Table 1. Continued.

Pt	Sex	Age at onset (year)	Age at diagnosis (year)	Sampling stage (months)	Seizure	Motor dysfunction	Mental retardation	Immunologic treatment	Age at surgical intervention and method/surgical outcome	Histologic findings	Elevated molecules in CSF	GluR ε2 (NR2B) in CSF (months)
20	M	7.5	7.8	4	D	r-HP	–	–			CD4, CD8, IFNγ, GrB	4+, 6+
				8	D	r-HP	–	IVIG			CD4, CD8, IFNγ, GrB	8+
				24	D	r-HP	–				IFNγ, TNF α, GrB	
				34	D	r-HP	–	Tacrolimus	–		IFNγ, TNF α, GrB	34–
21	M	7.8	10.0	50	W	l-HP	–	–	–		IFNγ, TNF α, GrB	
22	F	8.8	9.8	108	D	r-HP	++	–			TNFα,	108–
				132	D	r-HP	++	Tacrolimus	–		TNFα, GrB	132–
23	F	9.0	12.0	38	D	–	–, aphasia	–			TNFα, GrB	38+, 47+
				72	D	r-HP+ll-MP	++	Tacrolimus			CD4,	72-
				74	D	r-HP+ll-MP	++	Tacrolimus	–		CD8, GrB	74+
24	M	12.6	16.0	84	D	QP	+++	–	–		TNFα,	84–
25	M	15.0	15.4	180	W	r-HP	+, Psychosis	–	26Y: left frontal resection/weekly SPS	MGN+VBS+EP +SD+PVC	CD4,	180–
26	M	16.1	16.4	1	W	–	–	–			TNFα, GrB	1–, 2–
				3	D	lu-MP	–	Pulse	–		IFNγ, TNF α, GrB	3+
27	F	28.0	34.7	72	D	–	+	–	34Y: right frontal resection/daily CPS	MGN+VBS+EP +SD+PVC		

Sampling stage, period between onset of epilepsy and sampling point of cerebrospinal fluid (CSF); seizure, frequency of seizure; D, daily; W, weekly; Y, yearly; HP, hemiplegia; MP, monoplegia; QP, quadriplegia; l, left; r, right; ll, left lower; lu, left upper; ru, right upper; rl, right lower; mental retardation +, mild MR; ++, moderate MR; +++, severe MR; EFD, eye field defect; pulse, steroid pulse therapy; IVIG, intravenous infusion of globulin; FH, functional hemispherectomy; MGN, microglia nodule; VBS, vascular genesis on brain surface; EP, endothelial proliferation; SD, spongy degeneration; PVC, perivascular cuffing; FCD, focal cortical dysplasia. Elevated molecules in CSF show molecules beyond mean + SD of disease controls in CD4, CD8, IFNγ, TNFα, and Granzyme B (GrB); GluR ε2 (NR2B) in CSF (months), presence or absence of autoantibodies against GluR ε2 (NR2B) in CSF at each sampling stage (month), 96+ shows presence of the autoantibodies against GluR ε2 (IgG or IgM) at 96 months after the onset.

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from 21 patients), and IL-12 levels in CSF (29 samples from 22 patients) were measured by ELISA (Human IFN γ ELISA kit; Endogen, Rockford IL, USA; Human IL-4 ELISA kit; Endogen; Human IL-12 (p70) ELISA kit; Endogen; Human TNF α ELISA kit; Endogen) (Ichiyama et al., 2008). Autoantibodies against GluR ε2 (NR2B) were examined qualitatively in 46 samples from 25 patients by immunoblot (Takahashi et al., 2003, 2005). The mean number of samples per patient was 1.8 ± 1.2 (range 1–5). Some CSF data were not available from some of the 27 patients, because CSF was collected only for the examination of autoantibodies in other hospitals, and some measurements were available only recently. Data are expressed in mean ± SD.

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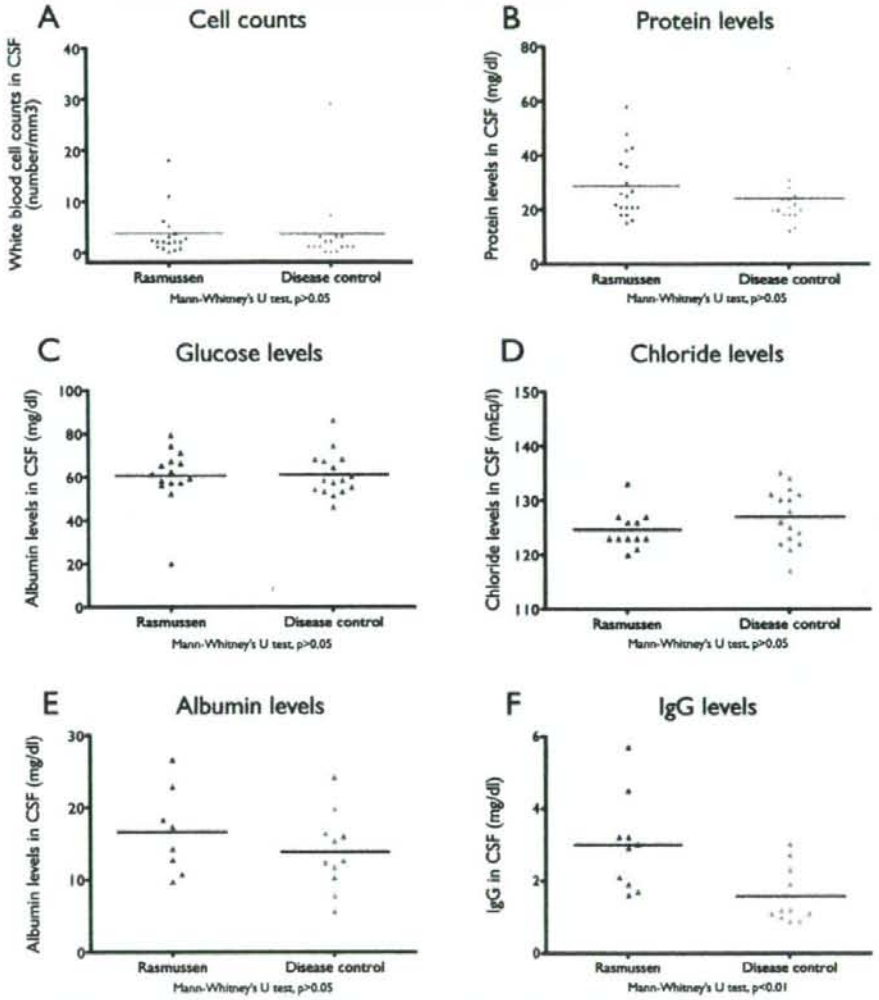

Figure 1.
Data of conventional and immunologic molecules at the first cerebrospinal fluid (CSF) examination. (**A**) White blood cell count in CSF; (**B**) protein level; (**C**) glucose level; (**D**) chloride level; (**E**) albumin level; (**F**) immunoglobulin G (IgG) level. Rasmussen: data from RS patients. Horizontal bars show mean levels. Mean interval between disease onset and first examination of white blood cell counts was 48.8 ± 46.4 months (mean ± SD).
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RESULTS

Conventional and immunologic data of the first CSF examination

Conventional and immunologic data of the first CSF examination of 19 and 24 patients before initiation of immunologic treatment were compared with those of disease controls to evaluate their roles in RS. At the first CSF examination, cell counts (3.6 ± 4.4, n = 18), protein levels (28.7 ± 12.1 mg/dl, n = 19), glucose levels (60.3 ± 13.3 mg/dl, n = 15), albumin levels (16.5 ± 5.9 mg/dl, n = 8), and chloride levels (124.6 ± 3.5 mEq/l, n = 12) in RS patients were similar to the levels in disease controls (Fig. 1). On the other hand, IgG levels were higher in RS patients (3.0 ± 1.3 mg/dl, n = 10) than in disease controls (1.6 ± 0.8 mg/dl, n = 11) (Mann-Whitney *U* test, $p < 0.01$) (Fig. 1F).

$CD4^+$ T cells were higher in RS patients (59.3 ± 21.4%, n = 7) than in disease controls (34.7 ± 15.0%, n = 12) (Mann-Whitney *U* test, p = 0.02) (Fig. 2). $CD8^+$ T cells were not significantly different between RS patients (34.8 ± 18.3%, n = 7) and disease controls (23.4 ± 7.0%, n = 11), whereas $CD3^+$ T cells were higher in RS patients (74.8 ± 15.5%, n = 7) than in disease controls (52.5 ± 18.4%, n = 12) (p = 0.01). IFNγ levels were not significantly different between RS patients (11.3 ± 6.0 pg/ml, n = 22) and disease controls (9.6 ± 9.1 pg/ml, n = 26) (Mann-Whitney *U* test, $p > 0.05$). TNFα levels were higher in RS patients (23.7 ± 34.8 pg/ml, n = 17) than in controls (4.0 ± 2.4 pg/ml, n = 13) ($p < 0.01$). Granzyme B levels were higher in RS patients (10.8 ± 15.5 pg/ml, n = 18) than in disease controls (1.2 ± 1.2 pg/ml, n = 13) (Mann-Whitney *U* test, $p < 0.01$). IL-4 and IL-12 levels were similar in RS patients and disease controls.

Clinical evolution and immune molecules in CSF

Evolutional changes of conventional laboratory data and immunologic molecules in CSF were evaluated in 27 RS patients using the data of the initial examination and subsequent follow-up examinations. White blood cell counts in CSF were elevated in two patients around onset, and were within normal limits in samples collected 5 months after onset (Fig. 3). Protein levels and albumin levels were higher in samples collected at the progressed stage, compared with those in the early stage. Half of the patients showed protein levels greater than 40 mg/dl 12.5 years after onset. Albumin levels were higher (>20 mg/dl) in half of the patients 5 years after onset. IgG levels were slightly elevated (>2.5 mg/dl) in two-thirds of patients at all stages of disease evolution.

$CD4^+$ T cell counts were elevated (>50%) in the majority of patients at all stages of disease evolution (Fig. 4). $CD8^+$ T cells were also elevated (>30%) in many patients at all stages of disease evolution, and the elevated levels declined evolutionally. $CD3^+$ T cells were elevated (~70%) in almost all patients at all stages of disease evolution. $CD4^+$ T cells and $CD3^+$ T cells were higher in samples collected at the progressed stage compared with the early stage.

IFNγ levels were elevated (>15.0 pg/ml) in many patients during the early stage, but the majority of patients had the same level as disease controls (~10.0 pg/ml) 5 years after onset (Fig. 5). IL-4 levels were higher in samples from the progressed stage compared with the early stage. IL-12 levels were elevated in several samples during the early stage. TNFα levels were elevated (>7.0 pg/ml) in many patients at all stages of disease evolution (Figure 6). Granzyme B levels were markedly elevated, especially in the early stages, and remained slightly elevated even in the progressed stage.

When patients without immunologic treatment were compared with patients after introduction of immunologic treatment, CD4, CD8, CD3, Granzyme B, IL-4, IL-12, TNFα, and IFNγ showed no statistically significant difference (data not shown, Mann-Whitney test).

Clinical evolution and autoantibodies against GluR ε2 (NR2B) in CSF

Anti-GluR ε2 (NR2B) autoantibodies in the CSF samples collected from a total of 25 RS patients were examined qualitatively by immunoblot, and evolutional changes were evaluated using the positive rates at various intervals after onset. In eight samples from five patients collected within 6 months of epilepsy onset, IgG autoantibodies against GluR ε2 (NR2B) were found in half of patients (50%), and IgM autoantibodies against GluR ε2 (NR2B) were found in one of eight patients (12.5%)

Figure 2.
Cytokines and Granzyme B at the first cerebrospinal fluid (CSF) examination. (**A**) Percentage of $CD4^+$ T cells in CSF; (**B**) percentage of $CD8^+$ T cells; (**C**) percentage of $CD3^+$ T cells; (**D**) interferon γ (IFNγ) level; (**E**) tumor necrosis factor α (TNFα) level; (**F**) interleukin 4 (IL-4) level; (**G**) Granzyme B level; (**H**) IL-12 level. Rasmussen: data from RS patients. Horizontal bars show mean levels. Mean interval between disease onset and first examination of Granzyme B was 37.8 ± 42.0 months (mean ± SD).
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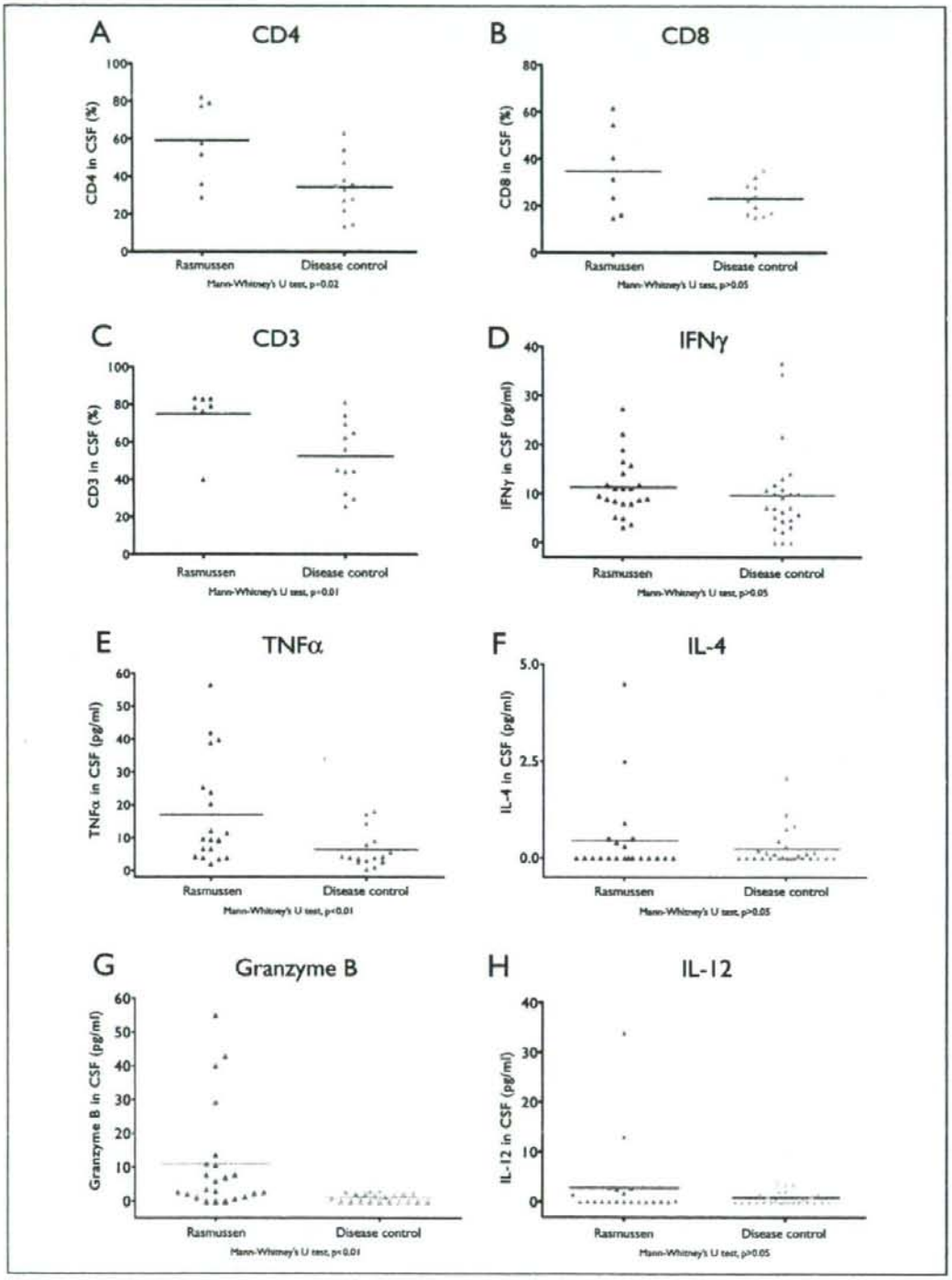

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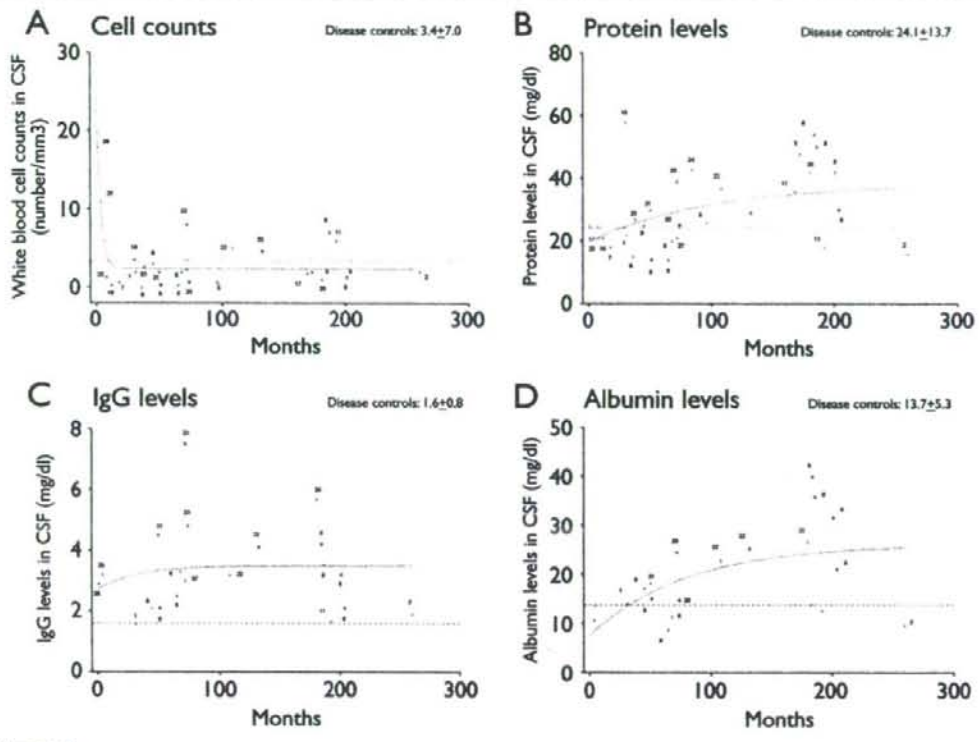

Figure 3.

Conventional and immunologic molecules in cerebrospinal fluid (CSF). (**A**) White blood cell count in CSF; (**B**) protein level; (**C**) immunoglobulin G (IgG) level; (**D**) albumin level. Horizontal axis in each panel shows time (months) from onset of epilepsy. Value (mean ± SD) for disease controls is shown above each panel. Numbers indicate patients' numbers in Table 1. Triangle symbols show data of patients without immunologic treatment; circle symbols show data of patients after introduction of immunologic treatment. Dotted lines show mean levels of disease controls. Solid lines show nonlinear regression curves produced by computer software, GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). In (**A**), nonlinear regression was done by equation of one phase exponential decay [95% confidence interval (CI) of plateau, 1.472–3.278]. In (**B**), nonlinear regression was done by equation of one phase association (95% CI of plateau, 16.49–59.78). In (**C**), nonlinear regression was done by equation of one phase association (95% CI of plateau, 2.328–4.659). In (**D**), nonlinear regression was done by equation of one phase association (95% CI of plateau, 12.33–39.91).

(Fig. 6C, D). As the clinical course evolved, the proportions of patients positive for autoantibodies against GluR ε2 (NR2B) (IgG) decreased. In five of eight patients (patients 1, 6, 8, 18, and 20), autoantibodies against GluR ε2 (NR2B) (IgG or IgM) disappeared evolutionally (Table 1). In two of five patients (patients 8 and 20), the autoantibodies disappeared after the initiation of tacrolimus treatment. In all three patients without immunologic treatment (patients 1, 6, and 18), the autoantibodies disappeared in ordinary epilepsy treatment.

Discussion

We studied 27 patients who were diagnosed with RS based on clinical criteria including (1) intractable partial seizures and (2) interictal symptoms and EEG, suggesting progressive involvement of unilateral hemisphere, independent of histologic characteristics. These criteria may exclude patients with RS at an early stage before deterioration, who may be included by European consensus criteria (Bien et al., 2005).

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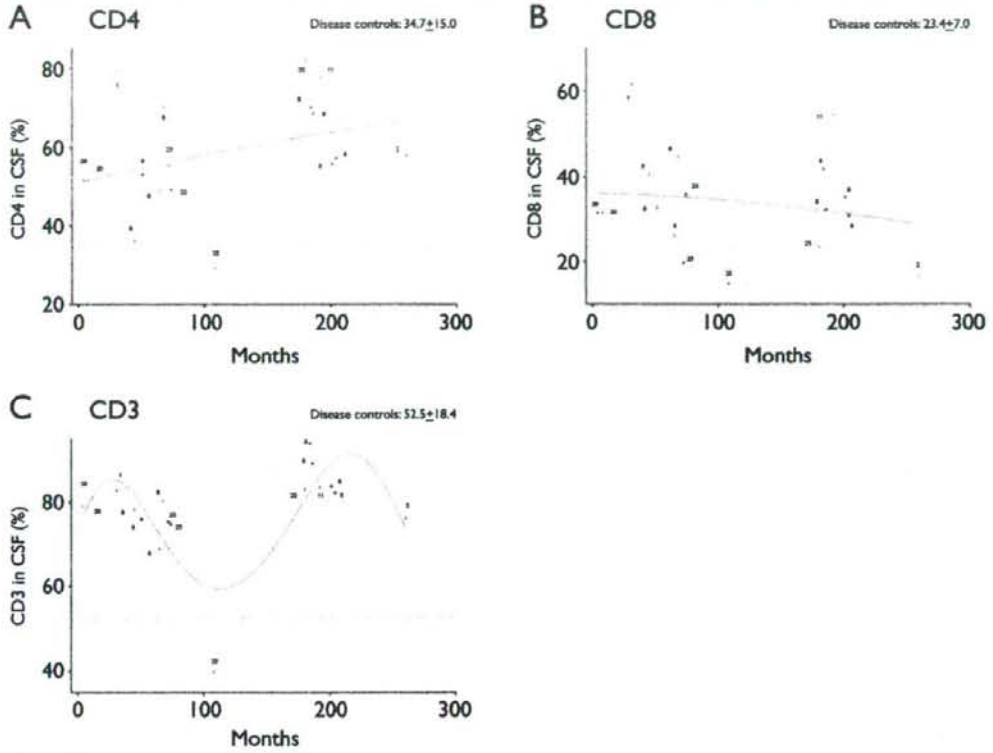

Figure 4.

Cell surface markers of lymphocytes in CSF. **(A)** Percentage of $CD4^+$ T cells; **(B)** percentage of $CD8^+$ T cells; **(C)** percentage of $CD3^+$ T cells. Horizontal axis in each panel shows time (months) from onset of epilepsy. Value (mean ± SD) for disease controls is shown above each panel. Numbers indicate patients' numbers in Table 1. Triangle symbols show data of patients without immunologic treatment; circle symbols show data of patients after introduction of immunological treatment. Dotted lines show mean levels of disease controls. Solid lines show nonlinear regression curves produced by computer software, GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). In **(A)**, nonlinear regression was done by equation of Gaussian distribution (95% CI of amplitude, −124.8 to 266.5). In **(B)**, nonlinear regression was done by equation of Gaussian distribution (95% CI of amplitude, 8.908 to 63.63). In **(C)**, nonlinear regression was done by equation of sum of two Lorentzian (95% CI of amplitude 1, 52.04–90.62).

Although an early diagnosis of RS is important to improve outcome, patients with RS are usually diagnosed with partial epilepsy at the onset of epilepsy. The possibility of RS is suspected only after epileptic seizures aggravate and brain function is impaired. We analyzed the conventional and immunologic test data of the first CSF examination, and found that IgG level, $CD4^+$ T cells, TNFα level, and Granzyme B level may contribute to a diagnosis of immune-mediated epilepsy including RS (Figs. 1 and 2). In patients with frequent partial seizures, these immune molecules should be measured for an early diagnosis of RS and the evolutional changes of these molecules should be followed to confirm the diagnosis of RS (Figs. 3–6). Clinical symptoms, MRI findings, and EEG findings are also essential for the diagnosis (Oguni et al., 1991; Bien et al., 2005).

Our cross-sectional data and nonlinear regression curves of immunologic molecules in CSF from early to progressed stages imply that white cell count and Granzyme B level are elevated in the early stage of RS around the onset of epilepsy, and decline within a few months to more or less constant levels (Figs. 3–6). $CD8^+$ T cells are

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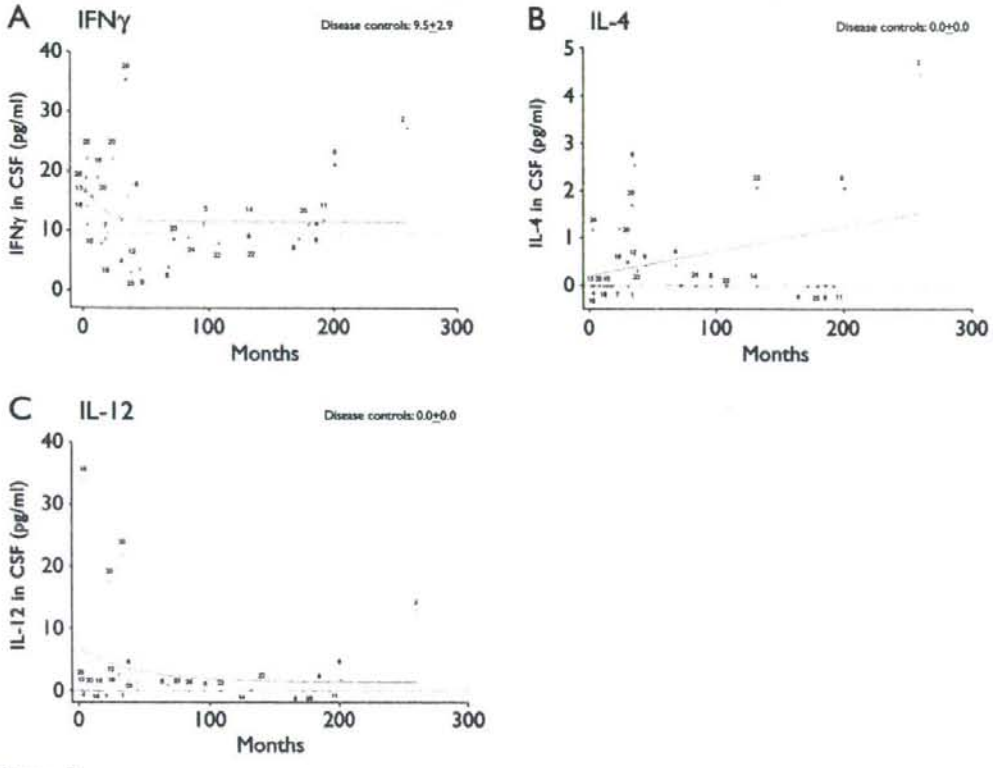

Figure 5.
Interferon γ 1 (IFNγ 1), interleukin 4 (IL-4), and IL-12 in cerebrospinal fluid (CSF). (**A**) IFNγ level in CSF; (**B**) IL-4 level; (**C**) IL-12 level. Horizontal axis in each panel (**A–C**) shows time (months) from onset of epilepsy. Value (mean ± SD) for disease control is shown above each panel. Numbers indicate patients' numbers in Table 1. Triangle symbols show data of patients without immunologic treatment; circle symbols show data of patients after introduction of immunologic treatment. Dotted lines show mean levels of disease controls. Solid lines show nonlinear or linear regression curves produced by computer software, GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). In (**A**), nonlinear regression was done by equation of one phase association (95% CI of plateau, 7.382–15.60). In (**B**), linear regression was done (95% CI of slope, −0.0001157 to 0.01034). In (**C**), nonlinear regression was done by equation of one phase association (95% CI of plateau, −5.106 to 7.900).

also elevated in the early stage and decline gradually toward the progressed stage. Granzyme B is usually secreted from CTLs, and sometimes from NK cells. Because the previous study of resected brain tissues has demonstrated that Granzyme B–secreting cells are not NK cells, but $CD8^+$ T cells (CTLs) in RS (Bien et al., 2002), we estimate that production of Granzyme B from $CD8^+$ T cells is activated more strongly in the early stage around onset, and continues even in the progressed stages (Figs. 4B and 6B). The cytotoxic mechanisms by Granzyme B may be very important, especially in the early stage around onset. Our data suggest that a crucial cytotoxic process contributes to the pathophysiologic mechanisms during the first few months of the disease, and declines in the progressed stage.

Our cross-sectional data and their nonlinear regression curves imply that IFNγ and IL-12 are produced especially in the early stage around onset, and $CD4^+$ T cells and TNFα level are elevated from the early stage to the progressed stage. IFNγ activates macrophage to secrete IL-12 and TNFα, and IL-12 facilitates the proliferation of Th1 cells in $CD4^+$ T cells. Therefore, differentiation and proliferation of autoreactive Th1 cells induced by cytokines in the early stage increase the ratio of $CD4^+$ T cells in CSF,

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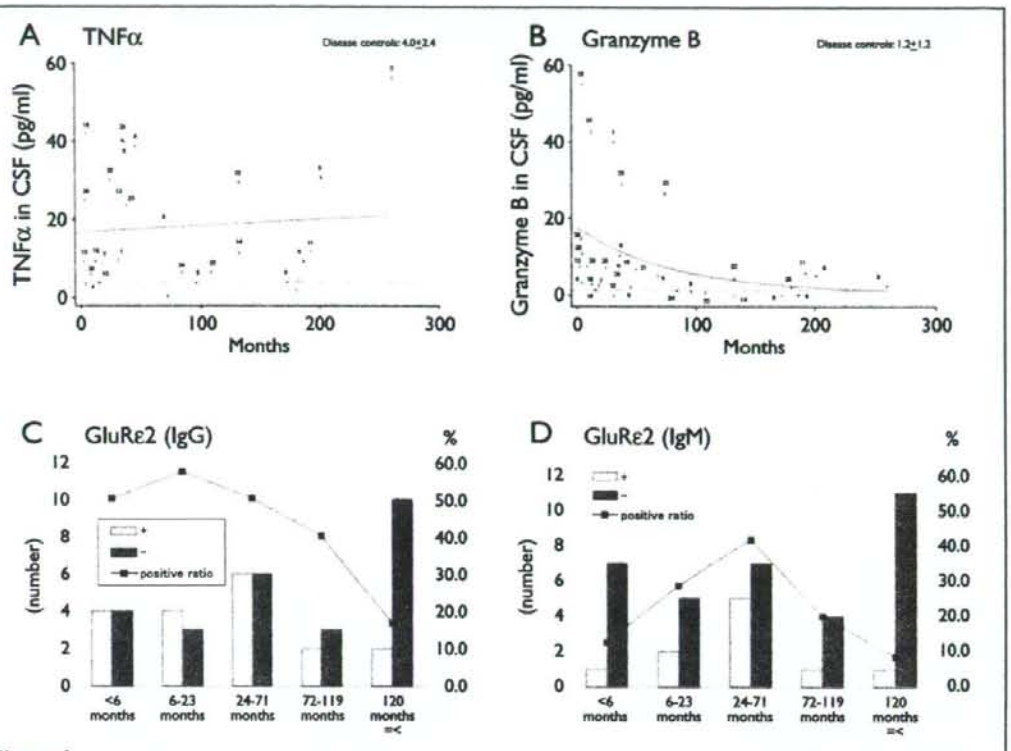

Figure 6.

Tumor necrosis factor α (TNFα), Granzyme B, and autoantibodies against GluR ε2 (NR2B) in cerebrospinal fluid (CSF). **(A)** TNFα level in CSF; **(B)** Granzyme B level; **(C)** immunoglobulin G (IgG) autoantibodies against GluR ε2 (NR2B); **(D)** IgM autoantibodies against GluR ε2 (NR2B). In panels **C** and **D**, yellow columns show the number of samples with antibodies against GluR ε2 (NR2B), and blue-green columns show the number of samples without autoantibodies against GluR ε2 (NR2B). Horizontal axis in each panel **(A–B)** shows time (months) from onset of epilepsy. Value (mean ± SD) for disease control is shown above each panel. Numbers indicate patients' numbers in Table 1. Triangle symbols show data of patients without immunological treatment; circle symbols show data of patients after introduction of immunological treatment. Dotted lines show mean levels of disease controls. Solid lines show linear or nonlinear regression curves produced by computer software, GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). In **(A)**, linear regression was done (95% CI of slope, −0.06487 to 0.09824). In **(B)**, nonlinear regression was done by equation of one phase association (95% CI of plateau, −20.17 to 21.29).

and initiate the subsequent autoimmune mechanisms. Prolonged production of TNFα from the early stage to the progressed stage leads to elevation of IL-6 in CNS. IL-6 may contribute to the inflammatory process and inhibition of regulatory T cells, resulting in augmentation of the autoimmune process. TNFα is reported to modulate AMPA-induced excitotoxicity (Bernardino et al., 2005), and to reduce GABA receptor (Stellwagen et al., 2005). Furthermore, transgenic mice of TNFα came to show epileptic seizures (Probert et al., 1995). These findings suggest that TNFα may contribute directly to epileptogenesis. Adding to the cytotoxic T-cell–mediated immune mechanisms, the autoimmune process mediated by Th1 cells and the epileptogenic effect of TNFα seem to be important also in RS. On the other hand, IFNγ induces the expression of MHC (class I+ II) and ICAM-1, and production of TNFα in microglia. These effects of IFNγ lead to the enhancement of the autoimmune cytotoxic process in CNS by $CD8^+$ T cells. TNFα induces the expression of MHC class I on astrocytes, and loosens capillary endothelial junctions. These effects of TNFα may also lead to apoptosis of astrocytes by $CD8^+$ T cell and inflammation. Brain tissues

of patients with RS are reported to show characteristic astrocytic apoptosis (Bauer et al., 2007).

Tacrolimus, an inhibitor of cytokine production, is effective in preserving neurologic function and delaying the progression of cerebral hemiatrophy in RS (Bien et al., 2004). Because IFNγ and Granzyme B are elevated, especially in the early stage, early initiation of tacrolimus may improve the outcome of RS. In patients 8, 20, and 22, development or progression of mental retardation was not observed after introduction of tacrolimus. However, in patient 23, progression of mental retardation was observed and higher levels of Granzyme B were sustained. Although our study showed no significant difference of CD4, CD8, CD3, Granzyme B, IL-4, IL-12, TNFα, or IFNγ between patients without immunologic treatment and patients after introduction of immunologic treatment, this may be attributed to the variety of clinical stage at the introduction of immunologic treatment and the variety of immunologic treatment. Further investigation is required to examine the effects of early initiation of tacrolimus in RS patients, which will depend on a correct early diagnosis using immunologic data in CSF and other methods.

Detrimental effects of autoantibodies against GluR mimicking excess of glutamate are reviewed in epilepsy, encephalitis, and other diseases (Levite & Ganor, 2008). Autoantibodies against GluR ε2 (NR2B) (IgG) were detected in half of patients within 6 months of epilepsy onset, and the autoantibody positive rate was lower in the progressed stage (Figure 6), and in all three patients without immunologic treatment, the autoantibodies disappeared evolutionally (Table 1). These data suggest that autoantibodies against GluR ε2 (NR2B) may be involved in the pathologic mechanisms from the early stage up to several years after onset of epilepsy. In a previous study in which we examined the effect of IgG antibodies against GluR ε2 (NR2B) in CSF from RS patients on excitatory postsynaptic current (EPSC) using patch clamp methods, both IgG in CSF from RS patients and rabbit IgG antibodies against mouse GluR ε2 (NR2B) had no effect on EPSC (Takahashi et al., 2006). However, anti-dsDNA antibodies in CSF from patients with systemic lupus erythematosus have been shown to cross-react with the N terminus of GluR ε2 (NR2B) and cause neuronal apoptosis in the rat hippocampus (DeGiorgio et al., 2001). It is possible that autoantibodies against GluR ε2 (NR2B) may also cause apoptosis in the rat hippocampus. Furthermore, autoantibodies against the N terminus of GluR ε2 (NR2B) have been reported to cause hippocampal neuron damage with ensuing memory impairment (Kowal et al., 2006), and amygdala neuron damage with emotional behavior impairment (Huerta et al., 2006). Therefore, autoantibodies against GluR ε2 (NR2B) may contribute to the cognitive and behavioral changes in RS through inducing apoptosis. A recent study has reported a causal relationship between autoantibodies against the N terminus of NMDA-type GluR hetero complexes and paraneoplastic encephalitis in patients with ovarian teratoma (Dalmau et al., 2007; Takahashi, 2008). Further investigations and a more suitable assay for the quantitative measurement of antibodies to NMDA receptors are required to elucidate the involvement of autoantibodies against GluR ε2 (NR2B) in the pathophysiologic mechanisms of RS.

In the later stages of RS, nonlinear regression curves of albumin levels in CSF imply that the CSF albumin level (protein level) increases evolutionally (Fig. 3). Direct brain exposure to serum albumin results in albumin uptake into astrocytes through transforming growth factor-beta receptors (TGF-β R), and induces NMDA-receptor–mediated neuronal hyperexcitability and subsequently epileptiform activity (Ivens et al., 2007). Therefore, elevated albumin levels in CNS may contribute to the pathologic mechanisms of intractable epilepsy, and TGF-β R may be a candidate therapeutic target for epilepsy in RS also.

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Statement of compliance with the Journal's guidelines for ethical standards in publishing and Disclosure of conflicts of interest.

We have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors has any conflicts of interest to disclose.

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Brief Communication

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Steroid-Responsive Chronic Cerebellitis With Positive Glutamate Receptor δ2 Antibody

Masaya Kubota, MD, PhD, and Yukitoshi Takahashi, MD, PhD

We report the clinical course of a 4-year-old girl with chronic cerebellitis (onset 2 days after diphtheria-pertussis-tetanus vaccination at 1 year and 7 months old) associated with anti-glutamate receptor δ2 antibody, who improved dramatically with steroid therapy (methylprednisolone pulse therapy plus oral prednisolone). Recently, it has been reported that the anti-glutamate receptor δ2 selectively expressed at the post-synaptic site of parallel fiber–Purkinje cell synapses has an important role in cerebellar function in the developing brain. The present case suggests that anti-glutamate receptor δ2 antibody plays a primary role in an immune-mediated process causing chronic cerebellar symptoms, and the lesion site seems to be localized to the parallel fiber–Purkinje cell synapse. Because the cerebellum is strongly involved in language acquisition as well as motor development, treatment must facilitate time for language learning while reducing the side effects of the corticosteroid therapy.

Keywords: chronic cerebellitis; glutamate receptor δ2

Because postinfectious acute cerebellar ataxia in childhood is generally benign and self-limited, no specific treatment is required. However, in some cases unlike acute cerebellar ataxia, unusual long-term cerebellar symptoms persist and therapeutic intervention is necessary. Recently, extensive molecular genetic studies have revealed the essential role of glutamate receptor δ2 in cerebellar functions, and glutamate receptor δ2 mutant mice or mice treated with specific antibody to glutamate receptor δ2 showed impairments in various cerebellar functions.[1–6] At present, the clinical relevance of glutamate receptor δ2 dysfunction is not fully understood. We herein report the clinical course of a 4-year-old girl with chronic cerebellitis associated with anti-glutamate receptor δ2 antibody who improved dramatically with steroid treatment, despite the absence of any therapy for 1 year and 8 months after onset.

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Case Report

This 4-year-old Korean girl had been quite healthy with normal psychomotor development until nystagmus and ataxic gait appeared at the age of 1 year and 7 months. Her family history was unremarkable. Two days later, she showed an unstable wide-based gait and could not sit steadily. She was given a diphtheria-pertussis-tetanus vaccination 2 days before onset of the cerebellar symptoms. Despite the diagnosis of acute cerebellar ataxia, the symptoms gradually worsened. A study of the cerebrospinal fluid showed cell count of 20/μL (15 lymphocytes), protein 10 mg/dL, and glucose 60 mg/dL. Two weeks after onset, prednisolone (1 mg/kg weight) was given every day for 2 weeks, but the symptoms persisted. Thereafter, the patient was not given any medication for 1 year and 8 months.

When she was admitted to our hospital for further evaluation at the age of 2 years and 9 months, she could not stand or walk without help due to cerebellar ataxia and could not speak at all. Excessive salivation was noticed. No definite nystagmus was observed. Basically, muscle tone of the extremities was hypotonic and intentional tremor of the upper extremities was observed. Deep tendon reflexes were induced normally, and no pathological reflexes were evident. On brain magnetic resonance imaging (MRI) and single-photon emission computed tomography (SPECT),

Table 1. Anti-Glutamate Receptor δ2 and ε2 Antibody Before and After Steroid Introduction (at 2 y and 9 mo, and 3 y and 9 mo)

	IgG-ε2	IgM-ε2	IgG-δ2	IgM-δ2
Cerebrospinal fluid (2 y and 9 mo)	−	−	−	+
Cerebrospinal fluid (3 y and 9 mo)	−	−	+	+
Serum (2 y and 9 mo)	−	−	−	−
Serum (3 y and 9 mo)	−	−	−	−

no structural or metabolic abnormalities were demonstrated. Urine vanilmandelic acid and homovanillic acid were within normal limits, and no abnormal mass was found on an abdominal ultrasound study. As shown in Table 1, anti-glutamate receptor δ2–immunoglobulin (Ig) M in cerebrospinal fluid was found to be positive and anti-glutamate receptor δ2-IgG was still negative. At that time, serum (simultaneously studied serum) anti-glutamate receptor-IgM and anti-glutamate receptor-IgG were negative. (For details regarding the detection of anti-glutamate receptor-IgM and -IgG, see the Methods section of Takahashi et al.[7]) Because the production of anti-glutamate receptor δ2 antibody in the central nervous system might be closely related to the patient's cerebellar symptoms, we selected corticosteroid therapy, with a high-dose methylprednisolone as a first-line treatment (at the age of 3 years and 3 months). Three weeks after the methylprednisolone pulse therapy (30 mg/kg weight divided for 3 days), the parents recognized a gradual improvement in truncal ataxia, resulting in walking with help more smoothly than before, and excess salivation disappeared. Subsequently, we started oral prednisolone (1 mg/kg weight, on alternate days) for further improvement. Surprisingly, 2 months later, she could walk without help and could eat food with a spoon by herself. Five months after the beginning of corticosteroid therapy, the truncal and limb ataxia almost disappeared so that she could walk fast and steadily. Concerning articulation, she could imitate a single phoneme or limited words and occasionally began to speak some words 6 months after therapy. One year later (at the age of 4 years and 2 months), she could run steadily and walk up stairs well, and began to speak two-word sentences. At present (4 years and 8 months of age), she has begun to speak intelligible Japanese and Korean. Then we started to taper the dose of corticosteroid. Thus, corticosteroid therapy improved the long-term cerebellar symptoms without any adverse effects, despite the treatment interval of 1 year and 8 months. At the age of 3 years and 9 months, anti-glutamate receptor δ2-IgM in the cerebrospinal fluid was found to be still positive and anti-glutamate receptor δ2-IgG had converted to positive (Table 1). Anti-glutamate receptor ε2 antibody was not detected in cerebrospinal fluid or serum.

Lymphocyte Stimulation Test by Glutamate Receptor δ2 and Diphtheria-Pertussis-Tetanus Vaccine

The patient's peripheral blood mononuclear cells were cultured in the presence of D33 (cell line expressing glutamate receptor δ2 subunits) alone and 6250 × diluted diphtheria-pertussis-tetanus vaccine alone, and in the presence of D33 and 6250 × diluted diphtheria-pertussis-tetanus vaccine at the age of 4 years. Responses were assessed by [^{3}H]thymidine incorporation in lymphocytes. Results were expressed as counts per minute (cpm) and as stimulation indexes (= cpm of cultures with drug/cpm of cultures without drug). Results were considered positive when the stimulation index was higher than 2. As shown in Table 2, stimulation index for the mixture of D33 (glutamate receptor δ2 subunits) and diphtheria-pertussis-tetanus vaccine was 5.17, whereas stimulation index for diphtheria-pertussis-tetanus vaccine alone was 2.7 and stimulation index for D33 alone was 1.32.

Discussion

Because the long-term cerebellar symptoms in our case differed from simple acute cerebellar ataxia, we diagnosed her as having chronic cerebellitis associated with anti-glutamate receptor δ2 antibody. Since Takahashi et al[7] reported opsoclonus-myoclonus syndrome with positive anti-glutamate receptor δ2 antibody, our case may be categorized in the broad opsoclonus-myoclonus syndrome spectrum, but the main symptoms in our case were limb and truncal ataxia and language developmental delay without apparent myoclonic movement in ocular and limb muscle.

The present case suggests that anti-glutamate receptor δ2 antibody plays a primary role in the pathogenesis of chronic cerebellar ataxia; furthermore, the lesion was functional and not destructive, because a dramatic improvement in cerebellar symptoms was brought about by corticosteroid therapy, despite the absence of any therapy for 1 year and 8 months after onset. The absence of structural and metabolic abnormality in MRI and SPECT also supports this idea. The anti-glutamate receptor δ2 antibody was generated exclusively in the central nervous system because the antibody was positive only in the cerebrospinal fluid before and after corticosteroid introduction. In cases with persistent cerebellar symptoms, the presence of anti-glutamate receptor δ2 antibody in cerebrospinal fluid should be checked.

The glutamate receptor δ2 was selectively expressed at the postsynaptic site of parallel fiber–Purkinje cell synapses,[1] and glutamate receptor δ2 mutant mice showed impairments in long-term depression at these synapses,[2] motor learning,[3,5] stabilization of the parallel fiber–Purkinje cell synapse,[2,4] and refinement of climbing fiber innervation

Table 2. Results of Lymphocyte Stimulation Test by Glutamate Receptor δ2 and Diphtheria-Pertussis-Tetanus Vaccine at the Age of 4

	Control	Phytohemagglutinin	D33 (glutamate receptor δ2 subunit) (400 μg)	6250 × diluted diphtheria-pertussis-tetanus	D33 (glutamate receptor δ2 subunits) (400 μg) + 6250 × diluted diphtheria-pertussis-tetanus
Count per minute	320	83 453	423	863	1654
Stimulation index			1.32	2.7	5.17

to Purkinje cells.[2] In addition to these developmental abnormalities, Hirai et al[6] showed that application of an antibody specific for glutamate receptor δ2 to cultured Purkinje cells induced α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor endocytosis, attenuated synaptic transmission and abrogated long-term depression; moreover, adult mice treated with this antibody revealed cerebellar dysfunction without apparent morphological changes in Purkinje cells.

Taken together with these basic data, and positive anti-glutamate receptor δ2 antibody and dramatic effect of corticosteroid therapy on cerebellar symptoms in our case, the lesion site seems to be localized at the parallel fiber– Purkinje cell synapse.

Sugiyama et al[8] reported a similar case of chronic cerebellitis associated with anti-glutamate receptor δ2 antibody but the cerebellar symptoms fluctuated, despite a high-dose intravenous immunoglobulin and corticosteroid pulse therapy. The long-term use of corticosteroids may be necessary to suppress the disease process, because short-term corticosteroid therapy 2 weeks after the onset in our patient was not effective for the cerebellar symptoms.

The lymphocyte stimulation test showed that the mixture of D33 (glutamate receptor δ2 subunits) and diphtheria-pertussis-tetanus vaccine activated lymphocytes more intensely than D33 (glutamate receptor δ2 subunits) or diphtheria-pertussis-tetanus vaccine alone. Lymphocytes stimulated by the lymphocyte stimulation test are usually T cells. Although we could not confirm a subset of stimulated T cells (CD4+ or CD8+) by glutamate receptor δ2, activated effector T cells that could invade the central nervous system beyond the blood-brain barrier definitively exist in peripheral blood circulation. We speculate that these activated T cells are produced by cross-reaction using molecular mimicry after a diphtheria-pertussis-tetanus vaccination and play an important role in the subsequent onset of chronic cerebellitis.

On the retarded language development in our case, we must not regard it as a simple dysarthria but as a learning problem in which the cerebellum is strongly involved. The internal model was introduced to extend the cerebellar functions in voluntary movement, perception, and language. Ito[9,10] maintains the hypothesis that reorganization of the neuronal circuit by error-driven induction of long-term depression constitutes the major memory and learning mechanisms of the cerebellum. Therefore, treatment must facilitate time for language learning while reducing the side effects of corticosteroid therapy.

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Cerebrospinal fluid levels of cytokines in non-herpetic acute limbic encephalitis: Comparison with herpes simplex encephalitis

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ABSTRACT

Background: Recently, non-herpetic acute limbic encephalitis (NHALE) was identified as a new subgroup of limbic encephalitis. The immunological pathophysiology of NHALE is still unclear. *Methods:* We measured the concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, IL-10, and soluble TNF receptor 1 (sTNFR1) in the cerebrospinal fluid (CSF) of 15 patients with NHALE and 13 with herpes simplex encephalitis (HSE) by cytometric bead array or ELISA. *Results:* The CSF concentrations of IL-6 in patients with NHALE and IFN-γ, IL-6, IL-10, and sTNFR1 in HSE patients were significantly higher than those of controls ($p < 0.001$, $p = 0.004$, $p < 0.001$, $p = 0.018$, and $p < 0.001$, respectively). There were significant correlations among CSF IL-6, IL-10, and sTNFR1 levels in HSE patients. The CSF concentrations of IFN-γ and sTNFR1 levels of patients with HSE were significantly higher than those with NHALE ($p = 0.001$ and $p = 0.002$, respectively). *Conclusions:* CSF cytokine levels in NHALE were relatively low compared with those in HSE. These results may be related to the favorable prognosis of NHALE.

1. Introduction

In Japan, non-herpetic acute limbic encephalitis (NHALE) was identified as a new subgroup of limbic encephalitis [1–3]. The clinical picture of NHALE is similar to that of herpes simplex encephalitis (HSE). However, the disease is not caused by herpes simplex virus (HSV) infection or a paraneoplastic disease process. Many previously reported patients with NHALE had a rather favorable neurological prognosis compared to those with HSE [2,4]. There have been a few reports on the autopsy cases with NHALE [4,5]. These reports demonstrated that there were neuronal loss and severe gliosis with inflammatory cell infiltrations in the hippocampus and amygdala. The pathogenesis of NHALE is still unclear.

To investigate the immunological pathogenesis of NHALE, we determined the cerebrospinal fluid (CSF) concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, IL-10, and soluble TNF receptor 1 (sTNFR1) as cytokines related to inflammation in patients with NHALE and HSE.

2. Patients and methods

Informed consent was obtained from the families of the patients and controls enrolled in this study.

2.1. NHALE

CSF samples were obtained from 15 patients with NHALE (five males and 10 females, aged from 12 to 82 years; median, 35 years) admitted to Yamaguchi University Hospital and seven collaborating research hospitals from July 1999 to February 2008 (Tables 1 and 2). The criteria for the diagnosis of NHALE were: (1) acute or subacute onset neurological disorder with limbic-associated symptoms, such as amnesia, delirium, panic, anxiety, excitation, etc., (2) negative HSV DNA in CSF by the nested polymerase chain reaction (PCR) and negative HSV antibodies in CSF determined by the enzyme-linked immunosorbent assay (ELISA), (3) lesions of the temporal lobe, especially hippocampi and amygdalae, on magnetic resonance imaging (MRI) (Fig. 1), (4) absence of malignancy, (5) no bacteria or fungi in CSF culture, and (6) the exclusion of all other neurological, vascular, metabolic, endocrine, toxic, and drug-induced disorders. CSF samples obtained during the acute stage were stored at −70 °C.

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2.2. HSE

CSF samples were obtained from 13 patients with HSE (eight males and five females, aged from 13 to 76 years; median, 61 years) admitted to Yamaguchi University Hospital and two collaborating research hospitals from October 2000 to December 2005 (Table 1). The diagnosis was based on the demonstration of HSV DNA in the CSF by nested PCR. CSF samples during acute stage were stored at −70 °C.

2.3. Control subjects

The control subjects for the CSF levels of the cytokines were 19 afebrile and non-infectious patients with neurological disorders, such as epilepsy, dementia, etc. (11 males and eight females, aged from 13 to 79 years; median, 55 years), as shown in Table 1. CSF samples were obtained from them on routine analysis and they all had normal CSF cell counts.

2.4. Clinical data

The clinical data including age, gender, clinical symptoms on admission, CSF findings at the time of specimen collection, MRI findings during the acute stage, and clinical outcomes in patients with NHALE and HSE were investigated. The outcomes were defined as follows: (1) normal resolution, (2) mild sequelae, (3) severe sequelae necessitating help with daily life activities, and (4) death [6].

2.5. Determination of cytokine concentrations

The concentrations of CSF IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were measured with a cytometric bead array (CBA) kit (BD PharMingen, San Diego, CA, USA) according to the manufacturer's manual, as previously described [7–9], with modification of the data analysis using GraphPad Prism software (GraphPad Prism Software, San Diego, CA, USA). Briefly, each series of beads exhibiting discrete fluorescence intensities is coated with a monoclonal antibody against a single cytokine, and a mixture of six series of beads can detect six cytokines in one sample. A secondary phycoerythrin-conjugated monoclonal antibody stains the beads proportionally to the amount of bound cytokine. After fluorescence intensity calibration and electronic color compensation procedures, standard and test samples were analyzed with a FACScan flow cytometer equipped with CellQuest software (BD PharMingen). The lower detection limits for IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were 7.1, 2.8, 2.6, 2.6, 2.5, and 2.8 pg/ml, respectively.

The CSF concentrations of sTNFR1 were determined with a sTNFR1 ELISA kit (Bender Medsystems, Vienna, Austria), as described previously [10]. The lower detection limit for sTNFR1 was 0.05 ng/ml.

2.6. Statistical analysis

All data were log transformed to obtain an approximately normal distribution. The differences in the results between groups were analyzed with a t-test and the χ^2 test, and those with a p-value of less than 0.05 were considered significant. Correlations were analyzed using Pearson's coefficient correlation. Analyses and calculations were performed using SPSS-12.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Clinical characteristics

Clinical data of patients with NHALE are shown in Tables 1 and 2. There were no significant differences in age or gender among patients with NHALE and HSE and controls (median age, 35, 61, and 55 years, respectively). The CSF cell counts of patients with NHALE were lower than those with HSE ($p = 0.015$, 9/μl vs. 32/μl as a median). The CSF protein levels of patients with NHALE were less than those with HSE ($p = 0.003$, 33 vs. 50 mg/dl as a median). Of the 15 patients with NHALE, 9 (67%) had mild sequelae and 6 (33%) survived without sequelae. Of the 13 patients with HSE, 1 (8%) died and 12 (92%) experienced disability (54% had severe and 38% had mild sequelae).

Table 1
Clinical data of patients with NHALE, HSE, and controls

	NHALE $N = 15$	HSE $N = 13$	Control subjects $N = 19$
Age (median, range)	35 yr, 11–82 yr	61 yr, 13–76 yr	55 yr, 13–79 yr
Sex (male: female)	5:10	8:5	11:8
Comorbid conditions	–	–	Epilepsy, 9; dementia, 5; psychosis, 4; Tic, 1
Prognosis	Normal, 6; mild sequelae, 9	Mild sequelae, 5; severe sequelae, 7; death, 1	–

NHALE, non-herpetic acute limbic encephalitis; HSE, herpes simplex encephalitis.

Table 2
Clinical characteristics of the 15 patients with non-herpetic acute limbic encephalitis

No./age/gender	Main symptoms on admission	Lesions on MRI	CSF findings		Neurological prognosis
			Cell (μl)	Protein (mg/dl)	
1/34 yr/M	Amnesia, delirium	Bilateral temporal lobes	12	39	Normal
2/73 yr/F	Somnolence, convulsion	Bilateral temporal lobes	32	24	Normal
3/35 yr/M	Amnesia, convulsion	Bilateral temporal lobes	9	39	Mild amnesia
4/11 yr/M	Convulsion, delirium	Right temporal lobe	187	33	Intellectual impairment
5/18 yr/F	Convulsion	Bilateral temporal lobes	39	31	Normal
6/49 yr/F	Amnesia, convulsion	Bilateral temporal lobes	42	50	Amnesia, psychopathy
7/31 yr/F	Convulsion	Bilateral temporal lobes	0	27	Epilepsy
8/47 yr/F	Insomnia, convulsion	Bilateral temporal lobes	9	47	Normal
9/82 yr/F	Amnesia, fugue	Bilateral temporal lobes	1	39	Amnesia
10/67 yr/M	Convulsion, delirium	Bilateral temporal lobes	1	35	Amnesia
11/75 yr/F	Convulsion, enuresis	Bilateral temporal lobes	0	32	Amnesia, psychopathy
12/51 yr/M	Amnesia, convulsion	Bilateral temporal lobes	0	28	Amnesia
13/14 yr/F	Panic	Left temporal lobe	121	27	Normal
14/19 yr/F	Excitation, convulsion	Bilateral temporal lobes	8	48	Intellectual impairment, epilepsy
15/12 yr/F	Anxiety, insomnia	Bilateral temporal lobes	14	25	Normal

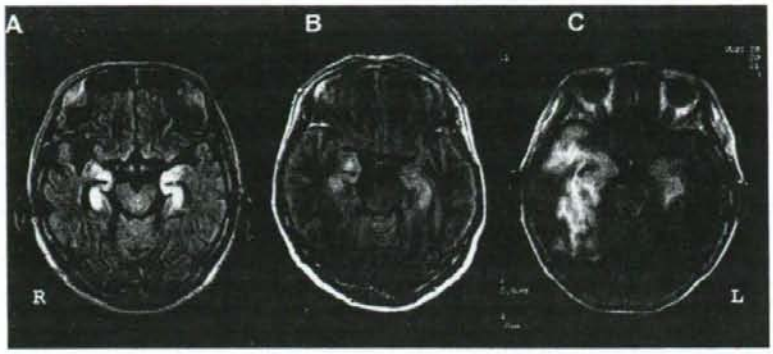

Fig. 1. FLAIR MRI of Patient 2 (A), Patient 6 (B), and Patient 9 (C) demonstrated high signal intensity lesions in the bilateral temporal lobes.

3.2. CSF concentrations of cytokines

In patients with NHALE, the CSF concentrations of IL-6 were significantly higher than those of controls ($p < 0.001$), but those of IFN-γ, TNF-α, IL-2, IL-4, IL-10, or sTNFR1 were not (Fig. 2).

In patients with HSE, the CSF concentrations of IFN-γ, IL-6, IL-10, and sTNFR1 were significantly higher than those of controls ($p = 0.004$, $p < 0.001$, $p = 0.018$, and $p < 0.001$, respectively), but those of TNF-α, IL-2, or IL-4 were not (Fig. 2). There were significant correlations among CSF IL-6, IL-10, and sTNFR1 levels in HSE patients (IL-6 and IL-10, $p = 0.008$; IL-6 and sTNFR1, $p < 0.001$; IL-10 and sTNFR1, $p = 0.030$) (Fig. 3).

The CSF concentrations of IFN-γ and sTNFR1 levels of patients with HSE were significantly higher than those with NHALE ($p = 0.001$, and $p = 0.002$, respectively) (Fig. 2).

4. Discussion

Main lesions in NHALE were in the bilateral temporal lobes, especially the hippocampus and amygdala, similar to those in HSE. However, HSV DNA or anti-HSV antibodies were not detected in the CSF of patients with NHALE. Previous reports on autopsy cases of NHALE revealed that HSV-1 or -2 were not detected in the brain [4,5]. Therefore, NHALE has been identified as a new type of encephalitis, especially in Japan [1–4]. Several autoantibodies, including those against the *N*-methyl-D-aspartate glutamate receptor and voltage-gated potassium channel, were detected in patients with NHALE [4,11–14]. Moreover, patients with limbic encephalitis associated with autoimmune disease, including Hashimoto's disease, Sjögren's syndrome, and systemic lupus erythematosus, have been reported [15–17]. These previous studies suggest that NHALE is immune-mediated encephalitis.

The clinical outcomes of patients with NHALE were relatively favorable compared with those with HSE. Moreover, CSF cell counts and protein concentrations of patients with NHALE were significantly less and lower than those with HSE, suggesting that inflammation in the CNS in NHALE is milder than that in HSE. In this study, we demonstrated CSF cytokine profiles of NHALE compared with HSE. In patients with NHALE, the CSF concentrations of IL-6 were significantly higher than those of controls, but those of IFN-γ, TNF-α, IL-2, IL-4, IL-10, or sTNFR1 were not. IL-6 is well-known as a cytokine that plays important roles in inflammatory re-

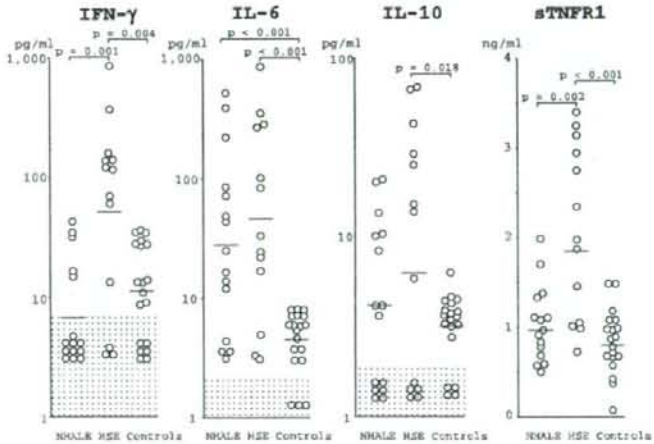

Fig. 2. The CSF concentrations of IFN-γ, IL-6, IL-10, and sTNFR1 in patients with NHALE, HSE, and controls. Horizontal lines indicate geometric means. Shaded areas indicate values below the detection limits.

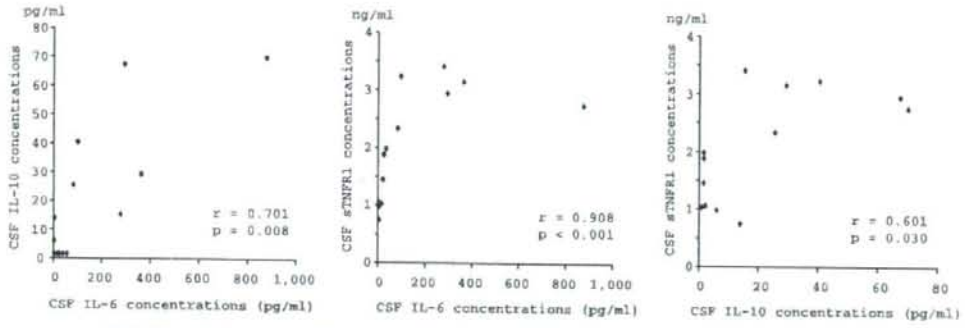

Fig. 3. The relationship among CSF IL-6, IL-10, and sTNFR1 concentrations in patients with HSE. r, Pearson's coefficient.

sponses [18,19]. Our results suggested that NHALE involves mild inflammation modified by IL-6 in the central nervous system (CNS). IFN-γ, which is produced by NK cells and $CD8^+$ and Th1 type $CD4^+$ T lymphocytes, plays an important role in host defense against viral infection, and inhibits viral replication [20]. We previously demonstrated that CSF IFN-γ levels were elevated in CNS disorders due to direct viral invasion, such as viral meningitis and HSE [2,21,22], but not in immune-mediated CNS disorders, such as acute disseminated encephalomyelitis, influenza-associated encephalopathy, acute encephalopathy following prolonged febrile seizures, and hemolytic uremic syndrome with encephalopathy [23–26]. Taking our findings into consideration, NHALE without elevated IFN-γ levels in the CSF in this study is not caused by direct viral infection.

In patients with HSE, the CSF concentrations of IFN-γ, IL-6, IL-10, and sTNFR1 were significantly higher than those with controls. Our present data that CSF IFN-γ levels were elevated in HSE were consistent with a previous study [2]. There were significant correlations among CSF IL-6, IL-10, and sTNFR1 levels in HSE patients. In addition, CSF sTNFR1 levels in HSE were significantly higher than those in NHALE. TNF-α increases blood–brain vascular permeability, injures vascular endothelial cells, and induces the necrosis of myelin and oligodendrocytes [29–31]. Previous studies have suggested that TNF-α mediates the pathogenesis of acute encephalitis/encephalopathy [23,32–35]. It is believed that sTNFR reflects the true biological activity of TNF-α [36–38]. CSF sTNFR1 levels are related to the neurological prognosis in bacterial meningitis and acute encephalopathy/encephalitis [10,33]. CSF sTNFR1 levels may reflect the neurological outcome in HSE. IL-10 as an anti-inflammatory cytokine decreases the production of IL-1, IL-6, and TNF-α induced by an endotoxin or bacteria [27,28]. Therefore we suggest that IL-10 is induced in the CNS to modulate pro-inflammatory cytokine-mediated inflammation in the CNS of patients with HSE. Patients with HSE showed elevated pro-inflammatory and anti-inflammatory cytokines in the CSF, suggesting that there was severe inflammation in the CNS of these patients.

In conclusion, the CSF concentrations of IL-6 in patients with NHALE and IFN-γ, IL-6, IL-10, and sTNFR1 in HSE patients were significantly higher than those in controls. Patients with HSE had many elevated cytokines in the CSF, but those with NHALE showed only an elevated CSF level of IL-6. These findings may be related to the fact that the clinical outcome of NHALE is relatively favorable compared with that of HSE.

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FULL-LENGTH ORIGINAL RESEARCH

Electroclinical features of epilepsy in patients with juvenile type dentatorubral-pallidoluysian atrophy

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Summary

Purpose: To clarify the electroclinical characteristics of epileptic seizures in patients with juvenile type dentatorubral-pallidoluysian atrophy (DRPLA).

Methods: Seventeen patients with juvenile type DRPLA confirmed by genetic analysis were studied retrospectively. The clinical records of all 17 patients and the ictal video electroencephalography (EEG) recordings from 12 of the 17 patients were reviewed.

Results: Electroclinical studies in 12 patients identified 11 habitual seizures in 6 patients as partial seizures on ictal video EEG recordings. Clinical manifestations composed mainly of versions of the head and loss of consciousness. These partial seizures were persistently recorded throughout the clinical course. Brief generalized seizures (atypical absence and myoclonic seizure) were observed in 6 of 12 patients at the early stage. In contrast, generalized tonic–clonic seizures (GTCS) were recorded in four advanced stage patients who were almost bedridden. Semiological studies in 17 patients showed that the prevalence of partial seizures was significantly higher in patients with younger epilepsy onset (below 10 years of age; χ^2 test, $p < 0.05$) and that the age of epilepsy onset was significantly lower in patients with partial seizures than in those without partial seizures (Mann-Whitney U test, $p = 0.02$). However, the number of CAG repeats and age at clinical onset were not significantly different between two groups.

Discussion: Partial seizure is one of the common epileptic features in juvenile type DRPLA, especially in patients with younger epilepsy onset. Seizure types may be affected in an age-dependent manner and change evolutionally during progression of the clinical stage.

KEY WORDS: Seizure, Progressive myoclonus epilepsy, Semiology, DRPLA.

Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant neurodegenerative disorder (Naito & Oyanagi, 1982) caused by expansion of CAG repeat in the DRPLA gene on chromosome 12p (Koide et al., 1994; Nagafuchi et al., 1994). Clinical phenotype varies depending on the degree of unstable expansion of the CAG repeat (Ikeuchi et al., 1995; Komure et al., 1995). Patients with more extensive expansion have earlier onset and present symptoms of progressive myoclonus epilepsies (PME) consisting of epileptic seizures, prominent myoclonus, and progressive mental retardation (PME phenotype). In contrast, patients with smaller expansion have later onset and their main manifestations are ataxia, choreoathetosis, and dementia (non-PME phenotype). Epilepsy may also exist but is much milder than that of the PME phenotype. Naito & Oyanagi (1982) defined such PME phenotype with onset before 20 years of age as "juvenile type" DRPLA. Juvenile type DRPLA usually becomes apparent at an earlier age in successive generations of the same pedigree (anticipation) (Ikeuchi et al., 1995; Komure et al., 1995).

DRPLA is most prevalent in Japan (Nagafuchi et al., 1994), but an increasing number of cases have been reported from other countries with the development of

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molecular methods for diagnosis (Munoz et al., 1999; Licht & Lynch, 2002; Brunetti-Pierri et al., 2006). However, the precise electroclinical features of epilepsy in juvenile DRPLA remain unclear. Only a few case reports evaluated epilepsy in patients with juvenile type DRPLA, and these reports analyzed the seizures usually based on clinical records without ictal electroencephalography (EEG) evaluation. Correct diagnosis of epileptic seizure types is essential to improve the outcome of epileptic seizures. To elucidate the characteristics of epilepsy in juvenile DRPLA, we evaluated the ictal video EEG recordings in 12 patients with juvenile DRPLA and studied the seizure semiology in 17 patients by conducting detailed interview.

Methods

The clinical records of 17 patients from 14 families (including three pairs of siblings) with genetically confirmed juvenile type DRPLA were reviewed retrospectively. Their clinical profiles are shown in Table 1. Three patients (patients 3, 8, and 10) were already diagnosed of DRPLA by DNA analysis before referral. The remaining patients were diagnosed of DRPLA during follow-up in our hospital by DNA analysis conducted after obtaining informed consent from their parents. Criteria for considering DNA analysis were: (1) the presence of clinical symptoms of PMEs including epilepsy, myoclonus, movement disorder, and developmental delay and (2) no findings indicative of other types of PME. Extending screening to family members of the probands differed from case to case for various reasons. Many families did not agree to the analysis of family members who showed no apparent symptoms, and in several families, the fathers were either dead or unavailable due to divorce. As a result, parental diagnosis was limited to only two families in our hospital. Both familial cases showed paternal transmission, and the fathers did not suffer from epilepsy. No siblings were newly diagnosed with the disease following DNA analysis.

Subjects comprised nine female and eight male patients. All patients were referred to our hospital between 1979 and 2006 because of refractory seizures. The ages at referral ranged from 7 to 28 years (mean, 15.0 years), and follow-up duration ranged from 0.3 to 28 years (mean, 6.8 years). Most of them had no remarkable clinical histories except patient 1 (asphyxia at birth without any subsequent complication) and patient 2 (simple febrile seizure). Brain magnetic resonance imaging (MRI) was conducted at our hospital, and no focal cortical lesion was found in any of the patients.

The clinical profile and seizure semiology of each patient were assessed from interviews with the parents conducted at our outpatient clinic by experienced epileptologists. All patients were evaluated by routine EEG with simultaneous video recording in the outpatient clinic using the standard international 10-20 system during awake and sleep, including activation by intermittent photic stimulation (IPS) with Grass PS22 or PS33 photic stimulator at a flash frequency of 18 Hz. Photoparoxysmal response (PPR) was defined as positive when the response consisted of bilateral diffuse (poly)spikes and slow wave complexes (classical type PPR) (Kasteleijn-Nolst, 1989). Long-term video EEG monitoring (more than 24 h) using the

Table 1. Clinical profiles analyzed from interviews

Patient No.	Sex	Epilepsy onset (year)	Clinical onset (year)	Initial symptom	No. of CAG repeats	Past history	Family history obtained at referral
1	M	4	4	Seizure	65	Asphyxia	Sibling of patient 16
2	M	5	4	Intellectual impairment	76	Simple Fc	n.r.
3	F	6	3	Intellectual impairment	75	n.r.	Grandmother: dementia
4	F	6	2	Intellectual impairment	71	n.r.	n.r.
5	M	6	3	Intellectual impairment	76	n.r.	n.r.
6	M	6	5	Intellectual impairment	69	n.r.	n.r.
7	F	6	6	Seizure	70	n.r.	Sibling of patient 10
8	F	7	5	Intellectual impairment	68	n.r.	n.r.
9	M	8	5	Intellectual impairment	71	n.r.	Sibling of patient 11 Grandmother: dementia
10	F	8	6	Intellectual impairment	70	n.r.	Sibling of patient 7
11	M	9	4	Intellectual impairment	73	n.r.	Sibling of patient 9 Grandmother: dementia
12	M	10	10	Seizure	64	n.r.	n.r.
13	F	11	7	Intellectual impairment	71	n.r.	Aunt: movement disorder
14	M	12	12	Seizure	68	n.r.	Grandfather: movement disorder
15	F	13	9	Intellectual impairment	69	n.r.	n.r.
16	F	18	18	Seizure	67	n.r.	Sibling of patient 1
17	M	18	18	Seizure	65	n.r.	n.r.

No., number; M, male; F, female; n.r., not remarkable; Fc, febrile convulsion.

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