changes on MRI is not included as its histopathological correlate has yet to be specified. Of note, histopathologically confirmed architectural abnormalities in the temporal lobe associated with HS should not be diagnosed as FCD Type I or “Dual Pathology” but FCD Type IIIa.

Double Pathology refers to two independent lesions affecting one or multiple lobes, but not including hippocampal sclerosis. This definition assumes that both lesions evolve from an independent pathogenesis, i.e. a cavernoma in one cerebral hemisphere and a ganglioglioma in the other. Electrophysiology will be necessary to characterize the “most likely” epileptogenic lesion.

Principal Lesions comprise any anatomical lesion with etiologically defined pathogenesis of either neoplastic, genetic, infectious, traumatic or metabolic origin. This includes the spectrum of epilepsy-associated tumors, vascular malformations, MCDs, encephalitis, traumatic scars, bleeding, vascular infarction, mitochondrial/metabolic dysfunction and genetic syndromes.

7. References

Andre VM, Wu N, Yamazaki I, Nguyen ST, Fisher RS, Vinters HV, Mathern GW, Levine MS, Cepeda C. Cytomegalic interneurons: a new abnormal cell type in severe pediatric cortical dysplasia. J Neuropathol Exp Neurol. 2007; 66:491–504. [PubMed: 17549009]

Andres M, Andre VM, Nguyen S, Salamon N, Cepeda C, Levine MS, Leite JP, Neder L, Vinters HV, Mathern GW. Human Cortical Dysplasia and Epilepsy: An Ontogenetic Hypothesis Based on Volumetric MRI and NeuN Neuronal Density and Size Measurements. Cereb Cortex. 2005; 15:194–210. [PubMed: 15297365]

Barkovich AJ, Kjos BO, Jackson DE Jr. Norman D. Normal maturation of the neonatal and infant brain: MR imaging at 1.5 T. Radiology. 1988; 166:173–180. [PubMed: 3336675]

Barkovich AJ, Kuzniecky RI, Bollen AW, Grant PE. Focal transmantle dysplasia: a specific malformation of cortical development. Neurology. 1997; 49:1148–1152. [PubMed: 9339707]

Barkovich AJ, Kuzniecky RI, Jackson GD, Guerrini R, Dobyns WB. A developmental and genetic classification for malformations of cortical development. Neurology. 2005; 65:1873–1887. [PubMed: 16192428]

Blumcke I. Neuropathology of focal epilepsies: a critical review. Epilepsy Behav. 2009; 15:34–39. [PubMed: 19248840]

Blumcke I, Giencke K, Wardelmann E, Beyenburg S, Kral T, Sarioglu N, Pietsch T, Wolf HK, Schramm J, Elger CE, Wiestler OD. The CD34 epitope is expressed in neoplastic and malformative lesions associated with chronic, focal epilepsies. Acta Neuropathol. 1999; 97:481–490. [PubMed: 10334485]

Blumcke I, Müller S, Buslei R, Riederer BM, Wiestler OD. Microtubule-associated protein-2 immunoreactivity: a useful tool in the differential diagnosis of low-grade neuroepithelial tumors. Acta Neuropathol (Berl). 2004; 108:89–96. [PubMed: 15146346]

Blumcke I, Pauli E, Clusmann H, Schramm J, Becker A, Elger C, Merschhemke M, Meencke HJ, Lehmann T, von Deimling A, Scheiwe C, Zentner J, Volk B, Romstock J, Stefan H, Hildebrandt M. A new clinico-pathological classification system for mesial temporal sclerosis. Acta Neuropathol. 2007; 113:235–244. [PubMed: 17221203]

Blumcke I, Pieper T, Pauli E, Hildebrandt M, Kudernatsch M, Winkler P, Karlmeier A, Holthausen H. Magnetic resonance imaging and neuropathological examination characterize a distinct variant of Focal Cortical Dysplasia Type I in children with severe epilepsies. Epileptic Dis. 2010 epub ahead of print.

Blumcke I, Vinters HV, Armstrong D, Aronica E, Thom M, Spreafico R. Malformations of cortical development and epilepsies. Epileptic Disord. 2009; 11:181–193. [PubMed: 19736171]

Cendes F, Cook MJ, Watson C, Andermann F, Fish DR, Shorvon SD, Bergin P, Free S, Dubeau F, Arnold DL. Frequency and characteristics of dual pathology in patients with lesional epilepsy. Neurology. 1995; 45:2058–2064. [PubMed: 7501159]

Cepeda C, Andre VM, Levine MS, Salanion N, Miyata H, Vinters HV, Mathern GW. Epileptogenesis in pediatric cortical dysplasia: The dysmature cerebral developmental hypothesis. Epilepsy & Behavior. 2006; 9:219–235. [PubMed: 16875879]

Chabardes S, Kahane P, Minotti L, Tassi L, Grand S, Hoffmann D, Benabid AL. The temporopolar cortex plays a pivotal role in temporal lobe seizures. Brain. 2005; 128:1818–1831. [PubMed: 15857932]

Chamberlain WA, Cohen ML, Gyure KA, Kleinschmidt-Demasters BK, Perry A, Powell SZ, Qian J, Staugaitis SM, Prayson RA. Interobserver and intraobserver reproducibility in focal cortical dysplasia (malformations of cortical development). Epilepsia. 2009; 50:2593–2598. [PubMed: 19817804]

Chandra PS, Salamon N, Nguyen ST, Chang JW, Huynh MN, Cepeda C, Leite JP, Neder L, Koh S, Vinters HV, Mathern GW. Infantile spasm-associated microencephaly in tuberous sclerosis complex and cortical dysplasia. Neurology. 2007; 68:438–445. [PubMed: 17283320]

Chassoux F, Devaux B, Landre E, Turak B, Nataf F, Varlet P, Chodkiewicz JP, Daumas-Duport C. Stereoelectroencephalography in focal cortical dysplasia: a 3D approach to delineating the dysplastic cortex. Brain. 2000; 123(Pt 8):1733–1751. [PubMed: 10908202]

Colombo N, Salamon N, Raybaud C, Ozkara C, Barkovich AJ. Imaging of malformations of cortical development. Epileptic Disord. 2009; 11:194–205. [PubMed: 19720583]

Crome L. Infantile cerebral gliosis with giant nerve cells. J Neurol Neurosurg Psychiatry. 1957; 20:117–124. [PubMed: 13429380]

Ding SL, Van Hoesen GW, Cassell MD, Poremba A. Parcellation of human temporal polar cortex: a combined analysis of multiple cytoarchitectonic, chemoarchitectonic, and pathological markers. J Comp Neurol. 2009; 514:595–623. [PubMed: 19363802]

Emery JA, Roper SN, Rojiani AM. White matter neuronal heterotopia in temporal lobe epilepsy: a morphometric and immunohistochemical study. J Neuropathol Exp Neurol. 1997; 56:1276–1282. [PubMed: 9413276]

Fauser S, Becker A, Schulze-Bonhage A, Hildebrandt M, Tuxhorn I, Pannek HW, Lahl R, Schramm J, Blumcke I. CD34-immunoreactive balloon cells in cortical malformations. Acta Neuropathol (Berl). 2004; 108:272–278. [PubMed: 15221338]

Fauser S, Schulze-Bonhage A. Epileptogenicity of cortical dysplasia in temporal lobe dual pathology: an electrophysiological study with invasive recordings. Brain. 2006; 129:82–95. [PubMed: 16317023]

Fauser S, Sisodiya SM, Martinian L, Thom M, Gumbinger C, Huppertz HJ, Hader C, Strobl K, Steinhoff BJ, Prinz M, Zentner J, Schulze-Bonhage A. Multi-focal occurrence of cortical dysplasia in epilepsy patients. Brain. 2009; 132:2079–2090. [PubMed: 19506069]

Ferrier CH, Aronica E, Leijten FS, Spliet WG, Boer K, van Rijen PC, van Huffelen AC. Electrocorticography discharge patterns in patients with a cavernous hemangioma and pharmacoresistent epilepsy. J Neurosurg. 2007; 107:495–503. [PubMed: 17886546]

Gambardella A, Palmini A, Andermann F, Dubeau F, Da Costa JC, Quesney LF, Andermann E, Olivier A. Usefulness of focal rhythmic discharges on scalp EEG of patients with focal cortical dysplasia and intractable epilepsy. Electroencephalogr Clin Neurophysiol. 1996; 98:243–249. [PubMed: 8641147]

Garbelli R, Meroni A, Magnaghi G, Beolchi MS, Ferrario A, Tassi L, Bramerio M, Spreafico R. Architectural (Type IA) focal cortical dysplasia and parvalbumin immunostaining in temporal lobe epilepsy. Epilepsia. 2006; 47:1074–1078. [PubMed: 16822257]

Garbelli R, Munari C, De Biasi S, Vitellaro-Zuccarello L, Galli C, Bramerio M, Mai R, Battaglia G, Spreafico R. Taylor's cortical dysplasia: a confocal and ultrastructural immunohistochemical study. Brain Pathol. 1999; 9:445–461. [PubMed: 10416985]

Hart YM, Andermann F, Robitaille Y, Laxer KD, Rasmussen T, Davis R. Double pathology in Rasmussen's syndrome: a window on the etiology? Neurology. 1998; 50:731–735. [PubMed: 9521265]

Hildebrandt M, Pieper T, Winkler P, Kolodziejczyk D, Holthausen H, Blumcke I. Neuropathological spectrum of cortical dysplasia in children with severe focal epilepsies. Acta Neuropathol. 2005; 110:1–11. [PubMed: 15965699]

Hyman MH, Whittemore VH. National Institutes of Health consensus conference: tuberous sclerosis complex. Arch Neurol. 2000; 57:662–665. [PubMed: 10815131]

Kahane P, Bartolomei F. Temporal lobe epilepsy and hippocampal sclerosis: lessons from depth EEG recordings. Epilepsia. 2010; 51(Suppl 1):59–62. [PubMed: 20331718]

Kremer S, De Saint Martin A, Minotti L, Grand S, Benabid AL, Pasquier B, Kahane P. Focal cortical dysplasia possibly related to a probable prenatal ischemic injury. J Neuroradiol. 2002; 29:200–203. [PubMed: 12447145]

Krsek P, Maton B, Korman B, Pacheco-Jacome E, Jayakar P, Dunoyer C, Rey G, Morrison G, Ragheb J, Vinters HV, Resnick T, Duchowny M. Different features of histopathological subtypes of pediatric focal cortical dysplasia. Ann Neurol. 2008; 63:758–769. [PubMed: 18571798]

Lerner JT, Salamon N, Hauptman JS, Velasco TR, Hemb M, Wu JY, Sankar R, Donald Shields W, Engel J Jr. Fried I, Cepeda C, Andre VM, Levine MS, Miyata H, Yong WH, Vinters HV, Mathern GW. Assessment and surgical outcomes for mild type I and severe type II cortical dysplasia: a critical review and the UCLA experience. Epilepsia. 2009; 50:1310–1335. [PubMed: 19175385]

Lombroso CT. Can early postnatal closed head injury induce cortical dysplasia. Epilepsia. 2000; 41:245–253. [PubMed: 10691125]

Maillard L, Vignal JP, Gavaret M, Guye M, Biraben A, McGonigal A, Chauvel P, Bartolomei F. Semiologic and electrophysiologic correlations in temporal lobe seizure subtypes. Epilepsia. 2004; 45:1590–1599. [PubMed: 15571517]

Marin-Padilla M, Parisi JE, Armstrong DL, Sargent SK, Kaplan JA. Shaken infant syndrome: developmental neuropathology, progressive cortical dysplasia, and epilepsy. Acta Neuropathol (Berl). 2002; 103:321–332. [PubMed: 11904751]

Martinian L, Boer K, Middeldorp J, Hol EM, Sisodiya SM, Squier W, Aronica E, Thom M. Expression patterns of glial fibrillary acidic protein (GFAP)-delta in epilepsy-associated lesional pathologies. Neuropathol Appl Neurobiol. 2009; 35:394–405. [PubMed: 19508443]

Marusic P, Tomasek M, Krsek P, Krijtova H, Zarubova J, Zamecnik J, Mohapl M, Benes V, Tichy M, Komarek V. Clinical characteristics in patients with hippocampal sclerosis with or without cortical dysplasia. Epileptic Disord. 2007; 9(Suppl 1):S75–82. [PubMed: 18319204]

Meroni A, Galli C, Bramerio M, Tassi L, Colombo N, Cossu M, Lo Russo G, Garbelli R, Spreafico R. Nodular heterotopia: a neuropathological study of 24 patients undergoing surgery for drug-resistant epilepsy. Epilepsia. 2009; 50:116–124. [PubMed: 18637832]

Mischel PS, Nguyen LP, Vinters HV. Cerebral cortical dysplasia associated with pediatric epilepsy. Review of neuropathologic features and proposal for a grading system. J Neuropathol Exp Neurol. 1995; 54:137–153. [PubMed: 7876884]

Nobili L, Cardinale F, Magliola U, Cicolin A, Didato G, Bramerio M, Fuschillo D, Spreafico R, Mai R, Sartori I, Francione S, Lo Russo G, Castana L, Tassi L, Cossu M. Taylor's focal cortical dysplasia increases the risk of sleep-related epilepsy. Epilepsia. 2009; 50:2599–2604. [PubMed: 19519797]

Palmini A, Gambardella A, Andermann F, Dubeau F, da Costa JC, Olivier A, Tampieri D, Gloor P, Quesney F, Andermann E, et al. Intrinsic epileptogenicity of human dysplastic cortex as suggested by corticography and surgical results. Ann Neurol. 1995; 37:476–487. [PubMed: 7717684]

Palmini A, Najm I, Avanzini G, Babb T, Guerrini R, Foldvary-Schaefer N, Jackson G, Luders HO, Prayson R, Spreafico R, Vinters HV. Terminology and classification of the cortical dysplasias. Neurology. 2004; 62:S2–8. [PubMed: 15037671]

Rakic P. Specification of cerebral cortical areas. Science. 1988; 241:170–176. [PubMed: 3291116]

Rojiani AM, Emery JA, Anderson KJ, Massey JK. Distribution of heterotopic neurons in normal hemispheric white matter: a morphometric analysis. J Neuropathol Exp Neurol. 1996; 55:178–183. [PubMed: 8786376]

Salamon N, Kung J, Shaw SJ, Koo J, Koh S, Wu JY, Lerner JT, Sankar R, Shields WD, Engel J Jr. Fried I, Miyata H, Yong WH, Vinters HV, Mathern GW. FDG-PET/MRI coregistration improves detection of cortical dysplasia in patients with epilepsy. Neurology. 2008; 71:1594–1601. [PubMed: 19001249]

Sisodiya SM, Fauser S, Cross JH, Thom M. Focal cortical dysplasia type II: biological features and clinical perspectives. Lancet Neurol. 2009; 8:830–843. [PubMed: 19679275]

Spreafico R, Blumcke I. Focal Cortical Dysplasias: clinical implication of neuropathological classification systems. Acta Neuropathol. 2010; 120:359–367. [PubMed: 20607544]

Talos DM, Kwiatkowski DJ, Cordero K, Black PM, Jensen FE. Cell-specific alterations of glutamate receptor expression in tuberous sclerosis complex cortical tubers. Ann Neurol. 2008; 63:454–465. [PubMed: 18350576]

Tassi L, Colombo N, Garbelli R, Francione S, Lo Russo G, Mai R, Cardinale F, Cossu M, Ferrario A, Galli C, Bramerio M, Citterio A, Spreafico R. Focal cortical dysplasia: neuropathological subtypes, EEG, neuroimaging and surgical outcome. Brain. 2002; 125:1719–1732. [PubMed: 12135964]

Tassi L, Garbelli R, Colombo N, Bramerio M, Lo Russo G, Deleo F, Milesi G, Spreafico R. Type I focal cortical dysplasia (FCD I): surgical outcome is related to histopathology. Epileptic Disord. 2010 epub ahead of print.

Taylor DC, Falconer MA, Bruton CJ, Corsellis JA. Focal dysplasia of the cerebral cortex in epilepsy. J Neurol Neurosurg Psychiatry. 1971; 34:369–387. [PubMed: 5096551]

Thom M, Eriksson S, Martinian L, Caboclo LO, McEvoy AW, Duncan JS, Sisodiya SM. Temporal Lobe Sclerosis Associated With Hippocampal Sclerosis in Temporal Lobe Epilepsy: Neuropathological Features. J Neuropathol Exp Neurol. 2009; 68:928–938. [PubMed: 19606061]

Thom M, Sisodiya S, Harkness W, Scaravilli F. Microdysgenesis in temporal lobe epilepsy. A quantitative and immunohistochemical study of white matter neurones. Brain. 2001; 124:2299–2309. [PubMed: 11673330]

Urbach H, Scheffler B, Heinrichsmeier T, von Oertzen J, Kral T, Wellmer J, Schramm J, Wiestler OD, Blumcke I. Focal cortical dysplasia of Taylor's balloon cell type: a clinicopathological entity with characteristic neuroimaging and histopathological features, and favorable postsurgical outcome. Epilepsia. 2002; 43:33–40. [PubMed: 11879384]

Wolf HK, Wiestler OD. Surgical pathology of chronic epileptic seizure disorders. Brain Pathol. 1993; 3:371–380. [PubMed: 8293193]

Yasin SA, Latak K, Becherini F, Ganapathi A, Miller K, Campos O, Picker SR, Bier N, Smith M, Thom M, Anderson G, Helen Cross J, Harkness W, Harding B, Jacques TS. Balloon cells in human cortical dysplasia and tuberous sclerosis: isolation of a pathological progenitor-like cell. Acta Neuropathol. 2010; 120:85–96. [PubMed: 20352236]

Figure 1. Histopathological findings in FCD Type Ia (abnormal radial lamination and abundant microcolumns)

11 year old girl with a 10 year history of drug-resistant seizures. **A:** Normal appearing neocortex adjacent to the lesion shown in B and C. Selective labelling of neuronal cell bodies using antibodies directed against NeuN reveals a characteristic layering of the human isocortex (L1-L6). Scale bar = 500 μm, applies also to B. **B:** Distinct microcolumnar arrangements of small diameter neurons can be detected in FCD Type Ia, when surgical specimen is cut perfectly perpendicular to the pial surface and 4 μm paraffin embedded sections were used. MRI showed smaller cortical (parieto-occipito-temporal) lobes in affected vs. non-affected hemispheres (Blumcke et al. 2010). High magnification in **C** reveals abundant microcolumns, which are composed of more than 8 neurons (arrow). In addition, Layer 4 is less clearly visible (arrowheads). Scale bar = 200 μm.

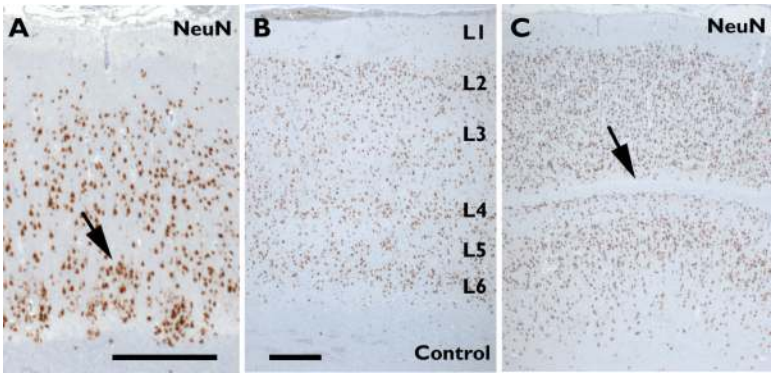

Figure 2. Histopathological findings in FCD Type Ib (abnormal tangential layer composition)

A: 3-year old girl with drug-resistant epilepsy originating from the left parieto-occipital lobe. The cortex is thin (hypoplastic) and no layering can be detected. NeuN immunoreactivity. MRI showed a smaller cortical region. Scale bar = 500 μm. **B:** NeuN immunoreactivity in a surgical case showing normal layer formation (L1-L6) with a sharp boundary between cortex and white matter (same image as Figure 1A). 4μm paraffin embedded section with haematoxylin counterstaining. Scale bar = 500μm, applies also to C. **C:** 23-year old male patient with drug-resistant focal epilepsy since birth and a hyperintense MRI signal at the parieto-occipital region. Note complete loss of Layer 4 (arrow). In addition, there is no distinction between supragranular Layers L2 and L3. The border towards the white matter is blurred.

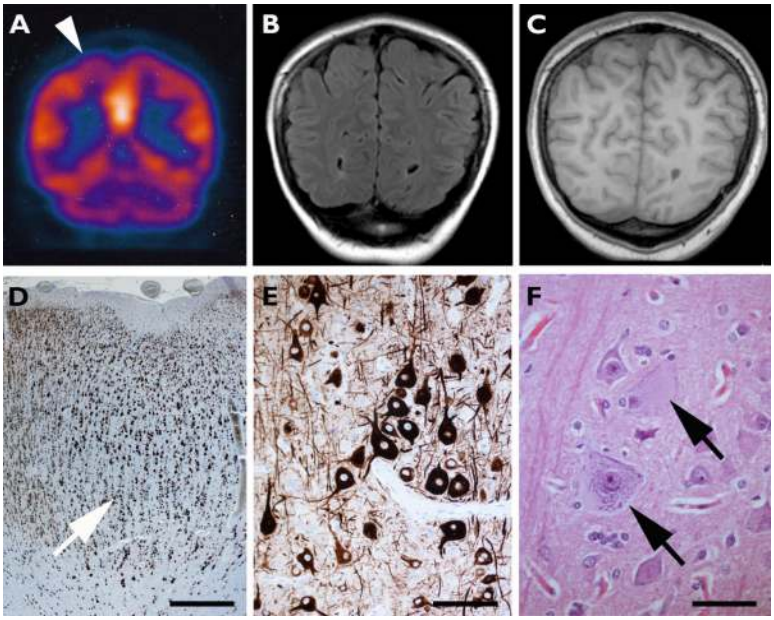

Figure 3. Imaging and histopathological findings in FCD Type IIa

FCD Type IIa in an 18 year old female patient with refractory seizures from age 5 years, that would start with sensory disturbance in the left foot. **A:** ^{18}F-FDG-PET showing an area of slight hypometabolism in the superior, medial right parietal lobe (arrowhead). **B:** Coronal T2 FLAIR did not reveal definitely abnormal signal intensities. **C:** Coronal T1-weighted MRI were also reported normal. Please note the different orientation of the planes of the PET and MR images. The MRI is oblique coronal, so that the inferior part of the image is posterior to that seen on the PET. **D:** Microscopic inspection of surgical specimen revealed severe cortical dyslamination (arrow) without distinguishable layer formation (except Layer 1). NeuN immunohistochemistry. Scale bar = 1000 μm. Section thickness = 15μm. E: Abundant dysmorphic neurons with dense accumulation of SMI32-neurofilament proteins can be identified. Scale bar = 100 μm. Section thickness = 7μm. F: High-power magnification of dysmorphic neurons (arrows) depicted from same area shown in E (H&E stain). Note their variable morphological appearance which may also result from plane of sectioning. No balloon cells can be recognized. Scale bar = 30 μm. Section thickness = 4μm.

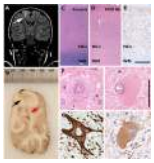

Figure 4. Imaging and histopathological findings in FCD Type IIb

A: The "transmantle-sign" in T2 FLAIR imaging is characterized by a funnel-like hyperintensity (arrow) tapering from the gyrus to the ventricle. **B:** Inspection of the surgical specimen reveals a distinct correlation between T2 FLAIR hyperintensity and lack of normal myelin content (black arrow points to greyish subcortical areas), which can be identified from the subcortical white matter to the ventricle (red arrow). **C:** H&E staining combined with Luxol-Fast blue (H&E-LFB) allows visualization of a sharp boundary between neocortex (NCX) and white matter (WM) in a control subject. **D:** H&E-LFB. In this FCD Type IIb specimen, the myelin content is significantly reduced (see also macroscopic image in B). **E:** NeuN immunohistochemistry, 4μm paraffin embedded serial section to D. Severe cortical dyslamination is visible (with the exception of Layer 1). In addition, cortical thickness is increased and not distinguishable from WM border (same magnification as C and D). Scale bar = 1 mm. **F:** In FCD Type IIb, enlarged dysmorphic neurons present with a huge nucleus and abnormal intracytoplasmic Nissl aggregates. **G:** Antibodies to non-phosphorylated neurofilament proteins (SMI32) reveal aberrant NFP accumulation in a dysmorphic neuron. **H:** Balloon cells are another hallmark of this FCD variant. Scale bar = 50 μm, applies also to F, G and I. **I:** Balloon cells express the intermediate filament vimentin. E, G and I: 4 μm paraffin embedded sections, counterstained with haematoxylin.

Figure 5. Histopathological findings in FCD Type IIIa

A: Normal cortical layering (L1-L6) observed adjacent to lesion shown in B . NeuN immunoreactivity. **B:** A characteristic finding in approx. 10% of MTLE patients is 'Temporal Lobe Slerosis' at the interface between Layers 2 and 3 (arrow) (Thom et al. 2009). In this patient, MRI signals within the temporal lobe were reported normal. Scale bar = 200 μm, applies also to A and C. **C:** There is laminar astrogliosis below temporal lobe sclerosis (arrow), indicating neuronal cell loss in Layers 2/3. GFAP immunoreactivity.

Figure 6. Histopathological findings in FCD Type IIIa (small "lentiform" heterotopia and heterotopic neurons with blurring of white matter boundary)

A: The boundary between gray and white matter is very sharp in normal appearing neocortex. **B:** Heterotopic neurons are a rare finding in normal deep white matter (Rojiani et al. 1996). **C:** Blurring of the gray-white matter boundary in a surgical temporal lobe specimen obtained from a 39 year old female patient with drug-resistant MTLE and hippocampal sclerosis. MRI signalling within the temporal lobe was reported normal. **D:** Increased numbers of heterotopic neurons can be often observed in deep subcortical white matter (Emery et al. 1997). Same patient shown in C. **E:** A rare finding is the observation of small "lentiform" heterotopias in the white matter of the temporal lobe obtained from a patient with HS. This abnormality was not reported by MRI prior to operation. **F:** Synaptophysin staining of "lentiform" heterotopias shown in E. Scale bar in B= 200 μm, applies also to A, C and D. Scale bar in F = 500μm, applies also to E. MAP2-immunoreactivity in A-D. 4μm paraffin embedded sections counterstained with haematoxylin.

Figure 7. Histopathological findings in FCD Type IIIb

A: CD34 immunoreactivity demarcated a ganglioglioma (GG). Abundant CD34 positive tumor aggregates can be identified within adjacent neocortex (arrows). This frequent infiltration pattern should not be confused with the diagnosis of FCD (no FCD). Scale bar = 1 mm. **B:** Cortical dyslamination and hypoplasia adjacent to a ganglioglioma, but not infiltrated by tumor clusters, are a hallmark of FCD Type IIIb. **C:** Normal cortical lamination adjacent to lesion shown in B at same magnification. **D:** Histopathological analysis identified a Dysembryoplastic Neuroepithelial Tumor (DNT). Tumor aggregates can be detected in close proximity to the mass lesion (arrow). H&E staining. Scale bar = 500 μm, applies also to B and C. **E:** Adjacent neocortex (NCx) revealed severely compromised cortical lamination (NeuN immunoreactivity). However, these clear cell tumor infiltrates contribute to the disrupted cortical architecture and should not be diagnosed as FCD (no FCD). Scale Bar = 200 μm. 4 μm paraffin embedded sections.

Figure 8. Histopathological findings in FCD Type IIIc associated with a vascular malformation

21 year old male patient with drug-resistant seizures and a leptomeningeal vascular malformation in the right temporo-occipital lobe. **A:** Histopathological specimen showing a vascular malformation (VM) in the subarachnoidal space. H&E staining. **B:** 4 μm paraffin embedded serial section to A. NeuN immunohistochemistry. The neocortex below the vascular malformation is atrophic and revealed severe tangential dyslamination with almost complete loss of Layers 3 and 4 (FCD Type IIIc). **C:** Adjacent to the vascular malformation and tangential dyslamination shown in B, microcolumnar (radial) dyslamination was also recognized in this patient (arrows). Arrowheads point to Layer 4, which is less clearly distinguishable. NeuN immunohistochemistry. Scale bar = 200 μm, applies also to A and B.

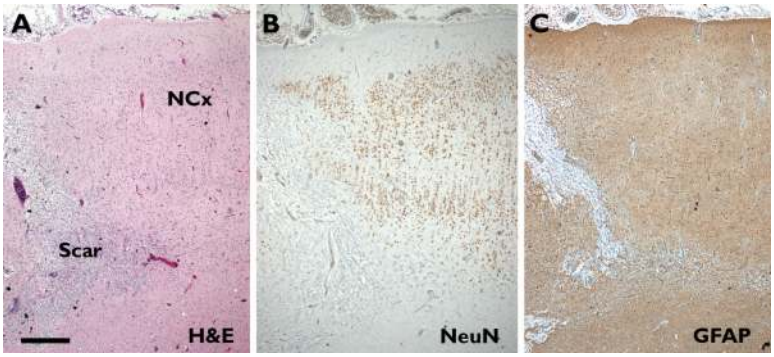

Figure 9. Histopathological findings in FCD Type IIId associated with a glio-mesodermal scar

A: Glio-mesodermal scarring in a 9-year old patient with perinatal hemorrhagic brain injury. NCx = Neocortex. HE = Haematoxylin-Eosin staining. **B**: NeuN labelling revealed disruption of cortical layering and abundant microcolumnar arrangement of cortical neurons. **C:** Pronounced reactive astrogliosis (GFAP) is a common finding in glio-mesodermal scarring. Scale bar in A = 500μm, applies also to B and C.

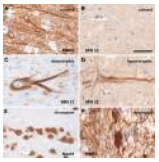

Figure 10. Abnormal cell types in FCD

Representative examples of abnormal cell types in FCD variants. All images were taken at same magnification (scale bar in B = 50 μm) using recommended immunohistochemical markers (see supplementary Table 3). 4μm paraffin embedded and formalin fixed specimens. **A – B:** biopsy control samples from Layer 3 (in A) and Layer 2 (in B). **C:** A dysmorphic neuron accumulating nonphosphorylated neurofilaments (antibody SMI 32) in a FCD Type IIb specimen. Also note significantly enlarged nucleus with prominent nucleolus. **D:** This hypertrophic pyramidal neuron was observed at the border between Layer 2 and Layer 3 in a FCD Type IIIa specimen. **E:** Microcolumn with alignment of immature, small diameter neurons. FCD Type Ia specimen. **F:** In gangliogliomas, dysplastic neurons often show bizarre morphology and multiple nucleoli.

Table 1
Three-tiered ILAE classification system for FCDs

The three-tiered ILAE classification system of Focal Cortical Dysplasia (FCD) distinguishes isolated forms (FCD Type I and II) from those associated with another principal lesion, i.e. hippocampal sclerosis (FCD Type IIIa), tumors (FCD Type IIIb), vascular malformations (FCD Type IIIc), or lesions acquired during early life (i.e., traumatic injury, ischemic injury or encephalitis, FCD Type IIId). Please note, the rare association between FCD Type IIa and IIb with hippocampal sclerosis, tumors or vascular malformations should not be classified as FCD Type III variant.

FCD Type I (isolated)	Focal Cortical Dysplasia with abnormal radial cortical lamination (FCD Ia)	Focal Cortical Dysplasia with abnormal tangential cortical lamination (FCD Ib)		Focal Cortical Dysplasia with abnormal radial and tangential cortical lamination (FCD Ic)
FCD Type II (isolated)	Focal Cortical Dysplasia with dysmorphic neurons (FCD IIa)		Focal Cortical Dysplasia with dysmorphic neurons and balloon cells (FCD IIb)	
FCD Type III (associated with principal lesion)	Cortical lamination abnormalities in the temporal lobe associated with hippocampal sclerosis (FCD IIIa)	Cortical lamination abnormalities adjacent to a glial or glio-neuronal tumor (FCD IIIb)	Cortical lamination abnormalities adjacent to vascular malformation (FCD IIIc)	Cortical lamination abnormalities adjacent to any other lesion acquired during early life, e.g., trauma, ischemic injury, encephalitis (FCD IIId)

FCD Type III (not otherwise specified, NOS): if clinically/radiologically suspected principal lesion is not available for microscopic inspection