DISCUSSION

Transmantle dysplasia is a very rare malformation of FCD, but its pathologic features are the same as those of FCD IIA or FCD IIB. Although very little is known about TD, this study may contribute to a new pathophysiologic understanding of FCD malformations that extend from the ventricular wall to the cortical surface. Our results indicate that cells expressing postnatal layer-specific markers are abnormally present and continuously distributed from deep WM to the cortical surface in TD. As previously described, neurons in these FCD lesions expressed layer-specific markers indicating immaturity (19). Moreover, we identified some specific marker expression patterns that differed between FCD IIA and FCD IIB pathologies.

TBR1-positive, CTIP2-positive, SATB2-positive, and FOXP1-positive cells, which are originally located in the deep cortex during the fetal period (10), were diffusely observed in the cortex and/or WM. In particular, TBR1-positive and FOXP1-positive cells in FCD IIB cases were mostly in the CxMoL, CxUL, and DWM, to a greater degree than in FCD IIA cases. This suggests that the malformation and pathologic onset of FCD IIB are more severe and earlier than those in FCD IIA. In mouse brain, Tbr1 is identified from E11.5 and is expressed at a low level in the upper layer and at high levels in the subplate and Layer VI, which extend axons to the thalamus and form callosal projections (14). Satb2 serves to form callosal projections (12). The FCD IIB malformation may be formed in the early fetal period and express immature markers. SATB2 is also known as a repressor of CTIP2 (12). However, our data show that the CTIP2-positive cell distribution is similar to that of SATB2. In the FCD brain, there may be different molecular mechanisms between SATB2 and CTIP2. On the other hand, the presence of many TBR1-positive and FOXP1-positive cells in DWM of FCD IIB might indicate a neuronal migration arrest in the very early fetal period.

The CUTL1-positive cell distribution pattern was similar to that of FOXP2-positive cell. In the cortex, concentrations of cells expressing these 2 markers tended to be larger in FCD IIA than those in FCD IIB, whereas the pattern was reversed in WM. In FCD IIB, the large numbers of CUTL1-positive and FOXP2-positive cells were in the ScWM, MWM and DWM, although there were no significant differences between FCD IIA and IIB, except for CUTL1 in MWM. In mouse brain, Cutl1 is expressed in the upper layer of the cortex from E16 and regulates late neuronal differentiation, such as dendrite development, spine formation, and synaptic function (15, 21). Persistent CUTL1 expression in FCD II suggests delayed synaptic maturation. Recently, *FOXP2*, a causative gene of developmental verbal dyslexia, was reported to negatively regulate sushi-repeat protein SRPX2 and plasminogen activator (uPAR), whose mutations lead to Rolandic epilepsy or perisylvian polymicrogyria (22). We speculate that FOXP2 overexpression contributes to regional cortical dysplasia via abnormal down-regulation of the SRPX2/uPAR complex.

Putative upper layer marker cells, FOXP1, FOXP2, and CUTL1, expressed the immature neuron marker Nestin/PROX1, whereas cells expressing the putative lower layer markers TBR1, CTIP2, and SATB2 only expressed MAP2/2B. This suggests that the cells expressing the putative upper layer markers may maintain their neuronal immaturity. Many CUTL1-positive, FOXP1-positive, and FOXP2-positive cells contained GFAP. Although we do not know the relationship of these markers to glial cell development, these findings may indicate abnormal neuronal progenitor cell differentiation into both the neuron and glial cells.

In conclusion, we demonstrate that neurons of FCD with TD had delayed maturation and abnormal differentiation. The remaining abnormal expression of various layer-specific markers in TD is evidence of immature neuronal differentiation in these cortical malformations. The present study may make it possible to clarify differences between FCD IIA and FCD IIB based on their layer-specific marker expression patterns.

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Imbalance of interneuron distribution between neocortex and basal ganglia: Consideration of epileptogenesis of focal cortical dysplasia

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ABSTRACT

Aim: The balance of excitation and inhibition of neurons and neuronal network is very important to perform complete neuronal function. Damage or loss of inhibitory γ-aminobutyric acid (GABA)-ergic interneuron is associated with impaired inhibitory control of cortical pyramidal neurons, leading to hyperexcitability and epileptogenesis. Ectopic neurons in the basal ganglia are to be one of the pathological features of epileptogenesis. In the present study, we investigated distribution of interneuron subtypes between neocortex and caudate nucleus.
Methods: We performed immunohistochemistry of GABA, glutamic acid decarboxylase (GAD), calretinin (CR), calbindin (CB), parvalbumin (PV) and neuropeptide. We used surgical materials of four focal cortical dysplasia (FCD) cases, having lesions of neocortex and caudate nucleus, and eight age-matched autopsy controls.
Results: The pathology showed three FCD IIa, containing dysmorphic neurons, and one FCD IIb, balloon cells. In the neocortex, the concentrations (each positive cell number/all cell numbers in the evaluated field) of GAD+, CR+ and CB+ cells were significantly lower in FCD than in controls. On the contrary, in the caudate nucleus those of CR+ and CB+ cells were significantly more in FCD than in controls.
Conclusion: The interneuron imbalance between the neocortex and basal ganglia may affect the epileptogenesis of FCD.

1. Introduction

To perform complete neocortical function, it is very important to have a balance between excitation and inhibition of neurons and neuronal network. Human neocortex mainly consists of projection neurons (excitatory) and interneurons (inhibitory), and glial cells. We know that approximately 35% of neocortical interneurons originate from the neocortical ventricular zone [1]. Also, 65% of them derive from the ganglionic eminence. Interneurons are characterized by the γ-aminobutyric acid (GABA) they contain and are divided by several specific markers, such as glutamic acid decarboxylase (GAD), calretinin (CR), calbindin (CB), parvalbumin (PV) and neuropeptide Y (NPY) [1].

It is thought that epilepsy results from molecular changes in glutamate and GABA receptors of aberrant neurons, causing a functional imbalance characterized by increased excitation and decreased inhibition [2,3]. Damage or loss of inhibitory GABAergic interneuron is associated with impaired inhibitory control of cortical pyramidal neurons, leading to hyperexcitability and epileptogenesis [3,4]. Malformations of cortical development (MCDs) are increasingly recognized as an underlying pathology in children with medically intractable epilepsy [5]. Focal cortical dysplasia (FCD), a distinct group of MCDs, is characterized mainly by disruption of the laminar architecture and/or the presence of specific abnormal cells [6,7]. The exact mechanism of epileptogenicity in FCD has not been elucidated so far. However, it may be based on the imbalance between the excitatory and inhibitory neuronal circuits, which seems to play an important role in the initiation and spread of epileptic seizures [8]. It is of considerable interest that the basal ganglia with dysmorphic neuron of FCD have been associated with epileptogenesis [9]. Recently, we reported that deep structure resection

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improved seizure control and resulted in no motor deficit after operation [9,10].

Although the number of inhibitory interneurons was decreased in the neocortex of FCD, very few studies of basal ganglia of FCD have been reported [11,12]. In the present study, we demonstrated the correlation of each interneuron subtype distribution in the neocortex and basal ganglia, using interneuron markers of GAD, CR, CB, PV and NPY. Our data may well provide a basic understanding of FCD epileptogenesis.

2. Materials and methods

2.1. Sample preparation

All cerebral tissues used in the present study were approved for research usage by the parents and ethical committees of the hospital and institute. Four cases of intractable epilepsy were selected for this study, with FCD and abnormal signals in the deep white matter on the imaging, and surgical resection of the lesions containing neocortex and deep cerebral structures. Their clinical features are summarized in Table 1. Average age of onset was 8.3 months of age, and age at surgical resection ranged from 3 months to 13 years. Studied samples were part of the tissues removed for therapeutic reasons after careful assessment of the epileptogenic areas, determined by analysis of seizure semiology, ictal and interictal electroencephalography, magnetoencephalography, electrocorticogram, MRI, fluorodeoxyglucose positron emission tomography and interictal single photon emission computed tomography. We then performed resection of the lesions including neocortex, white matter and deep brain structure. We pathologically evaluated the serial section including malformed cortex and the underlying caudate nucleus. The age-matched controls used were summarized in Table 1. After resection, all brains were fixed in 10% buffered formalin or 4% paraformaldehyde and embedded in paraffin. The serial sections were cut 4–6 μm thickness for histological and immunohistological examination. For pathological diagnosis, the sectioning tissues were stained by hematoxylin and eosin (HE) and Klüver–Barrera (KB) methods. The tissues diagnosed as FCD by two individual neuropathologists were classified by ILAE classification [7]. Two parts of the resection tissue, neocortex and basal ganglia, were analyzed on immunohistochemistry.

2.2. Immunohistochemistry

Our immunohistochemistry technique was previously described in detail [13]. The primary antibodies were incubated at 4 °C for 16–72 h. To investigate the presence of interneuron in the neocortex and caudate nucleus of the basal ganglia, we used the mouse monoclonal antibodies against Calretinin (CR, clone5A5, dilution of 1:100, Thermo Fisher Scientific Anatomical Pathology, Fremont, CA) and Calbindin D-28K (CB, clone CB955, 1:300, Sigma, St. Louis, MO), glutamic acid decarboxylase (GAD, 1:100, Enzo life Sciences, Plymouth Meeting, PA), parvalbumin (PV, 1:500, Sigma), and the polyclonal rabbit antibody against Neuropeptide tyrosine (NPY, 1:100, Phoenix Pharmaceuticals, Burlingame, CA).

2.3. Comparison of immunopositive cell counts between cortex and basal ganglia

Various immunopositivities of cells comprising the neocortex and caudate nucleus were observed in all cases. The number of immunopositive cells, excluding glial cells and endothelial cells, was counted in 5 fields of each region (neocortex and caudate nucleus) at a magnification of 200 times. The number was corrected per 100 nuclei in each region for each case as the immunopositive cell density. For statistical analysis, Student-*t* test was used for comparison between two parts. Student-*t* test was applied using statistical software (SPSS; SPSS Inc., Illinois, USA) at a significance level of $P<0.05$.

3. Results

All FCD cases were suspected by MRI and the other examinations before surgery and resected lesions (Fig. 1). We examined neurological and mental status before and after operation. As a result, no changes were found in all patients. The neuropathology of our cases showed abnormal lamination of the cortices, dysmorphic neurons and/or balloon cells, some heterotopic neurons in the white matter, and some normal-looking neurons, and was diagnosed as FCD IIa or IIb (Table 1, Fig. 2).

The resected caudate nucleus histologically identified the small number of dysmorphic neurons or balloon cells (Fig. 2C and F). Some neurons in the neocortex and caudate nucleus displayed GAD+, CR+, CB+, PV+ and NPY+ in each case (Fig. 3). In the neocortex, the concentration of each cell type was less in FCD than in controls (Fig. 4). The concentrations of GAD+, CR+ and CB+ cells showed significant differences between FCD and controls. That of GAD+ cells was $4.8\pm0.8\%$ (average ± standard deviation) in controls and $1.8\pm0.6\%$ in FCD ($P=0.0194$). That of CR+ cells was $10.6\pm4.3\%$ in controls and $2.8\pm2.2\%$ in FCD ($P=0.0472$). That of CB+ cells was $6.0\pm2.2\%$ in controls and $2.8\pm2.1\%$ in FCD ($P=0.0385$). That of PV+ cells was $7.6\pm6.5\%$ in controls and $3.6\pm2.7\%$ in FCD ($P=0.6311$), while that of NPY+ cells was $0.7\pm0.6\%$ in controls and $0.2\pm0.1\%$ in FCD ($P=0.1802$).

Table 1
Clinicopathological profiles of FCD patients and age-matched control.

Case		Sex	Age		Lesion	Pathology	Seizure	Intelligence (DQ or IQ)	Cause of death
			Onset	Surgery					
FCD	1	f	11M	13Y	F	FCD IIa	GTC	43	
	2	f	10M	5Y9M	F	FCD IIa	GTC	59	
	3	m	0M	3M	F	FCD IIa	GTC	ND	
	4	m	1Y	1Y1M	F	FCD IIb	CPS	91	
CTL	1	m	0M			BE, SAH		ND	Neonatal asphyxia
	2	f	2M			SAH		ND	CHD
	3	m	2M			HIE, PE, PVL		ND	BPD, pneumonia
	4	f	6M			PVL, HM		ND	SIDS
	5	m	1Y			BE, HE		ND	DIC, hyperlactemia
	6	f	1Y4M			SAH, SDH		ND	Dehydration, diarrhea
	8	f	2Y			BE, HE		ND	HUS
	7	f	8Y			CbI		ND	ALL

m; male, f; female, Y; year(s), M; month(s), GTC; generalized tonic–clonic seizure, CPS; complex partial seizure, IQ; intelligent quotient, DQ; developmental quotient, F; frontal lobe, FCD; focal cortical dysplasia, CTL; control, ND; not done, Pathological diagnosis was due to the recent international classification (see in text). BE; brain edema, SAH; subarachnoid hemorrhage, HIE; hypoxic–ischemic encephalopathy, PE; porencephaly, PVL; periventricular leukomalacia, HM; hypomyelination, HE; hemorrhage, SDH; subdural hemorrhage, CbI; cerebellar infarction, CHD; congenital heart disease, BPD; bronchopulmonary dysplasia, SIDS; sudden infant death syndrome, HUS; hemolytic uremic syndrome, ALL; acute lymphoblastic leukemia.

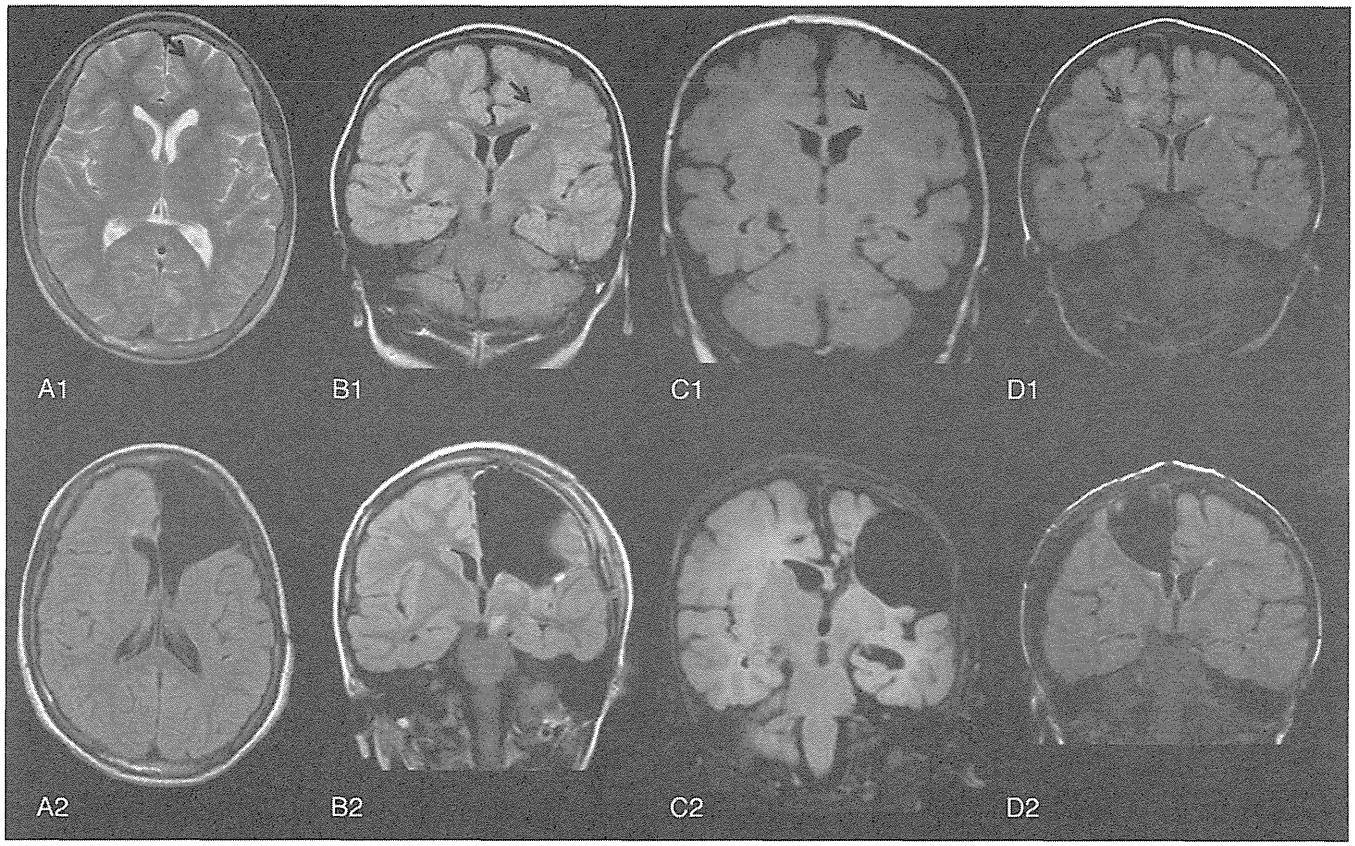

Fig. 1. MRI of pre-operation and post-operation of subjects. A1 is pre-operation MRI, axial view/T2-weighted image, and A2 is post-operation, axial view/FLAIR image of case 1 in Table 1. B1 is pre-operation MRI, coronal view/FLAIR image, and B2 is post-operation, coronal view/FLAIR image of case 2 in Table 1. C1 is pre-operation MRI, coronal view/FLAIR image, and C2 is post-operation, coronal view/FLAIR image of case 3 in Table 1. D1 is pre-operation MRI, coronal view/FLAIR image, and D2 is post-operation, coronal view/FLAIR image of case 4 in Table 1. Arrows indicate abnormal signals in the white matter and/or neocortex. In B1, there is definitive laterality of the caudate nuclei.

On the contrary, in the caudate nucleus the concentration of each cell type was higher in FCD than in controls (Fig. 4). The concentrations of CR+ and CB+ cells showed significant differences between FCD and controls. The GAD+ cell level was $3.0 \pm 2.1\%$ in controls and $4.2 \pm 2.8\%$ in FCD ($P = 0.5409$). The CR+ cell level was $1.2 \pm 0.4\%$ in controls and $4.2 \pm 2.6\%$ in FCD ($P = 0.0475$). The CB+ cell level was $0.1 \pm 0.2\%$ in controls and $7.6 \pm 4.1\%$ in FCD ($P = 0.0017$). The PV+ cell level was $1.8 \pm 2.0\%$ in controls and $4.1 \pm 3.2\%$ in FCD ($P = 0.2583$). The NPY+ cell level was $0.6 \pm 0.3\%$ in controls and $0.7 \pm 0.4\%$ in FCD ($P = 0.2386$).

4. Discussion

Neocortical interneurons play an important role in modulating neocortical plasticity and output [14]. GABAergic interneurons appear to perform important regulatory functions on developmental processes of neuronal migration, proliferation, and the postnatal development of cortical circuitry [15]. GABAergic inhibitory interneurons are morphologically distinct from excitatory pyramidal cells and account for 20–25% of all neocortical neurons. In the cortical dysplasia of epilepsy patients, there were reduced numbers of CR+ and PV+ interneurons [12,16,17]. Recent studies reported that several genes were involved in the reduction of the interneurons in the cortex [13]. Mice lacking the transcription factors responsible for regulation of differentiation (*Dlx1*, *Dlx2* or *Mash1*) and regionalization (*Nkx2.1*) in the basal telencephalon have reduced numbers of cortical GABAergic interneurons at the time of birth [18]. It is known that mice with targeted mutation of the gene encoding urokinase plasminogen activator receptor have a 50% reduction in neocortical GABAergic interneurons at embryonic and perinatal ages. This strain exhibits spontaneous seizure activity and higher susceptibility to pharmacologically induced convulsions [19]. *Dlx1*-lacking mutant mice showed CR- and somatostatin (SST)-positive interneuron loss in neocortex and hippocampus, cortical dysrhythmia, and generalized electrographic seizures [20]. Reduction of GABAergic interneurons may directly result in epileptogenesis. GABA is also co-localized with CB, CR and NPY. CB and CR belong to the large family of calcium-binding proteins (CBPs), which are characterized by the presence of a variable number of helix–loop–helix motives binding Ca^{2+} ions with high affinity. These proteins are involved in regulating calcium pools important for synaptic plasticity [21]. Neuropeptides differ from classical neurotransmitters in size, synthesis and mechanism of action. GABAergic interneurons co-localized with one or more neuropeptides are specifically targeted by serotoninergic and catecholaminergic afferents. NPY+ neurons in the neocortex are GABAergic, medium-sized, spiny and exhibit ultrastructural characteristics typical for neurons producing and releasing peptides.

In previous studies, the number of inhibitory neurons in patients with intractable epilepsy and mammalian models shows a variable aberrant pattern, such as a decreased or increased number of GABAergic interneurons [3,22]. Interestingly, approximately 35% of neocortical interneurons in the human brain originate in the subventricular zone [1]. Moreover, mutation of ARX, an initiation factor of interneuron migration, demonstrated many residual GABAergic interneurons in the white matter and striatum of the human brain [13]. Development of interneurons occurs from deep structures. Many cortical GABA-containing interneurons originate in the subpallial telencephalon and migrate tangentially to reach their final destination [23]. CR-containing

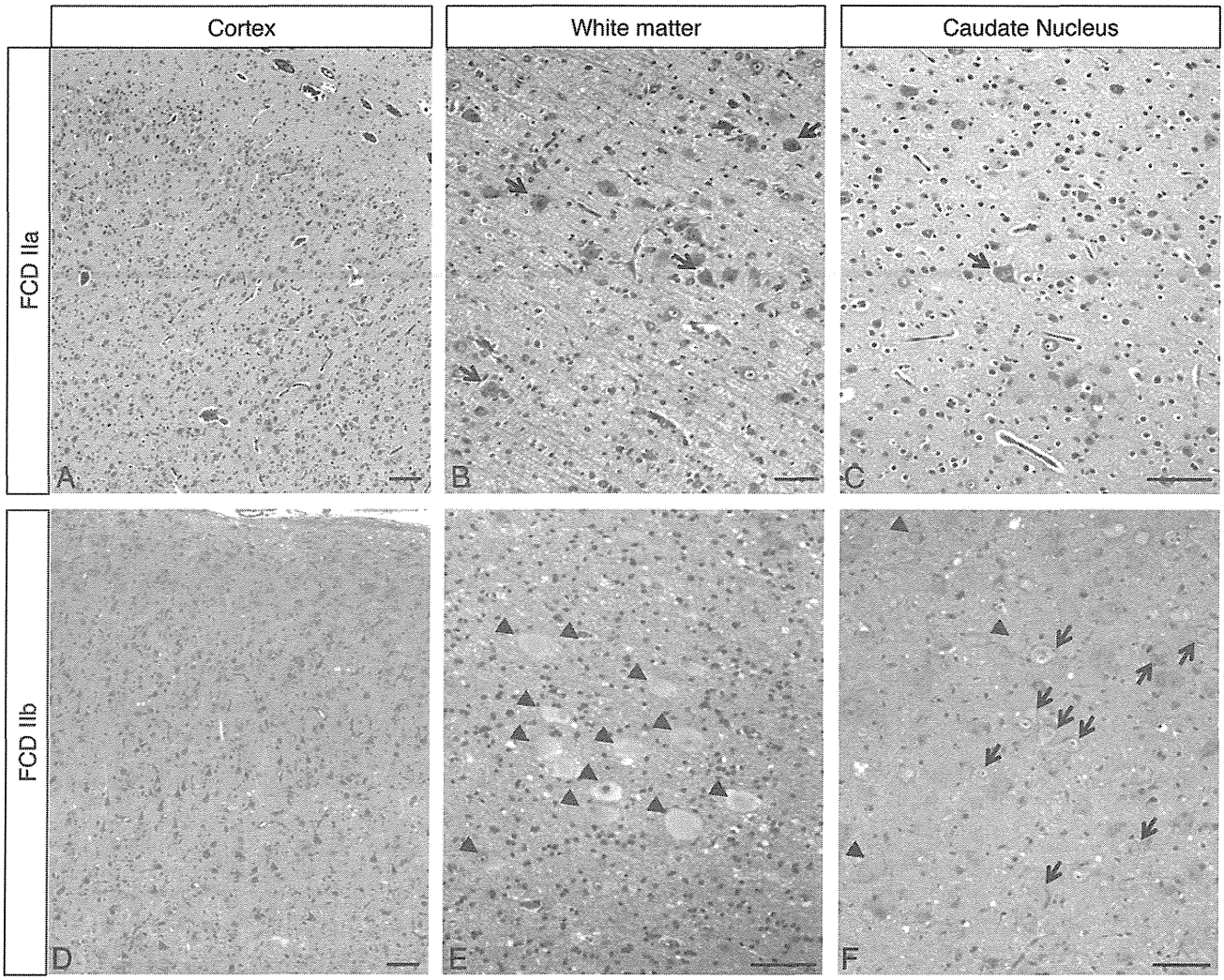

Fig. 2. Characteristic pathology. A shows unlayer neocortex and some large dysmorphic neurons (arrows) (case 2 in Table 1). B exhibits many heterotopic neurons in the white matter, which are variously sized and disorientated (the same case of A). C shows some dysmorphic neurons in the caudate nucleus (the same case of A). D, E and F are the neocortex, white matter and caudate nucleus of case 4 in Table 1, respectively. D shows unlayer neocortex and numerous disoriented neurons. E reveals many balloon cells (arrowheads). F demonstrates not only balloon cells (arrowheads) but also many dysmorphic neurons (arrows). Each scale bar indicates 100 μm.

interneurons primarily arise from the dorsal caudal ganglionic eminence. SST- or PV-containing interneuron progenitors primarily migrate from the Nkx2.1-expressing domain of the medial ganglionic eminence [24].

A factor which directs interneurons to the cortex or the striatum on migration, was discovered recently. Migrating interneurons expressing neuropilins, receptors for semaphorins, are directed to the cortex; those lacking them go to the striatum. Loss of neuropilin function increases the number of interneurons that migrate into the striatum [25]. Our results demonstrated that interneurons in patients were fewer in the cortex and more numerous in the striatum compared with controls. Interestingly, even our small case number indicated a significantly different distribution of interneurons between the neocortex and deep gray matter. The results suggest the dysfunction of interneuron migration factors in our cases. The abnormalities in the numbers of interneurons in the striatum would also serve to explain the pathophysiology in terms of striatal disorders. Besides a decreased number of interneurons in the cortex, an abnormally increased number of interneurons in the striatum may be related to the epileptogenesis of cortical dysplasia. Normal-looking neurons of our cases may have abnormalities of neuronal maturation and differentiation [17]. The recent experimental study in rat hippocampal CA1 slices demonstrated that the prototypic form of seizure activity was driven by fast-spiking interneurons [26]. The previous study suggested that fast-spiking network alone could drive the prototypic form of electrically-induced seizure-like oscillations through their excitatory GABAergic transmissions through gap junction-mediated communications [26]. Meanwhile, partial removal of the striatum with the cortical margin and the insular cortex in the present cases, especially at the reoperations, resulted in seizure freedom. Therefore, the imbalance of striatal and neocortical interneurons might be one of the causes of epileptogenesis.

In this study, the density of CR+ and CB+ interneurons had a significant increase in the caudate nucleus, but decreased in the neocortex. Surprisingly, the rate of GAD+ and PV+ interneurons also evidenced the same pattern of those of CR+ interneurons unless there was a significant difference. On the other hand, the rate of NPY+ interneurons exhibited no change between cerebral cortex and basal ganglia. We could not elucidate the reasons why the density of CB+ interneurons is higher in the FCD than in controls. From our data, the abnormal CBPs+ interneurons might influence not only the neocortex but also the basal ganglia. Recently, dysfunction and decreased number of interneurons are known to play an important role in epileptogenesis [27,28]. We speculate that the imbalance of

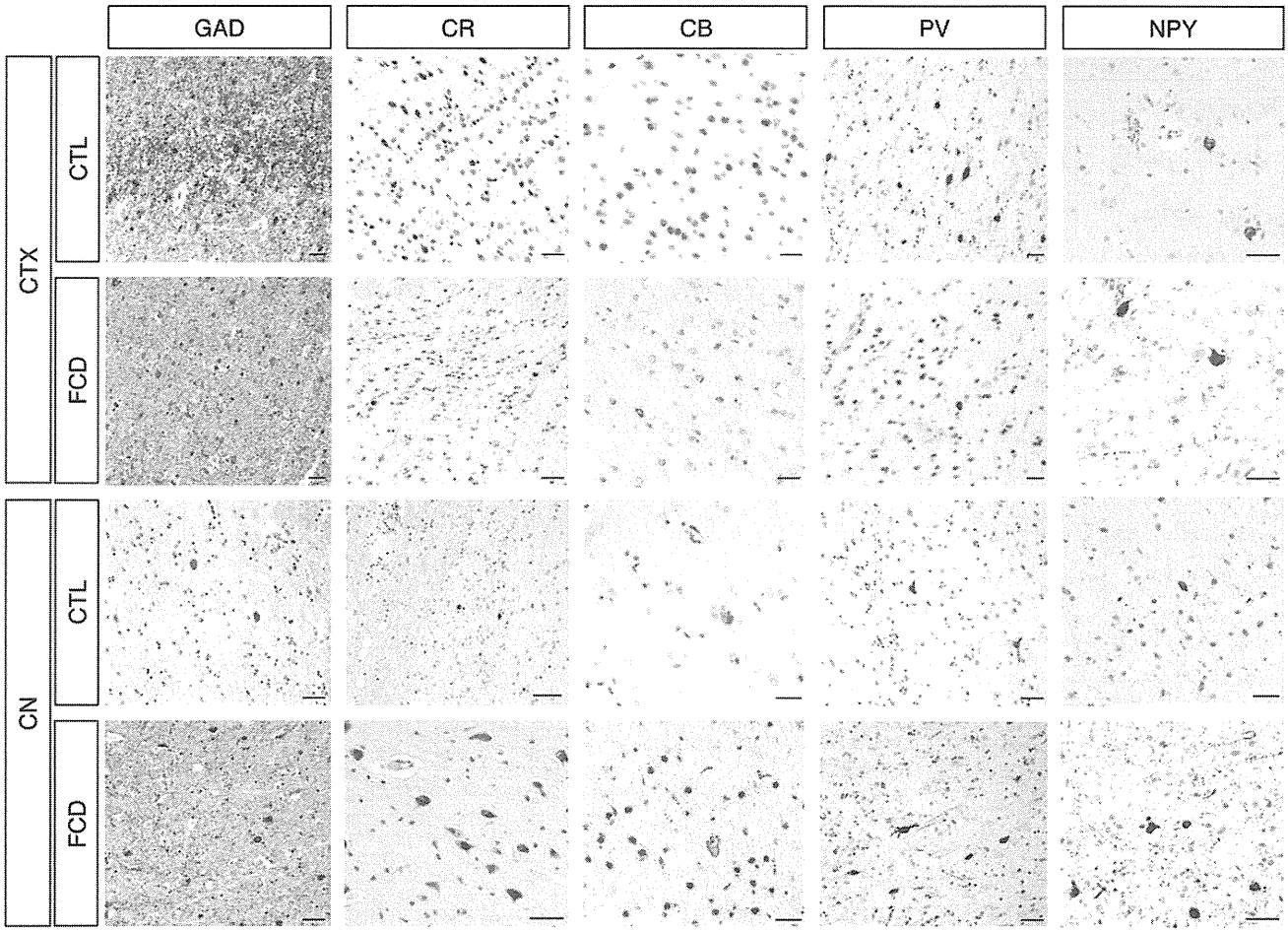

Fig. 3. Immunoreactivities for interneuron subtype markers. In neocortex, the numbers of immunoreactive cells of controls are relatively more than those of FCD. On the contrary, in caudate nucleus, the numbers of immunoreactive cells of controls are relatively less than those of FCD. Moreover, we noticed that immunoreactive cells of FCD are generally small in size. Each scale bar indicates 100 μm.

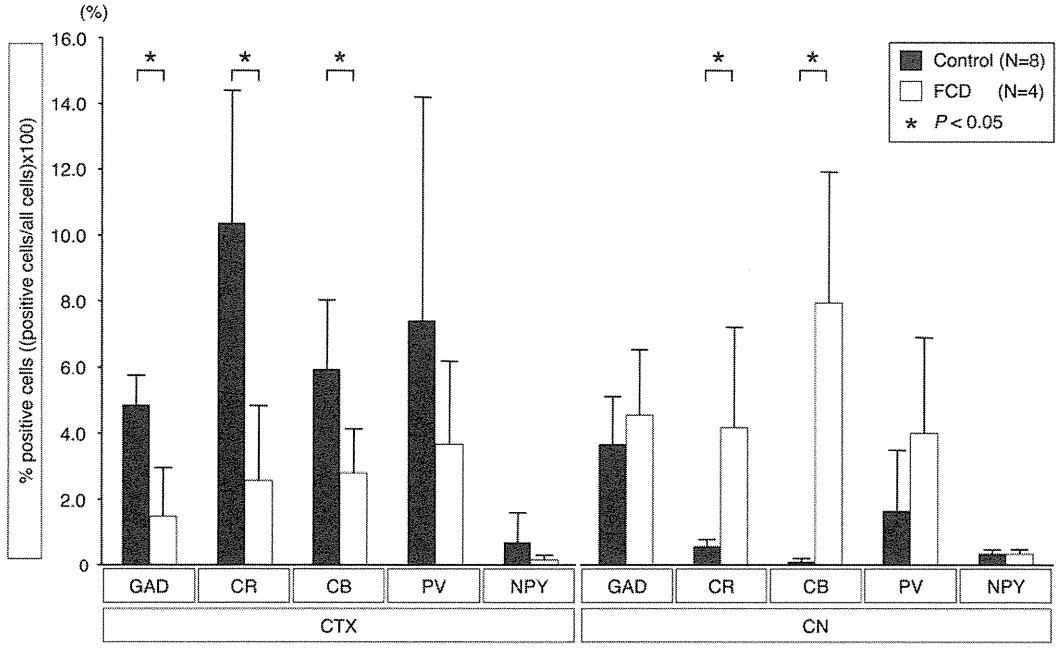

Fig. 4. Correlation between controls and FCD cases of interneuron subtype marker expression. In neocortex, there are significant differences in GAD+, CR+ and CB+ cell concentrations between controls and FCD. On the contrary, CR+ and CB+ cell concentrations in the caudate nucleus show a reverse significant difference.

interneurons, especially in the CBP-positive interneurons, may affect the epileptogenesis of FCD.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Novel pathological abnormalities of deep brain structures including dysplastic neurons in anterior striatum associated with focal cortical dysplasia in epilepsy

Clinical article

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Object. Some patients are not seizure free even after epileptogenic cortical resection. The authors recently described a case of frontal lobe epilepsy cured after the resection of periventricular white matter and striatum, in which dysplastic neurons were revealed. The authors attempted to confirm similar cases.

Methods. They reviewed the records of 8 children with frontal lobe epilepsy who had daily (7) or monthly (1) seizures and underwent resections including deep brain structures.

Results. Five patients underwent multiple resections. Neuroimaging of the deep structures showed the transmantle sign in 3 patients, ictal hyperperfusion in 6, reduced iomazenil uptake in 2, and spike dipole clustering in 6. All patients became seizure free postoperatively. Focal cortical dysplasia of various types was diagnosed in all patients. Dysmorphic neurons were found in the cortex and subcortical white matter of 5 patients. The striatum was verified in 3 patients in whom dysmorphic neurons were scattered. In the periventricular white matter, prominent astrocytosis was evident in all cases.

Conclusions. Pathological abnormalities such as dysmorphic neurons and astrocytosis in deep brain structures would play a key role in epileptogenesis.

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Key Words • **epilepsy** • **deep white matter** • **striatum** • **dysmorphic neuron**

Epilepsy is one of the major neurological disorders that involves seizures and affects numerous people of all ages worldwide. The prevalence of epilepsy in the US, Europe, and Asia is 5–9 cases per 1000 individuals.[3] Malformations of cortical development, such as FCD, are the main causes of refractory epilepsy.[21] Surgical removal is generally useful in treating medically refractory epilepsy.

Identifying the epileptogenic lesion is necessary for deciding on radical treatment for refractory focal epilepsy. Multiple imaging and monitoring modalities, such as EEG, video-EEG monitoring, MRI, SPECT, PET, and MEG, can be used to evaluate the pathogenesis of refractory epilepsy. Chronic and/or intraoperative ECoG is also an important approach in identifying epileptic discharges when deciding on a resection area.

However, some patients, despite undergoing preoperative examinations, do not become seizure free after resection of an assumed epileptogenic cortical lesion, and reoperation may be necessary. In many cases of reoperation for focal epilepsy, the postoperative cortical margins are subjected to additional resections after reexamination. Seizures recur in a small percentage of these patients even after reoperation, and the assumed epileptogenic lesion is frequently seen on the cortical margin.

Abbreviations used in this paper: ECoG = electrocorticography; EEG = electroencephalography; FCD = focal cortical dysplasia; GABA = γ-aminobutyric acid; MEG = magnetoencephalography.

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Recently, epileptogenic lesions have been extensively explored in cases of medically refractory focal epilepsy. Lesions of the insular cortex[16] and periventricular heterotopia[1] are foci of nonsuperficial epileptogenicity. A number of electrophysiological and histopathological studies[1,16] have confirmed epileptogenicity in the insular cortex and periventricular area. We recently described our earlier experiences with 2 cases of frontal lobe epilepsy that were cured without incurring any neurological deficit after resection of the periventricular white matter[15] and striatum[11] following prior resections. Both cases revealed pathological abnormalities and suggested possible epileptogenicity in the deep brain structures, which have not been reported as regions of epileptogenic lesions. A remaining deep lesion can become epileptogenic. Neurons generated in the neocortical ventricular zone migrate radially along glial cell guides to the cortex, and interneurons generated in the proliferative zone of the striatum migrate tangentially to the cortex.[13] Dysplastic neurons can also originate from these deep structures and some may migrate to the cerebral cortex while others may stay ectopically in the white matter and become involved in the potentially epileptogenic circuit.[2] From the viewpoint of cortical development, we hypothesized that cases with dysplastic neurons in deep structures other than those previously described can exist.

To validate our hypothesis, we searched for similar cases of epileptogenesis in the deep brain structures. The "epileptogenic zone" is defined as the brain region(s) that generates seizures.[22] The surgical suppression of seizures along with histological abnormalities in the resected lesion should be necessary to prove the epileptogenicity of the lesion. Surgical cases of frontal lobe epilepsy were investigated for epileptogenicity of deep brain structures, for example, the striatum and periventricular white matter.

Our aim in the present study was to discuss epileptogenesis in deep brain structures in a consecutive series of pediatric patients with malformations of cortical development who demonstrated frontal lobe epilepsy after the resection of deep brain structures. Note that there are several limitations to this study, that is, a small number of analyzed cases and a retrospective study design.

Methods

The institutional review board of the National Center of Neurology and Psychiatry approved our study, and the parents of all patients provided written informed consent.

We retrospectively reviewed the medical records of patients with extratemporal lobe epilepsy who had been treated with resection in our department between October 2006 and May 2010. Potential candidates for surgery for medically refractory frontal lobe epilepsy were examined by epileptologists, and they underwent interictal EEG (8 patients), video-EEG monitoring (8 patients), brain MRI (T1-weighted, T2-weighted, and FLAIR sequences; 8 patients), interictal (8 patients) and ictal (7 patients) technetium-99m ethyl cysteinate dimer SPECT, subtraction ictal SPECT coregistered with structural MRI (7 patients), ^{123}I-iomazenil SPECT (4 patients), PET (7 patients), and MEG (6 patients). Magnetoencephalography was performed using a 204-channel MEG system (Vectorview, Neuromag Co.). Our general criteria for resecting deep brain structures in patients were as follows: 1) frequent medically refractory seizures with strong semiology of the lesion; 2) potent pre- and/or intraoperative findings that suggested epileptogenicity in deep brain structures, such as stereo-EEG spikes, hyperperfusion in SPECT, and spike dipoles on MEG; 3) probable pathological diagnoses of developmental malformations of the brain; and 4) pediatric cases. We regarded the transmantle sign, which is a signal abnormality of the white matter linking the gray matter and the ventricle, as proof of dysmorphic neurons. We included the age criterion because plasticity in the pediatric brain is so great that a severe postoperative neurological complication would rarely occur.[10] For example, a previous article reported that 6 children, ages 7–14 years, underwent left hemispherectomy for Rasmussen syndrome, and their receptive functions were comparable with or surpassed their presurgical performance.[5]

Chronic intracranial ECoG was generally performed (4 patients) except in children younger than 5 years of age or with attention deficit hyperactivity behavior. Intraoperative ECoG was performed in all resections for neocortical epilepsy, except those involving functional hemispherotomy.

Surgical specimens were fixed with phosphate-buffered formalin and embedded in paraffin wax. Serial 4-μm-thick sections were cut and stained with H & E and Klüver-Barrera Luxol fast blue stain. Variations in FCD were evaluated according to the histological classification scheme proposed by Palmini et al.[17] and were as follows: FCD Type IA, isolated architectural abnormalities; FCD Type IB, architectural abnormalities plus giant neurons; FCD Type IIA, architectural abnormalities with dysmorphic neurons but without balloon cells; and FCD IIB, architectural abnormalities with dysmorphic neurons and balloon cells.

Neurological findings were evaluated before and after surgery. Patients were followed up in our department's outpatient clinic. In the present study, we evaluated seizure outcome using the Engel classification,[24] and neurological findings and pathological diagnoses were investigated.

Results

Patient Characteristics

During the study period, 73 children (0–15 years of age) with medically refractory epilepsy underwent surgeries at our institute. Sixteen patients underwent resections for frontal lobe epilepsy. Eight children underwent resection of the frontal lobe without any deep brain structures and attained seizure freedom. Another 8 children (1–13 years old, mean age 6 years, boys/girls 5:3) with medically refractory frontal lobe epilepsy underwent resection that did include deep brain structures (Table 1; previously reported Cases 1[11] and 2[15]). Seven patients suffered daily seizures and 1 (Case 3) suffered monthly seizures. One of the patients (Case 6) suffered a medically