ELSEVIER

Brain & Development xxx (2013) xxx–xxx

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood

Yukitoshi Takahashi [a,b,*], Etsuko Yamazaki [a], Jun Mine [a], Yuko Kubota [a], Katsumi Imai [a], Yuki Mogami [a], Koichi Baba [a], Kazumi Matsuda [a], Hirokazu Oguni [c], Kenji Sugai [d], Yoko Ohtsuka [e], Tateki Fujiwara [a], Yushi Inoue [a]

[a] *National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorder, Shizuoka, Japan*
[b] *Department of Pediatrics, Gifu University School of Medicine, Japan*
[c] *Department of Pediatrics, Tokyo Women's Medical University, Japan*
[d] *Department of Child Neurology, National Center of Neurology and Psychiatry, Japan*
[e] *Department of Pediatric Neurology, Okayama University School of Medicine, Japan*

Received 9 November 2012; received in revised form 14 January 2013; accepted 15 January 2013

Abstract

We examined seizure, cognitive, and motor outcomes in patients with Rasmussen syndrome or Rasmussen encephalitis (RS), after recent initiation of immunomodulatory therapies. Among 53 patients with a diagnosis of RS referred from all over Japan, 49 patients (male 22, female 27) with symptoms and findings characteristic of RS were evaluated. Regular intravenous immunoglobulin (IVIg) therapy was administered at a dose of 100 mg/kg/day, etc. Regular steroid pulse therapy was conducted with methylprednisolone at a dose of 30 mg/kg/day (children) or 1000 mg/day (adults) for 3 days. Tacrolimus was given at an initial dose of 0.1 mg/kg/day (children). Mean onset age was 8.7 ± 10.5 years. Seizure-free rate was 71% after treatment by functional hemispherectomy (FH), and response rate for seizures was 81% by regular steroid pulse therapy, 42% by tacrolimus therapy, and 23% by regular IVIg therapy. Rate of patients with IQ higher than 80 (R80) was 50% by regular steroid pulse therapy, 43% by regular IVIg therapy, 29% by tacrolimus therapy, and 0% by FH. R80 after regular steroid pulse therapy was 100% in patients without MRI lesions, and 37% in those with advanced MRI lesions. Improvement of motor function (paresis) was observed only by immunomodulatory therapy. Motor function was aggravated in 100% of patients treated by FH, 62% by regular IVIg, and 10% by regular steroid pulse therapy. We suggest a new treatment strategy for RS using early immunomodulatory therapy: initiation of regular steroid pulse therapy after early diagnosis indicated by biomarkers, then switching to tacrolimus therapy after several months.

Keywords: Rasmussen syndrome; Steroid-pulse therapy; IVIG therapy; Tacrolimus; Functional hemispherectomy; Seizure outcome; Cognitive outcome; Motor outcome

1. Introduction

Rasmussen syndrome or Rasmussen encephalitis (RS) is a slowly progressive, autoimmune chronic inflammatory disease of the central nervous systems [1–3]. Preceding infection occurring around two weeks before onset is observed in 38% of patients [3]. Histological examination usually shows inflammatory lesions with T cell infiltration. Cytotoxic T cells (CTLs) contribute to the immunopathology of RS [4]. The IFNγ, IL-12, and granzyme B levels in CSF are elevated suggesting immunological involvement, especially in the early stage of the disease [5].

* Corresponding author. Address: National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, 886 Urushiyama, Aoi-ku, Shizuoka 420-8688, Japan. Tel.: +81 54 245 5446; fax: +81 54 247 9781.
E-mail address: takahashi-ped@umin.ac.jp (Y. Takahashi).

0387-7604/$ - see front matter © 2013 The Japanese Society of Child Neurology. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.braindev.2013.01.010

Please cite this article in press as: Takahashi Y et al. Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood.. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.01.010

In RS, the initial symptom is usually intractable partial seizures, and epilepsia partialis continua (EPC) develops in 58.8% of the patients. Soon after the onset of RS, treatment with antiepileptic drugs (AEDs) is usually initiated because partial seizures are predominant symptoms. In a few years after onset, unihemispheric cortical dysfunctions (such as hemiplegia and cognitive deficit) become apparent [6,7]. RS is suspected when unilateral cortical deficit, unihemispheric EEG slowing, and unihemispheric cortical atrophy on MRI appear evolutionally. Before the availability of immunopathology, functional hemispherectomy (hemispherotomy) (FH) was the only treatment to achieve complete control of epileptic seizures. Therefore, in patients with involvement of the non-dominant hemisphere, FH is considered after the appearance of motor deficits. On the other hand, in those with disease involving the dominant hemisphere, immunomodulatory therapies using corticosteroids, intravenous immunoglobulin (IVIg), plasma pheresis (PEX) or immunoabsorption, and tacrolimus have been tried [2].

In considering treatment strategies for RS, comprehensive consideration of seizure outcome, neurological outcome, cognitive outcome, and motor outcome is necessary. In making a decision to undergo FH, the parents of patients with RS desire to achieve complete control of seizures and normal cognitive development at the sacrifice of hemiplegia.

With recent developments of many kinds of immunomodulatory therapies, we compared the treatment results of Japanese RS patients treated by surgery and/or immunomodulatory therapies, by evaluating their seizure, cognitive, and motor outcomes.

2. Methods

2.1. Patients

We identified 53 patients with a diagnosis of RS referred to the National Epilepsy Center from all over Japan between 1991 and 2010, and reviewed them basically according to the European diagnostic criteria for RS (Fig. 1) [2]. Of 53 patients, three patients who had no frequent partial seizures, and eight patients who had no unihemispheric cortical dysfunction were initially excluded from a diagnosis of RS. From the eight patients without unihemispheric cortical dysfunction, seven patients were subsequently diagnosed as having RS based on characteristic histology, elevated granzyme B in CSF, or high intensity lesion on MRI characteristic of RS [2,8]. RS was staged into three MRI categories: no lesion, high intensity lesion, and advanced MRI lesion.

2.2. Evaluation

Seizure outcome was classified according to the change in seizure frequency before and after treatments

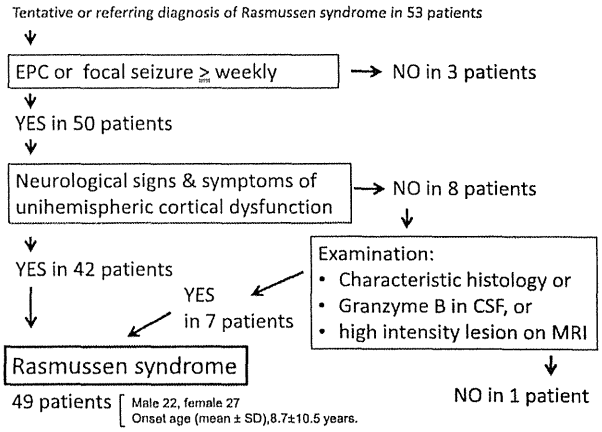

Fig. 1. Patient disposition. EPC, epilepsia partialis continua.

into seizure-free (free), >50% seizure reduction (responder) (decreased), between <50% reduction and <50% increase (stable), >50% seizure increase (aggravated). In patients with EPC and solitary partial seizures, change in frequency of solitary partial seizures was evaluated. Cognitive outcome was measured by intelligence quotient (IQ) or developmental quotient (DQ). IQ was measured by Tanaka–Binet, WISCIII, and WAISIII, dependent on the age at examination. DQ was measured by MCC-baby test, KIDS-test, and other scales. We used full scale IQ (FSIQ) measured by WISC or WAIS for evaluation. Cognitive outcome was classified into FSIQ/DQ increase >10 (improved), between <10 increase and <10 decrease (stable), FSIQ/DQ decrease >10 (aggravated), and uncertain (uncertain). Rate of FSIQ/DQ preservation was calculated as number of patients (improved + stable)/number of patients (improved + stable + decreased). Motor outcome was classified into improved, stable and aggravated.

2.3. IVIg therapy

The protocol for regular IVIg therapy was a dose of either 100 mg/kg/day for several days, 400 mg/kg/day for several days, or 1 g/kg for one day, at an interval of once a month for several months to several years depending on response.

2.4. Steroid pulse therapy

The protocol for regular steroid pulse therapy with methylprednisolone was doses ranging from 30 mg/kg/day (for children) to 1000 mg/day (for adults) for 3 days, at an interval of once in a month for several months to several years depending on response. Only patients who had received more than 3 cycles were evaluated.

Please cite this article in press as: Takahashi Y et al. Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.01.010

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

Please cite this article in press as: Takahashi Y et al. Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood.. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.01.010

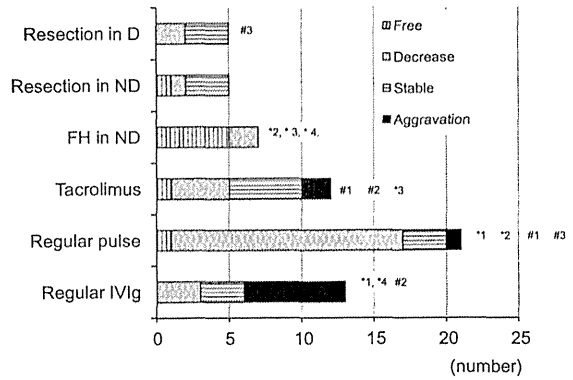

Fig. 2. Seizure outcome after surgery or immunomodulatory therapies. Horizontal axis shows the number of patients with each category of seizure outcome. Resection in D, surgical resection in dominant hemisphere; ND, non-dominant hemisphere; FH, functional hemispherectomy; IVIg, intravenous immunoglobulin. 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 (#) ($^{\#}1$, $p = 0.3080$; $^{\#}2$, $p = 0.2036$; $^{\#}3$, $p = 0.1646$).

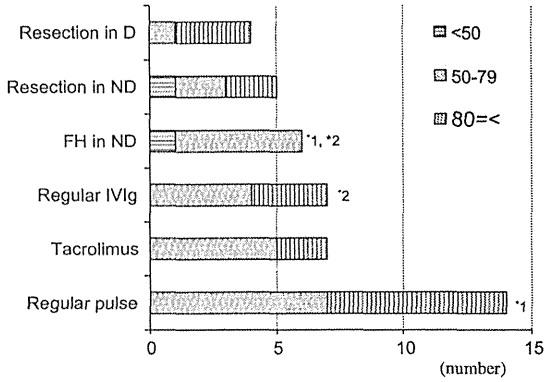

Fig. 3. The last FSIQ/DQ after surgeries or immunomodulatory therapies. Horizontal axis shows the number of patients with each category of FSIQ/DQ. Resection in D, surgical resection in dominant hemisphere; ND, non-dominant hemisphere; FH, functional hemispherectomy; IVIg, intravenous immunoglobulin. 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.

Please cite this article in press as: Takahashi Y et al. Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.01.010

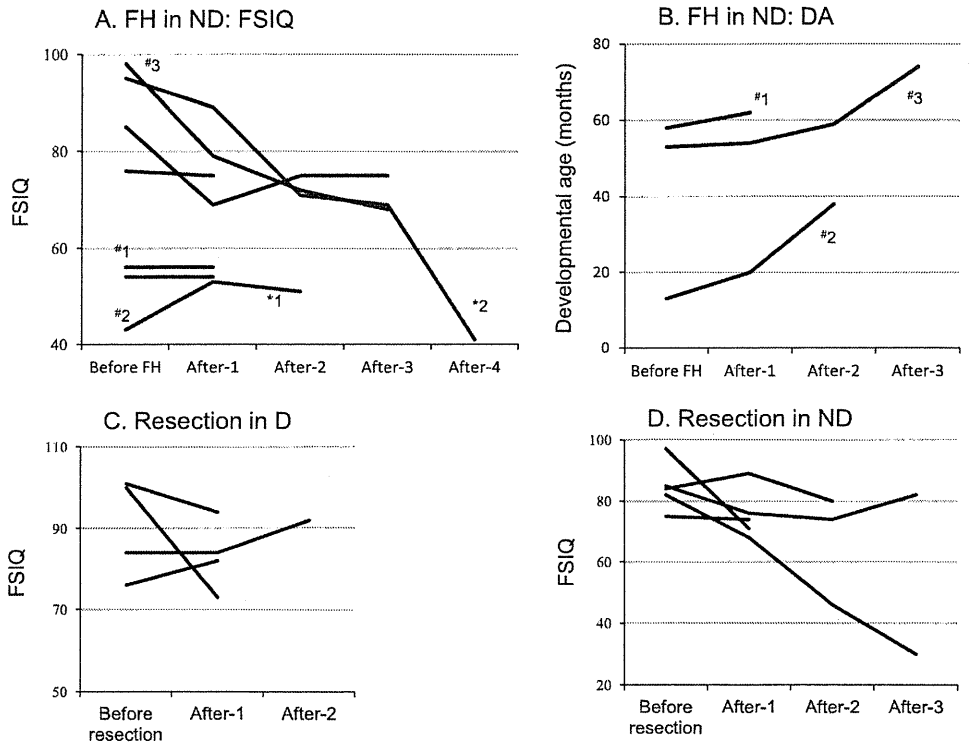

Fig. 4. Evolution of FSIQ/DQ and developmental age after surgical interventions. FSIQ: full scale intelligent quotient; DA: developmental age; after-1, -2, -3 and -4: first, second, third and fourth examinations, respectively, after surgery. A. "FH in ND: FSIQ" shows the evolution of FSIQ/DQ in seven 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)

Please cite this article in press as: Takahashi Y et al. Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood.. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.01.010

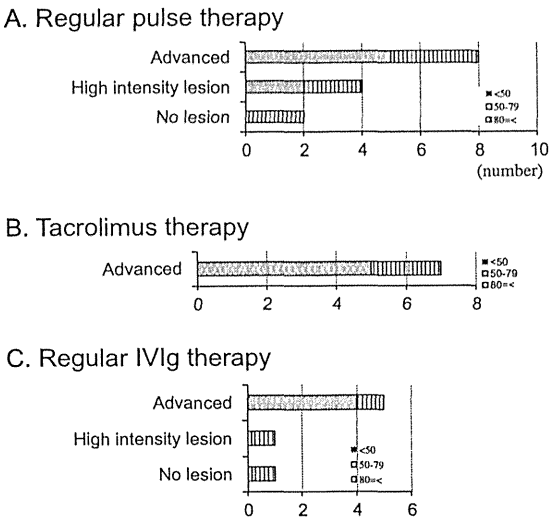

Fig. 5. MRI stage & the last IQ/DQ after immunomodulatory therapies. Horizontal axis shows number of patients with each category of FSIQ/DQ. Advanced, advanced lesion on MRI; High intensity lesion, high intensity MRI lesions; No lesion, without MRI lesion.

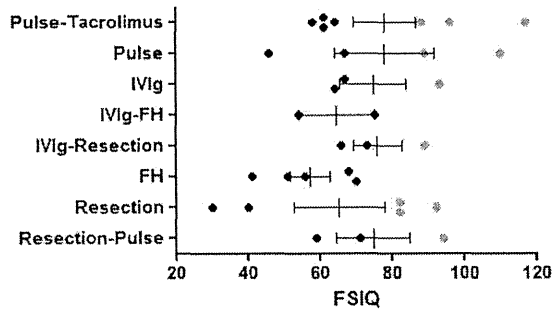

Fig. 6. Treatment modalities and the last FSIQ/DQ. Horizontal axis shows FSIQ. Pulse-Tacrolimus, regular steroid pulse therapy followed by tacrolimus therapy; Pulse, regular pulse therapy; IVIg, regular IVIg therapy; FH, functional hemispherectomy; Resection, surgical resection; Bars show mean ± SE. 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

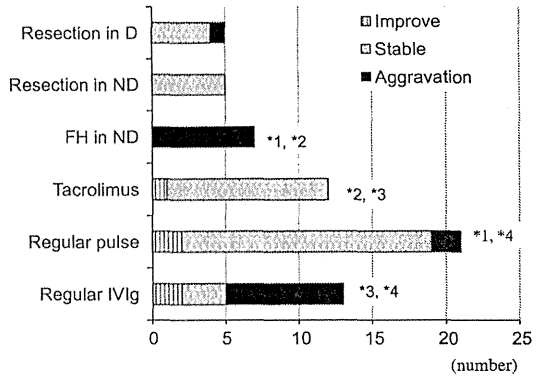

Fig. 7. Motor outcome after surgeries or immunomodulatory therapies. Horizontal axis shows number of patients with each category of cognitive changes. Resection in D, surgical resection in dominant hemisphere; ND, nondominant hemisphere; FH, functional hemispherectomy; Tacrolimus, tacrolimus therapy. 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.

Please cite this article in press as: Takahashi Y et al. Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.01.010

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

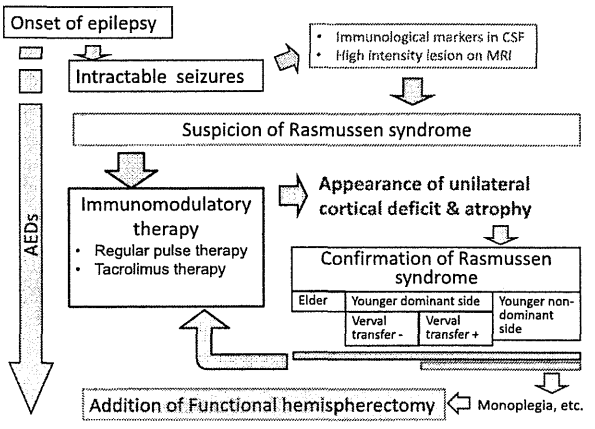

Fig. 8. New treatment strategy for Rasmussen syndrome with pediatric onset.

Please cite this article in press as: Takahashi Y et al. Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.01.010

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.

Please cite this article in press as: Takahashi Y et al. Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.01.010

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.

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; 5:e8
doi:10.4081/ni.2013.e8

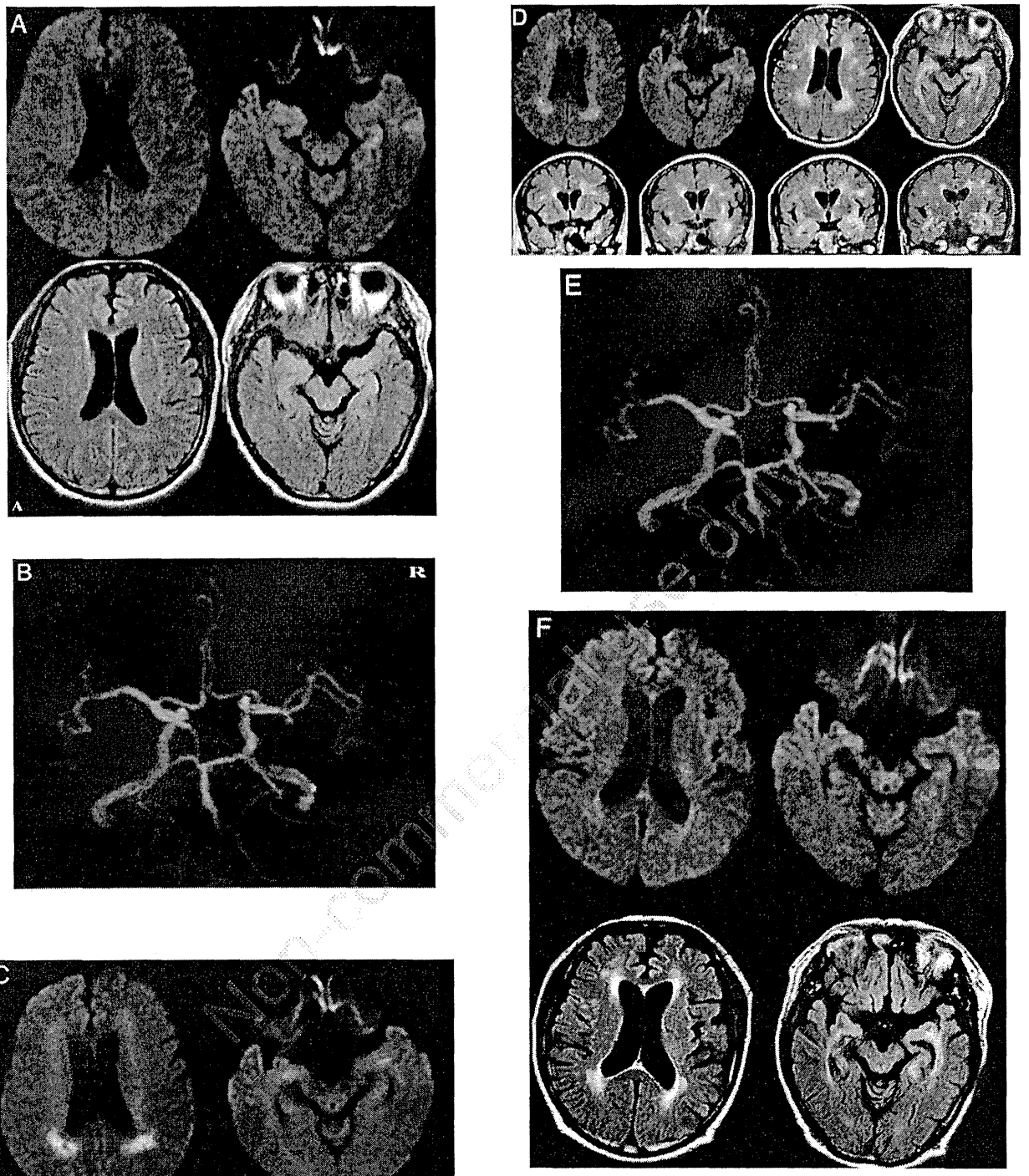

Figure 1. (A) Magnetic resonance imaging on Day 2 showed hyperintensity in the bilateral medial temporal cortex on diffusion-weighted imaging and fluid attenuated inversion recovery. (B) Magnetic resonance angiography showed no abnormalities. (C) Diffusion-weighted imaging and fluid attenuated inversion 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] Using PCR, we did not detect the DNA of HSV type 1, HSV type 2, and human herpes virus 6 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

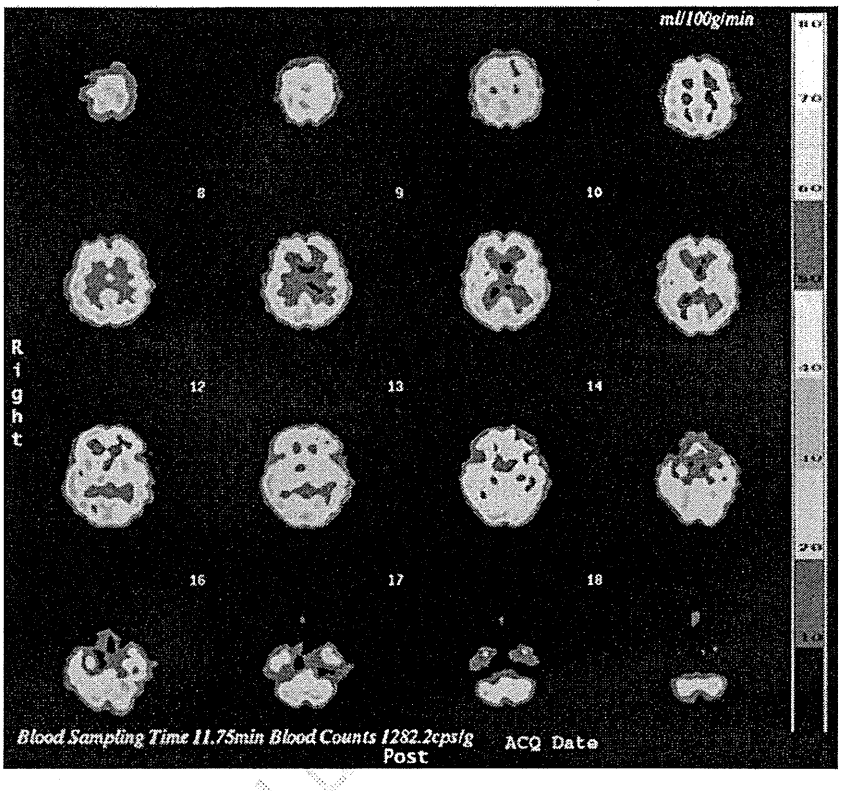

Figure 2. N-isopropyl-[^{123}I]p-iodoamphetamine single photon emission computed tomography showed hypoperfusion in the whole brain, which was predominant in the left frontal lobe.

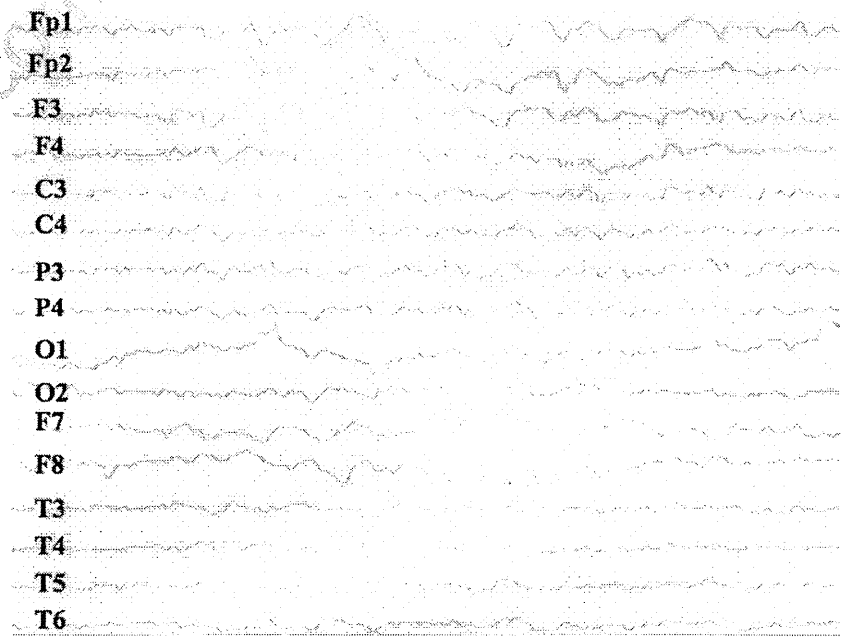

Figure 3. An electroencephalogram showed theta and delta waves in the frontotemporal regions bilaterally.

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

2006;46:322-7. [Article in Japanese].
13. Akman CI, Patterson MC, Rubinstein A, Herzog R. Limbic encephalitis associated with anti-GAD antibody and common variable immune deficiency. Dev Med Child Neurol 2009;51:563-7.
14. Okamoto S, Hirano T, Takahashi Y, et al. Paraneoplastic limbic encephalitis caused by ovarian teratoma with autoantibodies to glutamate receptor. Intern Med 2007;46:1019-22.
15. Shindo A, Ii Y, Sasaki R, et al. [Non-herpetic acute limbic encephalitis-like manifestation in a case of Hashimoto's encephalopathy with positive autoantibodies against ionotropic glutamate receptor epsilon2]. Rinsho Shinkeigaku 2007;47:629-34. [Article in Japanese].
16. Takado Y, Shimohata T, Tokunaga J, et al. [Patient with limbic encephalitis associated with anti-voltage-gated potassium channel antibodies who presented with insomnia and hand tremor]. Rinsho Shinkeigaku 2008;48:338-42. [Article in Japanese].
17. Takahashi Y, Kubota Y, Yamasaki E, Matsuda K. [Rasmussen encephalitis and non-herpetic acute limbic encephalitis]. Rinsho Shinkeigaku 2008;48:163-72. [Article in Japanese].
18. Kikuchi M, Watanabe S, Takahashi Y. [A case of non-herpetic acute limbic encephalitis in childhood--comparison with acute encephalitis with refractory repetitive partial seizures (AERRPS)]. No To Hattatsu 2007;39:221-5. [Article in Japanese].
19. Ishida H, Hattori H, Takaura N, et al. [A child with non-herpetic acute limbic encephalitis affecting the claustrum and hippocampus]. No To Hattatsu 2006;38:443-7. [Article in Japanese].
20. Mochizuki Y, Mizutani T. [Neuropathological findings of non-herpetic limbic encephalitis]. Brain Nerve 2010;62:861-8. [Article in Japanese].

Epilepsy Research (2013) 107, 238–243

journal homepage: www.elsevier.com/locate/epilepsyres

Genetic variations of immunoregulatory genes associated with Rasmussen syndrome

Yukitoshi Takahashi[a,b,*], Yukiko Mogami[a], June Mine[a], Katsumi Imai[a], Yasumichi Koide[a], Kazumi Matsuda[a], Noriyuki Akasaka[c], Takashi Konishi[d], Atsushi Imamura[e], Yushi Inoue[a]

[a] *National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorder, 886 Urushiyama, Aoi-ward, Shizuoka, Japan*
[b] *Department of Pediatrics, Gifu University School of Medicine, Yanagido, Gifu, Japan*
[c] *Department of Pediatrics, Nishi-Niigata Chuo National Hospital, Masago, West-ward, Niigata, Japan*
[d] *Department of Neurology, Japanese Red Cross Shizuoka Hospital, Aoi-ward, Oute-machi, Shizuoka, Japan*
[e] *Department of Pediatrics, Gifu Prefectural General Medical Center, Nagamori, Gifu, Japan*

Received 7 May 2013 ; received in revised form 29 August 2013; accepted 17 September 2013
Available online 5 October 2013

KEYWORDS
Rasmussen syndrome;
CTLA4;
PDCD1;
T-bet;
Epilepsy

Summary
Objective: To elucidate the genetic predisposition of Rasmussen syndrome (RS).
Methods: In 29 Japanese patients, we examined the genome sequences of *cytotoxic T-lymphocyte-associated protein 4 (CTLA4)*, *programmed cell-death 1 (PDCD1)*, and *T-bet (TBX21)* genes by direct sequencing, and evaluated the significance of SNPs (single nucleotide polymorphism) by comparison with Hap Map data.
Results: In all patients, no disease-causative mutations were found in *CTLA4*, *PDCD1*, and *T-bet*. However, rs231775 SNP in exon 1 of *CTLA4* showed significant positive genotypic ($p = 0.0363$) and allelic associations ($p = 0.0137$) with onset of RS compared with Japanese controls, as did rs231779 SNP in intron 1 of *CTLA4* ($p = 0.0467$ and 0.0188, respectively). Also, rs2227982 SNP in exon 5 of *PDCD1* showed significant positive genotypic and allelic associations with RS ($p = 0.0145$ and 0.0114, respectively). Poor cognitive outcome (IQ below 50) was found in 0% of wild type (C/C), 9% of heterologous (C/T) and 25% of homologous (T/T) genotype of rs2227982. Quadriplegia was found only in homologous (T/T) genotype, and hemiplegia was in heterologous (C/T) and homologous (T/T) genotype of rs2227982. No association between SNPs of *T-bet* and RS onset

* Corresponding author at: National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, 886 Urushiyama, Aoi-ku, Shizuoka 420-8688, Japan. Tel.: +81 54 245 5446; fax: +81 54 247 9781.
E-mail addresses: takahashi-ped@umin.ac.jp (Y. Takahashi), yukimoga@mch.pref.osaka.jp (Y. Mogami), jmine@med.shimane-u.ac.jp (J. Mine), imaik@szec.hosp.go.jp (K. Imai), koide12201974@yahoo.co.jp (Y. Koide), matsuda@szec.hosp.go.jp (K. Matsuda), akashin@masa.go.jp (N. Akasaka), tkonishi@hama-med.ac.jp (T. Konishi), aimamura30@hotmail.com (A. Imamura), yinoue-jes@umin.net (Y. Inoue).

0920-1211/$ – see front matter
http://dx.doi.org/10.1016/j.eplepsyres.2013.09.004

was found. Regarding SNPs in promoter regions (rs4794067 and rs17250932) of *T-bet*, however, IQ below 50 was found in 19% of wild type (T/T) and 0% of heterologous (T/C) genotype of rs4794067, and in 19% of wild type (T/T) and 0% of heterologous (T/C) genotype of rs17250932. Quadriplegic patients were found only in wild-type patients (rs4794067 and rs17250932).
Conclusions: We identified three SNPs (rs231775, rs231779, rs2227982) as some of the SNPs associated with onset of Japanese RS. We need further studies in other populations to confirm these genetic predispositions in RS.

Rasmussen syndrome or Rasmussen encephalitis (RS) is a slowly progressive, autoimmune neurological disease, and shows intractable epileptic seizures including epilepsia partialis continua (EPC) (Rasmussen et al., 1958; Bien et al., 2005; Takahashi, 2006). Infection occurring around two weeks prior to onset is observed in 38% of the patients (Takahashi, 2006). Histological examination usually shows inflammatory lesions with T cell infiltration. The immunopathology of RS is attributed mainly to activated cytotoxic T cells (CTLs) (Bien et al., 2002). The CSF levels of IFNγ, IL-12, and granzyme B levels are elevated in the early stage, suggesting Th1 and CTL involvement (Takahashi et al., 2009). Immunomodulatory therapies using intravenous immunoglobulin, plasmapheresis and tacrolimus have been reported to improve outcome (Bien et al., 2005; Takahashi et al., 2013). These data suggest that incomplete inhibition of CTLs activated by acute infection may contribute to the pathophysiology of RS.

Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) (MIM:123890) is a negative regulator of the immune system, resulting in inhibition of activated T cells. Ctla4 knockout mice show lethal lymph-proliferative inflammation (Waterhouse et al., 1995). Gene association studies reported a strong association of polymorphism of *CTLA4* with autoimmune diseases (Ghaderi, 2011). Programmed cell-death 1 (PDCD1) (MIM:600244) is also a negative regulator of the immune system, resulting in inhibition of activated T cells. *Pdcd1* knockout mice are susceptible to autoimmune diseases (Keir et al., 2008a). These mice have regulatory T cell dysfunction, resulting in susceptibility to experimental autoimmune encephalomyelitis (Wang et al., 2010). T-bet (TBX21) (MIM:604895) promotes differentiation of naïve T cells into Th1 cells that are essential for autoimmunity (Lazarevic and Glimcher, 2011), and expression of granzyme B in $CD8^+$T cells (Ji et al., 2011). We examined the associations of disease-causative mutations and polymorphisms in these immunoregulatory genes with Japanese RS.

Patients and methods

This retrospective study was performed at the National Epilepsy Center, Japan, after obtaining approval from the ethical committee.

Patients

We identified 57 Japanese patients who presented with a diagnosis of RS and were referred to the National Epilepsy Center from all over Japan between 1991 and 2012. We reassessed the diagnosis basically according to the European diagnostic criteria for RS (Figure A1) (Bien et al., 2005). Of 57 patients, two patients who had no frequent partial seizures, and six patients who had no unihemispheric cortical dysfunction were initially excluded from a diagnosis of RS. Of the six patients without unihemispheric cortical dysfunction, five were subsequently diagnosed as having RS based on characteristic histology, elevated granzyme B in CSF, or high intensity lesion on MRI characteristic of RS (Bien et al., 2005; Yamazaki et al., 2011). Of 49 patients with unihemispheric cortical dysfunction, one patient was excluded by histological findings. From the 53 patients with a confirmed diagnosis of RS, we examined only 29 patients who were actively treated in our epilepsy center and Nishi-Niigata hospital by the experimental costs. All 29 patients gave informed consent by the methods approved by the ethical committee.

Methods

Clinical characteristics were examined based on clinical records and referral letters from other hospitals. Outcome was evaluated by findings at the last observation. Seizure outcome in surgically treated patients was evaluated by the findings just before surgical intervention. Intelligence quotient (IQ) was measured by Tanaka—Binet, WISCIII, and WAISIII, dependent on the age at examination. We used full scale IQ (FSIQ) for evaluation.

Genomic DNA was extracted from EDTA blood samples using MagNA Pure (Roche Applied Science, Tokyo) and sent as anonymous samples to a commercial laboratory that performed genome sequencing (Takara bio, Co LTD, Yokkaichi). *CTLA4* from 5′ non-coding region to 3′ non-coding region was divided into 10 regions, and each region was amplified by PCR using primers (Table A1). *PDCD1* from the promoter region to 3′ non-coding region was divided into 16 regions, and subjected to PCR amplification using primers (Table A1). The promoter region, six exons and 3′ non-coding region of *T-bet* were divided into 10 regions, and amplified by PCR using primers (Table A1). PCR reaction was performed in a final volume of 20 μl containing 2 μl of genomic DNA (10 ng/μl) by the following cycling conditions: initially 94 °C for 4 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 59 °C and 1 min at 72 °C. Thereafter, PCR products were purified with exonuclease and alkaline phosphatase, and the purified PCR products were subjected to forward and the reverse reactions using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA, USA). The reaction solution was purified by XTerminator (Applied Biosystems, Carlsbad, CA, USA). Sequencing was done using ABI3730×1 (Applied Biosystems, Carlsbad, CA, USA). Mutations and polymorphisms were detected using Phred/Phrap/PolyPhred software (CodonCode Corporation, MA, USA).

Data of mutations and polymorphisms were compared with the data of Japanese and other populations obtained from Hap Map data (http://hapmap.ncbi.nlm.nih.gov/index.html.en). Hap Map project is a public international resource that will help researchers find genes associated with human disease. As HapMap data provide no phenotypic information about the samples from volunteers, we do not know their medical conditions. However, ages of volunteers were restricted above 20 years old, and they could understand the context of informed consent. As the age of volunteers is much older than the RS patients (6.8 ± 10.7), we suggest that the volunteers have few risks of RS. Current data from Japanese controls by HapMap may be used as tentative controls, until we will collect many new controls, to facilitate the genomic association study of RS.

For statistical analyses, chi-square test for trend and chi-square test were used. A p value less than 0.05 was considered as indicating a significant difference.

Results

Mean onset age of epilepsy in 29 patients (14 males, 15 females) was 6.8 ± 10.7 (mean $\pm$ SD) years (Table A2). The dominant hemisphere was involved in 15 patients, and non-dominant hemisphere in 14. Ten patients underwent surgical intervention and histological examination revealed typical features including microglia nodule, vasculogenesis on brain surface, endothelial proliferation, spongy degeneration and perivascular cuffing in ten patients; focal cortical dysplasia in four patients; and mesial temporal sclerosis in one patient with status epilepticus.

For *CTLA4*, the region downstream of exon 4 could not be examined by the (AT) 28 repeat at position 54947468–54947524, in spite of several modifications of sequencing conditions. Genomic sequencing revealed no disease-causative mutation, but detected two significant single nucleotide polymorphisms (SNPs) in *CTLA4* (Table 1). The rs231775 SNP in exon 1 (Thr 17 Ala) and rs231779 SNP in intron 1 showed significant positive genotypic and allelic associations with RS compared with Japanese controls. Mean age of onset was not significantly different between heterologous (A/G) and homologous (G/G) genotypes of rs231775. Frequencies of patients with EPC were not significantly different between heterologous (A/G) and homologous (G/G) genotypes of rs231775. The rs231776 (Intron 1), rs231777 (intron 1), rs231778 (intron 1), rs231780 (intron 1) and rs231721 (3′near) SNPs showed no significant association compared with Japanese controls. The rs231781 SNP (intron 3) had no significant association with RS onset compared with Asian controls. Rs231775 and rs231779 formed haplotypes in RS patients. In 25 patients (excluding two patients with deterioration after prolonged anesthesia) analyzed for cognitive outcome, mean IQ was not different between heterologous (A/G) and homologous (G/G) genotypes of rs231775 (Fig. 1). In 22 patients (excluding five patients with functional hemispherectomy and two patients with deterioration after prolonged anesthesia) analyzed for motor outcome, the proportion of patients without motor impairment was 4/16 (25%) in homologous genotype (G/G) and 1/6 (17%) in heterologous genotype (A/G) of rs231775.

Table 1 Single nucleotide polymorphisms (SNPs) in *CTLA4*, and *PDCD1*.

SNP	Subject	Genotype			Allele		HWE	Genotype	Allele frequency	
					Reference	SNP	χ^2	p-Value	p-Value	Odds ratio (95%CI)
rs231775		A/A	A/G	G/G	A	G				
(*CTLA4*, Exon 1,	RS	0	12	17	12	46	1.97	$p=0.0363$	$p=0.0137$	2.344 (1.175–4.676)
Thr17Ala)	Controls	15	55	42	85	139	0.20			
rs231779		C/C	C/T	T/T	C	T		$p=0.0467$	$p=0.0188$	2.257 (1.131–4.503)
(*CTLA4*, Intron 1)	RS	0	12	17	12	46	1.97			
	Controls	14	55	43	83	141	0.31			
rs34819629		G/G	G/A	A/A	G	A			$p=0.0195$	2.301 (1.134–4.671)
(*PDCD1*, Intron 2)	RS	4	9	9	17	27	0.41			
	Controls				49(CHB + JPT)	71(CHB + JPT)				
rs2227982		C/C	C/T	T/T	C	T		$p=0.0145$	$p=0.0114$	2.151 (1.179–3.924)
(*PDCD1*, Exon 5,	RS	4	12	13	20	38	0.21			
Ala215Val)	Controls	33	54	26	120	106	0.19			
rs10204525		G/G	G/A	A/A	G	A		$p=0.2979$	$p=0.2584$	1.57 (0.7145–3.450)
(*PDCD1*, Exon 5	RS	2	5	15	9	35	2.00			
(3′UTR))	Controls	10	45	58	65	161	0.09			

RS, Rasmussen syndrome; Controls, Japanese in Tokyo (JPT); CHB, Han Chinese in Beijing; HWE, Hardy-Weinberg Equilibrium values; p, chi square test.

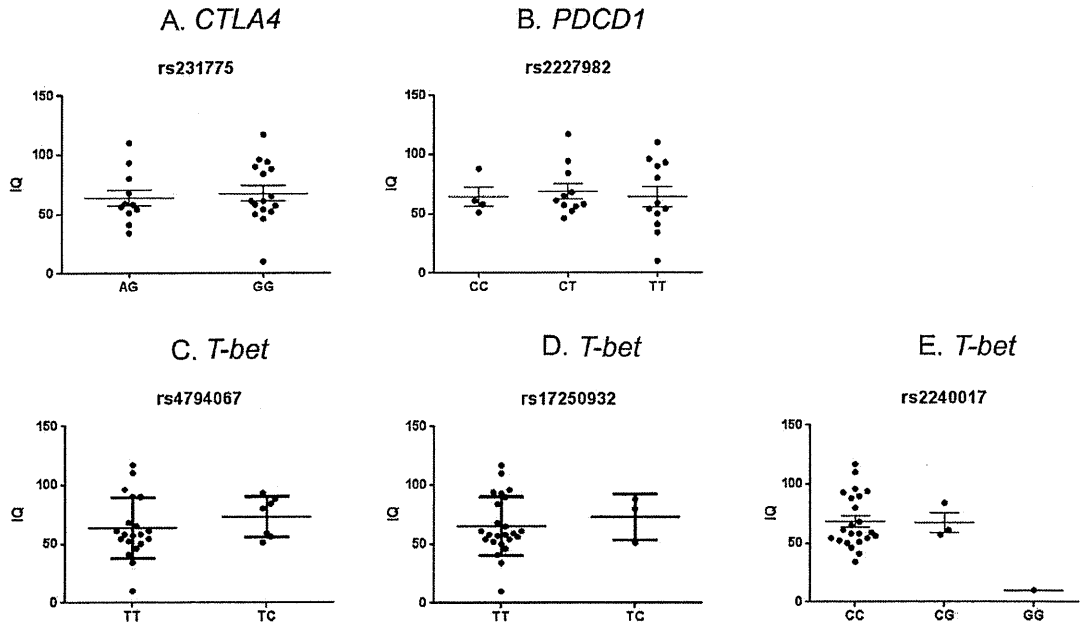

Figure 1 Cognitive outcome. *CTLA4*, cytotoxic T-lymphocyte-associated protein 4; *PDCD1*, programmed cell-death 1; *T-bet* (TBX21); IQ, intelligence quotient.

For *PDCD1*, the regions upstream of exon 1, a part of intron 1, exon 3 and exon 4 of gene could not be sequenced despite several modifications of PCR conditions. Genomic sequencing revealed no disease-causative mutation, but identified three candidate SNPs in *PDCD1* (Table 1). Rs34819629 (intron 2) showed significant allelic association compared with Asian controls [Han Chinese in Beijing (CHB) + Japanese in Tokyo (JPT)]. The rs2227982 SNP in exon 5 (Ala 215 Val) showed significant positive genotypic and allelic associations with RS compared with Japanese controls. Mean age of onset was not significantly different between patients with wild type (C/C) and those with T alleles in genotypes of rs2227982. Frequencies of patients with EPC was not significantly different between patients with wild type (C/C) and those with T alleles in genotypes of rs2227982. The rs10204525 SNP (exon 5) showed no significant genotypic and allelic associations compared with Japanese controls. The rs6705653 (intron 4) and rs2227981 (exon 5) SNPs showed significant positive genotypic association with RS compared with non-Japanese controls (Central South Africa), but no significant allelic association compared with Asian controls (CHB + JPT). The rs7419870 SNP (intron 1) showed no significant allelic association compared with Asian controls (CHB + JPT). The rs11568821 SNP (PD-1.3) was not found in RS patients. Rs6705653 and rs2227981 formed haplotypes in RS patients. In 27 patients (excluding two patients with deterioration after prolonged anesthesia) analyzed for cognitive outcome, IQ below 50 was found in 0% of wild type (C/C), 9% of heterologous (C/T), and 25% of homologous (T/T) genotype of rs2227982 (Fig. 1). In 22 patients (excluding five with functional hemispherectomy and two with deterioration after prolonged anesthesia), motor outcome was more favorable in wild-type (C/C) genotype of rs2227982 (monoplegia was the worst outcome) compared with homologous (T/T) genotype (five of whom had hemiplegia or quadriplegia).

The initial half of exon 1, exon 3 and exon 6 of *T-bet* could not be sequenced in spite of several modifications of PCR conditions. Genomic sequencing revealed no disease-causative mutation, but identified five candidate SNPs in *T-bet* (Table 2). The rs4794067 (promoter-1993), rs2240017 (exon 1, His 33 Gln) and rs2074190 (exon 1, Gly 130 Gly) SNPs showed no significant genotypic and allelic associations with RS compared with Japanese controls. The genotype data of rs2240017 in Japanese controls from Hap Map does not fit the Hardy-Weinberg equilibrium (HWE). The rs17250932 (promoter-1514) SNP showed no significant allelic associations compared with Yoruban controls. One of 26 patients had T/C genotype at NT11082994 (promoter). In 27 patients (excluding two patients with deterioration after prolonged anesthesia) analyzed for cognitive outcome, IQ below 50 was found in 19% of wild type (T/T) and 0% of heterologous (T/C) genotype of rs4794067; in 19% of wild type (T/T) and 0% of heterologous (T/C) genotype of rs17250932; and in 15% of wild type (C/C), 0% of heterologous (C/G), and 100% of homologous (G/G) genotype of rs2240017 (Fig. 1). Regarding SNPs in promoter regions (rs4794067 and rs17250932), none of the heterologous patients were quadriplegic, whereas wild-type patients (T/T) included a quadriplegic.

Discussion

Our study revealed that RS is associated with rs231775 in exon 1 of *CTLA4*, and rs2227982 in exon 5 of *PDCD1*. The rs231775 SNP in *CTLA4* involves a change of amino acid from Thr to Ala, resulting in reduced localization of CTLA4 in endoplasmic reticulum (Mäurer et al., 2002). Alteration of

Table 2 Single nucleotide polymorphisms (SNPs) in *T-bet* gene.

SNPs	Subject	Genotype			Allele		HWE	Genotype	Allele frequency	
					Reference	SNP	χ^2	*p*-Value	*p*-Value	Odds ratio (95%CI)
rs4794067		T/T	T/C	C/C	T	C				
(Promoter-1993)	RS	23	6	0	52	6	0.39	$p=0.6127$	$p=0.561$	1.333 (0.5040–3.527)
	Controls	96	16	1	208	18	0.13			
NT11082994		G/G	G/A	A/A	G	A				
(Promoter)	RS	25	1	0	51	1	0.01			
	Controls									
rs17250932		T/T	T/C	C/C	T	C			$p=0.99$	0.9909 (0.2386–4.115)
(Promoter-1514)	RS	26	3	0	55	3	0.09			
	Controls				109 (YRI)	9 (YRI)				
rs2240017		C/C	C/G	G/G	C	G		$p=0.737$	$p=0.3552$	0.5975 (0.1987–1.797)
(Exon 1	RS	25	3	1	53	5	3.42			
His33Gln)	Controls	35	6	3	76	12	7.80			
rs2074190		A/A	A/G	G/G	A	G		$p=0.7029$	$p=0.6891$	1.238 (0.4339–3.534)
(Exonc1	RS	24	5	0	53	5	0.26			
Gly130Gly)	Controls	98	14	1	210	16	0.38			

RS, Rasmussen syndrome; Controls, Japanese in Tokyo (JPT); YRI, Yoruban in Ibadan, Nigeria; HWE, Hardy-Weinberg Equilibrium values; *p*, chi square test.

intracellular storage of CTLA4 causes reduced expression of CTLA4 on the surface of T cells, subsequently leading to peripheral tolerance dysfunction. This exon SNP (rs231775) may contribute to prolonged excretion of granzyme B from CTLs in CSF after acute infection, subsequently inducing the onset of RS. This exon SNP has been associated with several autoimmune diseases including insulin-dependent diabetes mellitus (IDDM), Graves disease, Hashimoto thyroiditis, rheumatoid arthritis, and multiple sclerosis (MS), and CTL involvement is reported in the pathogenesis of IDDM and MS (Neumann et al., 2002; Ankathatti Munegowda et al., 2011; Niland et al., 2010). Therefore, in RS also, CTLs with poor tolerance related to this exon SNP may play a pivotal role in prolonged activation of CTLs after preceding infection of RS, resulting in apoptosis of neurons by excretion of granzyme B from CTLs (Takahashi et al., 2009).

Cleavage by granzyme B is reported to play a key role in the autoantigenicity of transaldolase (TAL) and contribute to the destruction of oligodendrocytes in MS (Niland et al., 2010). Sustained cleavage of proteins by granzyme B in RS may expose cryptic epitopes to autoreactive CTLs, resulting in progression of the disease.

The rs2227982 SNP in *PDCD1* (PD-1.9) involves a change of amino acid from Ala to Val in exon 5. Exon 5 determines the cytoplasmic domain of PDCD1 (192aa-288aa), including the immunoreceptor tyrosine-based inhibitory motif (ITIM) (I/L/VXYXXL/V; 221aa-226aa) and immunoreceptor tyrosine-switch motif (ITSM) (TEYATIV; 247aa-2512aa). Ligation of the cell surface domain of PDCD1 with PD ligand leads to phosphorylation of cytoplasmic tyrosines at the ITIM and ITSM, and increases the association of src homology 2-domain containing tyrosine phosphatase 2 (SHP-2) with the ITSM in PDCD1. Recruitment of SHP-2 dephosphorylates the signaling pathway through P13K and the downstream signals through Akt, resulting in decreased production of PDCD1 and cytokines in T cells (Keir et al., 2008b). We hypothesize that the rs2227982 SNP in *PDCD1* at 215aa may affect the binding of SHP-2 to ITSM, resulting in reduced peripheral tolerance. PDCD1 deficiency in knockout mice leads to autoimmune disorders, and SNPs have been associated with several autoimmune disorders (Nishimura et al., 1999; Okazaki and Honjo, 2007). The association of rs2227982 with human diseases has been reported only in Asian populations: ankylosing spondylitis in Korean and Chinese, and type 1 diabetes mellitus in Japanese (Lee et al., 2006; Yang et al., 2011; Ni et al., 2007). These data suggest that rs2227982 is one of the risk factors for autoimmune diseases including RS in Asian populations. The fact that rs2227982 is associated with autoimmune diseases only in Asian populations suggest that the pathogenetic effect of SNP in *PDCD1* may vary depending on the genetic background, as is shown by the various effects of *PDCD1* knockout in different strains of mice (Nishimura et al., 1999; Okazaki and Honjo, 2007). More patients homologous (T/T) for rs2227982 and less wild-type patients (C/C) had poor cognitive outcome (IQ < 50). We need further studies to confirm the relationship between rs2227982 and cognitive outcome in RS.

Our analysis revealed no association between RS and SNPs of *T-bet* that may cause reduction of T-bet expression. Because T-bet is essential for the differentiation of Th0 to Th1 cells and is involved in the early induction of IFN-γ in the development of cytotoxic CD8+ effector T cells,