Takahashi Yukitoshi, Inoue Yushi	Influence of Uridine Diphosphate Glucuronosyltransferase 2B7 -161C>T Polymorphism on the Concentration of Valproic Acid in Pediatric Epilepsy Patients	Therapeutic Drug Monitoring			in press
Yukitoshi Takahashi	Neuronal Antibodies in Creutzfeldt–Jakob Disease	JAMA Neurology			in press
高橋幸利、	Antibody Update　グルタミン酸受容体自己抗体	Brain and Nerve	65	345-353	2013
高橋幸利、 馬場好一、 井上有史	小児てんかん外科　早期手術患者の発見と利点　－発達の観点から－.	脳と発達	45	199-205	2013
高橋幸利	伝染性単核球症に続発し脳脊髄液に抗グルタミン酸受容体δ2 抗体をみとめた急性小脳失調症.	臨床神経学	53(7)	555-558	2013
高橋幸利	難治 epileptic spasm を有する症例における ACTH 療法反復施行の検討.	脳と発達	45	281-287	2013
高橋幸利	GluRε2 抗体（NR2B 抗体）-神経疾患における意義.	神経内科	79(3)	354-362	2013
高橋幸利	卵巣奇形腫を合併し抗 NMDA 受容体抗体陽性の glioblastoma の 1 例.	臨床神経学	53(9)	712-715	2013
高橋幸利	てんかん－基礎・臨床研究の最新知識-Ⅲ-10.抗てんかん薬の副作用.	日本臨床			印刷中
高橋幸利	難治性てんかんの病態を探る-脳炎後てんかんと免疫.	脳と発達			印刷中
高橋幸利	急性無菌性髄膜脳炎の経過中に局所性皮質反射性ミオクローヌスを呈し抗グルタミン酸受容体抗体が検出された 2 例.	臨床神経学			印刷中
Kobayashi K	Cortical contribution to scalp EEG gamma rhythms associated with epileptic spasms.	Brain Dev	35 (8)	762-770	2013

Kobayashi K	Successful treatment of early myoclonic encephalopathy using lidocaine and carbamazepine.	Epileptic Disord	15 (3)	352-357	2013
Kobayashi K	Questionnaire-based assessment of behavioral problems in Japanese children with epilepsy.	Epilepsy Behav	27 (2)	238-242	2013
Ikeda A	Anterior temporal lobe white matter abnormal signal (ATLAS) as an indicator for laterality of seizure focus in temporal lobe epilepsy: a comparison among double inversion-recovery, FLAIR and T2WI at 3. T.	Eur Radiol	23(1)	3-11	2013
Ikeda A	Bereitschaftspotential augmentation by neuro-feedback training in Parkinson's disease.	Clin Neurophysiol	124(7)	1398-405	2013
Ikeda A	Pre-SMA actively engages in conflict processing in human: a combined study of epicortical ERPs and direct cortical stimulation.	Neuropsychologia	51	1011-7	2013
Ikeda A	Internal structural change in the hippocampus using 3 Tesla MRI in mesial temporal lobe epilepsy.	Int Med	52(8)	877-85	2013
Ikeda A	Prolonged ictal monoparesis with parietal PLEDs.	Epi Disord	15(2)	197-202	2013
Ikeda A	Role of posterior parietal cortex in reaching movements in humans. Clinical implication for 'optic ataxia'.	Clin Neurophysiol	124(11)	2230-4	2013
Ikeda A	Higher degree of clinical anticipation in maternal transmission in benign adult familial myoclonus epilepsy in Japan.	Epi Disord			2013
Ikeda A	Evaluation of focus laterality in temporal lobe epilepsy: A quantitative study comparing double inversion-recovery MR imaging at 3T with FDG-PET.	Epilepsia			2013

Ikeda A	Long-term seizure outcome following epilepsy surgery: to be or not to be cured?	Neurol Med Chir	53(11)	805-13.	2013
Ikeda A	Evaluation of seizure foci and genes in the Lgi1 L385R/+ mutant rat.	Neurosci Res			2013
Ikeda A	Automatic reference selection for quantitative EEG interpretation: Identification of diffuse/localised activity and the active earlobe reference, iterative detection of the distribution of EEG rhythms.	Med Eng Phys			2013
池田昭夫	Faciobrachial dystonic seizureで初発したくすぶり型の抗leucine-rich glioma-inactivated 1 (LGI1)抗体陽性辺縁系脳炎の1例	臨床神経	53	706-11	2013
齊藤祐子	嗜銀顆粒性認知症の鑑別診断	最新医学	68(4)	820-826	2013
齊藤祐子	筋強直性ジストロフィーの中枢神経病理	Clinical neuroscience	31(8)	872-873	2013

Ⅲ. 研究成果の刊行物・別刷

BRAIN RESEARCH 1470 (2012) 89–97

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Abnormal maturation and differentiation of neocortical neurons in epileptogenic cortical malformation: Unique distribution of layer-specific marker cells of focal cortical dysplasia and hemimegalencephaly

Asako Arai[a,b,c,f,1], *Takashi Saito*[a,b,c,1], *Sae Hanai*[a,b,c], *Sayuri Sukigara*[a,b], *Shin Nabatame*[a,b], *Taisuke Otsuki*[a,d], *Eiji Nakagawa*[a,c], *Akio Takahashi*[a,d], *Yuu Kaneko*[a,d], *Takanobu Kaido*[a,d], *Yuko Saito*[a,e], *Kenji Sugai*[a,c], *Masayuki Sasaki*[a,c], *Yu-ichi Goto*[a,b], *Masayuki Itoh*[a,b,1,*]

[a]*Epilepsy Center, National Center of Neurology and Psychiatry, Tokyo, Japan*
[b]*Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan*
[c]*Department of Child Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan*
[d]*Department of Neurosurgery, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan*
[e]*Department of Pathology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan*
[f]*Department of Pediatrics, Tokyo Women's Medical University Medical Center East, Tokyo, Japan*

ARTICLE INFO

Article history:
Accepted 8 June 2012
Available online 1 July 2012

Keywords:
Focal cortical dysplasia
Hemimegalencephaly
Migration
Differentiation
SATB2
FOXP1
TBR1

ABSTRACT

Focal cortical dysplasia (FCD) and hemimegalencephaly (HME) are major causes of intractable epilepsy in children. The probable pathogenesis of FCD and HMG is the abnormal migration and differentiation of neurons. The aim of the present study was to clarify the abnormal cytoarchitecture, based on neuronal immaturation. Tissue samples were obtained from 16 FCD and seven HME patients, aged between 2 months and 12 years, who had been diagnosed as typical FCD and HME, following surgical treatment for intractable epilepsy. Paraffin-embedded sections were stained with the antibodies of three layer-markers that are usually present only during the fetal period, namely SATB2 (expressed in the upper layer of the normal fetal neocortex), FOXP1 (expressed in the 5th layer), and TBR1 (expressed in the 6th layer). In FCD, SATB2-positive (+) cells located in the middle and deep regions of FCD Ia and Ib, but only in the superficial region of FCD IIa and IIb. FOXP1+ cells diffusely located in the neocortex, especially the upper layer of FCD IIa and IIb. TBR1+ cells mainly located in the middle and deep regions, and also white matter. In FCD IIb, TBR1+ cells were in the superficial region. In HME, SATB2+ and FOXP1+ cells were found diffusely. TBR1+ cells were in the middle and deep regions. On the basis of continued expression of fetal cortical layer-specific markers in FCD and HME brains, the

*Corresponding author at: Department of Mental Retardation and Birth Defect Research, National Center of Neurology and Psychiatry, Kodaira, Tokyo 187-8502, Japan. Fax: +81 423461743.
E-mail address: itoh@ncnp.go.jp (M. Itoh).
[1] Equal contribution to this study.

0006-8993/$ - see front matter
http://dx.doi.org/10.1016/j.brainres.2012.06.009

abnormal neocortical formation in both is likely to be the result of disrupted neuronal migration and dysmaturation. The expression pattern is different between FCD and HME.

1. Introduction

Focal cortical dysplasia (FCD) and hemimegalencephaly (HME), rare epileptogenic brain malformations are usually accompanied by severe epilepsy and occasionally by mental retardation. The incidence of FCD epilepsy identified in surgical series varies between 12% and 40% (Blümcke et al., 1999; Nordborg et al., 1999; Prayson et al., 2002), while that of HME is lower. These diseases are of relatively high frequency in surgical epilepsy, but have a low morbidity rate (Prayson and Estes, 1995; Prayson, 2000). FCD and HME are usually diagnosed by neuropathological findings in specimens undergoing cortical resection for the treatment of refractory epilepsy. As common features, mental development problems sometimes remain, in spite of well-controlled epilepsy.

FCD was recognized as a pathologic substrate associated with epilepsy (Taylor et al., 1971). It is known that FCD has columnar and laminar disorganization with various cellular abnormalities, including dysmorphic neurons, giant neurons, and balloon cells (Prayson et al., 1996; Yamanouchi et al., 1996; Palmini et al., 2004; Alonso-Nanclares et al., 2005; Blümcke et al., 2011). On the other hand, HME mainly shows cortical laminar abnormality, such as polymicrogyria and neuronal heterotopia. Together with these pathological findings, it is thought that FCD and HME may result from erroneous migration, maturation, or cell death during ontogenesis (Crino and Eberwine, 1997; Cotter et al., 1999; Najm et al., 2007). However, a common pathogenesis remains unknown.

On the other hand, some molecules are useful to detect layer formation of human neocortex. We recently demonstrated that human malformed brains have unique layer patterns (Saito et al., 2010). In the present study, we seek to detect abnormal neuronal migration and differentiation in FCD and HME that will lead to greater understanding of the pathophysiology of the epileptogenic malformed brain.

2. Results

2.1. *Histological distribution*

Histopathological results were summarized in Table 1. We obtained 4 FCD Ia, 4 FCD Ib, 4 FCD IIa and 4 FCD IIb from the international classification (Blümcke et al., 2011), and seven HME. FCD Ic was relatively rare. Although we could not examine this subtype, it was enough to investigate FCD Ia and Ib for the aim of the present study because FCD Ic pathologically showed the combination of FCD Ia and Ib. All HME cases showed polymicrogyria and/or unlayered neocortex with neuronal heterotopia and mineralization.

2.2. *Immunohistochemistry of FCD*

Immunohistochemistry results were summarized in Table 2. SATB2+ cells were dominant in the middle and deep regions of the neocortex in FCD Ia and Ib, although they were widely distributed (Figs. 1 and 2, Table 2). Interestingly, SATB2+ cell distribution of FCD IIa and IIb was limited to the superficial region of the neocortex (Figs. 3 and 4, Table 2). FOXP1 immunoreactivity was diffusely intense (Table 2). Only FCD Ib revealed FOXP1+ cells in the white matter. FCD IIa and IIb demonstrated no FOXP1+ cells in the white matter, and FOXP1+ cells tended to appear in the superficial region of the neocortex. TBR1+ cells were the most prominent in the middle and deep regions of the neocortex (Fig. 4). Notably, TBR1+ cells exhibited a unique localization of the superficial region of the neocortex in FCD IIb (Fig. 4, Table 2). The immunopositive cells for SATB2+, FOXP1 and TBR1 were confirmed as neurons with NeuN-immunopositivity (data not shown). There were no significant differences in the marker expression patterns in each lobe.

2.3. *Immunohistochemistry of HME*

SATB2+ cells in HME were diffused in the neocortex, but relatively dense in the superficial region of the neocortex (Fig. 5). FOXP1 immunoreactivity also diffusely distributed, but was occasionally negative (Fig. 5, Table 2). TBR1+ cells were limited to the middle and deep regions of the neocortex (Fig. 5, Table 2). The immunopositive cells for SATB2+, FOXP1, and TBR1 in HME were also confirmed as neurons with NeuN-immunopositivity (data not shown). There were also no significant differences in the marker expression patterns in each lobe.

3. Discussion

In the normal developing cortex, the localization of SATB2, FOXP1, and TBR1 is restricted to specific cortical layers, and the expression of all three markers disappears in the postnatal brain (Saito et al., 2011). In the present series, SATB2, FOXP1, and TBR1 were diffusely expressed throughout the cortex in samples from all cases. The result indicates that FCD and HME consist of immature cells. Moreover, we identified that these layer-marker immunopositive cells were neurons by a neuron marker, NeuN. There was an observable tendency for SATB2+cells to be distributed in the middle and deep regions of the neocortex of FCD Ia and Ib, and limited to the superficial region of FCD IIa and IIb. It is quite interesting that SATB2+ and FOXP1+ cells in HME were diffusely distributed and TBR1+ cells were localized in the middle and deep regions of the neocortex. To evaluate the expression patterns of those specific markers in FCD subtypes or HME, we divided them into three regions of the neocortex in terms of thickness.

Although there is little evidence regarding the mechanisms responsible for human FCD, it has been reported that FCD neurons originate from abnormal migration, maturation, and

Table 1 – Clinicopathological profile of FCD and HME patients.

Case	Sex	Age at surgery	Age at seizure onset	Seizure	Intelligence	FCD location on imaging	Pathological findings	
							Main pathology	Others
FCD								
1	M	2 Y	2 m	CPS+GTC	100 (IQ)	P	FCD Ia	Mild gliosis
2	F	3 Y	3 m	CPS	50 (DQ)	F	FCD Ia	HN, gliosis
3	M	6 Y	4 m	CPS	33 (DQ)	F	FCD Ia	Mild gliosis
4	M	7 Y	11 m	CPS	58 (IQ)	F	FCD Ia	Mild gliosis
5	M	6 M	1 m	CPS	40 (DQ)	P	FCD Ib	Mild gliosis
6	M	2 Y	20 d	CPS	18 (DQ)	F	FCD Ib	HN, gliosis
7	F	3 Y	3 m	CPS	15 (DQ)	P	FCD Ib	HN, gliosis
8	F	12 Y	11 m	CPS	43 (IQ)	F	FCD Ib	Mild gliosis
9	F	3 Y	6 m	CPS+GTC	40 (DQ)	F+P	FCD IIa	HN, gliosis
10	F	5 Y	2 y 9 m	CPS	81 (IQ)	F	FCD IIa	HN, gliosis
11	M	6 Y	3 d	CPS	16 (DQ)	T+P+O	FCD IIa	HN, gliosis
12	M	7 Y	7 m	CPS	50 (IQ)	P	FCD IIa	HN, gliosis
13	M	10 Y	1 y 11 m	CPS+GTC	22 (IQ)	F+T+P	FCD IIb	HN, gliosis
14	M	3 Y	2y 9 m	GTC	15 (DQ)	T+P+O	FCD IIb	HN, gliosis
15	M	8 Y	3 m	CPS+GTC	30 (IQ)	F+P	FCD IIb	HN, gliosis
16	F	19 Y	9 m	CPS+GTC	25 (IQ)	P	FCD IIb	HN, gliosis
HME								
1	F	3 M	2 d	CPS+GTC	30 (DQ)	rt-hemisphere	Polymicrogyria	HN, M, gliosis
2	F	3 M	7 d	CPS+GTC	35 (DQ)	rt-hemisphere	DN, BC	HN, M, gliosis
3	M	3 M	14 d	CPS+GTC	30 (DQ)	lt-hemisphere	Polymicrogyria	HN, M, gliosis
4	M	6 M	7 d	CPS+GTC	50 (DQ)	lt-hemisphere	DN	HN, M, gliosis
5	M	3 M	14 d	EIEE	50 (DQ)	rt-hemisphere	DN, BC	HN, M, gliosis
6	F	4 M	1 d	EIEE	30 (DQ)	rt-hemisphere	Polymicrogyria	HN, M, gliosis
7	M	7 M	3 d	EIEE	20 (DQ)	lt-hemisphere	Polymicrogyria	HN, M, gliosis

M: male, F: female, M (m): month (s), Y (y): year (s), d: days, CPS: complex partial seizure, GTC: generalized tonic–clonic convulsion, EIEE: early infantile epileptic encephalopathy, IQ: intelligence quotient, DQ: development quotient, F: frontal lobe, P: parietal lobe, T: temporal lobe, O: occipital lobe, rt: right side, lt: left side, DN: dysmorphic neuron, BC: balloon cell, HN: heterotopic neuron, M: mineralization.

cell death during ontogenesis (Spreafico et al., 1998a; 1998b; Andres et al., 2005). Our results may support this theory, indicating the persistence of immature neurons in the white matter. A recent report shows that markers of neuronal immaturity were overexpressed to excess in FCD (Hanai et al., 2010). Moreover, the previous studies have also reported that FCD neurons exhibited various degrees of neuronal maturation, glial cells or a combination of neuronal and glial characteristics (Crino and Eberwine, 1997; Yamanouchi et al., 1998; Aronica et al., 2003; Fauser et al., 2004; Ying et al., 1999). FCD and HME may retain certain characteristics indicative of immaturity.

SATB2 is a DNA-binding protein that regulates chromatin organization and gene expression. In the developing brain, SATB2 is expressed in cortical projection neurons. In a previous study, SATB2 has expressed predominantly in the upper layer, and not in the deep layer, of the cortex (Britanova et al., 2008). This expression pattern suggests that SATB2 may be involved in the control of early aspects of upper layer neuron specification. Interestingly, the SATB2 expression pattern can be clearly divided into three types in the present study. The first pattern is SATB2 expression in the middle and deep regions of the neocortex and some in the white matter. The second is SATB2 expression in the superficial region of the neocortex. The third pattern is diffused SATB2 expression. The first was identified in FCD Ia and Ib, the second in FCD IIa and IIb, and the third in HME.

FOXP1+ cells are supposed to be projection neurons. FOXP1 is a member of a conserved family of genes that shares a common DNA-binding domain, namely the T-box (Tamura et al., 2004). The T-box genes encode transcription factors involved in the regulation of developmental processes. A similar protein that is highly expressed in the 4th and 5th layers has been reportedly disrupted in mice and shown to be critical for early cortical developmental processes (Takahashi

Table 2 – Summary of immunohistochemistry of FCD and HME patients.

Case	Pathological classification	SATB2				FOXP1				TBR1			
		Superficial	Middle	Deep	WM	Superficial	Middle	Deep	WM	Superficial	Middle	Deep	WM
FCD													
1	Ia	+	++	++	+	+	++	−	−	+	++	++	++
2	Ia	−	++	++	−	+	+	−	−		++	++	++
3	Ia	−	++	++	+	−	++	++	−	−	++	++	++
4	Ia	−	++	++	+	−	++	++	−	−	++	++	−
5	Ib	−	++	++	+	+	++	+	+	−	+	+	+
6	Ib	−	++	++	+	−	++	++	+	−	+	+	+
7	Ib	++	++	++	++	−	++	++	++	−	++	++	++
8	Ib	++	++	+	−	+	++	−	+	−	++	++	−
9	IIa	+	−	−	−	++	+	−	−	−	+	−	−
10	IIa	++	−	−	−	+	+	+	−	−	+	−	−
11	IIa	+	+	−	+	+	++	−	−	−	+	−	−
12	IIa	++	−	−	−	++	+	+	−	−	+	−	−
13	IIb	++	++	−	−	++	++	+	−	+	+	++	+
14	IIb	+	−	−	−	++	+	+	−	+	+	++	+
15	IIb	+	−	−	−	++	+	−	−	+	+	++	+
16	IIb	++	−	−	−	++	−	−	−	+	+	++	+
HOME													
1	rt-hemisphere	++	+	+	+	+	+	+	+	+	++	+	−
2	rt-hemisphere	++	+	+	+	+	+	+	+	+	++	+	+
3	lt-hemisphere	++	+	+	+	+	−	+	+	−	++	+	−
4	lt-hemisphere	++	+	+	+	−	+	−	−	−	++	+	−
5	rt-hemisphere	++	+	+	+	+	+	+	+	−	++	+	−
6	rt-hemisphere	++	+	+	+	+	+	+	+	−++	+	+	
7	lt-hemisphere	++	+	+	+	+	+	+	−	+	++	+	−
Control													
23–29 GW		+	++	−	−	−	−	++	−	++	++	+	−
1 M–8 Y		−	−	−	−	−	−	−	−	−	−	−	−

−: No immunopositive cells, +: less than 5 cells in 1 mm^2, ++: more than 5 cells in 1 mm^2.

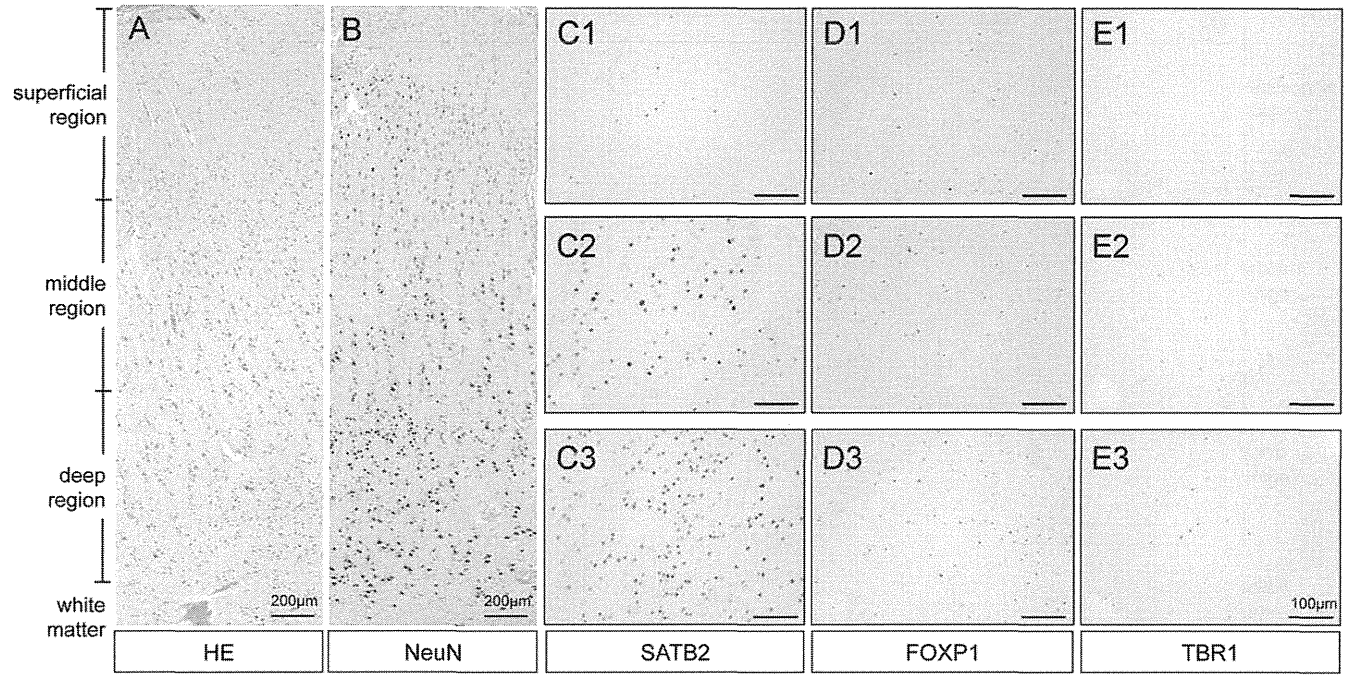

Fig. 1 – Histology and immunohistochemistry of FCD Ia. FCD Ia demonstrates abnormal radial lamination and abundant microcolumns of the neocortex (A, B). SATB2+ cells scattered in the superficial (C1), but at a high concentration in the middle (C2), and deep (C3) regions of the neocortex. FOXP1+ cells diffusely locate in the neocortex (D1–D3). TBR1+ cells diffusely locate in the neocortex (E1–E3) and those concentrations are low.
(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.

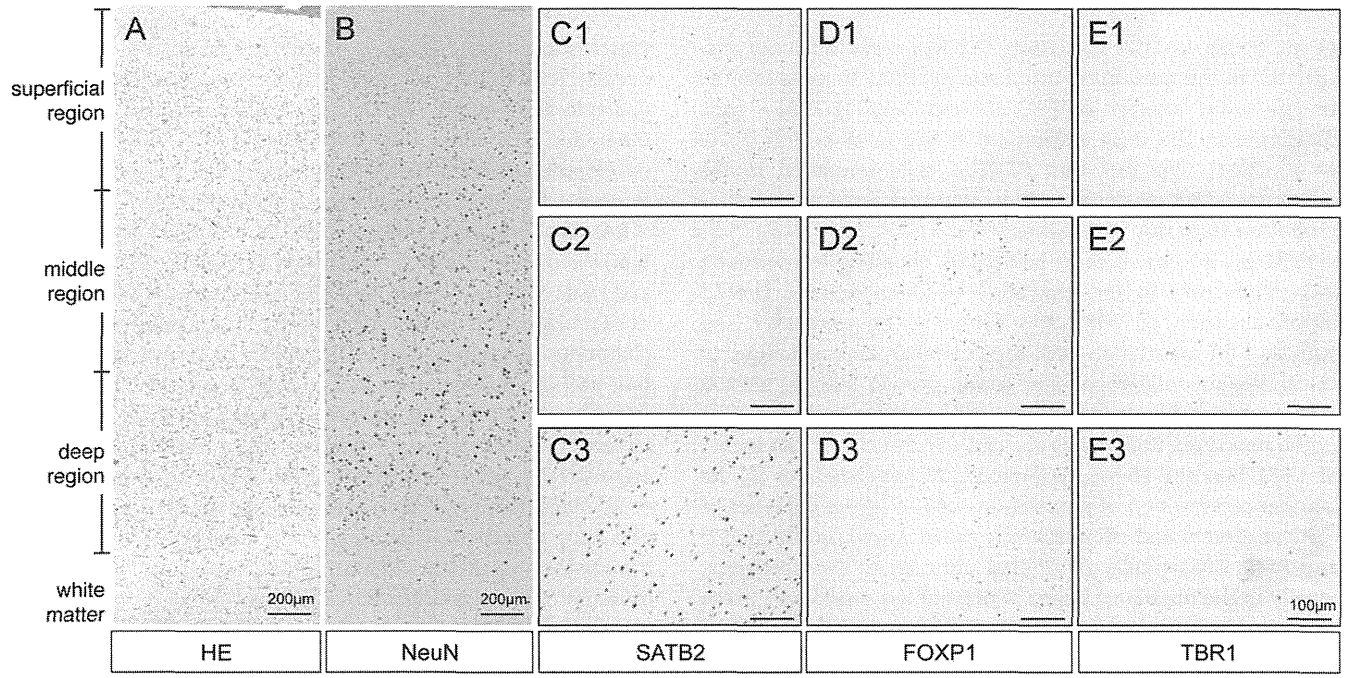

Fig. 2 – Histology and immunohistochemistry of FCD Ib. FCD Ib demonstrates abnormal tangential layer composition of the neocortex (A, B). SATB2+ cells scattered in the superficial (C1), but at a low concentration in the middle (C2) and a high concentration in the deep (C3) regions of the neocortex. FOXP1+ cells diffusely locate in the neocortex (D1–D3). TBR1+ cells diffusely locate in the neocortex, but those concentrations are low (E1–E3).
(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.

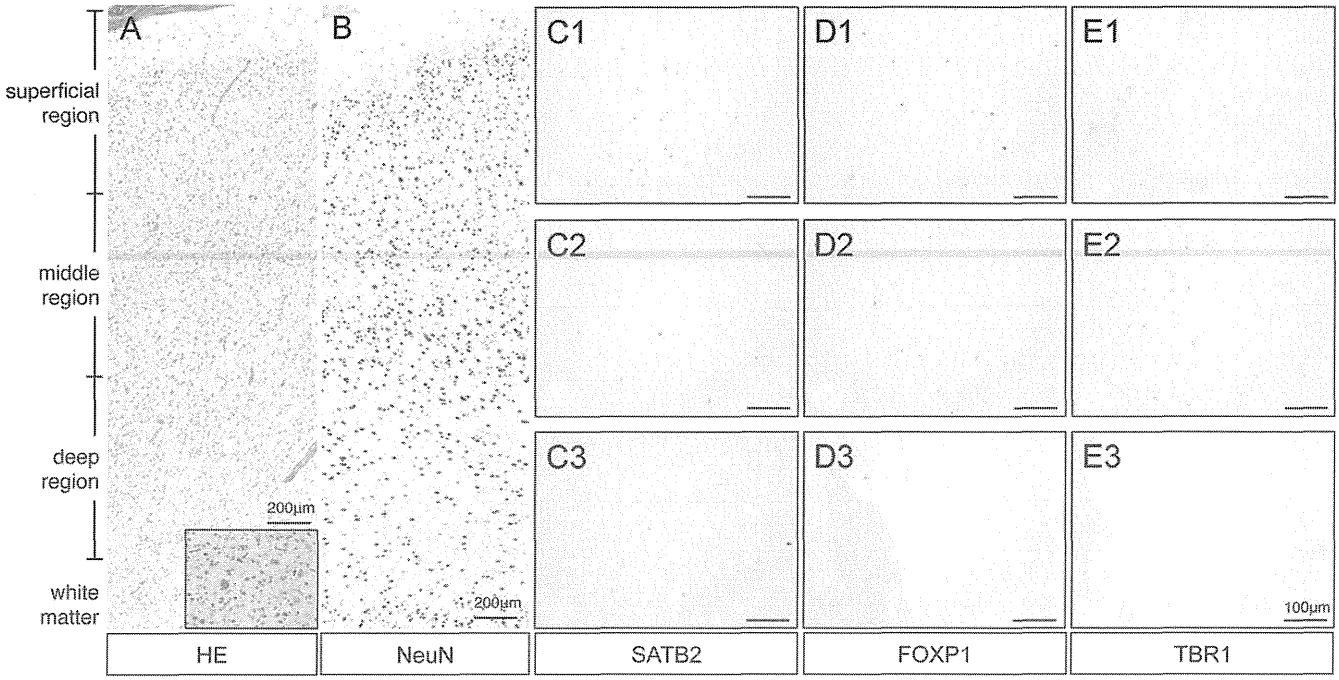

Fig. 3 – Histology and immunohistochemistry of FCD IIa. FCD IIa demonstrates unidentified layer-formation of the neocortex and a high neuronal concentration (A, B), and contains dysmorphic neurons (small window in A). SATB2+ cells scattered in the superficial (C1) and middle (C2) regions, but those concentrations are very low in the deep (C3) region of the neocortex. FOXP1+ cells diffusely locate in the neocortex (D1–D3), and evidence a relatively low concentration in the deep region (D3). TBR1+ cells diffusely locate in the neocortex, but those concentrations are low (E1–E3).
(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.

et al., 2008). In FCD and HME, FOXP1+ cells diffusely distributed in the neocortex. Interestingly, FOXP1+ cells located in the white matter of FCD Ib and HME. FOXP1+ cells distribute in the deep neocortex in the fetal period (Saito et al., 2011). The fact that FOXP1+ cells remained in the postnatal white matter may indicate more delayed neuronal migration than the other types.

TBR1 is also one of the T-box genes encoding transcription factors involved in the regulation of development. The C-terminal region of TBR1 was found to be necessary and sufficient for association with the guanylate kinase domain of calcium/calmodulin-dependent serine protein kinase. TBR1 is highly expressed in early neurons of the preplate and deep layer of the neocortex (Bulfone et al., 1995). Furthermore, the cortex of TBR1 mutants shows developmental abnormalities in the laminar organization of neurons, as well as in the guidance of cortical afferent and efferent axons (Hevner et al., 2001). In FCD and HME, TBR1+ cells distributed diffusely in the neocortex, mainly in the middle and deep regions of the neocortex. These data may be supported by the previous study (Hadjivassiliou et al., 2010). Moreover, TBR1+ cells were observed in the superficial region of the neocortex of FCD IIb and in the white matter of FCD Ia and Ib.

SATB2, FOXP1, and TBR1 are normally expressed in immature neurons. Based on our results indicating the diffuse expression of all three markers throughout the neocortex of FCD and HME, it appears that immature cells are present in the cortex of both. Furthermore, our results confirm that abnormal migration occurs, and this is supported by the well-known fact that epileptic malformed brains often have neuronal migration disruption. Our data may indicate that FCD and HME are developmental brain disorders characterized by abnormalities in neuronal migration and crucially differentiation.

It is of considerable interest that the immature transcription markers of the neocortex are useful to identify FCD subtypes and HME. SATB1-, FOXP1-, and TBR1-positive cells are expected to be projection neurons, which use excitatory neurotransmitter glutamate. Electrophysiological experiments have demonstrated that immature and potentiated excited-GABAergic neurons are strongly related to FCD epileptogenesis (Cepeda et al., 2007). Moreover, dysmorphic neurons and balloon cells are thought to originate from the neocortical subventricular zone (Lamparello et al., 2007). From our data, glutaminergic neurons of FCD may also have neuronal immaturity. The pathological causes of the intractable seizures in FCD and HME may thus include the immaturity and dysfunction of neurons.

4. Experimental procedures

4.1. *Human tissue preparation*

Human neocortical tissues were obtained from 16 FCD and seven HME patients (Table 1) who underwent surgical treatment