2.5. Tacrolimus therapy

The usual protocol for tacrolimus therapy was a starting dose of 0.1 mg/kg/day (for children) or 3 mg/day (for adults) with dose escalation after 2 months, depending on blood levels of tacrolimus. Only patients who had received treatment for more than 6 months were evaluated.

2.6. Statistical analyses

Non-parametric Mann–Whitney *U*-test was used to compare the quantitative variables between two groups. Chi-square test for trend was used to compare the seizure outcome. A p value <0.05 was considered as indicating a significant difference.

3. Results

3.1. Patients background

Mean onset age of epilepsy in 49 patients (male 22, female 27) was 8.7 ± 10.5 years. Twelve patients had preceding infection, seven had preceding vaccination, and four had preceding head trauma before onset of epilepsy. Dominant hemispheres were involved in 24 patients, and non-dominant in 25 patients.

Regular IVIg therapy was evaluated in 13 patients (dominant side, 7; non-dominant side, 6) (Table 1). Mean onset age was 13.6 ± 16.3 years, and mean lag period from onset to IVIg therapy was 4.0 ± 5.7 years. Regular steroid pulse therapy was evaluated in 21 patients (dominant side, 12; non-dominant side, 9). Mean onset age was 8.2 ± 11.7 years, and mean lag period from onset to steroid pulse therapy was 5.7 ± 6.2 years. Tacrolimus therapy was evaluated in 12 patients (dominant side, 9; non-dominant side, 3). Mean onset age was 8.8 ± 10.4 years, and mean lag period from onset to tacrolimus therapy was 6.4 ± 7.2 years.

Of 49 patients, 30 patients had received at least one kind of immunotherapy during the course of treatment. In these patients, cognitive outcome (the last IQ) was not related to onset age, treatment lag period, or disease duration.

Selection of treatments was determined by the attending doctors (Table 1). Among 24 patients with dominant hemisphere involvement, nine received regular pulse therapy, seven had regular IVIg therapy, and three underwent surgery as the initial therapy, in addition to AED therapies. In 25 patients with non-dominant hemisphere involvement, six had regular pulse therapy, five received regular IVIg therapy, and nine underwent surgery as the initial therapy, in addition to AED therapies. A total of 12 patients were treated with tacrolimus, 11 of whom received tacrolimus as a replacement of regular IVIg or pulse therapy.

3.2. Seizure outcome

Seizure-free rate (SFR) was 71% in patients who underwent FH of the non-dominant hemisphere, 20% surgical resection in the non-dominant hemisphere, and 0% surgical resection in the dominant hemisphere

Table 1
Treatment flow.

Involved hemisphere	1st Treatment	2nd Treatment	3rd Treatment	Number of patients
Dominant: 24 patients	Regular pulse therapy	Regular pulse		3
		Tacrolimus		5
		Surgery	Regular pulse-tacrolimus-regular pulse	1
	Regular IVIg therapy	Regular IVIg		3
		Regular pulse		1
		Tacrolimus		1
		Tacrolimus	Regular pulse	1
		Surgery		1
	Tacrolimus therapy	Regular pulse		1
	Surgery			3
	AEDs only			3
	Others			1
Nondominant: 25 patients	Regular pulse therapy	Regular pulse		3
		Tacrolimus		2
		Regular IVIg		1
	Regular IVIg therapy	Regular IVIg		1
		Regular pulse	Surgery	1
		Tacrolimus	Surgery	1
		Surgery		2
	Surgery	Surgery		7
		Regular pulse		2
	AEDs only			3
	Others			2

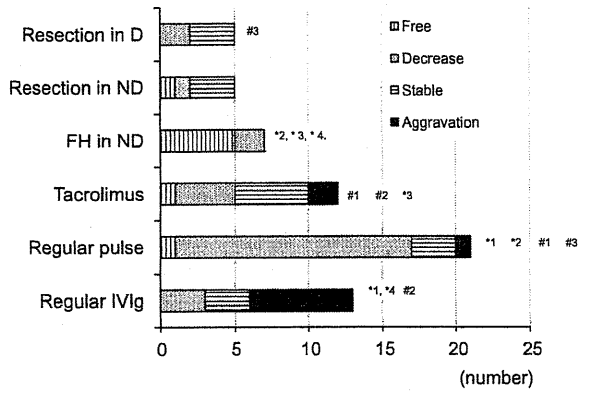

Fig. 2. Seizure outcome after surgery or immunomodulatory therapies. Horizontal axis shows the number of patients with each category of seizure outcome. Resection in D, surgical resection in dominant hemisphere; ND, non-dominant hemisphere; FH, functional hemispherectomy; IVIg, intravenous immunoglobulin. Chi-square test for trend detected significant differences in seizure outcome between two groups marked by (*) (*1, $p = 0.0003$; *2, $p = 0.0023$; *3, $p = 0.0033$; *4, $p = 0.0021$), and non-significant differences between two groups marked by (#) (#1, $p = 0.3080$; #2, $p = 0.2036$; #3, $p = 0.1646$).

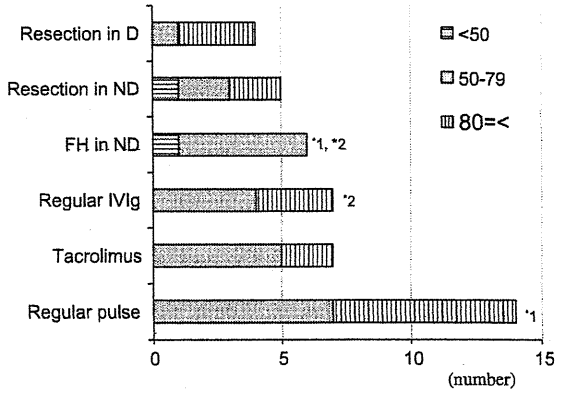

Fig. 3. The last FSIQ/DQ after surgeries or immunomodulatory therapies. Horizontal axis shows the number of patients with each category of FSIQ/DQ. Resection in D, surgical resection in dominant hemisphere; ND, non-dominant hemisphere; FH, functional hemispherectomy; IVIg, intravenous immunoglobulin. Chi-square test for trend detected significant differences in seizure outcome between two groups marked by (*) (*1, $p = 0.0141$; *2, $p = 0.0447$).

(Fig. 2) (Table 2). In two of seven patients treated by FH, seizures relapsed at three and six years after FH. SFR was 8% by tacrolimus therapy, 5% by regular pulse therapy, and 0% by regular IVIg therapy. Greater than 50% reduction rate (response rate, RR) was 81% by regular pulse therapy, 42% by tacrolimus therapy, and 23% by regular IVIg therapy. FH of the non-dominant hemisphere had better seizure outcome compared with regular pulse therapy ($p = 0.0023$), tacrolimus therapy ($p = 0.0033$) and regular IVIg ($p = 0.0021$). Seizure outcome by regular pulse therapy was better than by regular IVIg ($p = 0.0003$), but was not different from tacrolimus therapy or resection in dominant hemisphere.

3.3. Cognitive outcome

We compared the changes in FSIQ/DQ before and after various treatment modalities. Preservation of cognitive function was defined as "improved" and "stable" changes in FSIQ/DQ. Preservation rate of FSIQ/DQ was 76% by regular pulse therapy, 75% by tacrolimus therapy, 60% by surgical resection in non-dominant hemisphere, 60% by surgical resection in dominant hemisphere, 57% by FH of non-dominant hemisphere, and 45% by regular IVIg therapy (data not shown). The changes in FSIQ/DQ before and after treatment were not significantly different among the treatment modalities.

Next, cognitive outcome among the various treatment modalities was compared by the last FSIQ/DQ (Fig. 3). The proportion of patients with FSIQ/DQ higher than 80 after therapy (R80) was 75% by surgical resection in dominant hemisphere, 50% by regular pulse therapy, 43% by regular IVIg therapy, 40% by surgical resection in non-dominant hemisphere, 29% by tacrolimus therapy, and 0% by FH of non-dominant hemisphere. Regular pulse therapy had significantly better FSIQ/DQ than FH of non-dominant hemisphere. Regular IVIg also had significantly better FSIQ/DQ than FH of non-dominant hemisphere.

Table 2
Summary of outcome in Rasmussen syndrome.

		Epileptic surgery			Regular IVIg	Regular pulse	Tacrolimus
		FH in ND	Res in ND	Res in D			
Number		7	5	5	13	21	12
Seizure outcome	SFR (%)	71	20	0	0	5	8
	RR (%)	100	40	40	23	81	42
Cognitive outcome	PR (%)	57	60	60	45	76	75
	R80 (%)	0	40	75	43	50	29
Motor outcome	AR (%)	100	0	20	62	10	0
Discontinuation (%)					100	62	17

FH, functional hemispherectomy; ND, non-dominant hemisphere; Res, resection surgery; D, dominant hemisphere; IVIg, intravenous immunoglobulin; SFR, seizure free rate; RR, response rate; PR, FSIQ/DQ preservation rate; R80, rate of patients with FSIQ/DQ higher than 80 after therapy; AR, rate of motor function aggravation.

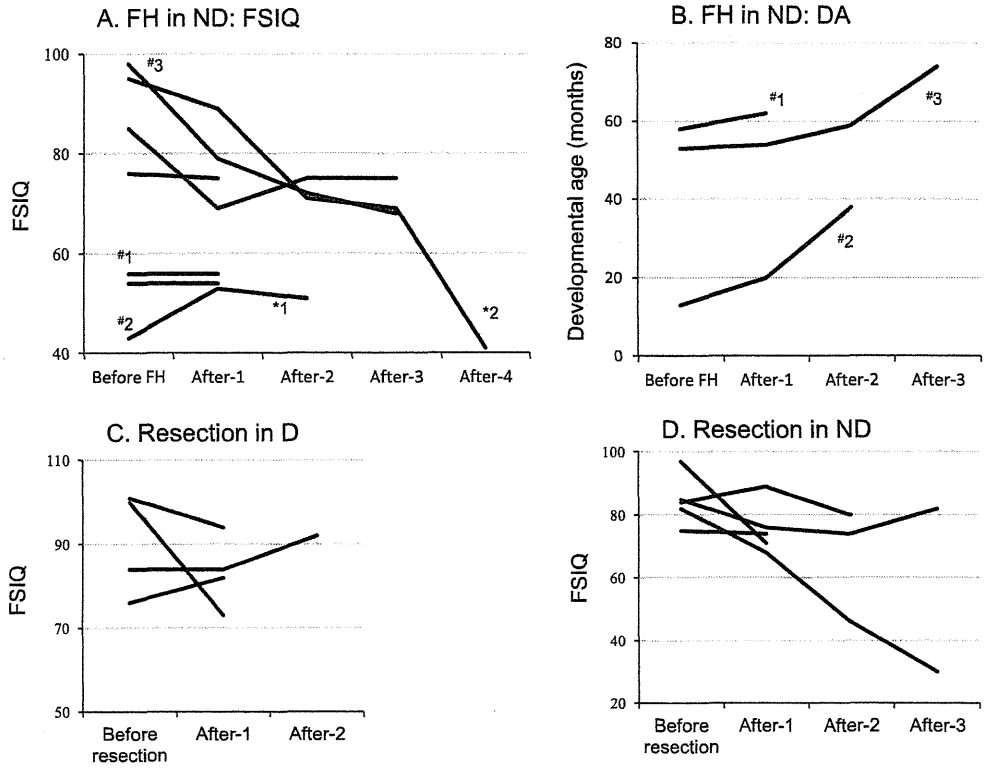

Fig. 4. Evolution of FSIQ/DQ and developmental age after surgical interventions. FSIQ: full scale intelligent quotient; DA: developmental age; after-1, -2, -3 and -4: first, second, third and fourth examinations, respectively, after surgery. A. "FH in ND: FSIQ" shows the evolution of FSIQ/DQ in seven patients treated by FH of the non-dominant hemisphere. *1, relapse of seizures at 3 years after FH; *2, relapse of seizures at 6 years after FH. #1, #2 and #3 in A and B denote the same patients. B. "FH in ND: DA" shows the evolution of DA measured by Tanaka–Binet test in three patients treated by FH. C. "Resection in D" shows the evolution of FSIQ in four patients treated by surgical resection in dominant hemisphere. D. "Resection in ND" shows the evolution of FS.

For precise evaluation of cognitive outcome of surgical intervention, evolution of FSIQ/DQ was studied (Fig. 4). In three patients with FSIQ/DQ higher than 80 before FH of non-dominant hemisphere, FSIQ/DQ decreased gradually after FH to below 80, during periods without seizure relapse (Fig. 4A). On the other hand, in four patients with FSIQ lower than 80 before FH, FSIQ/DQ was maintained at pre-FH levels. In younger patients whose cognitive function was evaluated by developmental age (DA), DA increased slightly after FH, although FSIQ/DQ did not improve (Fig. 4B). Two of four patients treated by surgical resection in dominant hemisphere had FSIQ/DQ higher than 90 before FH, and one showed FSIQ decrease greater than 10 after surgical intervention, without seizure control (Fig. 4C). On the other hand, in two patients with FSIQ lower than 90 before FH, FSIQ was maintained at pre surgical levels. In five patients treated by surgical resection in non-dominant hemisphere, four had FSIQ/DQ higher than 80, two of whom had FSIQ/DQ decrease greater than 10 after surgical intervention, without seizure control (Fig. 4D). On the other hand, in one patient with FSIQ/DQ lower than 80 before surgery, FSIQ/DQ was maintained at the pre surgical level.

R80 after regular pulse therapy was 100% in patients without MRI lesions, 50% in patients with high intensity lesions, and 37% in patients with advanced MRI lesions (Fig. 5A). R80 after tacrolimus therapy was 28% in patients with advanced MRI lesions (Fig. 5B). R80 after regular IVIg therapy was 100% in patients without MRI lesions and patients with high intensity lesions, and 20% in patients with advanced MRI lesions (Fig. 5C).

The relationship between treatment modalities and cognitive outcome is shown in Fig. 6. R80 was 43% by regular pulse therapy followed by tacrolimus therapy, 50% by regular pulse therapy, 33% by regular IVIg therapy, 0% by regular IVIg therapy followed by FH, 33% by regular IVIg therapy followed by surgical resection, 0% by FH of non-dominant hemisphere, 60% by surgical resection, and 33% by surgical resection followed by regular pulse therapy. No patient achieved IQ > 80 by treatments including FH (FH or IVIg followed by FH), but more than 50% of patients achieved IQ > 80 by regular pulse therapy and surgical resection. Mean IQ achieved by IVIg followed by FH (65 ± 15) tended to be higher than that by FH (57 ± 12), and that by surgical resection preceded by IVIg (76 ± 12) tended to be higher than that by surgical resection (65 ± 28)

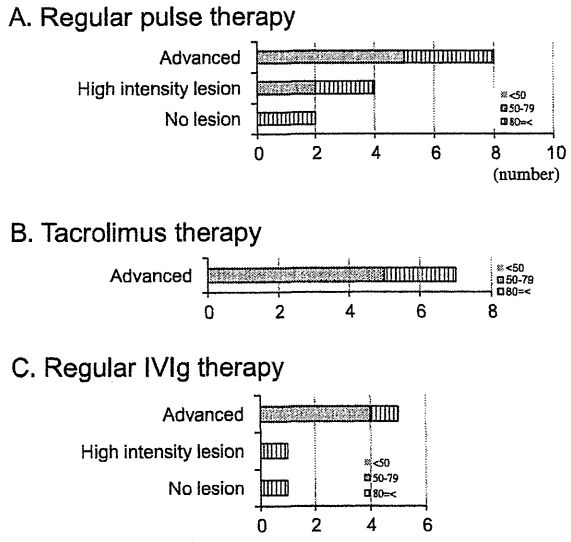

Fig. 5. MRI stage & the last IQ/DQ after immunomodulatory therapies. Horizontal axis shows number of patients with each category of FSIQ/DQ. Advanced, advanced lesion on MRI; High intensity lesion, high intensity MRI lesions; No lesion, without MRI lesion.

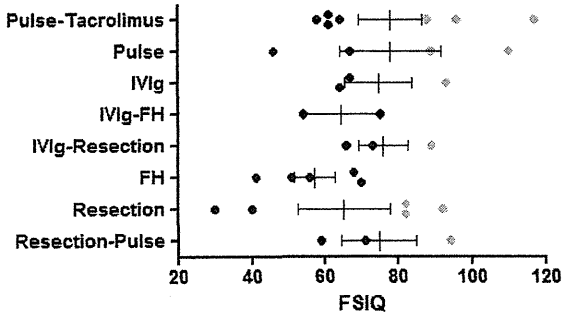

Fig. 6. Treatment modalities and the last FSIQ/DQ. Horizontal axis shows FSIQ. Pulse-Tacrolimus, regular steroid pulse therapy followed by tacrolimus therapy; Pulse, regular pulse therapy; IVIg, regular IVIg therapy; FH, functional hemispherectomy; Resection, surgical resection; Bars show mean ± SE. Purple dots mean data of FSIQ above 80. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

($p > 0.05$). Mean IQ achieved by resection followed by regular pulse therapy (75 ± 18) tended to be higher than that by surgical resection (65 ± 28) ($p > 0.05$).

3.4. Motor outcome

Improvement of motor dysfunction (paresis) was observed in 15% of patients treated by regular IVIg therapy, 10% of patients treated by regular pulse therapy, and 8% of patients treated by tacrolimus therapy (Fig. 7). Aggravation of motor function (progression of motor dysfunction) was observed in 100% of patients

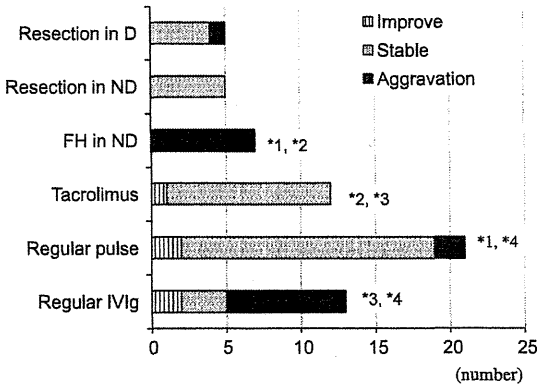

Fig. 7. Motor outcome after surgeries or immunomodulatory therapies. Horizontal axis shows number of patients with each category of cognitive changes. Resection in D, surgical resection in dominant hemisphere; ND, nondominant hemisphere; FH, functional hemispherectomy; Tacrolimus, tacrolimus therapy. Chi-square test for trend detected significant difference in cognitive changes between two therapies marked by (*) (*1, $p < 0.0001$; *2, $p < 0.001$; *3, $p = 0.0314$; *4, $p = 0.314$).

treated by FH, 62% of patients treated by regular IVIg, 20% of patients treated by surgical resection in dominant hemisphere, and 10% of patients treated by regular pulse therapy. Motor outcome by regular pulse therapy was significantly better than that by FH of non-dominant hemisphere and regular IVIg therapy. Motor outcome by tacrolimus therapy was significantly better than that by FH of non-dominant hemisphere and regular IVIg therapy.

3.5. Discontinuation of immunomodulatory therapies and adverse events

Regular IVIg therapy was discontinued in 100% (13/13) of the patients (Table 2), and treatment was switched to regular steroid pulse therapy in two patients, tacrolimus therapy in three, surgical intervention in three, and AEDs only in five. Significant adverse events were not observed. The reasons for discontinuation included aggravation of seizures, aggravation of motor dysfunction, and medical costs.

Regular pulse therapy was discontinued in 62% (13/21) of the patients, and treatment was switched to regular IVIg therapy in one patient, tacrolimus therapy in seven, surgical intervention in one, and AEDs only in four. Significant adverse events were not observed. The reason for discontinuation was disturbance of quality of life due to regular hospitalization for longer periods.

Tacrolimus therapy was discontinued in 17% (2/12) of the patients, and treatment was switched to regular pulse therapy in one patient and surgical intervention in one. Significant adverse events were not observed. The reasons for discontinuation included aggravation of seizures and aggravation of motor dysfunction.

4. Discussion

FH has been the major treatment for RS. In the current treatment strategy for RS, the indication of FH is considered as soon as RS is diagnosed [2]. From the viewpoint of seizure outcome, FH is the only treatment to achieve complete seizure control in RS, but the seizure-free rate of FH is not 100%; the rate was reported to be 62.5–85% in the literature [2]. From the viewpoint of cognitive outcome, we found that all patients with FSIQ/DQ higher than 80 before FH experienced reduction in IQ/DQ to levels below 80 after FH, but R80 after immunomodulatory therapy was 29–50%. Regarding motor outcome, FH inevitably results in deterioration of motor function. Although FH is an important beneficial treatment, many issues await solutions. We need to establish innovative treatment strategies that can improve seizure outcome as well as preserve cognitive and motor functions. Evidence of the efficacy of immunomodulatory treatments has accumulated, in this study we compared the outcomes among surgical intervention, regular IVIg therapy, regular pulse therapy and tacrolimus therapy, mainly in patients with pediatric onset RS. Based on the results of analyses, we attempted to propose innovative treatment strategies.

Among the various immunomodulatory therapies and surgical interventions, regular IVIg therapy showed relatively poor seizure outcome, average cognitive outcome, and poor motor outcome (Table 2). Regular IVIg therapy was discontinued, mainly because of aggravation of seizures, and/or deterioration of motor functions. These data suggested that regular IVIg therapy in patients with pediatric onset had disadvantages in seizure control and preserving motor functions. On the other hand, favorable responses in adult cases have led to the proposal of IVIg as first-line treatment especially in late onset cases [9,10]. Further studies on the efficacy of IVIg considering the age at treatment are needed.

Regular steroid pulse therapy showed relatively good seizure outcome, good cognitive outcome, and good motor outcome (Table 2). Regular pulse therapy had the highest response rate for seizure outcome among the immunomodulatory therapies, and this therapy reduces frequent intractable seizures in the acute stage. Regular steroid pulse therapy also had the best cognitive outcome among all treatments other than surgical resection in dominant hemisphere, although this treatment does not achieve complete seizure control. Motor outcome was good and deterioration of motor function was infrequent. Although short-term intravenous bolus administration of methylprednisolone has been reported to be effective in blocking status epilepticus [10,11], the efficacy of regular pulse therapy administered for several months has not been reported. Cognitive outcome of regular pulse therapy seemed to be better in earlier stages, because R80 was higher in patients without MRI lesions, compared to patients with advanced MRI lesions. These data suggest that regular pulse therapy may contribute to seizure control and cognitive preservation in early-stage RS. However, the treatment was discontinued in 62% of patients, mainly due to frequent hospitalization which disturbs school life. The therapy was replaced by tacrolimus therapy in seven of 13 patients. These data suggest that quality of life has to be considered in planning treatment strategies for RS patients.

Tacrolimus therapy showed moderately good seizure control, relatively good cognitive outcome, and very good motor outcome (Table 2). In a previous study, tacrolimus-treated patients had superior outcome in neurological and cognitive functions, but no better seizure outcome compared to untreated patients [12]. These data suggest that tacrolimus therapy can maintain cognitive and motor function, in spite of relatively inferior seizure control.

The diagnostic criteria of RS include clinical symptoms, EEG findings, and MRI characteristics suggesting unilateral cortical deficit [2]. Therefore, many patients have already more or less permanent disturbance of motor and cognitive functions when RS is confirmed, and FH is accepted mainly by patients with non-dominant hemisphere involvement. However, FH has the issues of not achieving 100% SFR and poor cognitive outcome, especially in patients with higher IQ. To improve the outcome of RS, we suggest a new treatment strategy using early immunomodulatory therapies (Fig. 8). Recent immunological studies in RS revealed a pivotal role of cytotoxic T cells, and proposed biomarkers such as CSF levels of granzyme B and IFNγ in early-stage RS without permanent neurological involvement [4,5]. Within one year of seizure onset, 60% of patients had high intensity lesions (HIL) on MRI [8]. Therefore HIL may be one of the early markers suggesting RS. While granzyme B and IFNγ in CSF

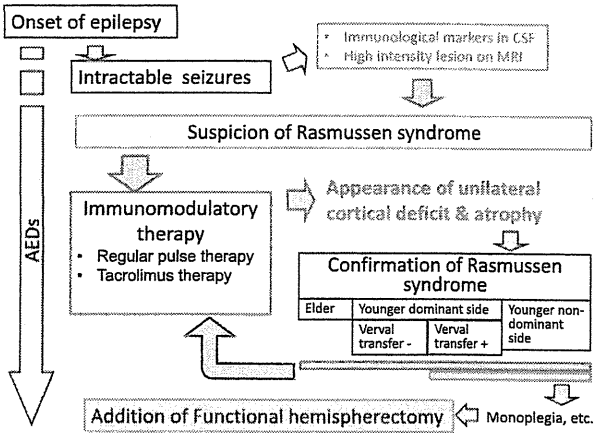

Fig. 8. New treatment strategy for Rasmussen syndrome with pediatric onset.

are early stage markers, HIL may also contribute to an early suspected diagnosis of RS and indicate the timing of starting immunomodulatory therapies before the appearance of unilateral cortical deficit. Because regular pulse therapy yields superior seizure outcome as well as better cognitive outcome in early stage before the appearance of MRI lesions than in later stage, we recommend regular pulse therapy as first-line immunomodulatory therapy in patients with suspected RS. After several to 12 months of regular pulse therapy when seizures become stable, switching to tacrolimus therapy is recommended so that therapy can be conducted mainly on an out-patient basis. In the course of immunomodulatory therapies, appearance of unilateral cortical deficits necessitates prompt addition of FH. When the neurological deficits manifested are equivalent to those that would inevitably result from FH, then FH is indicated mainly in patients with disease involving the non-dominant hemisphere. In patients with disease involving the dominant hemisphere, FH can be considered if verbal transfer is possible.

5. Conclusion

For the improvement of outcome of RS, there seems to be a place for immunomodulatory treatments in pediatric patients, and the treatments are recommended in the early stages, preferably before any motor or cognitive dysfunction and among no MRI lesions.

Statement

We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure of conflicts of interest

All authors have no conflict of interest to disclose.

Acknowledgements

This study was funded in part by grants-in-aid for Scientific Research I Nos. 21591342, 23591238 and 24591537; Comprehensive Research on Disability Health and Welfare; Research on Rare and Intractable Diseases; Intramural Research Grant (22-3) for Neurological and Psychiatric Disorders of NCNP; and grants from The Japan Epilepsy Research Foundation.

Part of this work has been presented on the International Symposium on Surgery for Catastrophic Epilepsy in Infants (ISCE), the 14 h Annual Meeting of ISS, Tokyo, February 18–19, 2012.

References

[1] Rasmussen T, Olszewski J, Lloyd-Smith D. Focal seizures due to chronic localized encephalitis. Neurology 1958;8:435–45.
[2] Bien CG, Granata T, Antozzi C, Cross JH, Dulac O, Kurthen M, et al. Pathogenesis, diagnosis and treatment of Rasmussen encephalitis: a European consensus statement. Brain 2005;128:454–71.
[3] Takahashi Y. Infections as causative factors of epilepsy. Future Neurol 2006;1:291–302.
[4] Bien CG, Bauer J, Deckwerth TL, Wiendl H, Deckert M, Wiestler OD, et al. Destruction of neurons by cytotoxic T cells: a new pathogenic mechanism in Rasmussen's syndrome. Ann Neurol 2002;51:311–8.
[5] Takahashi Y, Mine J, Kubota Y, Yamazaki E, Fujiwara T. A substantial number of Rasmussen syndrome patients have increased IgG, CD4+ T cells, TNFα, and granzyme B in CSF. Epilepsia 2009;50:1419–31.
[6] Oguni H, Andermann F, Rasmussen TB. The syndrome of chronic encephalitis and epilepsy. A study based on the MNI series of 48 cases. Adv Neurol 1992;57:419–33.
[7] Bien CG, Widman G, Urbach H, Sassen R, Kuczaty S, Wiestler OD, et al. The natural history of Rasmussen's encephalitis. Brain 2002;125:1751–9.
[8] Yamazaki E, Takahashi Y, Akasaka N, Fujiwara T, Inoue Y. Temporal changes in brain MRI findings in Rasmussen syndrome. Epileptic Disord 2011;13:229–39.
[9] Villani F, Spreafico R, Farina L, Giovagnoli AR, Bernasconi P, Granata T, et al. Positive response to immunomodulatory therapy in an adult patient with Rasmussen's encephalitis. Neurology 2001;56:248–50.
[10] Granata T, Fusco L, Gobbi G, Freri E, Ragona F, Broggi G, et al. Experience with immunomodulatory treatments in Rasmussen's encephalitis. Neurology 2003;61:1807–10.
[11] Hart YM, Cortez M, Andermann F, Hwang P, Fish DR, Dulac O, et al. Medical treatment of Rasmussen's syndrome (chronic encephalitis and epilepsy): effect of high-dose steroids or immunoglobulins in 19 patients. Neurology 1994;44:1030–6.
[12] Bien CG, Gleissner U, Sassen R, Widman G, Urbach H, Elger CE. An open study of tacrolimus therapy in Rasmussen's encephalitis. Neurology 2004;62:2106–9.

Influence of CYP2C19 Polymorphism and Concomitant Antiepileptic Drugs on Serum Clobazam and *N*-Desmethyl Clobazam Concentrations in Patients With Epilepsy

Yoshiaki Yamamoto, MS Pharm,† Yukitoshi Takahashi, MD, PhD,* Katsumi Imai, MD,* Kou Miyakawa, BS,* Shigeko Nishimura, PhD,* Risa Kasai,* Hiroko Ikeda, MD,* Rumiko Takayama, MD,* Yukiko Mogami, MD,* Tokito Yamaguchi, MD,* Kiyohito Terada, MD, PhD,* Kazumi Matsuda, MD, PhD,* Yushi Inoue, MD, PhD,* and Yoshiyuki Kagawa, PhD†*

Objective: The aims of this study were to identify the factors influencing the metabolism of clobazam (CLB) and its active metabolite [*N*-desmethyl clobazam (NCLB)] and to evaluate the NCLB concentration as an indicator for CYP2C19 polymorphism in epileptic patients.

Methods: A total of 302 serum samples from 238 Japanese patients were evaluated. The ratios of the serum CLB and NCLB concentrations to the CLB dose (CD ratios) were calculated and compared with CYP2C19 phenotypes.

Results: The mean CD ratio of NCLB in extensive metabolizers (EM: *1/*1), intermediate metabolizers (IM: *1/*2 or *1/*3), and poor metabolizers (PM: *2/*2, *3/*3, or *2/*3) was 3.1, 4.9, and 21.6 (μg/mL)/(mg/kg), respectively. In the EM and IM groups, the concomitant use of hepatic enzyme inducers (phenytoin and carbamazepine) reduced the CD ratio of CLB and increased that of NCLB. In the PM group, these inducers also decreased the CD ratio for CLB but did not elevate the CD ratio for NCLB. Using multiple regression analysis, body weight showed a positive correlation with an increased CD ratio for NCLB. The concomitant use of zonisamide and stiripentol also elevated the CD ratio for NCLB in the EM and IM groups, but that of the PM group was almost unchanged. When the cut-off value of the CD ratio for NCLB was set as 10.0 (μg/mL)/(mg/kg) for predicting the CYP2C19 PM status, the sensitivity and specificity were 94.4% and 95.7%, respectively.

Conclusions: The interaction between NCLB and other antiepileptic drugs showed marked differences among CYP2C19 phenotypes. Measurement of the serum NCLB concentration is clinically useful for identifying the PM phenotype.

Received for publication June 6, 2012; accepted December 18, 2012.

From the *Department of Clinical Research, National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Ulushiyama, Aoi-ku; and †Department of Clinical Pharmaceutics, Graduate School of Pharmaceutical Sciences, University of Shizuoka, Yada, Suruga-ku, Shizuoka-shi, Shizuoka, Japan.

The authors declare no conflict of interest.

Correspondence: Yoshiaki Yamamoto, MS Pharm, Department of Clinical Research, National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders 886, Ulushiyama, Aoi-ku, Shizuoka-shi, Shizuoka, 420-8688, Japan (e-mail: yamamoty@szec.hosp.go.jp).

Key Words: clobazam, *N*-desmethyl clobazam, CYP2C19, drug interaction, stiripentol, therapeutic drug monitoring

(*Ther Drug Monit* 2013;35:305–312)

INTRODUCTION

Cytochrome P450 (CYP) 2C19 plays an important role in the metabolism of the proton pump inhibitors, clopitogrel, sertraline, escitalopram, moclobemide, and voriconazol.[1] CYP2C19 activity is decreased in individuals with mutant alleles, which are CYP2C19*2 (681 G>A) and CYP2C19*3 (636 G>A). The frequency of CYP2C19*2 and *3 is 30% and 5%, respectively, in the Japanese population, which are higher rates than those of 15% and 0.04% in whites.[2] These 2 mutant alleles are associated with the poor metabolizer (PM) phenotype, which can influence both the toxicity and efficacy of drugs.[1] For this reason, it is important to identify the CYP2C19 phenotype. Previous studies have suggested that measurement of the blood concentrations of mephenytoin, omeprazole, or lansoprazole (and its metabolites) could be useful for identifying the CYP2C19 phenotype.[3]

Clobazam (CLB) is widely used for the treatment of refractory epilepsy. Figure 1 shows the metabolic pathways of CLB. It is metabolized mainly by CYP3A4 and partly by CYP2C19 and CYP2B6 to yield an active metabolite [*N*-desmethyl-clobazam (NCLB)]. NCLB is, itself, metabolized chiefly by CYP2C19 to an inactive metabolite (4′-hydroxynorclobazam).[4] In a previous study, a mutation of CYP2C19 was associated with the serum concentration of NCLB.[5] Kosaki et al[6] and Seo et al[7] reported that Japanese epileptic patients with the PM phenotype had a high NCLB concentration-to-dose ratio (CD ratio) and an elevated NCLB/CLB concentration ratio, but it remains unclear whether these ratios can be used to predict the CYP2C19 phenotype. One of the reasons for this is because the metabolism of CLB and NCLB is influenced by concomitant use of other antiepileptic drugs (AEDs). In particular, hepatic enzyme inducers (inducers) such as phenytoin (PHT), carbamazepine (CBZ), and phenobarbital (PB) induce CYP3A4, resulting in a decrease of the CLB concentration and an increase of the NCLB concentration.[8–10] Stiripentol (STP) is an inhibitor of CYP3A4 and CYP2C19 that is used for the treatment of the

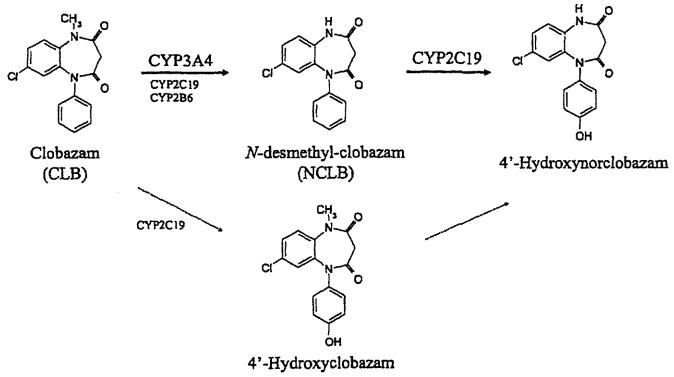

FIGURE 1. Metabolism of CLB. The major metabolic pathway of CLB is shown with bold arrows. CLB is demethylated mainly by CYP3A4 and partly by CYP2C19 or CYP2B6 to yield NCLB (an active metabolite). NCLB is hydroxylated chiefly by CYP2C19 to form an inactive metabolite (4'-hydroxynorclobazam). The minor metabolic pathway is shown by the thin arrows.

Dravet syndrome.[11,12] It is known that topiramate (TPM), oxcarbazepine, and felbamate also inhibit CYP2C19,[13–16] whereas zonisamide (ZNS) and diazepam are metabolized by CYP2C19.[17,18] Unfortunately, there is limited information available about interactions between CLB and these drugs, and it remains unknown whether these interactions differ among CYP2C19 phenotypes. In addition, there have only been a small number of studies on the pharmacokinetics of CLB in pediatric patients,[4,9] and the differences in metabolism between adults and children remain poorly understood. Giraud et al[4] reported that the NCLB/CLB concentration ratio was higher in pediatric patients carrying the CYP2C19*2 allele than in patients with the wild-type allele, but the influence of the CYP2C19 PM phenotype or concomitant AEDs was not investigated.

In general, therapeutic drug monitoring (TDM) is recommended when AEDs are administered to optimize drug doses, but the usefulness of TDM in patients receiving CLB is not established. Bardy et al[19] reported that measuring concentrations of CLB and NCLB shows limited value and could not establish the therapeutic target range or intoxication range. On the other hand, Kinoshita et al[20] reported that achieving a higher concentration of NCLB was associated with increased efficacy for refractory epilepsy, whereas Seo et al[7] suggested that a mutation of CYP2C19 can influence the efficacy of long-term CLB therapy. Thus, it may be important to monitor the plasma concentration profile of NCLB to achieve individualized therapy.

Accordingly, the aims of this study were to identify factors influencing the serum NCLB concentration and to determine whether NCLB can be used as a marker to estimate the CYP2C19 phenotypes in Japanese patients with epilepsy.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the ethics committee of the National Epilepsy Center (Shizuoka, Japan). A total of 238 patients with epilepsy who were on treatment with CLB (Mystan tablets or fine granules; Dainippon Sumitomo Pharma, Osaka, Japan) at our hospital between January 2008 and December 2011 were evaluated. Informed consent was obtained from each patient or guardian before blood collection. Patients using lansoprazole or clarithromycin (CYP2C19 or 3A4 inhibitors) and those suffering from hypoalbuminemia (albumin<3.0 mg/dL) were excluded. Patients under the age of 16 years were defined as pediatric patients. A total of 15 pediatric patients were taking STP (Diacomit; Biocodex, Gentilly, France), even though it was not approved in Japan in 2011 (compassionate use[21] or private import). None of the patients were using diazepam, oxcarbamazepine, or felbamate, which are potential inhibitors of CYP2C19.

Blood Sampling and Analysis of CLB and NCLB

Blood samples were obtained from the patients between 2 and 12 hours after the administration of CLB and were centrifuged at 3500 rpm. Serum concentrations of CLB and NCLB were analyzed by high-performance liquid chromatography with a reversed-phase column.[22] The steady-state concentration was measured after CLB treatment had continued with the same dosing schedule for at least 28 days. If inducers (PHT, PB, or CBZ) or possible CYP2C19 inhibitors (TPM, ZNS, or STP) were added or discontinued during the study period, we obtained 2 blood samples from the relevant patient. When 2 or more blood samples were obtained from a single patient with an unchanged AED regimen, the average value obtained at the highest maintenance dose of CLB was analyzed. Data on the age and body weight were treated in the same manner. Overall, we obtained 302 analyzable samples from 238 patients.

Genotyping for CYP2C19

DNA was isolated from blood samples by using a MagNA Pure Compact (Roche Diagnostics, Mannheim, Germany). Real-time polymerase chain reaction was performed with a Light-Cycler (Roche, Mannheim, Germany) using fluorogenic hybridization probes. The polymerase chain reaction conditions, primers, and hybridization probes were described previously.[23]

Based on the CYP2C19 genotype, patients were classified into 3 groups: extensive metabolizers (EM—CYP2C19*1/*1), intermediate metabolizers (IM—CYP2C19*1/*2 or *1/*3), and PM (CYP2C19*2/*2, *3/*3, or *2/*3).

Statistical Methods

For comparisons between 2 groups, the unpaired *t*-test was performed. For multigroup comparisons, analysis of variance or the Kruskal–Wallis test was used, followed by a Games/Howell post hoc test. Relationships between the CD ratio for NCLB and a variety factors were examined by multiple regression analysis, with the partial regression coefficient (B) and standardized partial regression coefficient (β) being obtained. A stepwise procedure was performed to assess the dependence between CD ratio for NCLB and 10 factors (age, gender, body weight, CLB concentration, and concomitant use of PHT, CBZ, PB, TPM, ZNS, or STP). Receiver operating characteristic (ROC) analysis was used to assess the CD ratio of NCLB or the NCLB/CLB concentration ratio as markers for

estimating the CYP2C19 phenotype. The results are expressed as the mean and 95% confidence interval (95% CI). Statistical analyses were conducted with IBM SPSS Statistics (Ver 19.0).

RESULTS

Patient Profile

Table 1 lists the characteristics of the patients, classified into 3 groups based on CYP2C19 phenotype. The gender and CLB dose showed significant differences among the 3 groups. Despite a significantly lower dose of CLB being used in the PM group, the NCLB concentration of this group was significantly higher than that of the EM group or the IM group ($P < 0.001$). The CD ratios for CLB and NCLB, and the NCLB/CLB concentration ratio, were also higher in the PM group than in the EM or IM groups ($P < 0.001$). The correlations between the CLB concentrations and NCLB concentrations in patients from the EM, IM, and PM groups were 0.40, 0.48, and 0.64, respectively ($P < 0.001$). The other AEDs used were as follows: valproic acid, clonazepam, bromide, lamotorigine, levetiracetam, gabapentin, and ethosuximide, which were not considered to have any significant effect on the concentrations of CLB or NCLB.

Relationship Between CD Ratios for CLB and NCLB in Children and Adults

Figure 2 shows the CD ratios for CLB and NCLB in adult and pediatric patients. In the EM and IM groups, the mean CD ratio for CLB was significantly higher in adults than in pediatric patients (EM group; 0.47 versus 0.36, $P < 0.05$ and IM group; 0.50 versus 0.36, $P < 0.005$, respectively). In contrast, there was no significant difference between adults and children in the PM group (0.58 versus 0.55). Also, the mean CD ratio for NCLB was higher in adult patients than in pediatric patients among all phenotypes (EM group: 3.9 versus 2.3, IM group: 6.3 versus 3.5 and PM group: 25.0 versus 19.2, $P < 0.005$, respectively).

Influence of Inducers on the CD Ratios

Figure 3 shows a comparison of the CD ratios for CLB and NCLB in patients with or without inducers. The mean CD ratios for CLB in patients from the EM, IM, and PM groups without inducers were 0.52, 0.50, and 0.72, respectively. Among patients using inducers, the mean CD ratio decreased significantly to 0.32 in the EM group, 0.38 in the IM group, 0.39 in the PM group, respectively. In contrast, the mean CD ratios for NCLB of patients without inducers in the EM, IM, and PM groups were 2.6, 3.7, and 21.4, respectively, whereas patients using inducers had CD ratios of 3.5, 6.2, and 21.9, respectively. In the IM group, the combined use of inducers significantly reduced the CD ratio for CLB and increased the CD ratio for NCLB, and the same trend was observed in the EM group. In contrast, the CD ratio for NCLB was almost unaffected by inducers in the PM group despite a large decrease of the CD ratio for CLB.

TABLE 1. Characteristics of the Subjects

	Mean (95% CI) or No. Patients			
	EM Group	IM Group	PM Group	*P*
Total no. samples	97	133	72	
Age (yrs)	21.51 (17.5–24.7)	22.7 (19.3–26.0)	17.3 (14.4–20.2)	NS
<15 yrs	49	63	42	NS
Gender (male/female)	49/48	60/73	47/25	<0.05
CLB dose (mg/d)	0.30 (0.27–0.34)	0.33 (0.30–0.37)*	0.25 (0.22–0.28)	<0.01
CLB concentration (μg/mL)	0.12 (0.10–0.14)	0.14 (0.12–0.16)	0.13 (0.11–0.15)	NS
CD ratio [(μg/mL)/(mg/kg)]	0.41 (0.36–0.47)*	0.44 (0.39–0.48)*	0.56 (0.49–0.64)	<0.005
NCLB concentration (μg/mL)	0.95 (0.74–1.16)†,‡	1.64 (1.40–1.88)‡	5.58 (4.70–6.45)	<0.001
CD ratio [(μg/mL)/(mg/kg)]	3.1 (2.5–3.6) †,‡	5.0 (4.5–5.5) ‡	21.6 (19.7–23.5)	<0.001
NCLB/CLB concentration ratio	10.3 (8.1–12.6)‡,§	16.0 (13.5–18.4)‡	53.6 (45.5–65.7)	<0.001
Concomitant AEDs				
PHT	25	39	20	NS
CBZ	26	36	16	NS
PB	14	16	3	NS
Inducers	52	71	33	NS
Topiramate	22	20	12	NS
ZNS	18	19	12	NS
Stirpentol	4	6	5	NS
Other AEDs	70	98	48	NS

Inducers were PHT, CBZ, and PB. Other AEDs were valproic acid, clonazepam, bromide, lamotorigine, levetiracetam, gabapentin, and ethosuximide. Significance was determined by the analysis of variance or the χ^2 test. Games-Howell post hoc test results are as follows:

*$P < 0.01$ versus PM.
†$P < 0.001$ versus IM.
‡$P < 0.001$ versus PM.
§$P < 0.005$ versus IM.

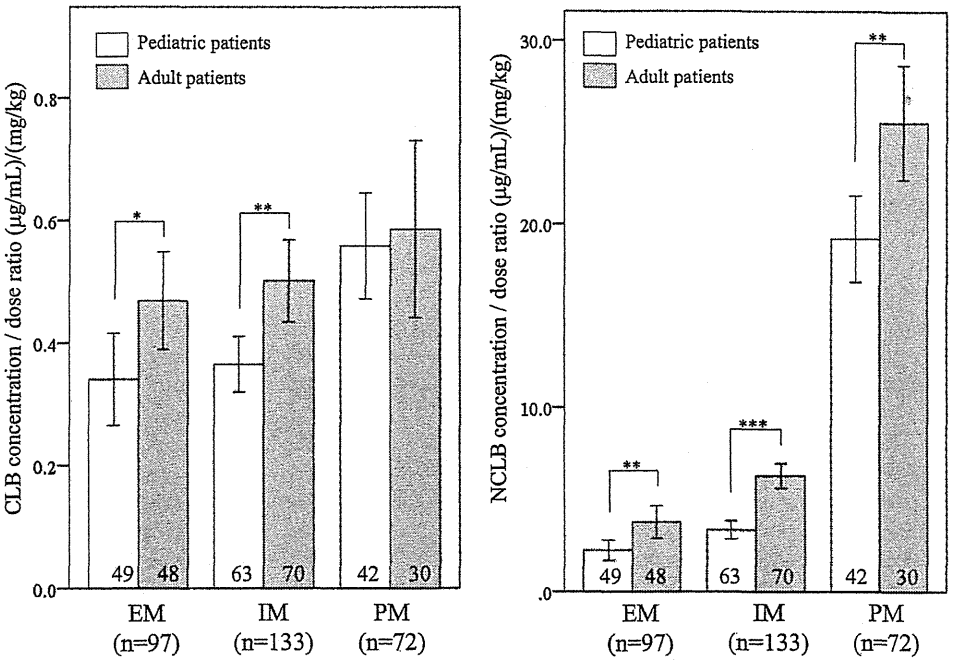

FIGURE 2. CD ratios for CLB and NCLB stratified by the CYP2C19 phenotype in adult and pediatric patients. Significance was determined by the unpaired *t*-test. $*P < 0.05$, $**P < 0.005$, $***P < 0.001$.

Factors Influencing the CD Ratio for NCLB on Multiple Regression Analysis

Tables 2 and 3 show the factors influencing the CD ratio for NCLB according to multiple regression analysis using the stepwise selection procedure. In adult patients, the concomitant use of PHT, CBZ, and ZNS showed a significant positive correlation in the EM and IM groups (Table 2). In contrast, the CD ratio for NCLB was not influenced by concomitant use of these AEDs in the PM group. Among the inducers, PHT showed the strongest positive correlation, whereas PB had no significant effect and was eliminated by stepwise selection. Age and TPM were also eliminated for all 3 groups. This model explained 48%, 47%, and 29% of the interindividual variability of the CD ratio for NCLB in the EM, IM, and PM groups, respectively.

Among pediatric patients, STP showed a significant positive correlation in the EM and IM groups, but not in the PM group (Table 3). Among the inducers, PHT was the only significant drug. In each group, ZNS had a similar influence to that shown in Table 2. Body weight had a significant positive correlation compared with that in adult patients. The model explained 76%, 46%, and 54% of the interindividual

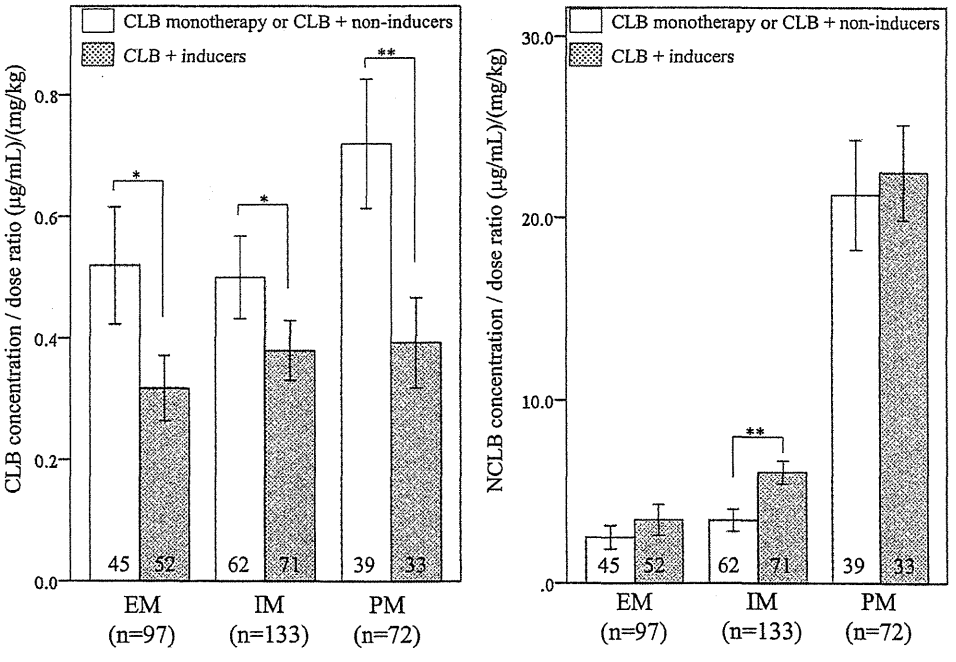

FIGURE 3. CD ratios for CLB and NCLB stratified by the CYP2C19 genotype in patients with or without inducers. Significance was determined by the unpaired *t*-test. $*P < 0.005$, $**P < 0.001$.

TABLE 2. Factors Influencing the CD Ratio for NCLB in Adult Patients Using Multiple Regression Analysis

Factor	*B*	β	*P*
EM group (R^2 = 0.48)			
Constant	1.0		0.09
PHT	3.7	0.57	<0.001
CBZ	1.5	0.24	<0.05
ZNS	4.7	0.59	<0.001
IM group (R^2 = 0.47)			
Constant	0.06		0.96
Body weight (kg)	0.06	0.33	<0.005
CLB concentration (μg/mL)	0.005	0.22	<0.05
PHT	3.2	0.56	<0.001
CBZ	1.9	0.31	<0.01
ZNS	3.1	0.31	<0.005
PM group (R^2 = 0.29)			
Constant	7.5		0.21
Body weight (kg)	0.18	0.34	<0.05
CLB concentration (μg/mL)	0.04	0.52	<0.005

Stepwise forward selection method: $P < 0.05$ to enter and $P < 0.10$ to remove.
B, partial regression coefficient; β, standardized partial regression coefficient; R^2, adjusted coefficient of determination.

TABLE 3. Factors Influencing the CD Ratio for NCLB in Pediatric Patients Using Multiple Regression Analysis

Factor	*B*	β	$P_{>}$
EM group (R^2 = 0.76)			
Constant	0.30		0.27
Body weight (kg)	0.05	0.34	<0.001
PHT	1.6	0.31	<0.001
ZNS	2.0	0.43	<0.001
STP	4.9	0.75	<0.001
IM group (R^2 = 0.46)			
Constant	0.34		0.64
Body weight (kg)	0.1	0.43	<0.001
PHT	1.5	0.21	<0.001
ZNS	2.6	0.43	<0.001
STP	3.2	0.39	<0.001
PM group (R^2 = 0.54)			
Constant	10.0		<0.001
Body weight (kg)	0.28	0.74	<0.001

Stepwise forward selection method: $P < 0.05$ to enter and $P < 0.10$ to remove.
B, partial regression coefficient; β, standardized partial regression coefficient; R^2, adjusted coefficient of determination.

variability of the CD ratio for NCLB in the EM, IM, and PM groups, respectively.

Changes of the CD Ratio for NCLB After Addition or Discontinuation of AEDs

Table 4 shows the effect on the CD ratio for NCLB with the addition or discontinuation of the inducers TPM, ZNS, or STP. In the EM and IM groups, adding PHT or CBZ to CLB increased the CD ratio for NCLB compared with that in the PM group, but there were no significant differences among the 3 groups. Because there was only 1 patient using PB in each group, it was not possible to compare the effect on the CD ratio of this drug. Addition of ZNS increased the CD ratio in the EM and IM groups. A few patients started or discontinued STP during the study period; addition of STP resulted in a marked increase of the CD ratio for NCLB in the EM and IM groups. These results were similar to those of multiple regression analysis.

Prediction of the CYP2C19 Phenotype From the CD Ratio (ROC Analysis)

Figure 4 shows the results of ROC analysis. For prediction of the CYP2C19 PM phenotype, the cut-off value of the CD ratio for NCLB was set at 10.0 (μg/mL)/(mg/kg). This achieved a sensitivity and specificity of 94.4% and 95.7%, respectively (Fig. 4A). Ten out of 230 patients with the EM or IM phenotypes had high CD ratios for NCLB (range: 10.4–16.4). They included 4 patients receiving inducers, and 5 patients receiving ZNS or ZNS plus inducers. The area under the ROC curve (AUC–ROC) was 0.991 (95% CI, 0.984–0.999), and this exhibited the best performance for predicting the PM phenotype of CYP2C19. When the NCLB/CLB concentration ratio was used as the dependent variable to predict the PM phenotype, the AUC–ROC value was 0.914 (95% CI, 0.880–0.949; data not shown). In contrast, when prediction of the IM phenotype was attempted in the EM and IM groups, the optimum cut-off value of the CD ratio for NCLB was 3.5 (μg/mL)/(mg/kg), achieving a sensitivity and specificity of 61.7% and 69.1%, respectively (Fig. 4B).

DISCUSSION

In this study, we investigated factors influencing the metabolism of CLB and NCLB in epilepsy patients classified by their CYP2C19 phenotype. Our data showed that the CD ratio for NCLB was markedly higher in the PM group than in the EM and IM groups, confirming previous reports.[6,7] We also found that the CD ratio for NCLB showed little change with the concomitant use of AEDs in the PM group and that this ratio could be employed as a marker for predicting the PM phenotype.

In addition, our study showed a higher CD ratio for CLB in the PM group (Table 1), and significant differences between pediatric and adult patients in the EM and IM groups, but not in the PM group (Fig. 2). Giraud et al[4] reported that CLB was metabolized by CYP3A4, CYP2B6, and CYP2C19 in vitro. Yanni et al[24] reported that the N-oxidation of voriconazole by CYP2C19 was 3-fold higher in children compared with that in adults, whereas *N*-oxidation by CYP3A4 did not differ. Our study suggested that CLB metabolism by CYP2C19 might be higher in pediatric patients compared with that in adults. Thus, pediatric patients from the PM group with the loss of CYP2C19 function may show a marked decrease of CLB metabolites.

Previous studies have suggested that the concomitant use of inducers reduces CLB concentrations and elevates NCLB concentrations because induction of CYP3A4 results in the increased conversion of CLB to NCLB.[5,6,8,10] In this study, similar results were observed for the EM and IM groups (Fig. 3). However, among the inducers, PB did not elevate the CD ratio for NCLB (Table 2). These findings agreed with

TABLE 4. Change of the CD for NCLB Ratio After the Addition or Discontinuation of AEDs

	EM		IM		PM		
Group	**n**	**Change Ratio Mean (95%CI)**	**n**	**Change Ratio Mean (95%CI)**	**n**	**Change Ratio Mean (95%CI)**	***P****
Additional AED							
PHT	2	2.2 (5.3–9.8)	6	1.9 (0.9–2.9)	2	0.7 (−0.01–1.5)	NS
CBZ	3	2.0 (−0.7–4.7)	8	1.4 (1.1–1.7)	3	0.9 (0.3–1.5)	NS
PB	1	2.6	1	0.90	1	0.9	—
Topiramate	11	1.0 (0.96–1.11)	9	0.99 (0.91–1.06)	5	0.99 (0.94–1.03)	NS
ZNS	2	2.7 (1.40–3.90)†,‡	7	1.66 (1.36–1.96)‡	4	1.08 (0.97–1.19)	<0.01
STP	1	10.3	2	4.48 (−9.23–18.2)	0	—	—

*Significance was determined by the Kruskal–Wallis test.
†$P < 0.05$ versus PM (Games/Howell post hoc test).
‡$P < 0.01$ versus PM (Games/Howell post hoc test).

the reports of Bun et al[8] and Sennoune et al.[10] NCLB is produced by the demethylation of CLB, whereas CLB can also be metabolized by direct hydroxylation. Thus, it is possible that PB might facilitate the hydroxylation of CLB by induction of hepatic enzymes. Our study also showed that PHT strongly increased the CD ratio for NCLB (Tables 2 and 3). In studies of the interaction between PHT and CYP3A substrates, the inducing effect of PHT on CYP3A4 has been found to be stronger than that of CBZ or PB.[25,26] Thus, PHT strongly induced CYP3A4, leading to the increased conversion of CLB to NCLB. However, despite a decrease of CLB in patients from the PM group receiving inducers, there was no change of the CD ratio for NCLB (Fig. 3). A possible explanation is that the high concentrations of NCLB might arise due to a shift of the CLB metabolism from desmethylation to another pathway (eg, hydroxylation).

According to multiple regression analysis, the concomitant use of ZNS and STP led to an increased CD ratio for NCLB in the EM and IM groups (Tables 2 or 3). This effect of ZNS and STP was also shown in patients who had commenced the use of these AEDs recently (Table 4). ZNS is metabolized mainly by CYP3A4, but Okada et al[17] have demonstrated that CYP2C19 polymorphism affects its metabolism. Thus, ZNS might competitively inhibit CYP2C19, resulting in an increase of the CD ratio for NCLB.

There were only 15 pediatric patients receiving CLB and STP, but the standardized partial regression coefficients were clearly different among the 3 groups (Table 3). In the STICLO study, Chiron et al[11] found that the CD ratio for NCLB increased about 3-fold after the addition of STP, and we showed the same trend in Japanese patients carrying the *2 or *3 alleles (IM group). Although the CYP2C19 polymorphism was not investigated in their study, 3 out of 20 patients had no increase in NCLB concentrations. Likewise, our multiple regression model did not show a positive correlation between the PM phenotype and concomitant use of STP. Yu et al[27] reported that metabolism of moclobemide (a CYP2C19 substrate) is extensively inhibited by omeprazole (a CYP2C19 inhibitor) in EM subjects, but not in PM subjects. Thus, the PM phenotype related to the loss of CYP2C19 function, results in little influence on the concomitant use of CYP2C19 inhibitors such as STP and ZNS.

The antiseizure effect of NCLB is only about one-fifth of that for CLB itself, but previous studies have indicated that the NCLB concentration has an influence on clinical efficacy in patients with refractory epilepsy.[7,20] Thus, the addition of ZNS or STP to CLB in EM or IM patients might produce a synergistic effect that could be clinically valuable. In particular, the EM phenotype has high enzyme activity, so the EM phenotype will be susceptible to CYP2C19 inhibitors compared with the

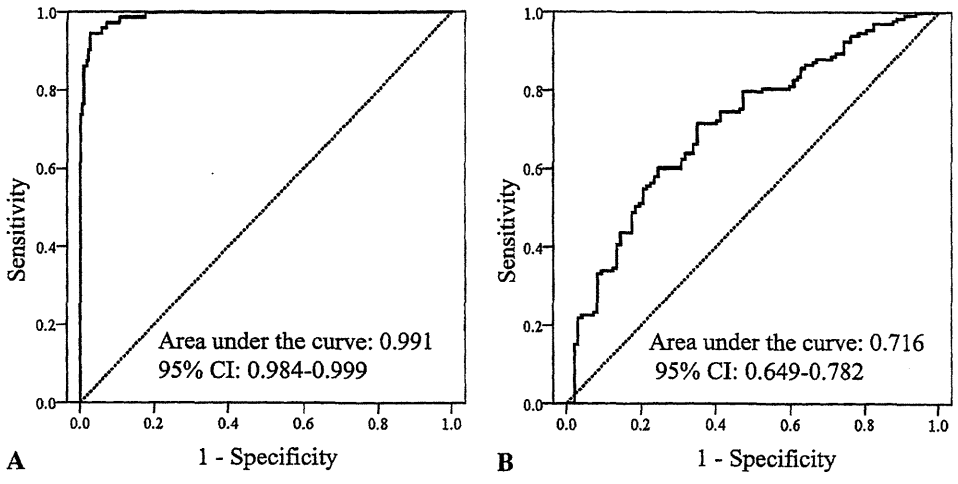

FIGURE 4. ROC curves used to determine optimum cut-off values of the CD ratio for NCLB to predict the CYP2C19 PM (A) and IM (B) phenotypes. A, To predict the CYP2C19 PM phenotype, the CD ratios for NCLB in the EM, IM, and PM groups were analyzed (n = 302). B, To predict the CYP2C19 IM phenotype, the CD ratios for NCLB in the EM and IM groups were analyzed (n = 230).

IM phenotype. Further studies on the clinical efficacy of CLB will be necessary to evaluate the influence of CYP2C19 polymorphism and concomitant use of STP and ZNS.

The metabolism of NCLB was hardly influenced by the concomitant use of TPM. Sachdeo et al[13] reported that a high dose of TPM inhibits CYP2C19, resulting in decreased clearance of PHT. In our study, only 2 out of 22 patients were on a high dose of TPM (>400 mg/d in adults or 8 mg/kg in children). This small number of patients using high-dose TPM may have contributed to the lack of impact on CYP2C19 activity.

Among pediatric patients, body weight had a strong influence in all 3 groups (Table 3). The liver weight/body weight ratio, hepatic blood flow, and renal blood flow all decrease as children grow up. Thus, an increase of body weight with growth reflects decreased the metabolism of NCLB.

In previous studies, the concentration of mephenytoin, omeprazole, or lansoprazole (and its metabolites) was found to be useful for identifying the CYP2C19 phenotype.[3] This is the first study to establish the CD ratio for NCLB as a marker that can predict the CYP2C19 PM phenotype in patients with epilepsy. If the CD ratio is >10 (μg/mL)/(mg/kg), the accuracy of predicting the PM phenotype is 95.3%. Several studies have demonstrated that patients with the PM phenotype have a high NCLB/CLB concentration ratio.[6,7] However, we found that the CD ratio for NCLB showed little change with the concomitant use of inducers in the PM group, whereas the NCLB/CLB concentration ratio was strongly influenced by inducers. For this reason, the AUC–ROC value was higher for the NCLB CD ratio than that for the NCLB/CLB concentration ratio. In contrast, it is difficult to predict whether patients have the EM or IM phenotypes, because these patients are susceptible to interactions with CYP3A4 inducers or CYP2C19 inhibitors.

In our study, the serum concentrations of CLB and NCLB were not measured at a specific time because of their long elimination half-life (31 hours for CLB and 70 hours for NCLB in a single-dose study).[28] In particular, the steady-state NCLB concentration remains unchanged for >7 days after withdrawal of CLB.[29] Thus, it was assumed that this CD ratio allows us to predict the PM phenotype of CYP2C19 regardless of the blood collection time, or the time interval since dosing. Further investigations will be needed to evaluate whether administering a single dose of CLB and analyzing the NCLB concentration can be used to predict the CYP2C19 phenotype.

This study had several limitations. First, we did not investigate the CYP2C19*17 allele, which is an ultrarapid metabolizer phenotype.[30] Because of its low frequency (1.3%) in the Japanese population,[31] we considered that it would be difficult to stratify a group carrying this allele. Second, there were only 3 patients using PB in the PM group, so the influence of PB could not be analyzed quantitatively. Also, only pediatric patients with Dravet syndrome received STP.

CONCLUSIONS

Our study identified factors influencing the CD ratio for NCLB and showed that interactions between CLB and PHT, CBZ, ZNS, and STP could be quantified by multiple regression analysis when patients were classified into EM, IM, and PM phenotypes. We recommend measuring the serum concentration of NCLB at least once during CLB therapy. The CD ratio for NCLB can be employed to predict the PM phenotype of CYP2C19, which is useful for predicting the impact on interactions between CLB and CYP3A4 inducers or CYP2C19 inhibitors.

REFERENCES

1. Swen JJ, Nijenhuis M, de Boer A, et al. Pharmacogenetics: from bench to byte—an update of guidelines. *Clin Pharmacol Ther.* 2011;89:662–673.
2. Gardiner SJ, Begg EJ. Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. *Pharmacol Rev.* 2006;58:521–590.
3. Streetman DS, Bertino JS Jr, Nafziger AN. Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogenetics.* 2000;10:187–216.
4. Giraud C, Tran A, Rey E, et al. In vitro characterization of clobazam metabolism by recombinant cytochrome P450 enzymes: importance of CYP2C19. *Drug Metab Dispos.* 2004;32:1279–1286.
5. Contin M, Sangiorgi S, Riva R, et al. Evidence of polymorphic CYP2C19 involvement in the human metabolism of *N*-desmethylclobazam. *Ther Drug Monit.* 2002;24:737–741.
6. Kosaki K, Tamura K, Sato R, et al. A major influence of CYP2C19 genotype on the steady-state concentration of *N*-desmethylclobazam. *Brain Dev.* 2004:26, 530–534.
7. Seo T, Nagata R, Ishitsu T, et al. Impact of CYP2C19 polymorphisms on the efficacy of clobazam therapy. *Pharmacogenomics.* 2008;9:527–537.
8. Bun H, Monjanel-Mouterde S, Noel F, et al. Effects of age and antiepileptic drugs on plasma levels and kinetics of clobazam and *N*-desmethylclobazam. *Pharmacol Toxicol.* 1990;67:136–140.
9. Theis JG, Koren G, Daneman R, et al. Interactions of clobazam with conventional antiepileptics in children. *J Child Neurol.* 1997;12:208–213.
10. Sennoune S, Mesdjian E, Bonneton J, et al. Interactions between clobazam and standard antiepileptic drugs in patients with epilepsy. *Ther Drug Monit.* 1992;14:269–274.
11. Chiron C, Marchand MC, Tran A, et al. Stiripentol in severe myoclonic epilepsy in infancy: a randomized placebo-controlled syndrome-dedicated trial. STICLO study group. *Lancet.* 2000;356:1638–1642.
12. Giraud C, Treluyer JM, Rey E, et al. In vitro and in vivo inhibitory effect of stiripentol on clobazam metabolism. *Drug Metab Dispos.* 2006;34:608–611.
13. Sachdeo RC, Sachdeo SK, Levy RH, et al. Topiramate and phenytoin pharmacokinetics during repetitive monotherapy and combination therapy to epileptic patients. *Epilepsia.* 2002;43:691–696.
14. Contin M, Riva R, Albani F, et al. Effect of felbamate on clobazam and its metabolite kinetics in patients with epilepsy. *Ther Drug Monit.* 1999; 21:604–608.
15. Soskin DP, Kane AJ, Stern TA. Phenytoin toxicity secondary to an oxcarbazepine–phenytoin 2C19 interaction. *Psychosomatics.* 2010;51:532–535.
16. Lakehal F, Wurden CJ, Kalhorn TF, et al. Carbamazepine and oxcarbazepine decrease phenytoin metabolism through inhibition of CYP2C19. *Epilepsy Res.* 2002;52:79–83.
17. Okada Y, Seo T, Ishitsu T, et al. Population estimation regarding the effects of cytochrome P450 2C19 and 3A5 polymorphisms on zonisamide clearance. *Ther Drug Monit.* 2008;30:540–543.
18. Fukasawa T, Suzuki A, Otani K. Effects of genetic polymorphism of cytochrome P450 enzymes on the pharmacokinetics of benzodiazepines. *J Clin Pharm Ther.* 2007;32:333–341.
19. Bardy AH, Seppälä T, Salokorpi T, et al. Monitoring of concentrations of clobazam and norclobazam in serum and saliva of children with epilepsy. *Brain Dev.* 1991;13:174–179.
20. Kinoshita M, Ikeda A, Begum T, et al. Efficacy of low-dose, add-on therapy of clobazam (CLB) is produced by its major metabolite, *N*-desmethyl-CLB. *J Neurol Sci.* 2007;263:44–48.
21. Inoue Y, Ohtsuka Y, Oguni H, et al. Stiripentol open study in Japanese patients with Dravet syndrome. *Epilepsia.* 2009;50:2362–2368.
22. Kunicki PK. Simple and sensitive high-performance liquid chromatographic method for the determination of 1,5-benzodiazepine clobazam and its active metabolite *N*-desmethylclobazam in human serum and urine with application to 1,4-benzodiazepines analysis. *J Chromatogr B.* 2001;750:41–49.
23. Yamamoto Y, Takahashi Y, Nishimura S, et al. Development of rapid genotyping methods for single nucleotide polymorphisms of cytochrome

P450 2C9 (CYP2C9) and cytochrome P450 2C19 (CYP2C19) and their clinical application in pediatric patients with epilepsy. *Yakugaku Zasshi.* 2011;131:809–815.
24. Yanni SB, Annaert PP, Augustijns P, et al. In vitro hepatic metabolism explains higher clearance of voriconazole in children versus adults: role of CYP2C19 and flavin-containing monooxygenase 3. *Drug Metab Dispos.* 2010;38:25–31.
25. Fukuoka N, Tsukamoto T, Uno J, et al. Influence of coadministered antiepileptic drugs on serum zonisamide concentrations in epileptic patients:quantitative analysis based on suitable transforming factor. *Biol Pharm Bull.* 2003;26:1734–1738.
26. May TW, Rambeck B, Jürgens U. Serum concentrations of topiramate in patients with epilepsy: influence of dose, age, and comedication. *Ther Drug Monit.* 2002;24:366–374.
27. Yu KS, Yim DS, Cho JY, et al. Effect of omeprazole on the pharmacokinetics of moclobemide according to the genetic polymorphism of CYP2C19. *Clin Pharmacol Ther.* 2001;69:266–273.
28. Pullar T, Edwards D, Haigh JR, et al. The effect of cimetidine on the single dose pharmacokinetics of oral clobazam and *N*-desmethylclobazam. *Br J Clin Pharmacol.* 1987;23:317–321.
29. Rupp W, Badian M, Christ O, et al. Pharmacokinetics of single and multiple doses of clobazam in humans. *Br J Clin Pharmacol.* 1979;7:51S–57S.
30. Sim SC, Risinger C, Dahl ML, et al. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther.* 2006;79:103–113.
31. Sugimoto K, Uno T, Yamazaki H, et al. Limited frequency of the CYP2C19*17 allele and its minor role in a Japanese population. *Br J Clin Pharmacol.* 2008;65:437–439.

Correspondence: Dr Esenwa, Department of Neurology, Columbia University, 710 W 168th St, 14th Flr, New York, NY 10032 (cce2117@columbia.edu).
Conflict of Interest Disclosure: None reported.

1. Gokhale S, Ghoshal S, Lahoti SA, Caplan LR, Caplan LR. An uncommon cause of intracerebral hemorrhage in a healthy truck driver. *Arch Neurol.* 2012; 69(11):1500-1503.
2. Qureshi AI, Tuhrim S, Broderick JP, Batjer HH, Hondo H, Hanley DF. Spontaneous intracerebral hemorrhage. *N Engl J Med.* 2001;344(19):1450-1460.
3. Kurth T, Kase CS, Schürks M, Tzourio C, Buring JE. Migraine and risk of haemorrhagic stroke in women: prospective cohort study. *BMJ.* 2010;341:c3659.

In reply

Sethi has raised some important points that merit attention and clarification. He points out that migraine-induced intracerebral hemorrhage (ICH) is indeed very rare, therefore, every case reported in the literature is a diagnosis of careful exclusion. We second this opinion; therefore, our case was worthy of report.[1]

Furthermore, he raised the possibility of an underlying arteriovenous malformation (AVM) in our case. First, he points out that the headaches were consistently lateralized to the left parietooccipital, questions the diagnosis of migraine, and suggests an underlying AVM as the possible diagnosis. He further suggests considering a repeat angiogram in a few weeks to rule out this possibility.

As mentioned in the case details, our patient had a long history of migraine headaches, sometimes with aura (frequent headaches 2 to 3 times a week for at least 20 to 25 years, with occasional severe headache once every 3 weeks or so).[1] *We would like to add that these headaches were indeed bilateral and sometimes also associated with migrainous aura. The headache that lead to ICH was located on the left parietooccipital region. Even after the ICH, he continued to have headaches on both sides and, as we have mentioned, occasionally involving the right retroorbital region as well. This clinical information is highly suggestive of diagnosis of classic migraine in our patient, with a prolonged episode that lead to ICH. Furthermore, the patient started having distinct episodes only after ICH, not to be confused with bilateral throbbing headaches, which he had preceding as well as following the ICH. Next, as pointed in the case details, the patient was seen in consultation nearly 2 years after the ICH and had numerous neuroimaging studies in the interim including computed tomography angiogram, magnetic resonance scans, and conventional arteriogram.*[1] *None of these studies showed any evidence of vascular malformation.*

The clinical distinction between migraines and AVM could be challenging, especially for parietooccipital AVMs.[2] *Although headache can be a presenting feature in a significant number of these patients, the headaches are almost always unilateral to the side of AVM and often associated with transient visual field loss on the opposite side.*[3,4] *Our patient had bilateral throbbing headaches with changing aura, both before and after ICH. This observation coupled with numerous neuroimaging-vascular studies confirmed the diagnosis of migraine-induced ICH in our patient.*

Sankalp Gokhale, MD

Author Affiliation: Department of Neurology, Duke University Medical Center, Durham, North Carolina.
Correspondence: Dr Gokhale, Department of Neurology, Duke University Medical Center, 2301 Erwin Rd, Durham, NC 27710 (sankalpsgokhale@gmail.com).
Conflict of Interest Disclosures: None reported.

1. Gokhale S, Ghoshal S, Lahoti SA, Caplan LR, Caplan LR. An uncommon cause of intracerebral hemorrhage in a healthy truck driver. *Arch Neurol.* 2012; 69(11):1500-1503.
2. Kupersmith MJ, Vargas ME, Yashar A, et al. Occipital arteriovenous malformations: visual disturbances and presentation. *Neurology.* 1996;46(4):953-957.
3. Waltimo O, Hokkanen E, Pirskanen R. Intracranial arteriovenous malformations and headache. *Headache.* 1975;15(2):133-135.
4. Sutton D. Radiology of cerebral angiomas. *Proc R Soc Med.* 1957;50:91-92.

A Mild Form of Adult-Onset Opsoclonus-Myoclonus Syndrome Associated With Antiglutamate Receptor Antibodies

We read with interest the article by Klaas and colleagues[1] on adult-onset opsoclonus-myoclonus syndrome (OMS). The authors reported the clinical characteristics of 21 patients at the Mayo Clinic and 116 patients in the literature, and they found 2 patients[2,3] with adult-onset OMS associated with anti–*N*-methyl-D-aspartate receptor antibodies in their literature review. Initial symptoms of these 2 patients were behavioral and/or mood changes, and they developed severe encephalopathy.

We have been following up a 39-year-old woman who developed OMS without preceding infections. This patient had dizziness, nausea, opsoclonus, upper-extremity myoclonus, and truncal ataxia. She showed no behavioral or mood changes. Results of brain magnetic resonance imaging and electroencephalography were normal. Extensive investigations, including whole-body positron emission tomography, revealed no evidence of neoplasia. In marked contrast to the 2 reported patients,[2,3] our patient demonstrated no symptoms of encephalopathy; cerebrospinal fluid examination revealed no pleocytosis. However, in cerebrospinal fluid, anti-*N*-methyl-D-aspartate receptor (antiglutamate receptors ε2-NT2 and ζ1-NT) antibodies and antiglutamate receptor δ2-NT antibody test results were positive, and their optical density levels (measured by enzyme-linked immunosorbent assay) were substantially increased in our patient (level [mean (SD) control value], ε2-NT2: 1.047 [0.162 (0.055)], ζ1-NT: 1.438 [0.231 (0.102)], and δ2-NT: 1.713 [0.172 (0.086)]).[4] After starting immunotherapy (intravenous immunoglobulin; 400 mg/kg/d for 5 days), all of the symptoms dramatically improved. No relapses have been recognized for 6 months, and she is receiving no treatment and working at her office.

Our findings suggest that OMS can be found in such a mild form of autoimmune disorders relating to antiglutamate receptor antibodies, and they further support the notion by Klaas et al[1] that comprehensive evaluations of autoantibodies should be conducted in patients

with adult-onset OMS. Measurements of these antibodies (including the antibody against glutamate receptor δ2 involved in cerebellar function[5]) may be helpful for early diagnosis and immunotherapy.

Taiki Kambe, MD
Yukitoshi Takahashi, MD
Yoshiaki Furukawa, MD

Author Affiliations: Department of Neurology, Juntendo Tokyo Koto Geriatric Medical Center (Drs Kambe and Furukawa); Department of Neurology, Faculty of Medicine, University and Postgraduate University of Juntendo (Drs Kambe and Furukawa), Tokyo; and National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka (Dr Takahashi), Japan.
Correspondence: Dr Furukawa, Department of Neurology, Juntendo Tokyo Koto Geriatric Medical Center, 3-3-20 Shinsuna, Koto, Tokyo 136-0075 Japan (furukawa@juntendo.gmc.ac.jp).
Conflict of Interest Disclosures: None reported.

1. Klaas JP, Ahlskog JE, Pittock SJ, et al. Adult-onset opsoclonus-myoclonus syndrome. *Arch Neurol.* 2012;69(12):1598-1607.
2. Kurian M, Lalive PH, Dalmau JO, Horvath J. Opsoclonus-myoclonus syndrome in anti-N-methyl-D-aspartate receptor encephalitis. *Arch Neurol.* 2010; 67(1):118-121.
3. Smith JH, Dhamija R, Moseley BD, et al. N-methyl-D-aspartate receptor autoimmune encephalitis presenting with opsoclonus-myoclonus: treatment response to plasmapheresis. *Arch Neurol.* 2011;68(8):1069-1072.
4. Yamaguchi Y, Wada M, Kurita K, Takahashi Y, Kato T. A case of acute limbic encephalitis associated with SLE accompanied with anti-glutamate receptor antibodies [in Japanese]. *Rinsho Shinkeigaku.* 2012;52(8):545-550.
5. Hirai H, Launey T, Mikawa S, et al. New role of δ2-glutamate receptors in AMPA receptor trafficking and cerebellar function. *Nat Neurosci.* 2003;6(8): 869-876.

In reply

We are grateful for the interest of Kambe and colleagues in our article regarding adult-onset opsoclonus-myoclonus syndrome (OMS).[1] In our literature search, we had encountered 2 patients with antibody targeting the GluN1 subunit (NR1 subunit) of the N-methyl-D-aspartate (NMDA) receptor.[2,3] In addition to the unusual finding of OMS in these 2 patients, both had a neuropsychiatric presentation typical of anti–NMDA receptor encephalitis.

In the first report demonstrating antibody specificity for the GluN1 subunit of the NMDA receptor, the encephalitic patients had a characteristic immunohistochemical pattern of antibody binding to rodent brain tissue, and antigenic specificity was confirmed by an immunofluorescence assay using a cell line transfected with the GluN1 subunit.[4] Enzyme-linked immunosorbent assay (ELISA) proved additionally useful in that report, but just for measuring antibody titers in patients established to be GluN1 antibody seropositive.[4] For quality assurance in the Mayo Clinic Neuroimmunology Laboratory practice, NR1 seropositivity is sought using both tissue and cell-based immunofluorescence assays. These assays are highly sensitive and specific, are approved by US regulatory agencies for clinical use, and have gone through a rigorous validation process at our own institution.

Kambe and colleagues describe a patient with OMS with additional evidence of cerebellar involvement (truncal ataxia) rather than encephalopathy. In contrast to more than 90% of patients with anti–NMDA receptor encephalitis,[4] no elevated white cell count was detected in the cerebrospinal fluid. The authors measured some reactivity of the patient's cerebrospinal fluid to GluN1 (GluRζ), GluN2B (GluRε2), and GluD1 (GluRδ2) glutamate receptor subunits[5] using ELISA. These findings are potentially novel and interesting. One area of concern is the potential lack of specificity of ELISA serological findings owing to a variety of endogenous and exogenous factors.[6] For that reason, ELISA has limited use as a primary investigational tool in autoimmune neurology. It would be informative if the authors could confirm their findings using tissue immunohistochemistry, Western blot, and glutamate receptor subunit-transfected HEK293 cell lines.

In our cohort of 21 adult patients with OMS, the diagnosis of an autoimmune etiology was supported by the clinical course, cerebrospinal fluid findings (in some), and responses to immunotherapy. The absence of a neural autoantibody in any of our patients demonstrates the need for novel antibody discovery in this area.

Andrew McKeon, MD
Sean Pittock, MD

Author Affiliations: Department of Neuroimmunology Laboratory, Mayo Clinic, Rochester, Minnesota.
Correspondence: Dr McKeon, Department of Neuroimmunology Laboratory, Mayo Clinic, 200 1st St SW, Rochester, MN (mckeon.andrew@mayo.edu).
Conflict of Interest Disclosures: Dr McKeon receives research support from the Guthy-Jackson Charitable Foundation. Dr Pittock is a named inventor on filed patents that relate to functional aquaporin 4/neuromyelitis optica–IgG assays and neuromyelitis optica–IgG as a cancer marker and receives research support from Alexion Pharmaceuticals Inc, the Guthy-Jackson Charitable Foundation, and the National Institutes of Health.

1. Klaas JP, Ahlskog JE, Pittock SJ, et al. Adult-onset opsoclonus-myoclonus syndrome. *Arch Neurol.* 2012;69(12):1598-1607.
2. Kurian M, Lalive PH, Dalmau JO, Horvath J. Opsoclonus-myoclonus syndrome in anti-N-methyl-D-aspartate receptor encephalitis. *Arch Neurol.* 2010; 67(1):118-121.
3. Smith JH, Dhamija R, Moseley BD, et al. N-methyl-D-aspartate receptor autoimmune encephalitis presenting with opsoclonus-myoclonus: treatment response to plasmapheresis. *Arch Neurol.* 2011;68(8):1069-1072.
4. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol.* 2008; 7(12):1091-1098.
5. Traynelis SF, Wollmuth LP, McBain CJ, et al. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev.* 2010;62(3):405-496.
6. Remaley A, Hortin GL. Protein analysis for diagnostic applications. In: Detrick B, Hamilton RG, Folds JD, eds. *Manual of Molecular and Clinical Laboratory Immunology.* Washington, DC: ASM Press; 2006:17-18.

Acute Unilateral Hearing Loss as an Early Symptom of Lateral Cerebral Sinus Venous Thrombosis

Gattringer et al[1] reported an interesting observation about the association of lateral venous sinus thrombosis and unilateral hearing loss in a cohort of 3 patients in a recent issue of the journal.

The presence of unilateral hearing loss is a rare presenting symptom of cerebral venous sinus thrombosis.

A discrepancy between clinical course and magnetic resonance imaging in a case of non-herpetic acute limbic encephalitis

Naoto Kohno,[1] Yuko Kawakami,[2] Chizuko Hamada,[2] Genya Toyoda,[2] Hirokazu Bokura,[2] Shuhei Yamaguchi,[3] Yukitoshi Takahashi[4]

[1]Department of Neurology, Ohda Municipal Hospital, Ohda; [2]Department of Neurology, Shimane Prefectural Central Hospital, Izumo; [3]Department of Neurology, Faculty of Medicine, Shimane University, Shimane; [4]Department of Research Shizuoka, National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan

Abstract

We report the case of a 64-year old man who presented memory disturbance, low-grade fever, weight loss, and bilateral hand tremors for three months. He was diagnosed with non-herpetic acute limbic encephalitis (NHALE). Follow-up magnetic resonance imaging (MRI) revealed new lesions after symptomatic improvement following steroid pulse therapy. This may indicate that there is a time lag between the disturbance or recovery of neurons and astrocytes. Thus, other lesions might occasionally appear during convalescence in patients with NHALE, even if only minimal lesions were found on the initial MRI.

Introduction

Kusuhara reported 4 cases of acute encephalitis in which T2-weighted magnetic resonance imaging (MRI) of the brain showed high signal intensities in both the hippocampi and amygdaloid bodies.[1] Polymerase chain reactions (PCR) for herpes simplex virus (HSV)-1 and HSV-2 DNA were negative, and paraneoplastic limbic encephalitis was excluded because of lack of malignancy. Fever and consciousness disturbances were found in all 4 cases, and convulsive seizures were reported in 3. The consciousness disturbances cleared within ten days, but severe amnestic syndrome remained as *sequelae*. These cases were considered to be non-herpetic acute limbic encephalitis (NHALE). Steroid pulse therapy and gamma-globulin were effective against NHALE with autoantibodies against glutamate receptors (GluR) delta 2 and epsilon 2. Follow-up MRI showed that the abnormal findings in bilateral hippocampi and amygdaloid bodies decreased or disappeared during convalescence.[2-5] We report a case of NHALE in which follow-up MRI revealed other lesions after symptomatic improvement by steroid therapy.

Case Report

A 64-year old man was admitted to our hospital for a memory disturbance. He had been treated for hypertension, hypercholesterolemia, gout, and gastroesophageal reflux disease, but he had no history of neurological or psychiatric illness. He occasionally drank alcohol. He had developed a 37-38°C low-grade fever and bilateral hand tremors three months previously and had lost 5 kg of body weight. He had become aware of the memory disturbance and his gait imbalance one month before admission. He showed no symptoms of focal infection such as a common cold or collagen disease, and hyperthyroidism was excluded. Laboratory data showed liver dysfunction and an inflammatory reaction. On admission, his body temperature was 36.9°C; however, a physical examination of the chest and abdomen showed no abnormalities. His state of consciousness was E4, V4, and M6 on the Glasgow Coma Scale. He had no apparent aphasia and presented no abnormalities in the peripheral cranial nerves or hemiparesis of the extremities. Deep tendon reflexes were normal without a pathological reflex. Nuchal rigidity and Kernig's sign were absent. He had bilateral 7-8 Hz action tremors of the hands. There was no intention tremor in the lower limbs but his gait was unsteady. He was topographically disorientated and could never find his way to the restroom on the ward. Laboratory data, including a complete blood count, renal function, electrolytes, ammonia, thyroid hormones, antinuclear acid, cytoplasmic antineutrophil cytoplasmic antibody, perinuclear antineutrophil cytoplasmic antibody, matrix metalloproteinase-3, rheumatoid arthritis test, immune complex, cytomegalovirus antigenemia, and electrocardiography, were all normal. Liver dysfunction and C-reactive protein had been normalized. A cerebrospinal fluid (CSF) examination showed elevated cell counts, with monocyte levels of 70 μL (polynuclear cells of 0 μL). The CSF protein level was 67 mg/dL. Diffusion-weighted imaging (DWI) and fluid attenuated inversion recovery (FLAIR) of his brain MRI on Day 2 showed hyperintensity in the bilateral medial temporal cortex (Figure 1A). Magnetic resonance angiography (MRA) showed no abnormalities (Figure 1B). N-isopropyl-[^{123}I]p-iodoamphetamine single photon emission computed tomography (SPECT) showed hypoperfusion in the whole brain, predominantly in the left frontal lobe (Figure 2). An electroencephalogram showed theta and delta waves in the frontotemporal regions bilaterally (Figure 3).

On Day 3, a neuropsychiatric assessment gave the patient a score of 15 on the Mini-Mental State Examination (MMSE). He was disoriented about time and experienced difficulty of recall. But he could draw interlocking pentagons. He scored 12 points on the Frontal Assessment Battery (range 0-18, cut-off score 15),[6] 82 points on the Kohs Block Design Test, and 32 out of 36 points on the Raven's Colored Progressive Matrices. The patient did not present consciousness disturbance or myelopathy, and the course of his illness was relatively slow. He did not seem to be suffering from acute disseminated encephalomyelitis. A clinical diagnosis of NHALE was made. He received 1000 mg/day methylprednisolone intravenously from Day 2 to Day 4, followed by oral 50 mg/day prednisolone for three days, 40 mg/day prednisolone for 14 days, 35 mg/day prednisolone for seven days, 30 mg/day prednisolone for seven days, and was taking 25 mg/day prednisolone when he was discharged on Day 38. He also received 5 g/day gamma globulin on Days 8 and 9, and 2.5 g gamma globulin on Day 10. On Day 7, he only had a slight tremor of the hands and his gait was almost normal. On Day 10, he was disorientated about time and place. On Day 12, he lost his way to the restroom on the ward. On Day 13, his disorientation improved. He could tell which month it was and when asked where he was could answer *in hospital*. After treatment, the clinical symptoms of memory disturbance, bilateral hand tremors, and unsteady gait gradually improved.

Correspondence: Naoto Kohno, Department of Neurology, Ohda Municipal Hospital, Yoshinaga 1428-3, Ohda-cho, Ohda, 694-0063, Japan.
Tel. +81.854.820330 – Fax: +81.854.847749.
E-mail: kohnonaoto@gmail.com

Key words: non-herpetic acute limbic encephalitis, steroid pulse therapy, magnetic resonance imaging, autoantibodies against glutamate receptors epsilon 2 and delta 2.

Conflict of interests: the authors declare no potential conflict of interests.

Received for publication: 17 April 2012.
Revision received: 19 February 2013.
Accepted for publication: 20 March 2013

Neurology International 2013; 4:e7
doi:10.4081/ni.2013.e7

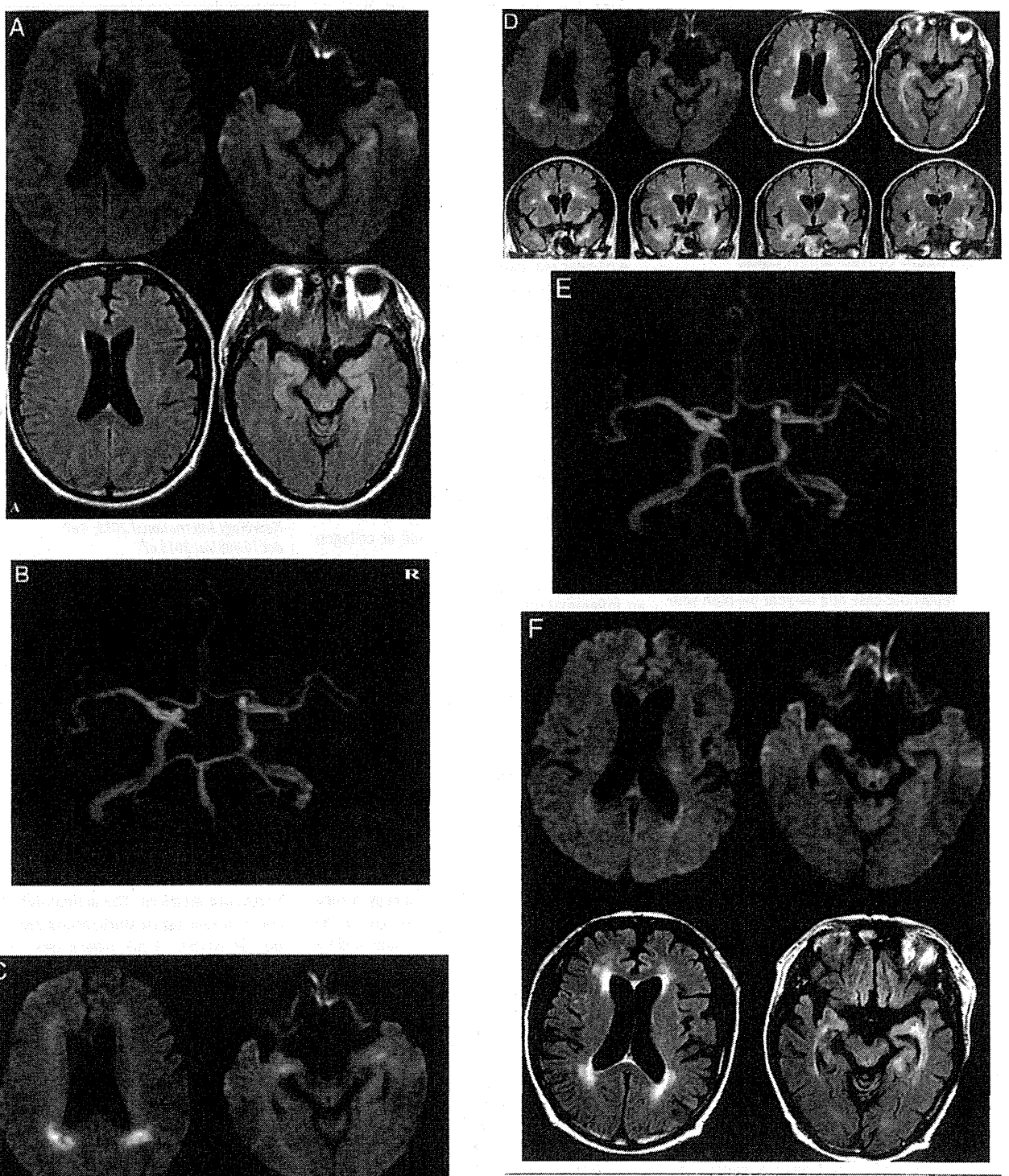

Figure 1. (A) Magnetic resonance imaging on Day 2 showed hyperintensity in the bilateral medial temporal cortex on diffusion-weighted imaging and fluid attenuated inversion recovery. (B) Magnetic resonance angiography showed no abnormalities. (C) Diffusion-weighted imaging and fluid attenuated inversion recovery on Day 14 revealed other hyperintense lesions around the lateral ventricles. (D) Diffusion-weighted imaging and FLAIR on Day 37 revealed that hyperintense lesions in the bilateral medial temporal cortex became more apparent in fluid attenuated inversion recovery image and lesions around the lateral ventricles remained. (E) Magnetic resonance angiography did not show any abnormal findings. (F) Magnetic resonance imaging 17 months later showed hyperintense lesions in the bilateral medial temporal cortex and on fluid attenuated inversion recovery the lesions around the lateral ventricles remained.

He was eventually able to walk steadily in the ward without losing his way. But DWI and FLAIR on Day 14 revealed other hyperintense lesions around the lateral ventricles (Figure 1C). There have been reports of antibodies to N-methyl-D-aspartate receptor (NMDAR), antibodies to NR2B- and NR2A-containing heteromers of the NMDAR detected by cell-based assay,[7] antibodies against full-length GluR epsilon 2 (B18) and GluR delta 2 subunits confirmed by Western blot,[8] and antibodies against peptides of GluR subunit of NMDAR quantified by an enzyme-linked immunosorbent assay (ELISA).[9] Using an ELISA, we examined serum and CSF antibodies against GluR epsilon 2, delta 2 and zeta 2. Serum and CSF antibodies against GluR epsilon 2-NT2, GluR epsilon 2-CT1, GluR delta 2-NT, GluR delta 2-CT were positive. Serum and CSF antibodies against GluR epsilon 2-M3-4, GluR zeta 1-NT, GluR zeta 1-CT were negative; thus, the patient was diagnosed with NHALE. We did not look for the other antibodies that have been found in patients with autoimmune limbic encephalitis, *i.e.* using ELISA on HEK293 cell lysates ectopically expressing NR1 or NR1-NR2B heteromers,[10] NMDAR GluR epsilon 2 (NR2B, GluN2B) or GluR zeta 1 (NR1, GluN1) subunits in this study.[11] HSV type 1, HSV type 2, and PCR did not detect human herpes virus 6 DNA in the CSF. Tumor markers, including carcinoembryonic antigen, carbohydrate antigen 19-9, and alpha-fetoprotein were within normal limits. Further examination with abdominal ultrasonography and enhanced computed tomography of the chest and abdomen revealed no abnormal findings.

A follow-up neuropsychiatric examination on Day 29 showed that the MMSE score had improved to 28 points. A neuropsychiatric examination on Day 37 revealed a full-scale intelligence quotient (IQ) score of 100, a verbal IQ score of 100, and a performance IQ of 99 on the Wechsler Adult Intelligence Scale. But DWI and FLAIR on Day 37 revealed that hyperintense lesions in the bilateral medial temporal cortex became more apparent in the FLAIR image and there were still lesions around the lateral ventricles (Figure 1D). MRA did not show any abnormal findings (Figure 1E). The patient was discharged with little *sequelae* on Day 38. He was taking glimepiride for diabetes mellitus and atorvastatin calcium hydrate for hypercholestemia in addition to prednisolone when he was discharged.

A follow-up MRI performed 17 months later showed no abnormalities on DWI, but revealed that hyperintense lesions in the bilateral medial temporal cortex and around the lateral ventricles on FLAIR remained (Figure 1F). We had not performed a whole body positron emission tomography (PET) scan. But the patient had presented no symptoms of malignancy and his symptoms of encephalitis had not recured

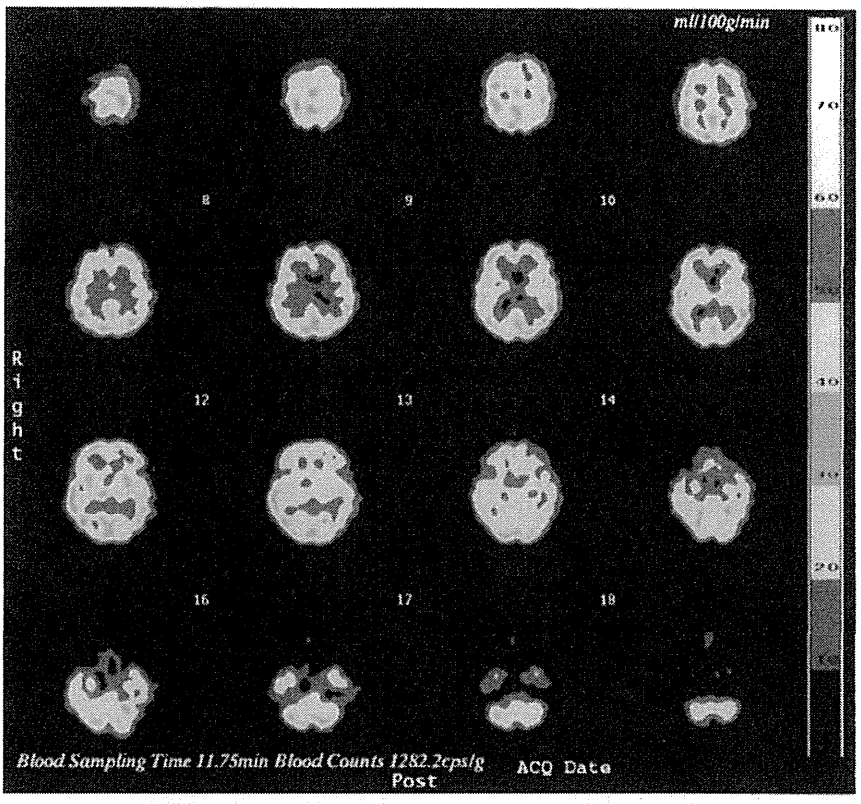

Figure 2. N-isopropyl-[123I]p-iodoamphetamine single photon emission computed tomography showed hypoperfusion in the whole brain, which was predominant in the left frontal lobe.

Figure 3. An electroencephalogram showed theta and delta waves in the frontotemporal regions bilaterally.

more than one year after he had stopped taking steroids. Thus, paraneoplastic limbic encephalitis has been ruled out.

Discussion

Takahashi reported 2 cases of NHALE and recognized four clinical features in both cases: i) an episode of preceding infection such as a common cold; ii) the appearance of reversible high signal intensity lesions in bilateral hippocampi and amygdaloid bodies on DWI; iii) elevation of only interleukin-6 in CSF; and iv) marked neurological improvement following intravenous administration of high-dose methylprednisolone.[4] Takahashi speculated that the immune reaction of the host might play a significant role in the pathogenesis of NHALE, based on these four clinical features. Our patient showed no symptoms of focal infection such as a common cold or collagen disease, and his low-grade fever might have been caused by NHALE.

Laboratory data in ambulant showed initial liver dysfunction and elevated C-reactive protein, but examination on admission showed that liver dysfunction and C-reactive protein had been normalized. The cause of the liver dysfunction was unknown, although a case of NHALE following a type-2 adenovirus infection that presented high fever and severe liver dysfunction has been reported.[12]

Steroid pulse therapy and gamma-globulin are effective against non-herpetic acute encephalitis with autoantibodies to GluR delta2 and epsilon2, and these antibodies in the CSF normalize during the clinical course.[2,3] Intravenous and oral steroids, in addition to an intravenous gamma globulin infusion, are also effective against acute-onset non-neoplastic limbic encephalitis with hypogammaglobulinemia.[13] Okamoto reported a case of paraneoplastic limbic encephalitis caused by an ovarian teratoma with autoantibodies to GluR in the CSF in which signs and symptoms improved following acyclovir and steroid pulse therapy.[14] Symptoms of Hashimoto's encephalopathy with antibodies against the amino terminus of -enolase in the serum and those against GluR epsilon2 in the serum and CSF,[15] as well as bilateral postural hand tremor, memory impairment, and insomnia caused by limbic encephalitis with the anti-voltage-gated potassium channel antibody,[16] also improved following steroid pulse therapy.

According to an MRI study in 91 adult patients with NHALE, DWI lesions were found in 20 of 49 (40.8%) patients at 12.5±9.4 days after onset, and FLAIR lesions were found in 32 of 59 (54.2%) patients at 14.7±17.5 days after onset.[17] Follow-up MRI in convalescence showed abnormal findings in bilateral hippocampi and amygdaloid bodies that were reversed on DWI.[4] T2-weighted and FLAIR imaging abnormalities decreased or disappeared followed by limbic atrophy during convalescence.[5] A T1-weighted MRI three months after onset in a 31-year old woman with NHALE showed a linear high-signal intensity in the hippocampi, and the lesions seemed to be a focal necrosis.[12] MRI abnormalities in children with NHALE are also reversible.[18,19] Takahashi *et al.*[17] reported that MRI lesions appear in various stages after onset. However, we are unaware of any other case of NHALE in which a follow-up MRI disclosed other lesions during convalescence.

The neuropathological lesions in patients with NHALE were limited to the hippocampus and amygdala. The rostral portion of the hippocampus showed small foci characterized by neuronal loss with neuronophagia co-existing with proliferation of microglia, macrophages, hypertrophic astrocytes, and a few lymphocytes. The caudal portion of the hippocampus and amygdala showed neuronal loss with astrocytosis and lymphocytic perivascular cuffing. No leptomeningitis, hemorrhagic necrosis, or evidence of any etiological agent was detected microscopically. The abnormal high intensity areas seen on the MRI corresponded well with the astrocytosis regions. These findings are more similar to those in cases of autoimmune limbic encephalitis cases than herpes simplex encephalitis. The mild neuropathological changes seem to reflect a good clinical outcome.[20]

Conclusions

To our knowledge, this is the first published case of NHALE in which a follow-up MRI revealed that other lesions became more prominent after symptomatic improvement following steroid pulse therapy. The MRI findings of lesions in the temporal cortices were not compatible with those of demyelinating lesions. Although SPECT showed hypoperfusion in the whole brain, MR angiography did not show any abnormal findings on Day 2 or on Day 37. The lesions around the lateral ventricles were irreversible and it is not appropriate to consider them ischemic changes. The appearance of additional lesions after steroid therapy did not match the course of clinical symptoms and may indicate that there is a time lag between the disturbance or recovery of neurons and astrocytes. Thus, other lesions might appear occasionally during convalescence in patients with NHALE, even if only a few lesions are found on the initial MRI.

References

1. Kusuhara T, Shoji H, Kaji M, et al. [Non-herpetic acute limbic encephalitis]. Rinsho Shinkeigaku 1994;34:1083-8. [Article in Japanese].
2. Hayashi Y, Matsuyama Z, Takahashi Y, et al. [A case of non-herpetic acute encephalitis with autoantibodies for ionotropic glutamate receptor delta2 and epsilon2]. Rinsho Shinkeigaku 2005;45:657-62. [Article in Japanese].
3. Wada Y, Takahashi R, Yanagihara C, et al. [Successful use of methylprednisolone therapy in a case of non-herpetic acute encephalitis with antibodies against ionotropic glutamate receptor epsilon2 and delta2]. Brain Nerve 2007;59:527-32. [Article in Japanese].
4. Takahashi T, Kamei S, Miki K, et al. [The analysis of cytokines in cerebrospinal fluid (CSF) in two cases of non-herpetic acute limbic encephalitis (NHALE)]. Rinsho Shinkeigaku 2003;43:162-9. [Article in Japanese].
5. Asaoka K, Shoji H, Nishizaka S, et al. Non-herpetic acute limbic encephalitis: cerebrospinal fluid cytokines and magnetic resonance imaging findings. Intern Med 2004;43:42-8.
6. Terada T, Obi T, Miyajima H, Mizoguchi K. [Assessing frontal lobe function in patients with amyotrophic lateral sclerosis by frontal assessment battery]. Rinsho Shinkeigaku 2010;50:379-84. [Article in Japanese].
7. Dalmau J, Tüzün E, Wu HY, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007;61:25-36.
8. Takahashi Y, Mori H, Mishina M, et al. Autoantibodies to NMDA receptor in patients with chronic forms of epilepsia partialis continua. Neurology 2003;61:891-6.
9. Fujita K, Yuasa T, Takahashi Y, et al. Antibodies to N-methyl-D-aspartate glutamate receptors in Creutzfeldt-Jakob disease patients. J Neuroimmunol 2012;25190-3.
10. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol 2008;7:1091-8.*
11. Takano S, Takahashi Y, Kishi H, et al. Detection of autoantibody against extracellular epitopes of N-methyl-D-aspartate receptor by cell-based assay. Neurosci Res 2011;71:294-302.
12. Nagasawa H, Wada M, Kurita K, et al. [A case of non-herpetic acute limbic encephalitis associated with a type-2 adenovirus infection]. Rinsho Shinkeigaku