

Fig. 4 – Histology and immunohistochemistry of FCD IIb. FCD IIb demonstrates unidentified layer-formation of the neocortex and high neuronal concentration (A, B), and contains balloon cells (small window in A). SATB2+ cells are at a high concentration in the superficial (C1), but scattered in the middle (C2) and deep (C3) region of the neocortex. The concentrations of FOXP1+ cells are high in the superficial (D1) and deep (D3) regions of the neocortex, and evidence a relatively low level in the middle region (D2). TBR1+ cells are at a relatively high concentration in the superficial (E1) and deep (E3) regions of the neocortex, but at a low concentration (E2) in the middle region. (A) Hematoxylin and eosin staining; (B) NeuN-immunostaining; (C) SATB2-immunostaining, superficial region (C1), middle region (C2), and deep region (C3); (D) FOXP1-immunostaining, superficial region (D1), middle region (D2), and deep region (D3); (E) TBR1-immunostaining, superficial region (E1), middle region (E2), and deep region (E3). Scales of A–B and C1–E3 indicate 200 µm and 100 µm, respectively.

of pharmacoresistant focal and/or general epilepsy after detailed examinations, including head magnetic resonance imaging (MRI), electroencephalogram (EEG), video monitoring EEG, single photon emission computed tomography (SPECT), positron emission tomography (PET), and magnetoencephalogram (MEG) at our hospital. The use of human tissue was approved by the Ethical Committee of our hospital and institute, and informed consent was obtained from the parents of all patients.

As control materials, we used six fetuses from 23 to 29 gestational weeks of age, and 10 children from 1 month to 8 years of age, in a previous study (Saito et al., 2011). All controls were autopsied. The average fixation time was within 14 days, and the time from death to starting fixation was within 12 h.

4.2. Histological and immunohistochemical examination

Fresh surgical tissues were fixed in 10% neutral buffered formalin, embedded in paraffin and sliced at 6 µm thickness. Serial sections were evaluated histologically using hematoxylin and eosin and Klüver-Barrera stainings, as well as immunocytochemically using the antibodies against NeuN and three layer-markers. Three neuropathologists independently diagnosed with FCD, using the recent international

classification (Blümcke et al., 2011) and HME, showing polymicrogyria, unlayered cortex and/or neuronal heterotopia.

Immunohistochemical examination was performed with the previously described methods. Briefly, after deparaffined, serial sections were autoclaved for antigen-retrieval, and endogenous peroxidase activity was blocked with 0.3% H₂O₂ and non-specific protein with 2% bovine serum albumin. They were then incubated with the four antibodies at 4 °C overnight. As the primary antibodies, we used NeuN, Neuronal nuclei (dilution of 1:100; Chemicon International Inc., Temecula, CA), SATB2, special AT-rich sequence-binding protein 2 (1:100; Bio Matrix Research, Japan), TBR1, T-box brain 1 (1:100; Abcam, UK), and FOXP1, Forkhead box P1 (1:100; Abcam). Previous studies demonstrate that SATB2, FOXP1, and TBR1 express only in the fetal neocortex, and distribute in the upper and middle layers, the deep layer and mainly the upper layer, respectively (Saito et al., 2011).

4.3. Cell density analysis

As FCD is histologically characterized by disorganization of cerebral neocortical layer-formation, it is difficult to determine separate layers in the neocortex. Therefore, we divided the neocortex thickness equally into three regions (superficial, middle, and deep) in order to evaluate the immunopositive cell distribution. This is because FCD and HME pathology usually

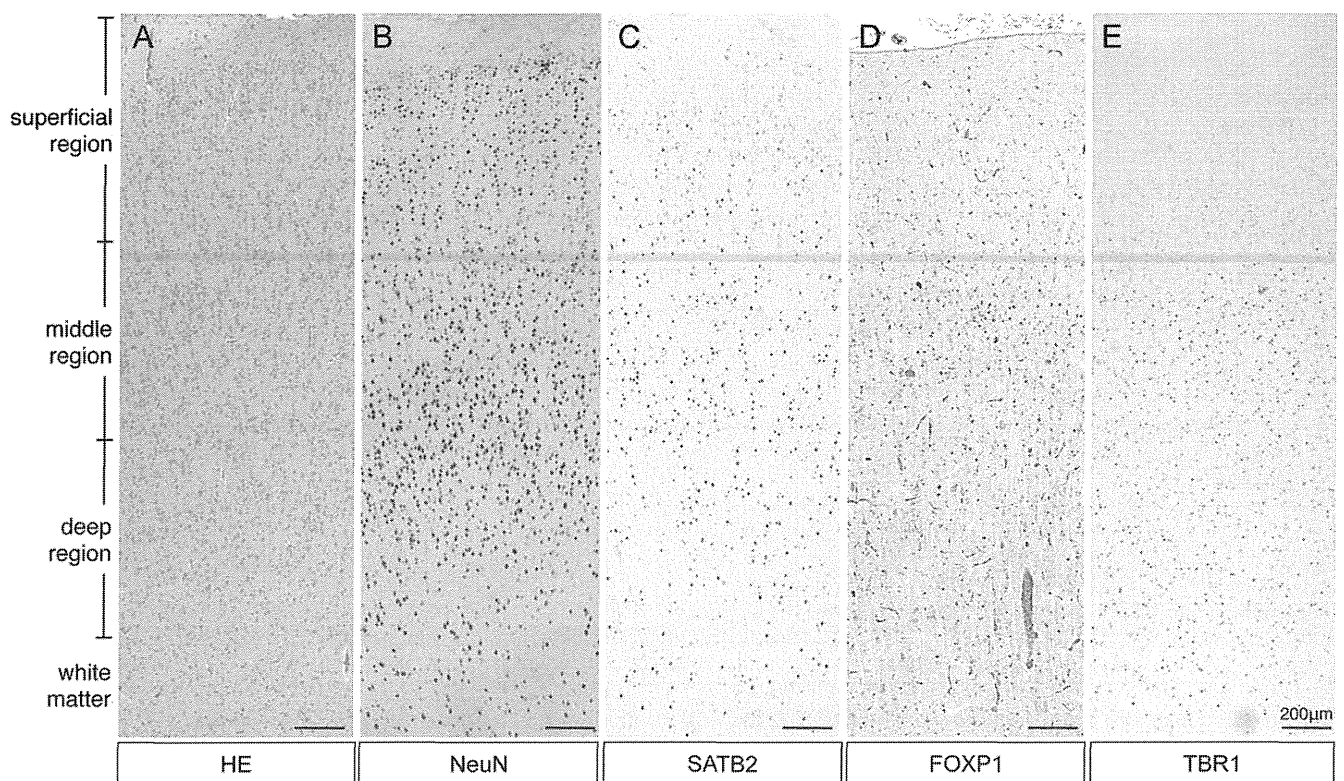


Fig. 5 – Histology and immunohistochemistry of HME. HME also demonstrates unidentified layer-formation of the neocortex, showing partially abundant microcolumns and abnormal tangential composition (A). NeuN+ cells are diffusely observed in the neocortex and white matter (B). SATB2+ cells show a high concentration in the superficial, middle and deep regions of the neocortex (C). FOXP1+ cells diffusely locate in the neocortex (D). TBR1+ cells diffusely locate in the middle and deep regions of the neocortex, but are absent in the upper region (E). (A) Hematoxylin and eosin staining; (B) NeuN-immunostaining; (C) SATB2-immunostaining; (D) FOXP1-immunostaining; (E) TBR1-immunostaining. Scales indicate 200 μ m.

show normal layer formation in the neocortex. The density of SATB2-, TBR1-, and FOXP1-positive (+) cells was determined in each brain region examined. The density of positively stained neurons was graded semiquantitatively as negative (-; no neurons stained), mild (+; <5 cells stained over an area of 1 mm² area), and marked (2+; >5 cells stained in a 1 mm² area) in each region. These layer-marker positive cells were confirmed as neurons with NeuN-immunostaining.

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The authors declare that they have no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with their guidelines.

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ORIGINAL ARTICLE

Delayed Maturation and Differentiation of Neurons in Focal Cortical Dysplasia With the Transmantle Sign: Analysis of Layer-Specific Marker Expression

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Abstract

Transmantle dysplasia is a rare type of focal cortical dysplasia (FCD) characterized by expansion of the cortex from the deep white matter to the surface and in which there is a FCD IIA or IIB pathologic pattern. To characterize possible mechanisms underlying this regional disorder of radial migrating cells, we studied the expression patterns of neocortical layer-specific markers using immunohistochemistry in surgical specimens from 5 FCD IIA and 4 FCD IIB cases in children. All neuronal cells expressed the mature neuron marker MAP2/2B but not the microglia markers Iba-1 and CD68. Some layer-specific markers showed distinct expression patterns. TBR1-positive, SATB2-positive, and FOXP1-positive cells were diffusely distributed in the cortex and/or the white matter. TBR1-positive and FOXP1-positive cells were generally more numerous in FCD IIB than in FCD IIA and were mostly in the cortical molecular and upper layers. FOXP1-, FOXP2-, and CUTL1-positive cells also expressed the immature neuron marker, Nestin/PROX1, whereas TBR1-, CTIP2-, and SATB2-positive cells only expressed MAP2/2B. These data highlight differences between FCD IIB and FCD IIA with more cells having the immature marker in upper layer markers in the former. By analyzing layer-specific marker expression patterns, we

identified apparent neuronal maturation differences between FCD IIA and FCD IIB in cases of transmantle dysplasia.

Key Words: Epilepsy, Focal cortical dysplasia, Layer-specific markers, Neural maturation, Transmantle dysplasia, Transmantle sign.

INTRODUCTION

Abnormal maturation and differentiation of neurons result in the formation of cerebral cortical malformations associated with epilepsy. Focal cortical dysplasia (FCD) is a distinctive malformation of cortical development that is a common cause of intractable epilepsy in children who present for surgical treatment (1–4). Recently, the International League Against Epilepsy proposed a new classification of FCD pathologic subtypes based on the identification of isolated dyslamination of the neocortex (FCD I), the presence of characteristic dysmorphic neurons (FCD IIA) or balloon cells (FCD IIB), or a combination of dysplasia associated with tumor, hippocampal sclerosis, vascular malformation, or perinatal injury (FCD III) (5). FCD IIA and FCD IIB also show abnormal neocortical lamination. The pathogenesis of FCD is thought to involve abnormal differentiation of neuron and glial cell in the early embryonic period (6). In the magnetic resonance image, some FCDs exhibit a transmantle sign or are termed *transmantle dysplasia* (TD), a funnel-shaped lesion from the cortex that passes through the white matter (WM) to the superolateral ventricle (3, 7). It is thought that a genetic abnormality in early-progenitor cells forms funnel-shaped lesions of FCD (6). However, TD is a very rare, little-reported entity. A detailed pathologic examination of TD cases would enhance understanding of the pathogenetic mechanisms in FCD.

Laminar formation of the neocortex is known to be highly programmed by genetic control during the embryonic period. Using layer-specific markers, it is possible to identify cell birth timing and cell maturation lineage stage. At first, postmitotic projection neurons migrate into the cortical plate from the subventricular germinal zone with an inside-out pattern. At the neuronal migration stage, some molecules (e.g., reelin, Lis1, and doublecortin) are required to form the neocortex (2, 8). Finally, mammalian brains show a 6-layer neocortex, each layer with a specific function. Specific genes have important roles at each step; the molecular mechanisms are

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TABLE 1. Clinicopathological Profile of FCD with TD Patients

Case	Sex	Age at Seizure Onset	Age at Surgery	Seizure Type	Intelligence (DQ or IQ)	TD Location on Imaging	Pathologic Diagnoses
1	F	1 mo	6 mo	GTC	53	P + O	FCD IIA
2	F	1 mo	8 mo	GTC + WS	86	T + P + O	FCD IIA
3	M	0 mo	1 mo	WS	100	Fr	FCD IIA
4	F	2 y 1 mo	6 y 4 mo	GTC	83	Fr	FCD IIA
5	F	1 mo	2 y 9 mo	GTC + WS + MS	10	T + P + O	FCD IIA
6	M	0 mo	6 mo	GTC + MS	95	Fr	FCD IIB
7	F	3 y 0 mo	10 y 9 mo	CPS	111	T + P	FCD IIB
8	M	1 y 0 mo	1 y 1 mo	CPS	91	Fr	FCD IIB
9	M	4 mo	2 y 1 mo	WS + CPS	100	P	FCD IIB

M, male; F, female; GTC, generalized tonic-clonic seizure; WS, West syndrome; MS, myoclonic seizure; CPS, complex partial seizure; IQ, intelligence quotient; DQ, developmental quotient; Fr, frontal lobe; T, temporal lobe; P, parietal lobe; O, Occipital lobe; TD, transmantle dysplasia; FCD IIA and FCD IIB, focal cortical dysplasia IIA and IIB. Pathologic diagnoses are based on the international classification.

well known in rodent brains (9) and, to some extent, in the developing human brain (10). Special AT-rich sequence binding protein 2 (Satb2), COUP-TF interacting protein 2 (Ctip2), T-box brain 1 (Tbr1), Cut-like homeobox 1 (Cutl1), and Forkhead box P1 and P2 (Foxp1 and Foxp2) are well-known neocortical layer-specific markers. Other layer-specific molecules also play very important roles in forming cortical lamination, and disruption of their genes leads to profound cortical malformation (8, 11). SATB2 generates callosal projections in Layers II to IV and makes subcortical connections (9, 12). Orthodenticle homeobox 1 (Otx1) is expressed in Layers V and

VI (13). Tbr1, a member of the T-box homeobox gene family, is expressed in preplate and Layer VI in fetal brain and makes corticocortical projection neurons (14). Cutl1, *Drosophila* homeobox CUT-like 1, is expressed in pyramidal neurons of the upper layer (15). Foxp1, a transcription factor of the winged-helix/forkhead family, is expressed in neocortical Layers III to V (16).

In a previous study, we reported that the neocortical layer formation of human developing brains is similar to that in mice using the layer-specific markers SATB2, CTIP2, TBR1, CUTL1, and FOXP1 (10). In addition, we found that

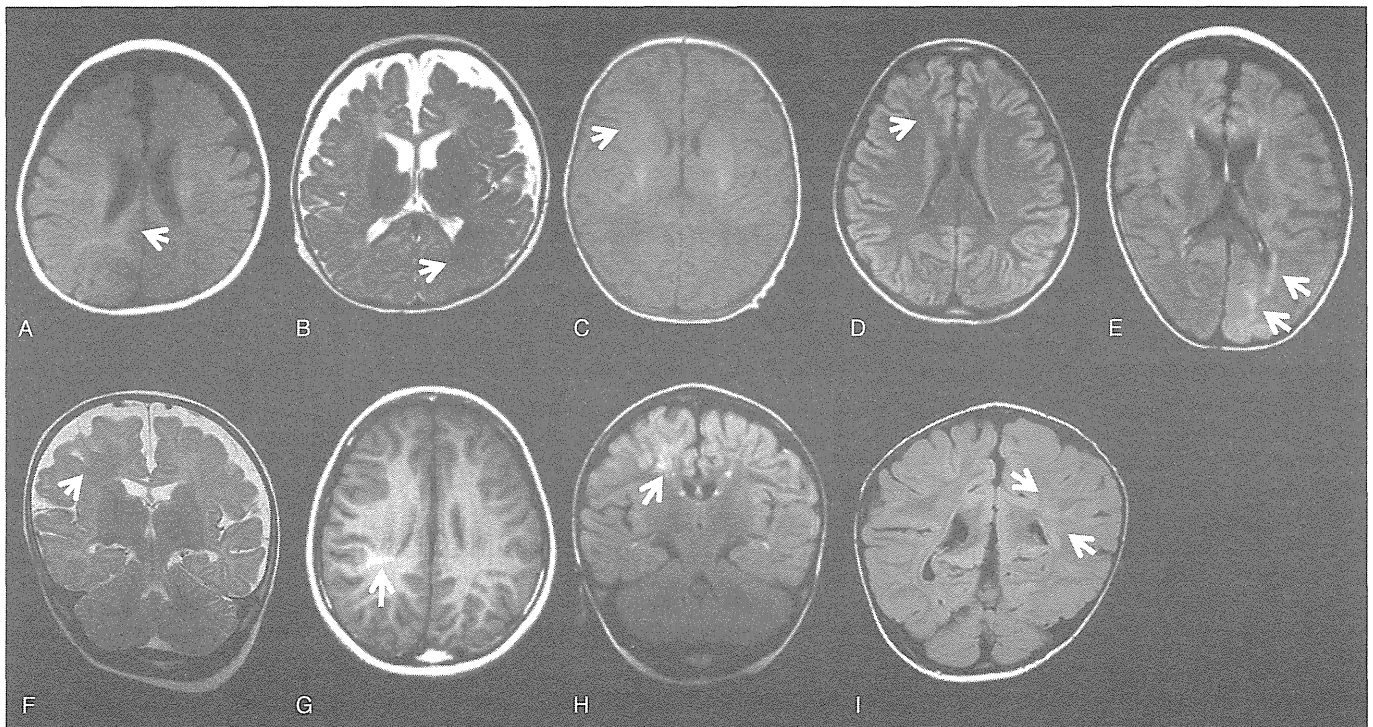


FIGURE 1. Head magnetic resonance images of all patients showing the “transmantle sign” of focal cortical dysplasia (arrows). **(A)** Axial view of a T1-weighted image of Case 1. **(B)** Axial view of a T2-weighted image of Case 2. **(C)** Axial view of a T1-weighted image of Case 3. **(D)** Axial view of a FLAIR image of Case 4. **(E)** Axial view of a FLAIR image of Case 5. **(F)** Coronal view of a T2-weighted image of Case 6. **(G)** Axial view of a T1-weighted image of Case 7. **(H)** Coronal view of a FLAIR image of Case 8. **(I)** Coronal view of a FLAIR image of Case 9.

various lissencephalies exhibited abnormal layer formation by random migration (10). However, very little is known about the expression patterns of layer-specific markers in human brain (10, 17), particularly in cortical dysplasia (18). To understand FCD formation and the origin of the component neurons, we investigated the expression of layer-specific markers from the deep WM to the cortex in FCD with TD.

MATERIALS AND METHODS

Tissue Samples

The use of all tissue samples for this research was approved by the patients' parents and by ethical committees of

the hospital and institute sources. The cases were selected based on the presence of TD, that is, abnormal intensity from the lateral ventricle to the cortex on the magnetic resonance image (7). Cerebral samples removed from patients with drug-resistant epilepsy and TD during surgery were studied (Table 1 and Fig. 1). The samples used were obtained from epileptogenic areas, as determined by analysis of seizure semiology, ictal and interictal electroencephalography, magnetic resonance imaging, fluorodeoxyglucose positron emission tomography, and interictal single photon emission computed tomography. For the age-matched control study, we used the same materials as previously described (10, 19). After resection, all samples were fixed in 10% buffered formalin or 4% paraformaldehyde and

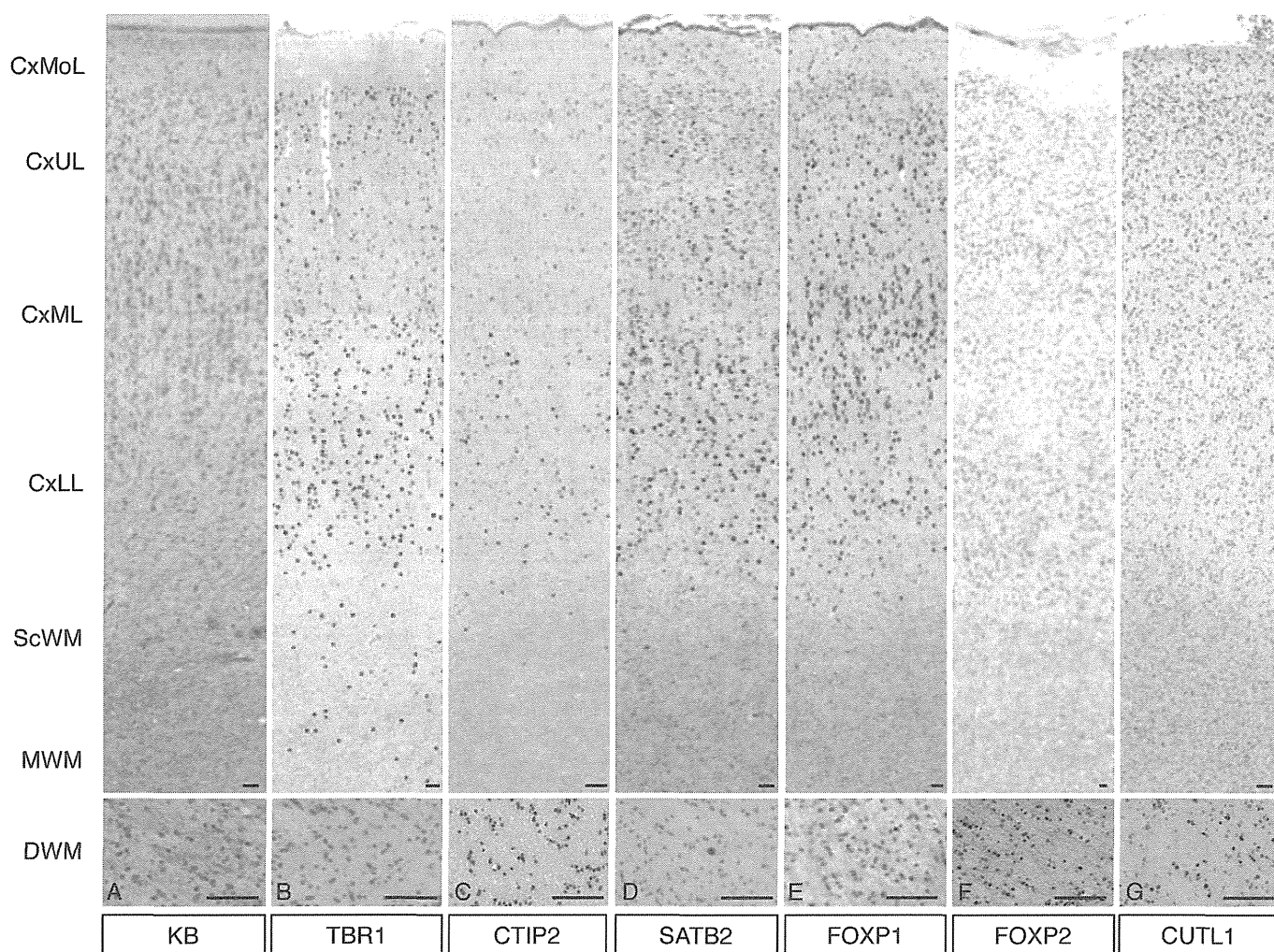


FIGURE 2. Expression patterns of layer-specific markers in focal cortical dysplasia (FCD) IIA. **(A)** FCD IIA shows cortical dyslamination, a high cell density, and many heterotopic neurons in the white matter. **(B)** TBR1-positive cells are distributed diffusely, particularly in the cortical upper (CxUL) and lower (CxLL) layers. **(C, D)** CTIP2-positive cells and SATB2-positive cells are predominantly in the CxLL. **(E)** FOXP1-positive cells are in the cortical middle layer (CxML) and CxUL. **(F)** FOXP2-positive cells are diffusely distributed in the cortex and white matter; there are a few in the deep white matter (DWM). **(G)** CUTL1-positive cells are also diffusely distributed. **(A)** KB indicates Klüver-Barrera. **(B)** TBR1 indicates T-box, brain, 1. **(C)** CTIP2 indicates COUP-TF interacting protein 2. **(D)** SATB2 indicates special AT-rich sequence binding protein 2. **(E)** FOXP1 indicates Forkhead box P1. **(F)** FOXP2 indicates Forkhead box P2. **(G)** CUTL1 indicates Cut-like homeobox 1. CxMoL indicates molecular layer of cortex; CxUL, upper layer (upper one third of the cortex); CxML, middle layer (middle one-third of the cortex); CxLL, lower layer (lower one-third of the cortex); ScWM, subcortical white matter; MWM, middle white matter; DWM, deep white matter (near ventricular wall). Bars = 100 μ m.

embedded in paraffin. Serial sections, 4 to 6 μm thick, were cut for histologic and immunohistologic examination. For diagnosis of the resected tissues, the sections were stained with hematoxylin and eosin and Klüver-Barrera stains. The cases were diagnosed as FCD IIA or FCD IIB by 3 neuropathologists based on in the recent classification (5).

Immunohistochemistry

Immunohistochemistry was performed as previously described (10, 20). The primary antibodies were incubated at 4°C for 24 to 72 hours. To investigate the presence of layer-specific markers in the cortex and WM, we used monoclonal antibodies against SATB2 (dilution of 1:100; Bio Matrix Re-

search, Inc., Chiba, Japan), CUTL1 (1:1000; Abnova, Taipei, Taiwan), CTIP2 (1:100; Abcam, Cambridge, UK), and polyclonal antibodies against TBR1 (1:100; Abcam), FOXP1 (1:200; Abcam), and FOXP2 (1:1000; Abcam). In addition, we performed double staining to confirm whether layer-specific marker cells were neuron or glial cells. We used anti-microtubule-associated protein 2 (MAP2, 1:100; Sigma, St. Louis, MO) or anti-MAP2B (1:100; BD Biosciences, San Jose, CA) as mature neuron markers. Antibodies to Nestin (1:100; Chemicon International, Inc., Billerica, MA) or Prospero homeobox protein 1 (PROX1, 1:100; Abcam) were used as immature neuron markers. Anti-gial fibrillary acidic protein (GFAP, 1:100; Cell Signaling Technology, Inc., Beverly, MA) or anti-GFAP (1:1000;

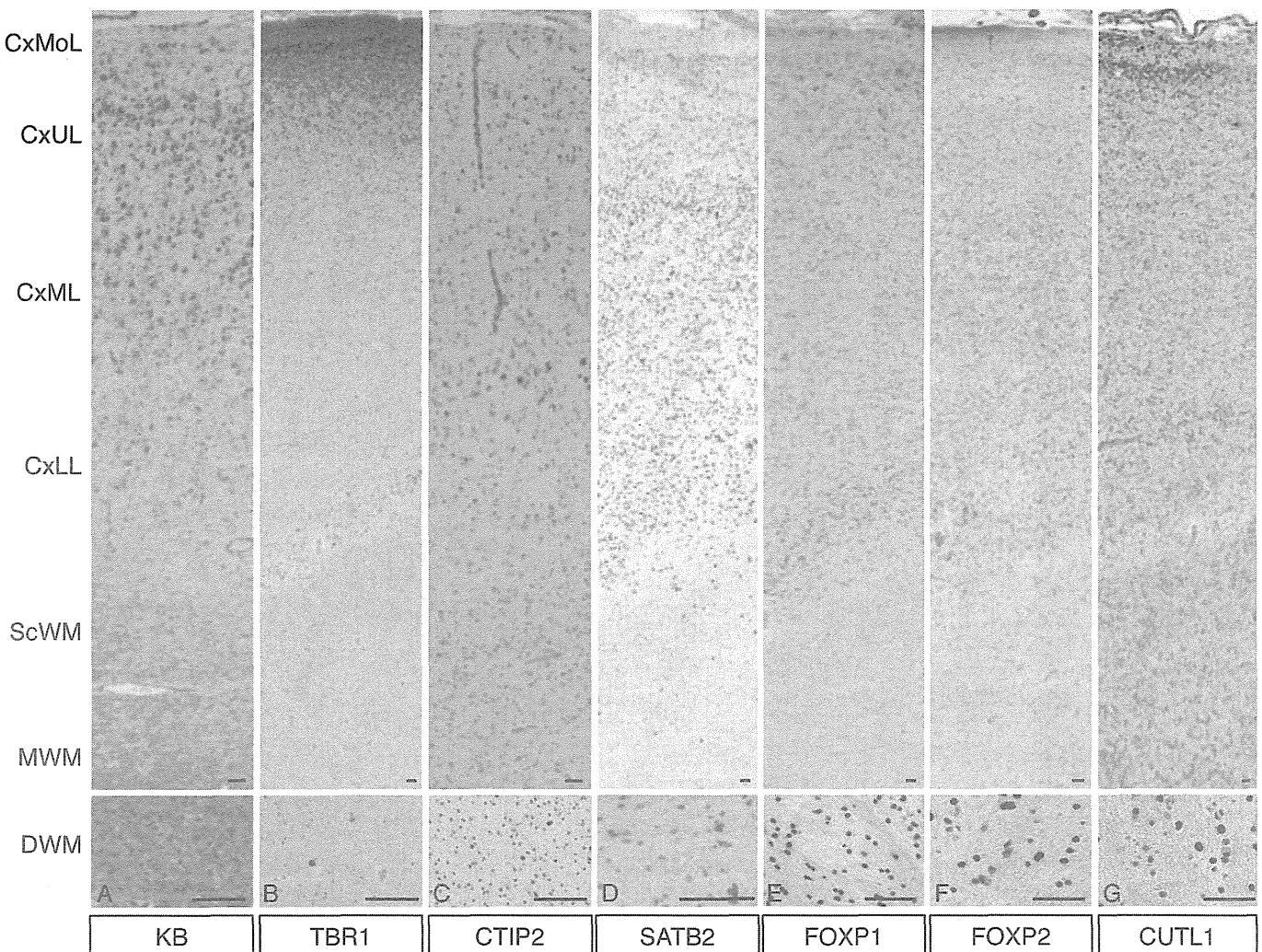


FIGURE 3. Expression patterns of layer-specific markers in focal cortical dysplasia (FCD) IIB. **(A)** FCD IIB also shows cortical dyslamination, high cell density, and many heterotopic neurons in the white matter. **(B)** TBR1-positive cells are diffusely distributed in the cortex and white matter. **(C)** CTIP2-positive cells are in the CxML and CxLL. **(D)** SATB2-positive cells are diffusely distributed in the cortex, but there are only a few in the white matter. **(E)** FOXP1-positive cells are in the cortex and DWM. **(F)** FOXP2-positive cells are also in the cortex and white matter. **(G)** CUTL1-positive cells are diffusely distributed; they are particularly numerous in the white matter. **(A)** KB indicates Klüver-Barrera. **(B)** TBR1 indicates T-box, brain, 1. **(C)** CTIP2 indicates COUP-TF interacting protein 2. **(D)** SATB2 indicates special AT-rich sequence binding protein 2. **(E)** FOXP1 indicates Forkhead box P1. **(F)** FOXP2 indicates Forkhead box P2. **(G)** CUTL1 indicates Cut-like homeobox 1. CxMoL indicates molecular layer of cortex; CxUL, upper layer (upper one-third of the cortex); CxML, middle layer (middle one-third of the cortex); CxLL, lower layer (lower one-third of the cortex); ScWM, subcortical white matter; MWM, middle white matter; DWM, deep white matter (near ventricular wall). Bars = 100 μm .

TABLE 2. Cell Type and Layer-Specific Marker Coexpression

	Cell-Type Marker	Layer-Specific Marker					
		TBR1	CTIP2	SATB2	FOXP1	FOXP2	CUTL1
FCD IIA	MAP2/2B	+	+	+	+	+	+
	Nestin/PROX1	-	-	-	+	+	+
	GFAP	-	-	-	-	-	+
	Iba-1/CD68	-	-	-	-	-	-
FCD IIB	MAP2/2B	+	+	+	+	+	+
	Nestin/PROX1	-	-	-	+	+	+
	GFAP	-	-	-	+	+	+
	Iba-1/CD68	-	-	-	-	-	-

+ indicates appearance of merged cells; -, no merged cell appearance.

MAP2/2B indicates microtubule-associated protein 2/2B; GFAP, glial fibrillary acidic protein; TBR1, T-box, brain, 1; CTIP2, COUP-TF interacting protein 2; SATB2, special AT-rich sequence binding protein 2; CUTL1, Cut-like homeobox 1, FOXP1 and FOXP2, Forkhead box P1 and P2; PROX1, Peospero homeobox protein 1.

Chemicon) were used as astrocyte markers. Antibodies against Iba-1 (1:100; Biocare Medical, Concord, CA) or CD68 (1:100; DAKO, Glostrup, Denmark) were used as microglia markers. 4',6'-Dimamidino-2-phenylindole was used as a cell nuclear marker.

Comparison of Immunopositive Cell Counts Between FCD IIA and FCD IIB with TD

Variable cell staining was observed in all cases. Because the FCD neocortex showed random layer formation, we divided

the cortex evenly into 4 parts: the molecular layer (CxMoL), cortical upper layer (CxUL, upper one-third), cortical middle layer (CxML, middle one-third), and cortical lower layer (CxLL, other deep cortex). The WM was evenly divided into the subcortical WM (ScWM; upper third), middle WM (MWM; middle third), and deep WM (DWM; periventricular region). Cells, excluding endothelial cells, were counted in each layer in 5 fields at a magnification of ×200. The number was corrected per 100 nuclei in each layer for each case as the immunopositive cell density.

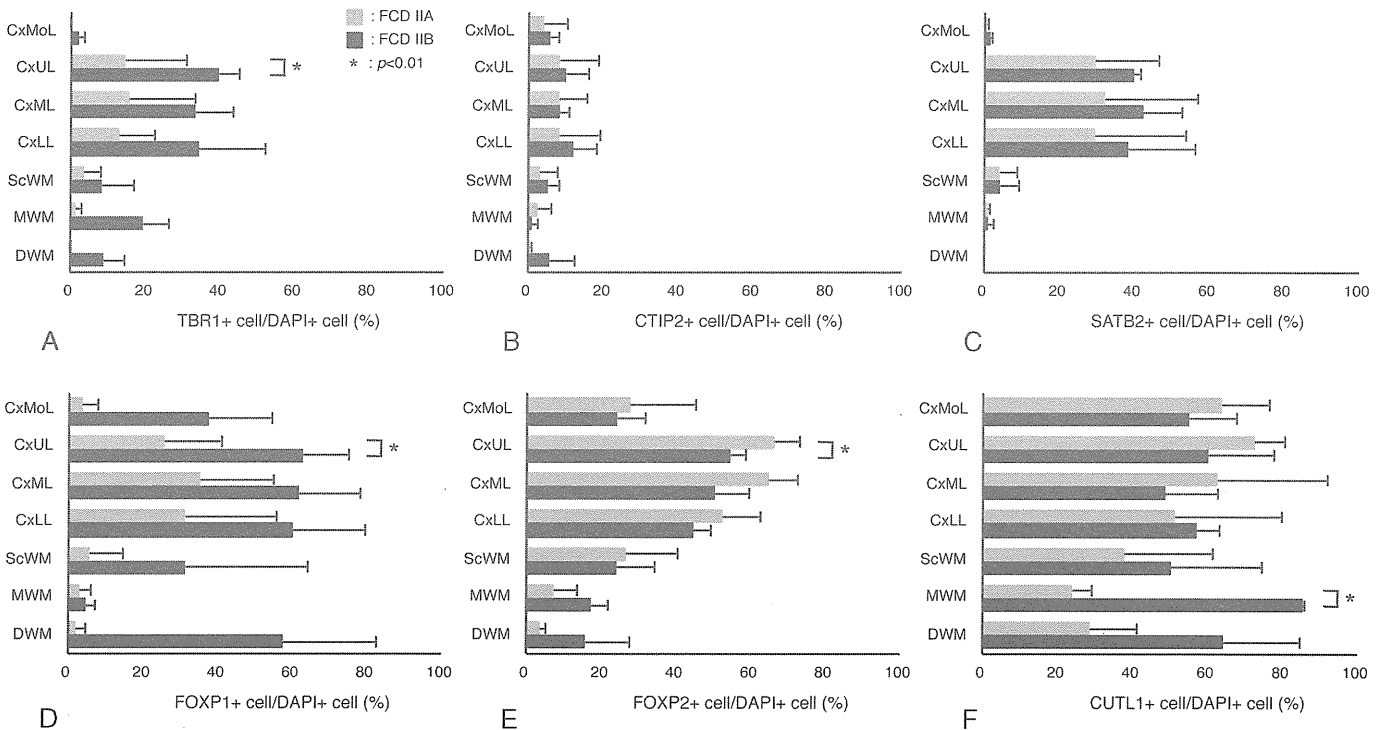


FIGURE 4. Distribution of layer-specific marker cell ratios (marker-positive cell number/total 4',6'-dimamidino-2-phenylindole-positive cell number) in regions of focal cortical dysplasia (FCD) IIA and IIB. **(A-F)** Compared with FCD IIA, FCD IIB has a higher TBR1 ratio **(A)** and FOXP1 ratio **(D)** in the cortical upper layer (CxUL), and CUTL1 ratio in the middle white matter (MWM) **(F)**. On the contrary, FCD IIA shows higher FOXP2 ratio in the CxUL **(E)**. Other region marker ratios are not significantly different between FCD IIA and FCD IIB. TBR1 indicates T-box, brain, 1; CIP2, COUP-TF interacting protein 2; SATB2, special AT-rich sequence binding protein 2; FOXP1 and 2, Forkhead box P1 and P2; CUTL1, Cut-like homeobox 1.

For statistical analyses, Student *t*-test was used for comparison between FCD IIA and FCD IIB in each region. The same test was applied using statistical software (SPSS Inc., Chicago, IL); $p < 0.05$ was considered significant.

RESULTS

The FCD samples showed abnormal lamination of the cortices and many ectopic neurons in the WM (Figs. 2 and 3). Our FCD series was composed of 5 FCD IIA and 4 FCD IIB, and the immunohistochemistry is summarized in Table 2. In previous studies, TBR1-, CTIP2-, SATB2-, FOXP1-, and CUTL1-containing cells appeared in the fetal period (10, 14, 21). After birth, SATB2-positive and CUTL1-positive cells are present until 3 months of age, although the other markers have already disappeared (10). In addition, FOXP2 appeared predominantly in Layers IV to V before birth and in Layer IV after birth (Figures: Supplemental Digital Content 1, <http://links.lww.com/NEN/A357>; Supplemental Digital Content 2, <http://links.lww.com/NEN/A358>; Supplemental Digital

Content 3, <http://links.lww.com/NEN/A359>; Supplemental Digital Content 4, <http://links.lww.com/NEN/A360>).

Distribution and Densities of Cells Expressing Layer-Specific Markers

In FCD IIA and FCD IIB, the layer-specific marker-positive cells were diffusely localized in the cortex; there were few positive cells in the WM (Figs. 2–4). In FCD IIA, CUTL1-positive and FOXP2-positive cells were generally more numerous than cells expressing the other markers (Fig. 2). Only TBR1-positive cells were not in the CxMoL. In FCD IIB, all marker cells were also widely distributed, although there were relatively few CTIP2-positive cells (Fig. 3). CUTL1-positive, FOXP1-positive, and FOXP2-positive cells were numerous in both the cortex and the WM.

Densities of TBR1-positive and FOXP1-positive cells tended to be higher in FCD IIB than those in FCD IIA (Figs. 4A, D). CTIP2-positive and SATB2-positive cell densities were not different between FCD IIA and FCD IIB

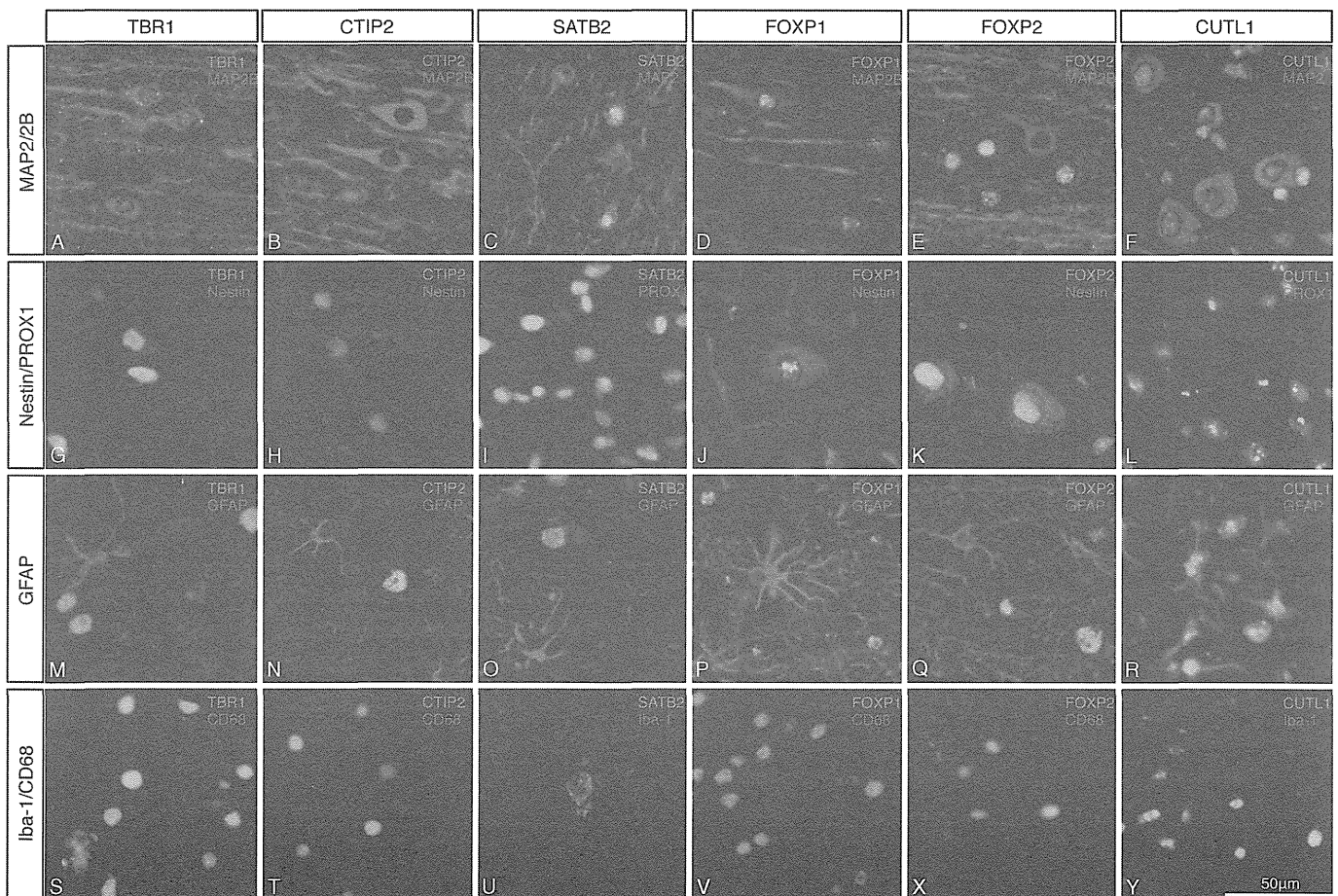


FIGURE 5. Types of cells expressing layer-specific markers in focal cortical dysplasia (FCD) IIA. (**A–F**) All cells expressing layer-specific markers express microtubule-associated protein (MAP) 2 or 2B. (**G–L**) Forkhead box P1 (FOXP1)-positive, Forkhead box P2 (FOXP2)-positive, and Cut-like homeobox 1 (CUTL1)-positive cells also express the immature neuron markers, Nestin or Prospero homeobox protein 1 (PROX1) (**J–L**), whereas cells expressing the other markers do not (**G–I**). (**M–R**) Only CUTL1-positive cells express glial fibrillary acidic protein (GFAP) (**R**). (**S–Y**) Layer-specific marker-positive cells do not express Iba-1 or CD68.

(Figs. 4B, C). In the cortex, the densities of FOXP2-positive and CUTL1-positive cells in FCD IIA tended to be higher than those in FCD IIB (Figs. 4E, F); however, in the WM, FOXP2-positive and CUTL1-positive cell densities in FCD IIB were higher than those in FCD IIA (Figs. 4E, F). White matter densities of FOXP1-positive and FOXP2-positive cells in FCD IIB were greater than those in FCD IIA (Figs. 4D, E).

Phenotypes of Cells Expressing Layer-Specific Markers

To investigate the phenotypes of cells expressing layer-specific markers, we performed double staining with mature neuron, immature or progenitor neuron, glial cell, and microglia markers. In both FCD IIA and FCD IIB, all TBR1-positive, CTIP2-positive, and SATB2-positive cells expressed MAP2 and/or MAP2B (MAP2/2B) but not Nestin, PROX1, or GFAP (Table 2). There were no Iba1- or CD68-positive cells that expressed the layer-specific markers (Figs. 5 and 6 and Table 2).

In FCD IIA, mean MAP2/2B-positive cell concentrations ranged from 20.0% to 30.3% among the layer-specific marker cells. FOXP1-positive, FOXP2-positive, and CUTL1-positive cells also expressed Nestin or PROX1 (Nestin/PROX1; Figs. 5J–L and Table 2). Mean Nestin/PROX1-positive cell concentrations ranged from 13.3% to 26.2%. FOXP1-positive and FOXP2-positive cells did not express GFAP, but some CUTL1-positive cells expressed GFAP (18.0% in all CUTL1-positive cells; Fig. 5R).

In FCD IIB, mean MAP2/2B-positive cell concentrations were 32.2%, 23.5%, and 11.7%, respectively, for FOXP1-positive, FOXP2-positive, and CUTL1-positive cells. Mean Nestin/PROX1-positive cell concentrations were 46.7%, 36.6%, and 59.1%, respectively (Fig. 6 and Table 2). Thus, there were more Nestin/PROX1-positive cells in FCD IIB than in FCD IIA. A few FOXP1-positive cells in FCD IIB expressed GFAP but more numerous FOXP2-positive and CUTL1-positive cells expressed GFAP (Figs. 6P–R). The mean concentrations of GFAP-expressing cells were 23.2% and 28.3% of cells, respectively.

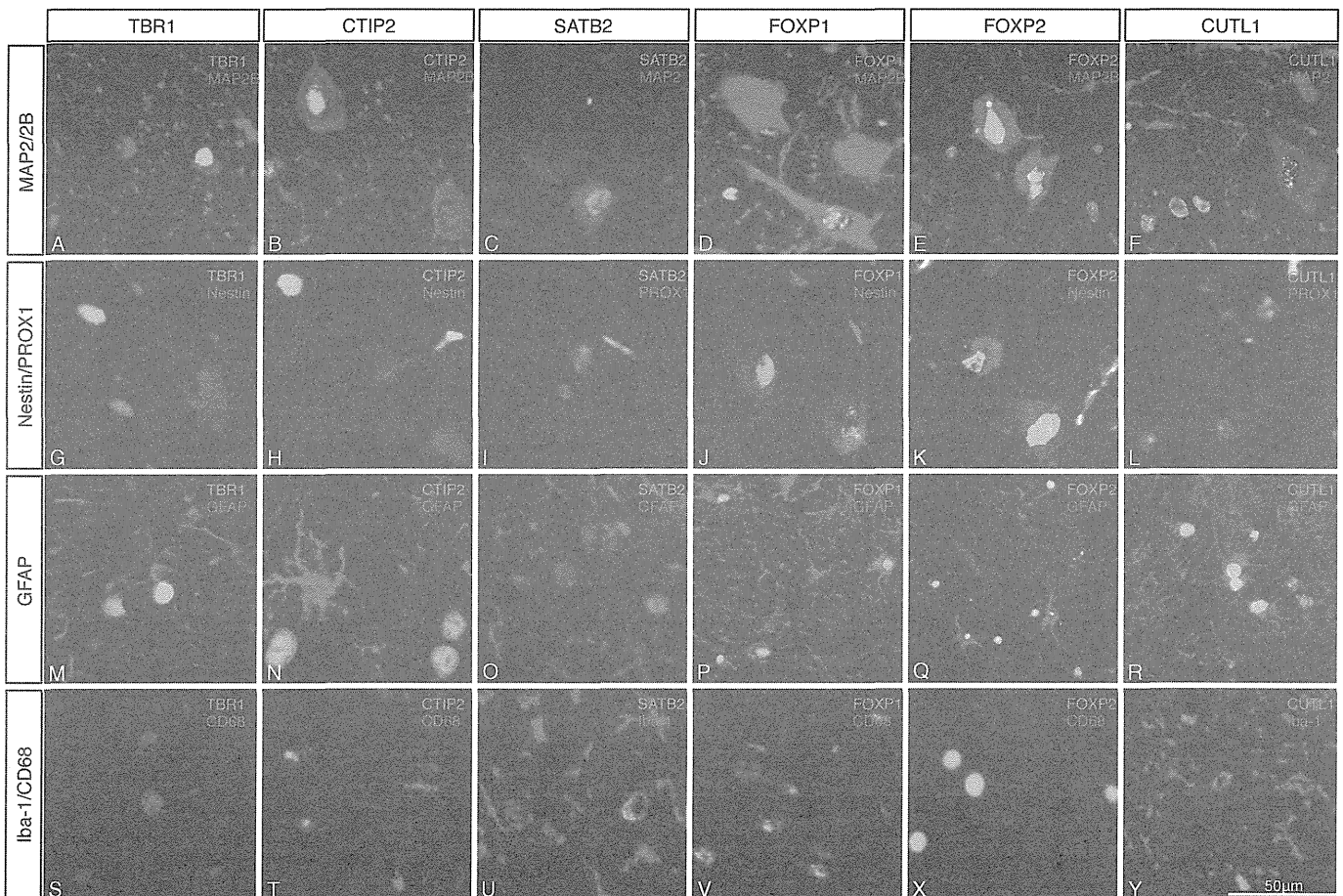


FIGURE 6. Types of cells expressing layer-specific markers in focal cortical dysplasia (FCD) IIB. **(A–F)** All cells expressing layer-specific markers express microtubule-associated protein (MAP) 2 or 2B. **(G–L)** Forkhead box P1 (FOXP1)-positive, Forkhead box P2 (FOXP2)-positive, and Cut-like homeobox 1 (CUTL1)-positive cells also express the immature neuron markers, Nestin or Prospero homeobox protein 1 (PROX1) (J–L), whereas cells expressing the other markers do not (G–I). **(M–R)** FOXP1-positive, FOXP2-positive, and CUTL1-positive cells express glial fibrillary acidic protein (GFAP) (P–R). **(S–Y)** Layer-specific marker-positive cells do not express Iba-1 or CD68.