NEUROSURGICAL FOCUS

Neurosurg Focus 53 (4):E6, 2022

# Focal cortical dysplasia pathology: diagnostic difficulty, classification, and utility for pathogenesis

**Ozge Kapar, MD,[1] Zahide Mail Gurkan, MD,[2] Muge Dolgun, MD,[3] Altay Sencer, MD,[4] Candan Gürses, MD,[5] and Bilge Bilgic, MD[1]**

[1]Department of Pathology, Istanbul University; [2]Department of Neurology and Clinical Neurophysiology, Istanbul University; [3]Department of Neurosurgery, Sultangazi Haseki Training and Research Hospital; [4]Department of Neurosurgery, Istanbul Faculty of Medicine, Istanbul University; and [5]Department of Neurology, Koc University, Istanbul, Turkey

**OBJECTIVE** In the histopathological examination of treatment-resistant epilepsy, focal cortical dysplasia (FCD) is the most common diagnosis in the pediatric group. FCD is classified histopathologically according to the International League Against Epilepsy (ILAE) classification. In the last decade since the ILAE classification has been released, molecular genetic studies have revealed mTOR pathway–related mutations as a major etiology. The objective of this study was to determine the incidence of FCD in treatment-resistant epilepsy patients, explore histomorphological and immunohistochemical features, examine clinicopathological correlation, demonstrate mTOR pathway activation using a pS6 antibody immunohistochemically, and try to introduce a candidate for possible targeted therapies.

**METHODS** Paraffin blocks and slides of tissue from patients with treatment-resistant epilepsy were reexamined retrospectively. Histopathological subtypes of FCD were determined according to the ILAE classification. NeuN and neurofilament H (NF-H) staining were performed, and additionally a pS6 antibody was used to demonstrate mTOR pathway activation.

**RESULTS** In 32 cases diagnosed with FCD, or 17.5% of 183 surgical epilepsy materials, there were no significant differences in the statistical analysis of clinical variables between the ILAE FCD subtypes. Recommended antibody NeuN revealed microcolumnar alignment in the FCD type Ia and IIIa groups and the loss of lamination in the type Ib group. Another recommended antibody, NF-H, was not found to be useful in discriminating between normal and dysmorphic neurons. pS6 expression, showing mTOR pathway activation, was observed in dysmorphic neurons and balloon cells in all FCD type II cases.

**CONCLUSIONS** Significant pS6 expression in FCD type II represents the genomic nature of the disease noted in the literature. Nevertheless, the known *MTOR* gene and mTOR pathway–related mutations remain behind proportionally to explain the mTOR pathway activation in all FCD type II cases. Clinicopathologically and genetically integrated classification and usage of mTOR pathway inhibitors in treatment are expected as a recent evolution.

https://thejns.org/doi/abs/10.3171/2022.7.FOCUS21731

**KEYWORDS** focal cortical dysplasia; treatment-resistant epilepsy; ILAE classification

Focal cortical dysplasia (FCD) is the localized malformation of cortical development and the most common etiology of treatment-resistant epilepsy in the pediatric population.[1] Most seizures are controlled by antiepileptic drugs, but in treatment-resistant cases, surgery remains an important option.[2,3] We have gained a lot of important knowledge from excised tissue obtained during epilepsy surgery. Standardized sampling is important, histochemical and immunohistochemical studies may help in making a diagnosis, and International League Against Epilepsy (ILAE) histopathological subtypes should be included in the final pathology report.[4]

FCD is histopathologically classified according to the ILAE classification. In the FCD classification, structural findings related to stratification of the cortex and associated cellular abnormalities are considered. FCD is histopathologically divided into different groups; it is claimed that these groups have distinctive clinical characteristics, such as age at seizure onset, seizure frequency, epilepsy duration, surgical age, imaging findings, and postoperative

**ABBREVIATIONS** DNET = dysembryoplastic neuroepithelial tumor; FCD = focal cortical dysplasia; HS = hippocampal sclerosis; ILAE = International League Against Epilepsy; LEATs = long-term epilepsy-associated brain tumors; NF-H = neurofilament H.

**SUBMITTED** December 12, 2021. **ACCEPTED** July 21, 2022.
**INCLUDE WHEN CITING** DOI: 10.3171/2022.7.FOCUS21731.

©AANS 2022, except where prohibited by US copyright law



Unauthenticated | Downloaded 10/04/22 07:27 PM UTC

**TABLE 1. ILAE consensus classification of FCD**

| | | | | |
|---|---|---|---|---|
| FCD Type I | Abnormal radial cortical lamination (Type Ia) | Abnormal tangential cortical lamination (Type Ib) | Abnormal radial and tangential cortical lamination (Type Ic) | |
| FCD Type II | Existence of dysmorphic neurons (Type IIa) | | Existence of dysmorphic neurons and balloon cells (Type IIb) | |
| FCD Type III | Abnormal cortical lamination associated with hippocampal sclerosis (Type IIIa) | Abnormal cortical lamination adjacent to a glial or glioneuronal tumor (Type IIIb) | Abnormal cortical lamination adjacent to vascular malformation (Type IIIc) | Abnormal cortical lamination adjacent to any other lesion during early life, e.g., trauma, ischemic injury, encephalitis (Type IIId) |

From: Blümcke I, Thom M, Aronica E, Armstrong DD, Vinters HV, Palmini A, Jacques TS, Avanzini G, Barkovich AJ, Battaglia G, Becker A, Cepeda C, Cendes F, Colombo N, Crino P, Cross HJ, Delalande O, Dubeau F, Duncan J, Guerrini R, Kahane P, Mathern G, Najm I, Özkara Ç, Raybaud C, Represa A, Roper SN, Salamon N, Schulze-Bonhage A, Tassi L, Vezzani A, Spreafico R. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia.* 2011;52(1):158-174, published by John Wiley & Sons. © 2010 International League Against Epilepsy.

survival rates.[5] ILAE classification has validity in routine practice, while there are still difficulties in FCD diagnosis, classification, and correlation with clinical findings.[6]

Besides the morphological changes found, molecular genetic studies have made great progress in recent years and mutations in *MTOR* and various genes regulating the mTOR pathway have been detected in cortical malformations.[7,8] Because the mTOR pathway suggests a role in epileptogenesis, targeted therapy is suggested to be an alternative to antiepileptic drugs.

Our aim in this study was to determine the incidence of FCD in the treatment-resistant epilepsy patients within our case series, attempting to determine histomorphological and immunohistochemical features and demonstrate the relation between the ILAE subtypes and the clinical features while trying to predict the prognosis of the patients. In addition, one of the objectives was to question the importance of mTOR pathway activation in different clinical and histological variables, examine the contribution to the diagnostic process, and determine the patient group for molecular targeted therapies.

## Methods

### Patients and Samples

All patients with epilepsy who underwent epilepsy surgery between 2002 and 2017 at Istanbul University were included in the study. This retrospective study was approved by the institutional ethics committee of Istanbul University, Istanbul Faculty of Medicine.

### Pathological Examination and Diagnosis

#### Macroscopic Examination and Sampling

Specimens for epilepsy surgery were obtained via cerebral lobectomy (temporal, frontal, or occipital), selective amygdalohippocampectomy, lesionectomy, and hemispherectomy. Cerebral lobectomy specimens were also obtained with hippocampectomy specimens in approximately 80% of hippocampal sclerosis (HS) cases. Each specimen was processed according to an established protocol for the cortex and hippocampus.[4] Cortex sampling was perpendicular to the arachnoid-pial surface to properly evaluate the cortical stratification. Anatomically intact hippocampal tissues were cut parallel to the coronal plane in the anterior-posterior axis at an interval of 5 mm and completely sequentially sampled and numbered. Fragmented tissues were not convenient to use for this protocol.

#### Diagnosis

All pathology slides of the cases were retrieved from the archive and the diagnoses were reviewed. The types of FCD cases according to the recommended ILAE classification were determined. The ILAE classification system is shown in Table 1. Principles of the Declaration of Helsinki were considered during the study, and the patients' confidential data were kept according to their respective guidelines. Due to the retrospective nature of the study, written informed consent could not be obtained and was not required.

#### Immunohistochemical Analysis

The most appropriate paraffin-embedded tissue blocks for immunohistochemical analysis were selected after all archived slides were reviewed. Immunohistochemistry was performed on paraffin-embedded tissue blocks with an automatic staining system (Ventana BenchMark XT, IHC/ISH automated staining platforms; Roche Diagnostics Ltd.).

The primary antibodies used in the study were 1) anti-NeuN, clone A60 (1:200, MAB377; EMD Millipore), the primary antibody for neurons; 2) anti–neurofilament H (NF-H), clone SMI-32 (1:800, SIG 801702; BioLegend), the primary antibody for layer specificity; and 3) anti-phospho-S6 ribosomal protein (S6, phosphorylated at ser235/236 [p-S6 S235/236]) antibody, polyclonal (1:800, E18070; Spring), the primary antibody for mTOR signaling activation.

### Clinical Data

Pathology reports and clinical records were investigated for clinical features. Gender, age at seizure onset, seizure duration, age at surgery, and seizure outcomes of these patients were retrospectively summarized and analyzed. Seizure outcomes were assessed after a minimum 1-year follow-up period after surgery. For the evaluation of postoperative seizure outcome, the Engel classification system was used: seizure free (class I), > 90% reduction in seizure frequency (class II), 50%–90% reduction in seizure frequency (class III), and < 50% reduction in seizure frequency (class IV).[9]



Unauthenticated | Downloaded 10/04/22 07:27 PM UTC

**TABLE 2. Comparisons of clinical parameters between HS and FCD**

| Parameter | HS, n = 121 | FCD, n = 32 | p Value |
|---|---|---|---|
| Mean age at seizure onset ± SD, mos | 146.1 ± 118.8 | 38.6 ± 46.2 | <0.001* (z = 5.11) |
| Mean epileptic seizure duration ± SD, mos | 185.5 ± 116.7 | 134.6 ± 142.5 | 0.021* (z = 2.30) |
| Mean surgical age ± SD, yrs | 29.7 ± 11.2 | 16.2 ± 14.7 | <0.001* (z = 4.61) |
| Gender, n (%) | | | |
| Male | 45 (37.2) | 20 (62.5) | 0.01† ($c^2$ = 6.64) |
| Female | 76 (62.8) | 12 (37.5) | |
| Engel class, n‡ | | | |
| I | 88 | 15 | 0.001§ |
| II–IV | 7 | 15 | |

* Mann-Whitney U-test.
† Chi-square test.
‡ The minimum follow-up duration was 1 year.
§ Fisher's exact test.

### Statistical Analysis

All statistical analyses were performed using SPSS software (IBM Corp.). Data are given as mean ± standard deviation or median (interquartile range) where appropriate. Proportions were written as percentages. Categorical variables were compared using Pearson chi-square and Fisher's exact tests. Continuous, nonparametric variables were analyzed using the Mann-Whitney U-test analysis and the Kruskal-Wallis test with independent samples. A p value < 0.05 was considered significant in all comparisons.

## Results

Histopathological examination of 183 patients included in the study revealed HS in 121 (66.1%), FCD in 32 (17.5%), tumor in 7 (3.8%), vascular malformation in 3 (1.6%), and encephalitis in 2 (1.1%) cases. The other diagnoses were hamartoma in 3 cases, heterotopia in 1 case, and cyst formation in 1 case. In 13 cases (6%) there were no histopathological findings.

Of the 183 patients, 55 (30.1%) were in the pediatric age group (1–17 years old). In the pediatric age group, 18 (32.7%), 14 (25.5%), and 6 (10.9%) patients were diagnosed with FCD, HS, and tumor, respectively. In adults, 107 (83.6%), 14 (10.9%), and 1 (0.8%) patient were diagnosed with HS, FCD, and tumor, respectively. The findings show that FCD in the pediatric age group and HS in the adult age group were the most frequent diagnoses.

Age at seizure onset in patients with FCD ranged from 0 to 11 years (mean 3.2 years, median 3 years). Seizure duration ranged from 0 to 46 years (mean 11.2 years, median 13 years). Surgical age ranged from 0 to 49 years (mean 16.2 years, median 12 years).

Results of the comparison of clinical parameters between the FCD and HS groups are shown in Table 2. The FCD group was associated with significantly earlier seizure onset (mean 3.2 years), shorter preoperative seizure duration (mean 11.2 years), and earlier surgical age (mean 16.2 years) compared with the HS group. The male gender ratio in the FCD group was significantly higher than in the HS group. Patients in the FCD group had poorer seizure control after surgery than those in the HS group, with a minimum 1 year of follow-up (p = 0.001).

There were 7 cases of FCD type Ia, 8 cases of FCD type Ib, 6 cases of FCD type IIa, 7 cases of FCD type IIb, and 4 cases of FCD type IIIa (Table 3). Diagnostic hallmarks of these types were a microcolumnar array in group Ia, dysmorphic neurons in group IIa, and balloon cells in group IIb. In the IIIa group, normal stratification of the cortex was lost. Pyramidal neuronal loss in the hippocampus was accompanied by microcolumnar arrays in the temporal cortex. Our study did not include any cases with a diagnosis of FCD type Ic, IIIb, IIIc, or IIId.

All 4 FCD type III cases were located in the temporal lobe. The other temporally located cases consisted of only 3 cases in the FCD type I group and 1 case in the FCD type II group. Localizations of FCD cases are shown in Table 3. Temporal lobe localization in FCD type IIIa showed a statistically significant difference compared with FCD type I (Fisher's exact test, p = 0.02).

NeuN, NF-H (SMI-32), and pS6 were studied immunohistochemically in all cases except 3 FCD type Ib cases, 1 FCD type IIa case, and 1 FCD type IIIa case because their paraffin blocks were not available. Accordingly, NeuN revealed the microcolumnar sequence in FCD type Ia and IIIa groups and the loss of lamination in the FCD type Ib group. Despite staining in normal neurons and some dysmorphic neurons, immunoreactivity was not observed in balloon cells. In 1 FCD type IIa case, neither dysmorphic neurons nor normal neurons were stained with NF-H. The other cases showed immunoreactivity in dysmorphic neurons and normal neurons. In contrast, immunoreactivity was not observed with NF-H in any balloon cells. Includ-

**TABLE 3. Localizations of the FCD cases**

| Location | FCD Type, n (%) | | | | |
|---|---|---|---|---|---|
| | Ia | Ib | IIa | IIb | IIIa |
| Temporal | 1 (14) | 2 (25) | — | 1 (14) | 4 (100) |
| Frontal | 2 (29) | 6 (75) | 4 (67) | 4 (57) | — |
| Parietal-occipital | 4 (57) | — | 2 (33) | 2 (29) | — |



Unauthenticated | Downloaded 10/04/22 07:27 PM UTC

**TABLE 4. Immunohistochemical expressions in cell groups**

| FCD Type | NeuN (n = 27) | NF-H (n = 4) | pS6 (n = 24) |
|---|---|---|---|
| I | | | |
| Reactive/normal neurons | 12/12 | 12/12 | 6/12* |
| IIa | | | |
| Reactive/normal neurons | 5/5 | 4/5 | 1/5 |
| Dysmorphic neurons | 5/5 | 4/5 | 3/5 |
| IIb | | | |
| Reactive/normal neurons | 7/7 | 7/7 | 0/7 |
| Dysmorphic neurons | 7/7 | 7/7 | 7/7 |
| Balloon cell | 0/7 | 0/7 | 5/7 |
| IIIa | | | |
| Reactive/normal neurons | 3/3 | — | — |

* Sparsely.

ing staining in some normal neurons, pS6 showed positivity in some of the dysmorphic neurons and weak staining in balloon cells. In balloon cells of 2 FCD type IIb cases and dysmorphic neurons of 2 FCD type IIa cases, there was no expression with pS6, but pS6 expressions were seen in all FCD type II cases of dysmorphic neurons and balloon cells (Table 4).

In our study, apart from FCD cases, pS6 immunoreactivity was observed in a ganglioglioma case.

## Discussion

We present these findings to illustrate the difficulties in making the diagnosis of FCD, evaluate the consistency of our cases after classification with respect to clinical variables, and review the utility of the recommended immunohistochemical markers.

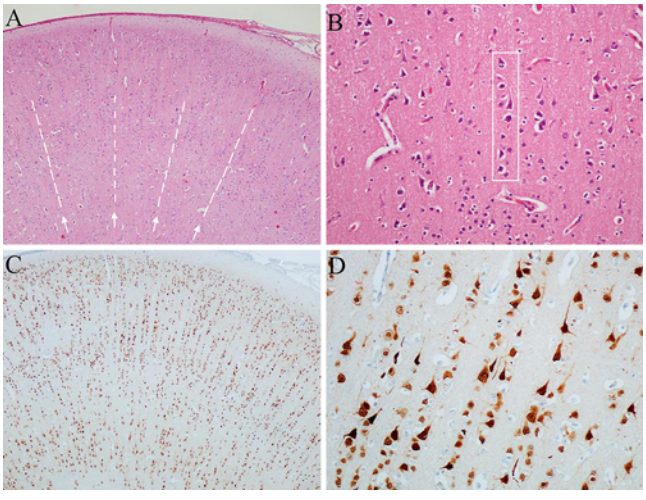



**FIG. 1.** Photomicrographs showing histopathological features of FCD type Ia. **A and B:** Abnormal radial cortical lamination (*arrows* and *dashed lines*, A) and microcolumnar sequences (*box*, B). H&E, original magnification ×20 (A) and ×200 (B). **C and D:** NeuN immunoreactivity showing microcolumnar sequences. NeuN, original magnification ×20 (C) and ×200 (D).

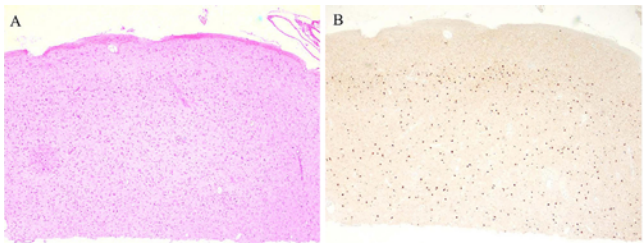



**FIG. 2.** Photomicrographs demonstrating histopathological features of FCD type Ib. **A:** Abnormal tangential cortical lamination. H&E, original magnification ×20. **B:** NeuN immunoreactivity. NeuN, original magnification ×20.

### Diagnosis and Classification

FCD cases have heterogeneous histomorphology characterized by structural findings related to stratification abnormality (abnormal cortical lamination) of the cortex and cellular anomalies.[10] The ILAE classification is based on these findings.[5] The FCD type I category shows lamination defects and is subdivided into 3 groups, i.e., type Ia, type Ib, and type Ic, in which the lamination defect exists in radial, tangential, and both directions, respectively.[5]

Microcolumnar sequences of small immature neurons associated with abnormal radial migration and neuronal maturation are a characteristic finding of FCD type Ia. Microcolumnar arrays can be described as the sequencing of 8 immature cells in a vertical direction (Fig. 1).[11] They are distinctly observed in the third layer of the cortex. This appearance in FCD type Ia is similar to the stage of radial migration during early fetal development of the cortex. The focus of attention is the columnar architecture, which continues at the top of the gyrus and deep in the sulcus in adults.[12] This architecture should not be considered a migration defect. However, the differentiation between a microcolumnar sequence in FCD or variation in these localizations can be challenging. In addition, findings can be difficult to diagnose in some cases.

In FCD type Ib, the microscopic appearance of layer stratification resembles the horizontal migration stage observed in the second half of gestation.[12] The six-layer histological structure of the cortex is lost because of the lamina-specific neuronal paucity. Although all the layers of the cortex can be affected, layers II and IV are predominantly affected and the borders with neighboring layers cannot be selected clearly (Fig. 2).

In FCD type II, dysmorphic cells and balloon cells are identified in addition to the lamination defect also noted in type I. The presence of dysmorphic cells in the diagnosis of FCD type IIa, and balloon cells in addition to dysmorphic cells in the diagnosis of FCD type IIb, are required. Dysmorphic cells have abnormal cytoplasm and cytoplasmic extensions. Phosphorylated (shown by the NF-H [2F11] antibody) and nonphosphorylated (shown by the NF-H [SMI32] antibody) accumulation of neurofilament isoforms are aggregated and concentrated toward the cell membrane. Balloon cells are large cells with homogenous, opaque, eosinophilic cytoplasm and an eccentrically located nucleus. The Nissl body cannot be



Unauthenticated | Downloaded 10/04/22 07:27 PM UTC

seen due to the accumulation of neurofilament proteins (Fig. 3).[13]

FCD type I and II groups are isolated forms of FCD. But in FCD type III, adjacent lesions accompany the lamination defect, which is seen in FCD type I. FCD type III is subgrouped according to adjacent principal lesions (HS in FCD type IIIa, glial or glioneuronal tumors in FCD type IIIb, vascular lesions in FCD type IIIc, and various lesions induced by trauma, ischemia, encephalitis, etc., in FCD type IIId).[5]

In the literature, temporal cortex involvement is noted in approximately 35% of patients with HS, and the presence of cortical dysplasia in the range of 10%–50% has been reported. Considering the number of HS cases, cases in the FCD type IIIa group indicate cortex involvement in 3.2% of HS cases. This rate is quite low compared with the rate reported in the literature.[14]

Several years following publication of the ILAE classification, good interobserver agreement for FCD types IIa, IIb, and IIIa was reported; a moderate agreement for FCD type Ib; and no agreement for FCD type Ic.[15]

For FCD type I and III groups, nonperpendicular cortex sampling causes diagnostic problems for observing lamination. Normal/dysmorphic neuronal differentiation is the most common challenge for FCD type IIa cases.

### Immunohistochemical Analysis

In the evaluation of FCD cases, some antibodies are recommended, i.e., NeuN to show cortical lamination defects, NF-H (SMI32) to show dysmorphic neurons, and vimentin and CD34 to show balloon cells.[4] NeuN has been reported as the most valuable marker for the diagnosis of FCD types I and III, indicating a cortical stratification defect. NF-H and vimentin have been found to be the most valuable markers in the diagnosis of FCD type II.[16] In our cases, NeuN revealed the microcolumnar sequence in FCD type Ia and IIIa groups and the loss of lamination in the FCD type Ib group. NF-H was observed in dysmorphic neurons and neuronal cells featuring normal morphology. Immunoreactivity was not observed with NF-H in any balloon cells. These findings show that NeuN may help to reveal cortical lamination abnormalities, but NF-H is not a crucial marker. NF-H expression has been indicated in aged pyramidal cells of cortex layers III and V.[4]

### Clinical Findings

In our cases, there was no significant difference in the age at onset, duration of seizure, and surgical age between the types of FCD ($p > 0.05$). Temporal lobe localization in FCD type IIIa showed a statistically significant difference compared with FCD type I (Fisher's exact test, $p = 0.02$).

ILAE classification is based on histopathological data, whereas clinical, EEG, and imaging differences led to specifying the different groups. The clinical, electrophysiological, and radiological features of FCD type II are more distinctively defined. Whereas FCD type II caused more frequent temporal lobe seizures, FCD type I caused frontal seizures, often affecting a larger area. FCD type II was associated with earlier seizure onset, early surgical age, short epilepsy duration, and more frequent seizures. Even

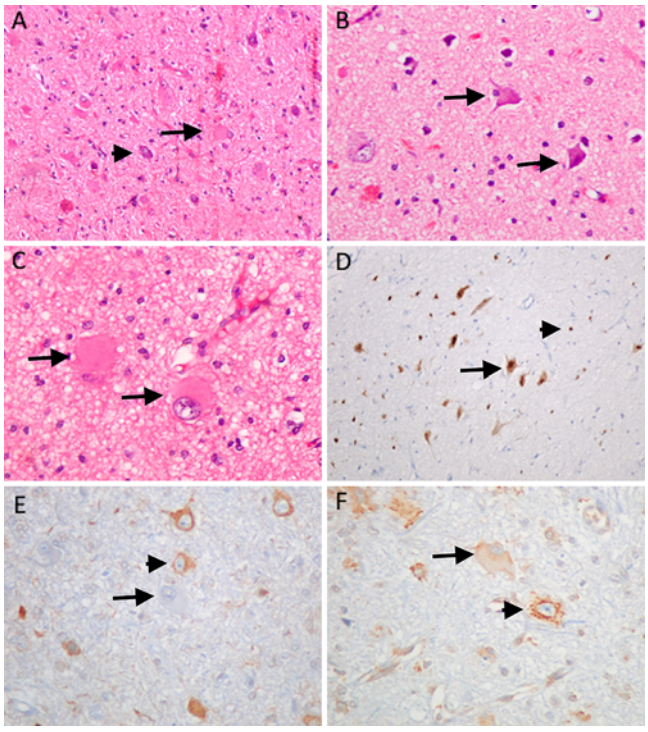



**FIG. 3.** Photomicrographs of histopathological features of FCD type II. **A–C:** Dysmorphic neurons (*arrowhead*) and balloon cells (*arrow*, A), dysmorphic neurons (*arrows*, B), and balloon cells (*arrows*, C) in an FCD type IIb case. H&E, original magnification ×100 (A), ×200 (B), and ×400 (C). **D:** NeuN immunoreactivity in normal (*arrowhead*) and dysmorphic (*arrow*) neurons. NeuN, original magnification ×100. **E:** NF-H immunoreactivity in dysmorphic neurons (*arrowhead*) and balloon cells (*arrow*). NF-H, original magnification ×200. **F:** More intense and granular pS6 immunoreactivity in dysmorphic neurons (*arrowhead*) and weak immunoreactivity in balloon cells (*arrow*). pS6, original magnification ×200.

though there are adverse results, FCD type II has had better postsurgical seizure outcomes. Clinical findings distinguishing HS with FCD (FCD type IIIa) from isolated FCD type I were presented by Tassi et al., showing HS with FCD was associated with less frequent seizures and better seizure control.[17] In subsequent studies, when nontemporal lobe epilepsy cases were excluded, no significant differences were found in terms of onset of epilepsy, surgical age, seizure frequency, temporal cortex MRI findings, and postoperative seizure rate; only febrile seizure history and presence of aura were more frequent in FCD type IIIa.[14,18,19]

These findings show that although the ILAE classification is widely accepted and developed with the aim of clinicopathological correlation, specifically for FCD types I and III, a classification with higher interobserver compliance and better reflecting clinicopathological differences between groups was expected.

### New Treatment Target: mTOR Pathway Activation?

The mTOR signaling pathway that is effective in the mechanisms of epileptogenesis, such as neural stem cell proliferation, neuroplasticity, and development of synaptic connections, is another topic that has been recently discussed.[20] The mTOR molecule has a central position



Unauthenticated | Downloaded 10/04/22 07:27 PM UTC

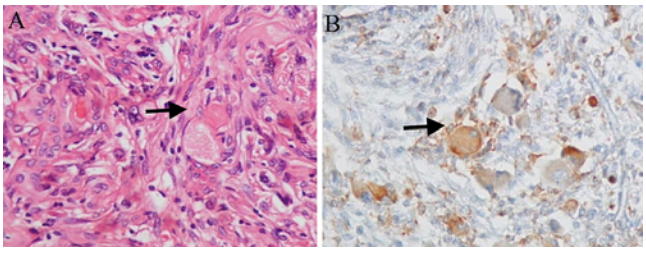



**FIG. 4. A:** Photomicrographs of neuronal (*arrow*) and glial cell components in a ganglioglioma case. H&E, original magnification ×200. **B:** pS6 immunoreactivity in neuronal cells (*arrow*). pS6, original magnification ×200.

in intracellular signaling cascades and is present as part of mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) with serine-threonine kinase activity. These complexes have central roles in the mTOR pathway. Signaling pathways such as PI3K/AKT and RAS/ERK act on TSC1 and TSC2 and affect the mTOR pathway.[21]

Hyperphosphorylation of mTOR pathway substrate ribosomal S6 protein (pS6) causes mTOR pathway activation. As a downstream molecule of mTOR intracellular signaling, pS6 antibody expression is related to mTOR pathway activation.

Activation of mTORC1 in FCD was first noted in 2004.[22] Detection of mTOR pathway activation in hemimegalencephaly also revealed the relationship between cortical malformations and mTOR.[8] As a result of genetic studies in recent years, mutations in the *MTOR* gene and various genes regulating the mTOR pathway have been detected in cortical malformations such as FCD and hemimegalencephaly. Somatic mutations in the *MTOR* gene are the most common cause of FCD. Loss of function mutations in mTORC1 inhibitor genes (*TSC1*, *TSC2*, *DEPDC5*, *NPRL2*, *NPRL3*, *NEDD4L*) and gain of function mutations in mTOR activating genes (*AKT3*, *PIK3CA*) are reported in cortical malformations.[7,23,24]

The "mTORopathy" term is proposed for abnormalities such as cortical tubers in tuberous sclerosis, hemimegalencephaly, and FCD type IIa/IIb related to the mTOR pathway that show similar morphology. In these conditions, cortical malformation accompanies abnormal cell proliferation. Tuberous sclerosis is known as autosomal dominant multisystemic disease and can present with epilepsy caused by mutation of TSC1 and TSC2 encoding hamartin and tuberin, respectively, and lead to activation of the mTOR pathway. Hemimegalencephaly, exhibiting similar morphology as FCD type II, is a more extended lesion compared with FCD.[25–27]

In addition to these entities, glioneuronal tumors such as ganglioglioma and dysembryoplastic neuroepithelial tumor (DNET) manifest neuronal and glial proliferation and show co-occurrence with FCDs. Tumoral lesions observed in epilepsy surgery are the most common feature noted in a group consisting of adult and pediatric patients. Luyken et al. suggested the term "long-term epilepsy-associated brain tumors" (LEATs) for drug-resistant epilepsy-related tumors with a history of epilepsy for more than 2 years.[28] LEATs refer to slow-developing tumors, mostly originating from the temporal lobe, presenting with an early-onset seizure.[29] Approximately 60% of these tumors are diagnosed as ganglioglioma and DNET.[30] Ganglioglioma is a biphasic tumor containing dysplastic neurons and glial cells. DNET, on the other hand, has a typical appearance of the myxoid matrix containing oligodendroglial cell–like cells and neurons extending along the vessels in the microcolumnar pattern.[8] mTOR pathway activation is also reported in these tumors.[31,32]

The pS6 antibody showed staining in most of the dysmorphic neurons and weak staining in balloon cells, including staining in some normal neurons. Negative staining in some cells was accepted to be related to the sparsity of balloon cells, and these were the old cases with long archived periods (14, 13, and 12 years). In the literature, almost 40% of FCD cases have mTOR-related gene mutations,[23] but pS6 immunoreactivity due to activation of the mTOR pathway presented in all FCD type II cases. This finding showed us that there are more mutations that need to be explored.

In our study, apart from FCD cases, pS6 immunoreactivity was detected in a ganglioglioma case as well (Fig. 4). In previous studies consisting of a variety of epilepsy-associated pathologies and varied cell types, pS6 immunopositivity has been reported to be less evident and absent in FCD type I or histologically normal cortex,[33–35] but also observed in astrocytes and microglia in HS, in microglia cells in Rasmussen encephalitis, and in dysplastic neurons in glioneuronal tumors.[36,37] We focused on pS6 immunoreactivity in cortical tissues. In our study, pS6 highlighted dysmorphic cells and balloon cells of FCD type II, which was consistent with the literature data. In addition to these findings, in a mouse study it was shown that intrauterine mTOR mutation caused neuronal migration defect and epilepsy, and these subjects had large neurons in the brain. At the same time, seizures decreased after rapamycin treatment.[38]

In the management of seizure control, antiepileptic drugs do not provide anything more than symptomatic treatment. Most antiepileptics act on ion channels, but epilepsy control cannot be achieved in 20%–30% of patients. This situation indicates that new treatment approaches are necessary. Information on the underlying molecular genetic etiology is important in the detection of targeted therapies. The mTOR pathway is involved in epileptogenesis and its relationship with epilepsy leads to new treatment options. The use of everolimus, which is the rapamycin (mTOR inhibitor) analog, is recommended for subependymal giant cell astrocytoma and angiomyolipoma without surgical options and in patients with tuberous sclerosis.[39,40] However, its effect is unclear both in patients with tuberous sclerosis–associated epilepsy and in other epilepsy causes in which mTOR pathway activation has been demonstrated. In the phase 2 study on tuberous sclerosis patients, everolimus treatment showed a decrease in seizures by more than 50% in 60% of patients.[41] In addition, mTOR inhibitors have been found to prevent and reduce seizures in patients with posttraumatic epilepsy, temporal lobe epilepsy, and absence epilepsy.[42–44]

Mutations in various genes that regulate the mTOR pathway detected in FCD and hemimegalencephaly and its



Unauthenticated | Downloaded 10/04/22 07:27 PM UTC

role in epileptogenesis indicate its importance as a putative therapeutic target in the future. The utility of pS6 immunohistochemistry as a biomarker is gaining importance due to its potential role as a diagnostic and predictive marker.

### Limitations of the Study

We analyzed only 32 cases diagnosed with FCD from a single institute, and further large-scale studies are required.

## Conclusions

This study provides insight into FCD cases that are encountered relatively occasionally but need comprehensive work. The FCD ILAE classification is widely used and designed according to heterogenous histomorphological findings. We did not show clinical differences between FCD groups in our small-scale study. Accordingly, diagnostic problems remain, both pathologically and clinically. Mostly small-scale studies are available for these diagnostic groups. Potential future updates on molecular genetic specifications in classification may help foster shared efforts toward a better understanding of FCD types. Meanwhile, these updates can contribute to the diagnostic process, reflect clinical differences, and guide treatment strategies. New mTOR pathway–related molecular genetic specifications have been identified mostly in the FCD type II group. In addition, significant pS6 antibody expressions in definitive cells of FCD type II (dysmorphic neurons and balloon cells) were consistent with genetic findings in the literature, even though the *MTOR* gene and mTOR pathway–related mutations remain behind proportionally to explain the mTOR pathway activation in all FCD type II cases. Subsequently, FCD type I cases had subtler findings and showed less relation with the mTOR pathway. These findings look promising for the usage of mTOR pathway inhibitors in the treatment of a group of FCDs.

## Acknowledgments

This study was funded by Istanbul University (project no. 28658).

## References

1. Blumcke I, Spreafico R, Haaker G, et al. Histopathological findings in brain tissue obtained during epilepsy surgery. *N Engl J Med*. 2017;377(17):1648-1656.
2. López González FJ, Rodríguez Osorio X, Gil-Nagel Rein A, et al. Drug-resistant epilepsy: definition and treatment alternatives. *Neurologia*. 2015;30(7):439-446.
3. Schulze-Bonhage A, Zentner J. The preoperative evaluation and surgical treatment of epilepsy. *Dtsch Arztebl Int*. 2014; 111(18):313-319.
4. Blümcke I, Aronica E, Miyata H, et al. International recommendation for a comprehensive neuropathologic workup of epilepsy surgery brain tissue: a consensus Task Force report from the ILAE Commission on Diagnostic Methods. *Epilepsia*. 2016;57(3):348-358.
5. Blümcke I, Thom M, Aronica E, et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia*. 2011;52(1):158-174.
6. Najm IM, Sarnat HB, Blümcke I. Review: The international consensus classification of Focal Cortical Dysplasia—a critical update 2018. *Neuropathol Appl Neurobiol*. 2018;44(1): 18-31.
7. Marsan E, Baulac S. Review: Mechanistic target of rapamycin (mTOR) pathway, focal cortical dysplasia and epilepsy. *Neuropathol Appl Neurobiol*. 2018;44(1):6-17.
8. Aronica E, Crino PB. Epilepsy related to developmental tumors and malformations of cortical development. *Neurotherapeutics*. 2014;11(2):251-268.
9. Engel J Jr, Van Ness PC, Rasmussen TB, Ojemann LM. Outcome with respect to epileptic seizures. In: Engel J Jr, ed. *Surgical Treatment of the Epilepsies*. 2nd ed. Raven Press; 1993:609-621.
10. Taylor DC, Falconer MA, Bruton CJ, Corsellis JA. Focal dysplasia of the cerebral cortex in epilepsy. *J Neurol Neurosurg Psychiatry*. 1971;34(4):369-387.
11. Hildebrandt M, Pieper T, Winkler P, Kolodziejczyk D, Holthausen H, Blümcke I. Neuropathological spectrum of cortical dysplasia in children with severe focal epilepsies. *Acta Neuropathol*. 2005;110(1):1-11.
12. Sarnat HB, Flores-Sarnat L. Radial microcolumnar cortical architecture: maturational arrest or cortical dysplasia? *Pediatr Neurol*. 2013;48(4):259-270.
13. Sisodiya SM, Fauser S, Cross JH, Thom M. Focal cortical dysplasia type II: biological features and clinical perspectives. *Lancet Neurol*. 2009;8(9):830-843.
14. Marusič P, Tomásek M, Kršek P, et al. Clinical characteristics in patients with hippocampal sclerosis with or without cortical dysplasia. *Epileptic Disord*. 2007;9(suppl 1):S75-S82.
15. Coras R, de Boer OJ, Armstrong D, et al. Good interobserver and intraobserver agreement in the evaluation of the new ILAE classification of focal cortical dysplasias. *Epilepsia*. 2012;53(8):1341-1348.
16. Cepeda C, Hurst RS, Flores-Hernández J, et al. Morphological and electrophysiological characterization of abnormal cell types in pediatric cortical dysplasia. *J Neurosci Res*. 2003; 72(4):472-486.
17. Tassi L, Garbelli R, Colombo N, et al. Type I focal cortical dysplasia: surgical outcome is related to histopathology. *Epileptic Disord*. 2010;12(3):181-191.
18. Fauser S, Essang C, Altenmüller DM, et al. Is there evidence for clinical differences related to the new classification of temporal lobe cortical dysplasia? *Epilepsia*. 2013;54(5):909-917.
19. Johnson AM, Sugo E, Barreto D, et al. Clinicopathological associations in temporal lobe epilepsy patients utilising the current ILAE focal cortical dysplasia classification. *Epilepsy Res*. 2014;108(8):1345-1351.
20. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell*. 2012;149(2):274-293.
21. Lipton JO, Sahin M. The neurology of mTOR. *Neuron*. 2014; 84(2):275-291.
22. Miyata H, Chiang ACY, Vinters HV. Insulin signaling pathways in cortical dysplasia and TSC-tubers: tissue microarray analysis. *Ann Neurol*. 2004;56(4):510-519.
23. Baldassari S, Ribierre T, Marsan E, et al. Dissecting the genetic basis of focal cortical dysplasia: a large cohort study. *Acta Neuropathol*. 2019;138(6):885-900.
24. Crino PB. mTORopathies: a road well-traveled. *Epilepsy Curr*. 2020;20(6_suppl):64S-66S.
25. Crino PB. Focal brain malformations: seizures, signaling, sequencing. *Epilepsia*. 2009;50(Suppl 9):3-8.
26. Mühlebner A, Bongaarts A, Sarnat HB, Scholl T, Aronica E. New insights into a spectrum of developmental malformations related to mTOR dysregulations: challenges and perspectives. *J Anat*. 2019;235(3):521-542.
27. Barkovich AJ, Guerrini R, Kuzniecky RI, Jackson GD, Dobyns WB. A developmental and genetic classification for malformations of cortical development: update 2012. *Brain*. 2012;135(Pt 5):1348-1369.



Unauthenticated | Downloaded 10/04/22 07:27 PM UTC

28. Luyken C, Blümcke I, Fimmers R, et al. The spectrum of long-term epilepsy-associated tumors: long-term seizure and tumor outcome and neurosurgical aspects. *Epilepsia*. 2003; 44(6):822-830.
29. Thom M, Blümcke I, Aronica E. Long-term epilepsy-associated tumors. *Brain Pathol*. 2012;22(3):350-379.
30. Blumcke I, Aronica E, Urbach H, Alexopoulos A, Gonzalez-Martinez JA. A neuropathology-based approach to epilepsy surgery in brain tumors and proposal for a new terminology use for long-term epilepsy-associated brain tumors. *Acta Neuropathol*. 2014;128(1):39-54.
31. Boer K, Troost D, Timmermans W, van Rijen PC, Spliet WG, Aronica E. Pi3K-mTOR signaling and AMOG expression in epilepsy-associated glioneuronal tumors. *Brain Pathol*. 2010; 20(1):234-244.
32. Becker AJ, Löbach M, Klein H, et al. Mutational analysis of TSC1 and TSC2 genes in gangliogliomas. *Neuropathol Appl Neurobiol*. 2001;27(2):105-114.
33. Baybis M, Yu J, Lee A, et al. mTOR cascade activation distinguishes tubers from focal cortical dysplasia. *Ann Neurol*. 2004;56(4):478-487.
34. Yao K, Duan Z, Zhou J, et al. Clinical and immunohistochemical characteristics of type II and type I focal cortical dysplasia. *Oncotarget*. 2016;7(47):76415-76422.
35. Patil VV, Guzman M, Carter AN, et al. Activation of extracellular regulated kinase and mechanistic target of rapamycin pathway in focal cortical dysplasia. *Neuropathology*. 2016; 36(2):146-156.
36. Liu J, Reeves C, Michalak Z, et al. Evidence for mTOR pathway activation in a spectrum of epilepsy-associated pathologies. *Acta Neuropathol Commun*. 2014;2:71.
37. Prabowo AS, Iyer AM, Veersema TJ, et al. BRAF V600E mutation is associated with mTOR signaling activation in glioneuronal tumors. *Brain Pathol*. 2014;24(1):52-66.
38. Lim JS, Kim WI, Kang HC, et al. Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy. *Nat Med*. 2015;21(4):395-400.
39. Bissler JJ, Franz DN, Frost MD, et al. The effect of everolimus on renal angiomyolipoma in pediatric patients with tuberous sclerosis being treated for subependymal giant cell astrocytoma. *Pediatr Nephrol*. 2018;33(1):101-109.
40. Kingswood JC, Jozwiak S, Belousova ED, et al. The effect of everolimus on renal angiomyolipoma in patients with tuberous sclerosis complex being treated for subependymal giant cell astrocytoma: subgroup results from the randomized, placebo-controlled, Phase 3 trial EXIST-1. *Nephrol Dial Transplant*. 2014;29(6):1203-1210.
41. Krueger DA, Wilfong AA, Holland-Bouley K, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Ann Neurol*. 2013;74(5):679-687.
42. Russo E, Citraro R, Donato G, et al. mTOR inhibition modulates epileptogenesis, seizures and depressive behavior in a genetic rat model of absence epilepsy. *Neuropharmacology*. 2013;69:25-36.
43. Guo W, Zhang CQ, Shu HF, Yang MH, Yin Q, Yang H. Expression of bone morphogenetic protein-4 in the cortical lesions of focal cortical dysplasia IIb and the tuberous sclerosis complex. *J Mol Neurosci*. 2013;50(1):7-13.
44. Zeng LH, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci*. 2009;29(21): 6964-6972.

## Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

## Author Contributions

Conception and design: Kapar, Bilgic. Acquisition of data: Kapar, Gurkan, Dolgun, Sencer, Gürses. Analysis and interpretation of data: Kapar, Gürses, Bilgic. Drafting the article: Kapar. Critically revising the article: Bilgic. Reviewed submitted version of manuscript: Bilgic. Approved the final version of the manuscript on behalf of all authors: Kapar. Administrative/technical/material support: Dolgun. Study supervision: Bilgic.

## Supplemental Information

### Previous Presentations

Portions of this work were presented in poster form at the 15th International Congress of American Pathology and Oncology Research in Chicago, Illinois, on December 3, 2018.

## Correspondence

Ozge Kapar: Istanbul University, Istanbul, Turkey. m.ozgekapar@gmail.com.



Unauthenticated | Downloaded 10/04/22 07:27 PM UTC