

ORIGINAL ARTICLE

# Expression of Astrocyte-Related Receptors in Cortical Dysplasia With Intractable Epilepsy

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## Abstract

Epilepsy is one of the major neurologic diseases, and astrocytes play important roles in epileptogenesis. To investigate possible roles of astrocyte-related receptors in patients with intractable epilepsy associated with focal cortical dysplasia (FCD) and other conditions, we examined resected epileptic foci from 31 patients, including 23 with FCD type I, IIa, or IIb, 5 with tuberous sclerosis complex, and 3 with low-grade astrocytoma. Control samples were from 21 autopsied brains of patients without epilepsy or neurologic deficits and 5 patients with pathologic gliosis without epilepsy. Immunohistochemical and immunoblot analyses with antibodies against purinergic receptor subtypes P2RY1, P2RY2, P2RY4, potassium channels Kv4.2 and Kir4.1, and metabotropic receptor subtypes mGluR1 and mGluR5 were performed. Anti–glial fibrillary acidic protein, anti-NeuN, and anti-CD68 immunostaining was used to identify astrocytes, neurons, and microglia, respectively. Most glial fibrillary acidic protein–immunopositive astrocyte cells in the brain samples from patients with epilepsy were P2RY1-, P2RY2-, P2RY4-, Kv4.2-, Kir4.1-, mGluR1-, and mGluR5-positive, whereas samples

from controls and pathologic gliosis showed lower expression levels of these astrocyte-related receptors. Our findings suggest that, although these receptors are necessary for astrocyte transmission, formation of the neuron-glia network, and other physiologic functions, overexpression in the brains of patients with intractable epilepsy may be associated with activation of intracellular and glio-neuronal signaling pathways that contribute to epileptogenesis.

**Key Words:** Astrocyte, Cortical dysplasia, Epilepsy, Metabotropic receptor, Potassium channels, Purinergic receptor.

## INTRODUCTION

Epilepsy is one of the major neurologic diseases characterized by recurrent seizures and characteristic psychomotor symptoms (1). The pathophysiology of epilepsy is generally recognized to be caused by abnormal excitation of neurons with spread or synchronization throughout the brain. Many studies have demonstrated physiologic dysfunction and morphologic abnormalities of neurons in the brains of patients with epilepsy. Recently, a significant role for astrocytes in the causation of neuronal firing has been described (2). Epileptic foci are populated by increased numbers of astrocytes, suggesting physiologic roles for glia in epilepsy pathogenesis (2–4). Recent studies demonstrate favorable long-term outcomes in patients with hippocampal sclerosis (5,6) and other types of intractable epilepsy (i.e. focal cortical dysplasia [FCD] and tuberous sclerosis complex [TSC]) after surgical resection of epileptic foci. Resection specimens from these patients facilitate studies of epileptogenesis.

In FCD and TSC, there are balloon cells that have some biologic characteristics of astrocytes but that may have several properties that differ from those in astrocytes in the normal brain. Indeed, astrocytes express various cell surface receptors that are similar to those of neurons (7). These receptors can be activated by synaptically released neurotransmitters, glial transmitters, and other molecules in the extracellular space (8,9). Among the receptors expressed on astrocytes are glutamate receptors and both ionotropic and metabotropic receptors (mGluR) (10). Metabotropic GluR2/3, mGluR4, and mGluR8 are expressed in reactive astrocytes of epileptic

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brains, and mGluR1, mGluR5, and mGluR8 are reported to be upregulated in the hippocampus in experimental animal models of temporal lobe epilepsy (11). Activation of these receptors leads to increased intracellular  $\text{Ca}^{2+}$  concentrations and wave propagation resulting in the release of glutamate from astrocytes (8,10). Several studies have also demonstrated the presence of voltage-dependent sodium, potassium, and calcium ion channels on astrocytes (12). The inwardly rectifying potassium ion (Kir) channels on astrocytes play a major role in the removal of potassium from the extracellular space (12). In specimens from patients with temporal lobe epilepsy, analysis of the ratio of their inward to outward potassium conductance showed that they were significantly smaller in astrocytes in lesions versus those of nonepileptic areas (13). The Kir 4.1 subunit is involved in this epileptic condition (13,14). Moreover, appreciation of the potential impact of astroglial ATP release on seizure activity is emerging (15).

Purinergic receptors are expressed in various mammalian cells and are activated by extracellular adenine and uridine nucleotides (16). Purinergic type 2 (P2) receptors expressed in brain cells regulate important physiologic functions, including neurotransmission, inflammation, cell growth, and apoptosis (17–19). Recently, the P2 receptor subtype Y1, P2RY1 in astrocytes, was reported to inhibit excitatory glutamatergic synaptic neurotransmission (20). Purinergic type 2 receptors are recognized as important components of glio-neuronal transmission in regulating neuronal excitation (17,18).

To facilitate the development of new treatments for epilepsy, it is important to understand the pathobiologic roles of astrocytes in epileptogenesis. Therefore, we investigated the expression of astrocyte receptors in epileptic lesions.

## MATERIALS AND METHODS

### Cases

Thirty-one patients with the diagnoses of FCD ( $n = 23$ ), TSC ( $n = 5$ ), or low-grade astrocytoma (LGA) ( $n = 3$ ) were selected from the registered repository of surgical material at our hospital. The patients were diagnosed as having intractable epilepsy and had had surgical resection of epileptic foci for treatment. For controls, 28 autopsy brain samples from patients without epilepsy or neurologic deficits were used (Table, Supplemental Digital Content 1, <http://links.lww.com/NEN/A618>). The surgical samples were obtained from epileptogenic areas, as determined by analysis of seizure semiology, ictal and interictal electroencephalography, and neuroimaging such as magnetic resonance imaging and positron emission tomography. The developmental quotient and intelligence quotient were evaluated with the Japanese Standard Infantile Development Test (Enjoji), Kinder Infant Development Scale, or Wechsler Intelligence Scale for Children-Third Edition before and 1 year after resection. In addition, a postoperative outcome score with the International League Against Epilepsy classification of outcome was recorded with respect to epileptic seizures 1 year after resection (21).

Immunohistochemical and histologic studies of human brain tissues and analyses of clinical data were performed with the informed consent of the adult patients or parents of the pediatric patients and the permission of the ethical committee

of our hospital and institute. The cases consisted of 23 FCD patients aged 1 month to 43 years, 5 TSC patients aged 5 months to 31 years, and 3 LGA patients aged 2 to 22 years (Table). Two neuropathologists routinely examined the resected specimens and determined the pathologic diagnosis according to international diagnostic criteria for FCD and brain tumors (22–24). Based on the pathologic diagnoses, the patients were divided into 3 FCD groups of FCD I (presenting abnormal cortical lamination), FCD IIa (dysmorphic neurons), FCD IIb (dysmorphic neurons and balloon cells), TSC (bizarre large neurons and balloon cells), or LGA (diffuse proliferation of astrocytes without malignant features) (Table).

### Pathologic Analysis

All samples had been fixed in 10% buffered formalin or 4% paraformaldehyde and embedded in paraffin. Four- or 6- $\mu\text{m}$ -thick serial sections were cut and stained with hematoxylin and eosin and with Luxol fast blue/cresyl violet (Klüver-Barrera).

For immunohistochemistry, sections were deparaffinized with xylene and ethanol and incubated at 90°C for 10 minutes with 0.1% succinate buffer for antigen retrieval. Nonspecific binding of IgG was blocked by incubation with 2% normal goat serum for 30 minutes at room temperature. Sections were then incubated with the primary antibodies overnight at 4°C. The primary antibodies used were polyclonal antibodies to P2RY1 (dilution of 1/100; MBL International Co., Woburn, MA), P2RY2 (1/100; MBL International Co.), P2RY4 (1/100; Acris Antibodies, San Diego, CA), Kv4.2 (1/1000; Abcam, Cambridge, UK), Kir4.1 (1/100; Abcam), mGluR1 (1/50; Abcam), and mGluR5 (1/100; Chemicon International Inc., Billerica, MA). Monoclonal anti-gial fibrillary acidic protein (GFAP) (1/100; Cell Signaling Technology Inc., Beverly, MA) was used as an astrocyte marker; anti-NeuN (1/500; Chemicon International Inc.) was used as a neuron marker; and anti-CD68 (1/50; Chemicon International Inc.) was used as a microglia marker. The sections were then sequentially incubated with the secondary rabbit IgG or mouse IgG antibodies (Nichirei Co., Tokyo, Japan), followed by additional staining with the chromogen 3-amino-9-ethyl carbazole substrate (Nichirei Co.), and counterstained with hematoxylin. For double staining, the secondary antibodies used were a fluorescein isothiocyanate-conjugated chicken anti-rabbit IgG and a tetramethyl rhodamine isothiocyanate-conjugated donkey anti-mouse IgG (Life Technologies Co., Grand Island, NY). The sections were mounted with a 4',6'-dimamidino-2-phenylindole (Vector Laboratories, Inc., Burlingame, CA) for labeling nuclei. The stained sections were observed with a fluorescent microscope (Olympus Co., Tokyo, Japan).

### Immunostaining Analysis

Immunopositive cells were counted, and the ratio of double-immunopositive cell numbers per GFAP-, NeuN- or CD68-positive cell was calculated from 3 randomly chosen areas of each section, under 100 $\times$  magnification of a light microscope. The number was corrected per 100 nuclei in each layer for each case as the immunopositive cell ratio. Mean ratios of double-marked cells were calculated for P2RY1, P2RY2, P2RY4, Kv4.2, Kir4.1, mGluR1, and mGluR5, respectively, and

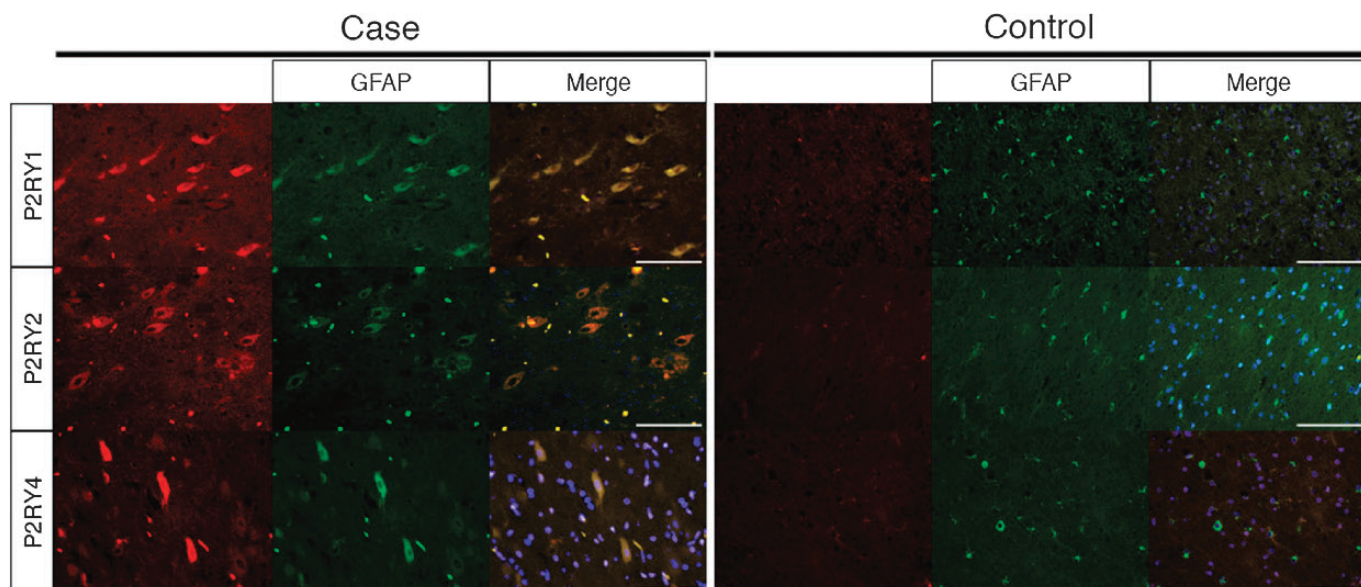
**TABLE. Patient Data**

Patient	Age at Seizure Onset				Before Resection				After Resection		
	Pathology	Mutation	Sex	Age at Seizure Onset	Seizure Type	Lesion	EEG	DQ/IQ	Age at Surgery	DQ/IQ*	Engel Score†
1	FCD I		M	3 m	Spasm and tonic seizure	LO-P	Spikes, SW at LO, P	14	1 y 0 m	19	1
2			M	3 y 6 m	GTC	RF	Sharp waves, SW at RF	98	4 y 4 m	108	3
3			M	3 y 5 m	CPS	RT	Spikes, SW at RT	62	13 y 4 m	72	1
4			M	11 y 0 m	CPS and GTC	RT	Spikes at RT	76	13 y 10 m	85	3
5			M	20 y	CPS	LT	Spikes at LT	68	43 y 4 m	100	1
6	FCD IIa		M	9 d	Asymmetric tonic spasm	RF	Spikes, SW at RF, C	125	1 m	112	1
7			M	1 d	Tonic seizure	LP	Slow waves, sharp, SB at L hemisphere	150	3 m 19 d	53	1
8			F	1 m	Tonic seizure	RT	Spikes, SW at R hemisphere	100	3 m 21 d	63	1
9			M	6 d	GTC	RO	SW at R hemisphere	175	3 m 22d	100	1
10			F	1 m	Spasm and GTC	LP	Hypsarrhythmia	49	8 m	71	1
11			F	1 m	R Upper limb clonic seizure	RF	Slow waves, spikes at RF	64	9 m	40	1
12			F	2 y 1 m	Tonic seizure	RF	Rhythmic fast waves at RF	nd	6 y 4 m	94	2
13			F	8 y 3 m	Hypermotor seizure	RT	Spikes at RT	83	8 y 10 m	90	1
14			F	1 y 6 m	Hypermotor seizure	RF	Spikes at LF, P	50	11 y 0 m	49	3
15	FCD IIb		M	6 d	Tonic seizure	RO	Slow waves at RO, T, F	78	3 m	93	1
16			M	9 m	Tonic seizure	LT	Spikes at LT	64	1 y 8 m	58	1
17			M	4 m	Atonic seizure	nd	Rhythmic slow waves at LC, P	100	2 y 2 m	112	1
18			F	1 y 0 m	GTC	RF	Slow waves at RF	20	2 y 10 m	79	3
19			F	6 y 6 m	R Tonic seizure	LF	Slow waves, sharp, SB at LF, C, P	96	8 y 3 m	92	3
20			M	6 y 11 m	R Clonic seizure	LF	Rhythmic slow waves at LF, C, P	76	8 y 3 m	82	1
21			M	1 y	CPS	RO	Rhythmic fast waves at RO	68	11 y 0 m	89	1
22			M	5 y 11 m	GTC	LF	SW at LF	75	12 y 2 m	96	1
23			M	3 y	GTC	RF	Spikes at RF	72	12 y 4 m	69	4
24	TSC	TSC2 +	F	1 m	Spasm and tonic seizure	LF	Spikes, SW at LF	87	5 m	35	3
25		Nd	F	1 m	Spasm and GTC	LC	Hypsarrhythmia	44	7 m	20	4
26		TSC2 +	M	11 m	Tonic seizure	RF	Spikes at RF	84	1 y 10 m	89	3
27		no mutation	F	6 m	GTC	LT	Spikes, slow waves at LT, O	58	2 y 5 m	68	1
28		Nd	F	Nd	Nd	Nd	Nd	Nd	31 y	Nd	Nd
29	LGA		M	1 y 7 m	Tonic seizure	RT	Spikes at RT	83	2 y 7 m	79	1
30			F	13 y	Nd	LT	Sharp at LT	97	18 y 10 m	Nd	1
31			M	13 y 8 m	Oral automatism	LT	Sharp, slow waves at LT	78	22 y 3 m	86	2

\*These scores evaluated at 1 year after resection.

†These scores were evaluated at 1 year after resection. Engel scoring has 4 categories: 1, seizure-free; 2, markedly decreased seizure frequency; 3, moderately decreased seizure frequency; and 4, no change after resection. FCD, focal cortical dysplasia; TSC, tuberous sclerosis complex; LGA, low-grade astrocytoma; M, male; F, female; m, month (s); y, year (s); GTC, generalized tonic clonic convulsion; CPS, complex partial seizure; Nd, not described; L, left; R, right; O, occipital; P, parietal; T, temporal; SW, spike and slow wave; SB, suppression-burst.





**FIGURE 1.** Astrocyte purigenic receptors (P2RY1, P2RY2, and P2RY4) (red) are expressed in glial fibrillary acidic protein (GFAP) (green)-positive cells in the brains of focal cortical dysplasia patients; there is little expression of purigenic receptors in control brains. The tissue for P2RY1/GFAP immunostaining is from a sample from Patient 18 (Table). P2RY2/GFAP immunostaining is from Patient 16. P2RY4/GFAP immunostaining is from Patient 15. The control panels are derived from a brain sample from a 3-day-old boy who died of progressive renal failure with congenital polycystic kidney disease (postmortem interval, 3 hours). DAPI, blue. Scale bar = 50  $\mu\text{m}$ .

compared with controls. Balloon cells were present in the FCD IIB and TSC lesions, but numbers of balloon cells were excluded because of their variable numbers of GFAP-immunostained and unstained balloon cells in each case and the unknown origin of those cells.

### Statistical Analysis

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; SPSS Inc., Chicago, IL) with a significance level of  $p < 0.05$ . In addition, regression analyses were performed to compare the ratios in the number of immunopositive cells per astrocyte with various clinical data, including onset age, seizure type, lesion, interval time between onset and surgery, developmental quotient or intelligence quotient, gestational age at birth, and Engel score at 1 year after resection (Table).

### Immunoblotting

Protein extraction from prepared tissues was carried out according to a previously described procedure (25). The tissues selected for analysis were adjacent to the areas with severe pathologic findings, and 50  $\mu\text{g}$  of the extracted proteins were loaded on each lane. After transfer to a polyvinylidene difluoride membrane, each immunoreacted band was detected using an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Buckinghamshire, UK) according to the manufacturer's instructions. As a reference,  $\beta$ -actin was detected using a specific antibody (Sigma, St. Louis, MO). The expression levels of detected bands were measured and calculated

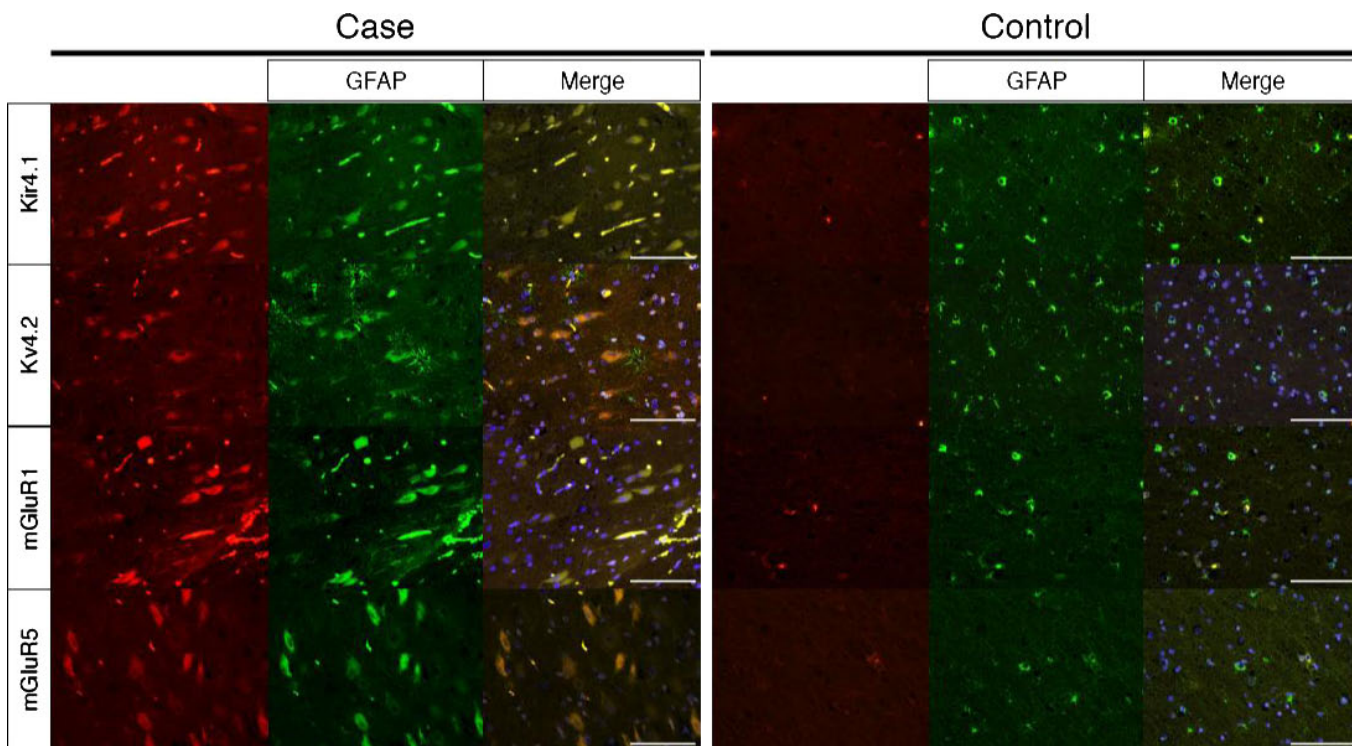
by ImageQuant TL 7.0 (GE Healthcare, Buckinghamshire, UK) and compared with those of each subgroup.

### RESULTS

The pathologic classification of FCD cases was done according to recent international criteria (24). There were 5 FCD I, 9 FCD IIA, and 9 FCD IIB patients (Table). The other types of cortical dysplasia, TSC and LGA, were diagnosed by brain magnetic resonance imaging and genetic analysis of *TSC1* (hamartin) and/or *TSC2* (tuberin) or by having a less than 1% measurement with Mib-1 index (Table).

To evaluate various receptors of astrocytes in epileptogenesis lesions of the brains, emphasis was placed on GFAP-immunopositive cells (Figs. 1, 2). The immunoreactivities for all antibodies appeared to be in the cytosol. These immunostaining patterns in autopsied control sections were the same patterns as those in surgical tissues for all antibodies (Figure, Supplemental Digital Content 2, <http://links.lww.com/NEN/A619>). Negative immunostaining for all antibodies was performed by omission of the primary antibodies (data not shown).

The ratios of the P2RY1-positive cell number per GFAP-positive cell number (P2RY1/GFAP) were  $69.5\% \pm 19.1\%$  (average  $\pm$  SD) in FCD I patient samples,  $49.9\% \pm 25.4\%$  in FCD IIA samples,  $57.0\% \pm 22.7\%$  in FCD IIB samples,  $66.1\% \pm 1.2\%$  in TSC samples,  $72.0\% \pm 11.8\%$  in LGA samples, and  $0.9\% \pm 2.8\%$  of control cases (Fig. 3A). The ratios of P2RY2/GFAP in patients samples were  $63.3\% \pm 20.7\%$  in FCD I,  $86.2\% \pm 17.4\%$  in FCD IIA,  $99.8\% \pm 0.4\%$  in FCD IIB,  $99.3\% \pm 1.6\%$  in TSC, 100.0% in LGA, and  $1.17\% \pm 0.5\%$  in control cases (Fig. 3B). The ratios of P2RY4/GFAP



**FIGURE 2.** Expression of potassium ion channels (Kir4.1, Kv4.2) and metabotropic receptor subtypes (mGluR1, mGluR5) (red) in the brains of patients with focal cortical dysplasia. All markers of astrocytic receptors are expressed in glial fibrillary acidic protein (GFAP) (green)-positive cells, whereas there is very little expression in a control brain. The case tissue samples are from Patient 15 (Table). The control panels are derived from a brain sample from a 3-day-old boy who died of progressive renal failure with congenital polycystic kidney disease (postmortem interval, 3 hours). DAPI blue. Scale bar = 50  $\mu$ m.

in patient samples were  $80.2\% \pm 11.6\%$  in FCD I,  $91.0\% \pm 11.3\%$  in FCD IIa,  $88.8\% \pm 21.8\%$  in FCD IIb,  $80.2\% \pm 27.4\%$  in TSC,  $90.1\% \pm 11.7\%$  in LGA, and  $3.9\% \pm 16.7\%$  in control cases (Fig. 3C). The ratios of Kv4.2/GFAP were  $57.2\% \pm 26.5\%$  in FCD I,  $40.7\% \pm 16.2\%$  in FCD IIa,  $49.0\% \pm 23.0\%$  in FCD IIb,  $59.3\% \pm 26.4\%$  in TSC,  $44.4\% \pm 7.5\%$  in LGA, and  $9.8\% \pm 22.5\%$  in control cases (Fig. 3D). The ratios of Kir4.1/GFAP were  $80.0\% \pm 38.0\%$  in FCD I,  $65.3\% \pm 33.7\%$  in FCD IIa,  $76.8\% \pm 30.9\%$  in FCD IIb,  $76.1\% \pm 17.7\%$  in TSC,  $98.1\% \pm 1.1\%$  in LGA, and  $9.8\% \pm 22.5\%$  in control cases (Fig. 3E). The ratios of mGluR1/GFAP were  $88.5\% \pm 9.2\%$  in FCD I,  $56.9\% \pm 30.3\%$  in FCD IIa,  $91.7\% \pm 12.1\%$  in FCD IIb,  $78.4\% \pm 28.3\%$  in TSC,  $88.8\% \pm 9.1\%$  in LGA, and  $1.2\% \pm 1.7\%$  in control cases (Fig. 3F). The ratios of mGluR5/GFAP were  $69.4\% \pm 9.4\%$  in FCD I,  $72.0\% \pm 29.5\%$  in FCD IIa,  $69.1\% \pm 25.0\%$  in FCD IIb,  $47.9\% \pm 30.0\%$  in TSC,  $85.1\% \pm 5.7\%$  in LGA, and  $2.4\% \pm 10.9\%$  in control cases (Fig. 3G).

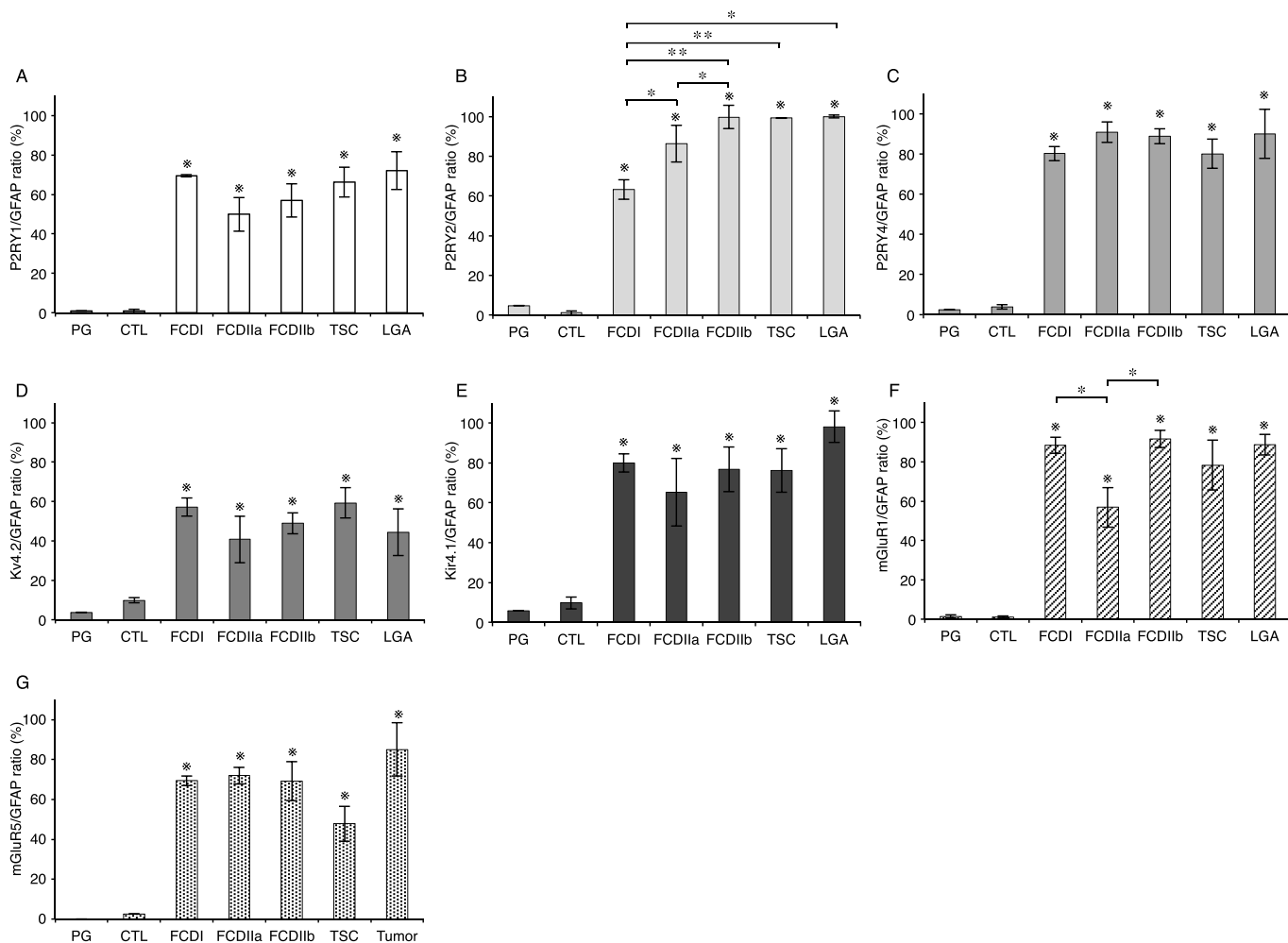
Compared with control cases, the ratios of P2RY1/GFAP, P2RY2/GFAP, P2RY4/GFAP, Kv4.2/GFAP, Kir4.1/GFAP, mGluR1/GFAP, and mGluR5/GFAP were significantly different among the FCD I, FCD IIa, FCD IIb, TSC, and LGA patient samples ( $p < 0.001$ ) (Fig. 3). For the ratios of P2RY2/GFAP, there were significant differences between FCD I and FCD IIa, FCD I and FCD IIb, FCD I and TSC, FCD I and LGA, and FCD IIa and FCD IIb ( $p < 0.05$ ).

The ratios of mGluR1/GFAP were significantly different between FCD I and FCD IIa and between FCD IIa and FCD IIb ( $p < 0.05$ ), but there were no significant differences between the pathologic subgroups for P2RY1, P2RY4, Kv4.2, Kir4.1, and mGluR5 expression. None of the receptors were found to be expressed by neurons or microglia (Figure, Supplemental Digital Content 3, <http://links.lww.com/NEN/A620> and Figure, Supplemental Digital Content 4, <http://links.lww.com/NEN/A621>). The pathologic gliosis patient samples were not different in any of the ratios versus those of the control samples (Fig. 3).

The immunohistochemical results were reflected in the immunoblotting data (Fig. 4). All receptors were detected at higher levels of expression versus the controls (Figure, Supplemental Digital Content 5, <http://links.lww.com/NEN/A622>). Moreover, the analysis suggested that, in epileptic foci specifically, there was overexpression of the astrocyte receptors. There were no significant relationships between any receptor-cell marker ratio with specific clinical parameters (Table).

## DISCUSSION

The number of glial cells in the cerebral cortex is estimated to be more than 3 times the number of neurons (26). Astrocytes have many brain homeostatic functions including modulating synaptic function and plasticity, regulating the perisynaptic environment and contributing to the permeability



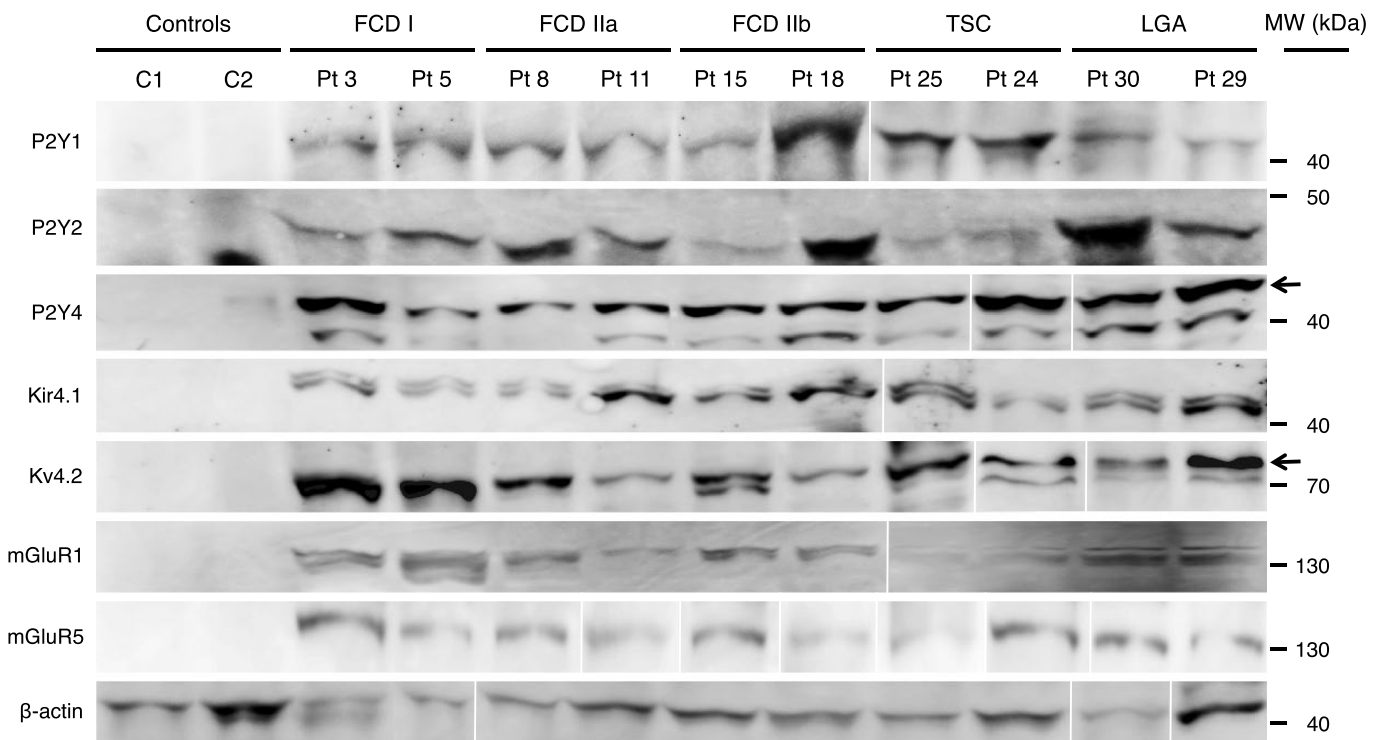
**FIGURE 3.** Ratio of astrocytic receptor–positive cell number per glial fibrillary acidic protein (GFAP)–positive cell number in the brains of patients with focal cortical dysplasia (FCD), tuberous sclerosis complex (TSC), and low-grade astrocytoma (LGA) and controls. Brains of FCD, TSC, and LGA patients show greater expression of all purinergic type 2 (P2) receptor subtypes. P2RY2/GFAP ratios are significantly altered between FCD I, FCD IIa, and FCD IIb, as well as TSC and LGA. Metabotropic GluR1/GFAP ratios are also significantly altered between FCD I, FCD IIa, and FCD IIb. PG, pathologic gliosis without epilepsy (n = 7); CTL, controls (n = 21); FCD type I, focal cortical dysplasia type I (n = 5); FCD type IIa, focal cortical dysplasia type IIa (n = 9); FCD type IIb, focal cortical dysplasia type IIb (n = 9); TSC, n = 5; LGA (n = 3). \* p < 0.05; \*\* p < 0.01, \* p < 0.05 vs. controls.

of the blood-brain barrier (3,27). On the other hand, disrupted glial functions can lead to epileptogenesis (8). Abnormal and increased numbers of glia, including astrogliosis and glial tumors, are a prominent feature of epileptic foci in human brains and in the brains of experimental epilepsy animals. One of the mechanisms by which glial cells may promote epilepsy is by increasing neuronal excitability that results in hyperexcitability and hypersynchrony. This may be caused by an imbalance of ions and neurotransmitters as a consequence of glial dysfunction. A few reports have suggested that epileptogenesis may result from dysfunction of astrocyte receptors (3). Alterations of many astrocytic functions in epilepsy, such as of K<sup>+</sup>-buffering and mGluR-mediated cell signaling, are well known (28,29). Abnormal Ca<sup>2+</sup>-dependent gliotransmission directly contributes to the excessive neuronal synchronization of seizures. Astrocytes from hyperexcitable networks respond to

neuronal signals with massive Ca<sup>2+</sup> elevations, thereby generating recurrent neuronal excitation and promoting seizures (10,30). Moreover, astrocyte transmission is necessary for forming the neuron-glia network and performing neuronal functions (31).

We found that most GFAP-positive cells in epilepsy brains overexpressed the astrocytic receptors of P2RY1, P2RY2, P2RY4, Kv4.2, Kir4.1, mGluR1, and mGluR5, although very little of this expression was found in neurons or microglia (Figures, Supplemental Digital Content 3–5, <http://links.lww.com/NEN/A620>, <http://links.lww.com/NEN/A621>, <http://links.lww.com/NEN/A622>). These receptors are known as astrocyte contributors to brain function (3), and the expression of the 7 receptors was found in human brains using both immunohistochemistry and immunoblotting techniques. P2RY1, P2RY2, and P2RY4, members of the purinergic receptor type Y





**FIGURE 4.** Immunoblotting of astrocyte receptors in the brains of patients (Pts) with focal cortical dysplasia (FCD), tuberous sclerosis complex (TSC) and low-grade astrocytoma (LGA) (Table). Expression of all receptors is not detected in control (C1, C2) brains, whereas all receptors are expressed at high levels in samples of patient brains. C1 was a 3-month-old who died suddenly with pneumonia (postmortem interval, 3 hours). C2 was 11 years old who died a suddenly with a myocardial infarction (post-mortem interval, 2 hours). Patients 3, 5, 8, 11, 15, 18, 25, 24, 29, and 30 in the Table are abbreviated to Pt 3, 5, 8, 11, 15, 18, 25, 24, 29, and 30, respectively.

family, are activated by ATP/ADP. P2RY1, P2RY2, and P2RY4 promote astrocyte proliferation, and P2RY2 interactions with integrins promote astrocyte migration (32). P2RY2 was previously reported to be upregulated in a pathologic astrocyte-proliferating condition (17). Extracellular released ATP, which activates P2 purinergic receptors, can induce the proliferation of astrocytes. Purinergic signaling may, therefore, be a major pathologic mechanism of astrocytes. Interestingly, the ratio of P2RY2/GFAP showed differences between FCD I and other types of FCD, TSC, and LGA, whereas 5 pathologic gliosis patient tissue samples indicated no difference in the ratio of P2RY2/GFAP versus controls. The high ratio of P2RY2/GFAP might, therefore, be characteristic of epileptic foci. In addition, we found a greater P2RY2/GFAP ratio in FCD IIb versus FCD IIa patient samples. This suggests that more severely malformed brains in intractable epilepsy patients have a greater expression of P2RY2. P2RY1 and P2RY2 were recently reported to contribute to neuronal proliferation and migration (7,33). High ratios of P2RY1/GFAP and P2RY2/GFAP may also reflect the extent of malformation of the brains. Indeed, the P2RY2/GFAP ratio grew gradually higher in more severe malformations or glial proliferation (TSC and LGA) (Fig. 3).

These receptors also play important roles in neuron-glia interactions. P2RY2 is expressed in neurons and glial cells and its signaling mediates neuroprotective responses (3,32,34). Astrocytes also release neurotrophic factors that contribute to neuronal

survival and sprouting and supply energy substrates to neurons (35). Although P2RY1, P2RY2, and P2RY4 have inhibitory roles in synaptic transmission, postsynaptic P2RY1 and P2RY4 receptors are involved in neuromodulation of transmitter release (36,37). Recently, it has been demonstrated that P2RY1 induction leads to the release of glutamate (38). Our data suggest that increases in the numbers of purinergic receptor-expressing cells may lead to epileptogenesis through the increase of glutamate release and hyperexcitability of neurons.

Metabotropic GluR5 is reported to be expressed in most balloon cells and intensively in astrocytes (39). One recent report indicated that mGluR5 is expressed in premature, but not in mature, astrocytes (40). Our finding that mGluR5-positive astrocytes were identified in epileptic brains may mean that there is immaturity or vulnerability to the environment, as with premature astrocytes. Moreover, mGluR1 is occasionally observed in glial cells (39). Glutamate released from astrocytes can also become a transmitter of neuron-glia communication. Outflow of glutamate from neuronal activity elicits calcium increase in astrocytes through mGluR5 receptors (41). Glutamate from astrocytes has been found to have various effects on neuronal activity through NMDA receptors or mGluR5 receptor, depending on astrocyte calcium levels (42,43), and activation of mGluR1 and mGluR5 in astrocytes results in potassium channel expression (42). Mutations of the human *Kir4.1* gene, the product of which is expressed in brain astrocytes, are

associated with epilepsy (44) 16 Kir channels, identified in human genome genes, have been identified in glial cells. Among them, the resting conductance of astrocytes is largely caused by Kir4.1. Loss of Kir4.1 function causes membrane depolarization and breakdown of potassium ion and glutamate homeostasis, which results in seizures (45). The Kir4.1-null epileptic mouse was shown to delay potassium ion clearance after synaptic activation (46). Kir4.1 inactivation in astrocytes impairs extracellular potassium ion and glutamate clearance and produces a seizure phenotype (47). Voltage-gated potassium (Kv) channels are suggested to play an important role in epilepsy. Kv4.2 is expressed in both neurons and astrocytes. Its regulation, which may involve potassium channels, is of interacting proteins, and alterations in the subcellular localization of the channel result in epilepsy (48). The glia-neuron communication is performed with complex transmitters, such as ATP/ADP and glutamate, and through ion channels.

Overexpression of those astrocytic receptors is considered to trigger activation of the related signaling pathways. Astrocytic receptor activation critically contributes to epileptogenesis. Many of the astrocytic functions involving K<sup>+</sup>-exchanging, along with adenosine- and mGluR-mediated signaling systems, are altered in epilepsy. Moreover, those functions may trigger the pathogenesis of intractable epilepsy associated with cortical malformations. Thus, astrocytes may become a new target for drug development for epilepsy therapy. In exploring advanced therapeutics, it is important to control the astrocytic receptor-related signaling pathways.

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