The maximum accelerations of the head were reduced by using wearable airbag in both backward fall and forward fall. In forward fall-down, shock absorption by front protecting airbag was significant, but shock absorption by back protecting airbag was small. This means the airbag is not versatile, and it is necessary to use suitable airbag of protecting purpose. At the first time, we thought the protection against backward fall was important because in case of backward fall it is difficult to protect for pedestrians by themselves. But, this doesn't mean the protection against forward fall is not important especially for elder persons and epilepsy patients. We continue investigation on what to be protected and improvement of airbag.

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Short communication

Autoantibodies to glutamate receptor GluRε2 in a patient with limbic encephalitis associated with relapsing polychondritis

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ABSTRACT

Limbic encephalitis is a rare central nervous system (CNS) manifestation of relapsing polychondritis (RP). Vasculitis is assumed to be the cause of CNS involvement in RP. Several studies, however, have described CNS involvement in RP with no evidence of vasculitis but with a more nonspecific inflammatory picture. We report a patient with limbic encephalitis associated with RP who presented with anti-glutamate receptor (GluR) ε2 (NR2B) autoantibodies in his cerebrospinal fluid and sera. Brain MRI showed a high signal intensity lesion in the medial temporal lobe and progressive atrophy without multifocal abnormality on fluid-attenuated inversion recovery scanning. Our patient's results raise the interesting possibility that anti-GluRε2 (NR2B) antibodies function in the development of limbic encephalitis in certain patients with RP.

1. Introduction

Relapsing polychondritis (RP) is a disorder of unknown etiology manifesting as episodic and progressive inflammation of cartilaginous structures throughout the body that include the ears, joints, nose, eyes, respiratory tract, cardiovascular system and skin [1]. The etiology and pathogenesis of RP are unknown, but autoantibodies to type II collagen restricted to cartilage have been detected in the sera of 30–50% of patients studied [2]. Rarely, the central nervous system (CNS) is involved in RP with clinical manifestations of headache [3–5], meningitis [5], encephalitis [3,6–8], cerebral infarction [9], hemiplegia [3], ataxia [10,11], seizures [3,5,7], confusion [3,8,10–12], psychosis [3,5,10,12] or dementia [4,6–9,11]. We report a patient with limbic encephalitis associated with RP who presented with anti-glutamate receptor (GluR) ε2 (NR2B) antibodies.

2. Case report

A 62-year-old man was admitted to our hospital because of generalized tonic–clonic convulsion followed by recurrent focal seizures. He had a previous history of bilateral ear swelling, erythema and tenderness which emerged at age 58, lasted 6 months and was followed for about 1 year by nasal deformity and conjunctivitis. At age 60, he experienced fever and headache. The diagnosis was aseptic meningitis, which subsided spontaneously in a month. At age 61, he again experienced fever and headache accompanied by delirium. Aseptic meningitis again was diagnosed and subsided in a month. Results of a cerebrospinal fluid (CSF) study and medication details given at these events are not available. No specific immunosuppressant therapy had been performed for his systemic symptoms. During exacerbation of his symptoms, finger deformity emerged bilaterally. Three months before admission, his family had noticed such psychiatric and behavioral abnormalities as visual and auditory hallucinations, agitation, disinhibition, cognitive decline and seizure emergence.

On admission, a neurological examination showed disturbed consciousness and recurrent clonic convulsion in the left face and upper and lower limbs. Both ears were swollen (Fig. 1A), and his nose and fingers (Fig. 1B) deformed. Deep tendon reflexes were reduced in his lower limbs. Laboratory blood tests revealed an inflammatory reaction that included increased C-reactive protein (CRP) of 19.1 mg/dl accompanied by a high body temperature of 38.0 °C. His white blood cell count was 7400/mm^3. He also had increased HbA1C, indicative of diabetes mellitus. Serum rheumatoid factor and antinuclear antibodies were negative. His CSF was sterile with an increased cell count of 39/mm^3 (59% lymphocytes) and an increased protein level of 68 mg/dl. A polymerase chain reaction DNA for herpes simplex virus was negative. A fluid-attenuated inversion recovery MRI scan of the brain at that time showed a slightly high signal intensity lesion in the medial temporal lobe that included the hippocampus and amygdala (Fig. 1C), and mild chronic ischemic change in the putamen. Diffusion-weighted MR images were normal. An electroencephalogram showed slowing of background activities to 4–5 Hz. Limbic

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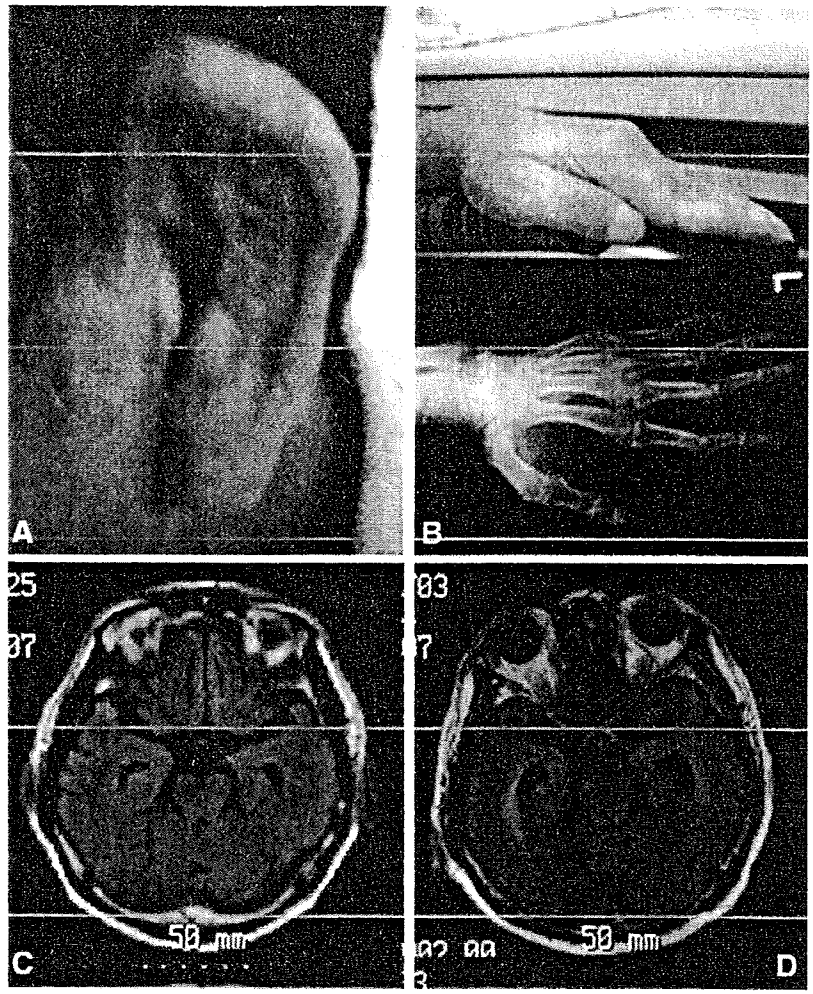

Fig. 1. The patient's clinical characteristics. A: Ear swelling, B: finger deformity, C: fluid-attenuated inversion recovery MR image of the brain at first admission showing a slightly high signal intensity lesion in the medial temporal lobe, and D: brain MR image 14 months later showing marked atrophy of the medial temporal lobe bilaterally.

encephalitis associated with relapsing polychondritis was diagnosed based on his clinical presentation of auricular signs, nasal chondritis, arthritis of the hands, previous history of conjunctivitis, systemic inflammatory reactions and the absence of any known collagen vascular disease. Intravenous injection of 500 mg/day methylprednisone 3 days a week for 3 weeks, together with phenytoin stopped his seizures, disturbed consciousness and inflammatory reaction. Once consciousness became normal, his hearing disturbance cleared up.

He was discharged on the 28th hospital day, but fever, headache and delirium recurred within a week. On readmittance his CSF showed increased protein of 64 mg/dl, an IgG level of 8.7 mg/L and a cell count of 512/mm^3 (30% lymphocytes). We found anti-GluRε2 (NR2B) IgG and IgM antibodies in his CSF and anti-GluRε2 (NR2B) IgG antibodies in his sera. No anti-GluRδ2 antibodies were detected in either fluid.

Intravenous administration of 500 mg/day methylprednisone 3 days a week for 4 weeks again was given followed by oral prednisone at 20 mg/day and tacrolimus at 3 mg/day. No fever or inflammatory reaction such as an increased white blood cell count or serum CRP level occurred thereafter, but cognitive decline continued, marked medial temporal lobe atrophy being detected 14 months after his first admission (Fig. 1D).

3. Discussion

Our patient presented with limbic encephalitis characterized by fever, headache, delirium, psychosis, seizures and dementia during the course of RP which rarely presents as limbic encephalitis [6–8] resulting in dementia [6]. Stewart et al. [5] reported extensive cerebral and systemic vasculitis to be the cause of CNS involvement in RP. In contrast, other authors have reported inflammatory changes non-specific to vasculitis in patients presenting limbic encephalitis associated with RP [7,8]. Multifocal neurological abnormalities and MRI lesions suggest that vasculitis is the cause of CNS manifestations in RP patients. Our patient's neurological symptoms and the MR findings of a medial temporal lobe lesion with progressive atrophy but no apparent asymmetrical multifocal lesion are similar to those of patients with non-herpetic limbic encephalitis rather than vasculitis. Because non-herpetic limbic encephalitis often is accompanied by anti-GluR antibodies, we tested his sera and CSF and found anti-GluRε2 (NR2B) antibodies. These antibodies have been detected in such human neurological disorders as non-herpetic, non-paraneoplastic limbic encephalitis, Rasmussen encephalitis, limbic encephalitis with ovarian teratoma, focal epilepsy, acute ischemic stroke and systemic lupus erythematosus [13–15]. The neuronal damage produced by some of these disorders may result in the release of GluR peptide [15]. In inflammatory disorders such as Rasmussen encephalitis, antigen presentation and autoantibody production could be initiated by immune cells already resident in the CNS [15]. The mechanism by which anti-GluRε2 antibodies produce limbic encephalitis remains to be clarified in our patient. Mice immunized with the GluR subtypes NR2/NR3 and gluR3 reportedly experienced CNS neuronal loss [16–18]. Gahring et al. [19] reported that GluR2 autoantibodies from a

patient with olivopontocerebellar atrophy activated α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors, a GluR subtype, in cultured mouse neurons. Dalmau [20] reported 100 patients with encephalitis-associated antibodies against N-methyl-D-aspartate receptors, another GluR subtype, and discussed the pathogenic role of these antibodies in developing encephalitis. In our patient, the release of glutamate receptor might have been caused by inflammation or ischemia of the central or peripheral nervous system associated with RP although there was no apparent ischemic change that paralleled the clinical course of CNS involvement. Alternatively, the autoantibodies could have been generated independent of the neuronal damage produced by RP. Various autoimmune diseases, including rheumatoid arthritis and Sjögren's syndrome, have been reported as being concurrent with RP [1].

The pathogenicity of anti-GluRε2 (NR2B) antibodies in limbic encephalitis has yet to be determined [15]. The anti-GluR ε2 (NR2B) antibodies generated in our patient may have caused limbic encephalopathy instead of vasculitis. This is the first report of the presence of these antibodies in RP-related CNS involvement. Our patient's case suggests that autoantibodies to the GluR ε2 (NR2B) subunit are associated with limbic encephalitis in certain patients with RP.

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BRIEF COMMUNICATION

HLA Class I markers in Japanese patients with carbamazepine-induced cutaneous adverse reactions

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Summary

Carbamazepine (CBZ) is frequently used for treating epilepsy, but this drug causes cutaneous adverse drug reactions (cADRs) that may range from mild to severe. It is reported recently that the human leukocyte antigen HLA-B*1502 is associated with Stevens-Johnson syndrome (SJS) induced by CBZ in Han Chinese. We examined HLA class I in 15 Japanese patients who fulfilled the diagnostic criteria for CBZ-induced cADRs (mild in 10 and severe = SJS in 5). HLA-B*1518, HLA-B*5901 and HLA-C*0704 alleles showed higher relative risks (above 10.0) for severe cADRs. The haplotype (HLA-A*2402-B*5901-C*0102) had high relative risk (16.09) for severe cADRs. In patients with severe cADRs, frequencies of HLA-A*1101, HLA-A*3303, HLA-B*1501, HLA-B*4403, HLA-B*5101, HLA-B*5201, HLA-C*0702, and HLA-C*1202 alleles are relatively lower than in the Japanese population. These data may suggest that HLA-B*5901 is one of the candidate markers for CBZ-induced SJS in Japanese.

KEY WORDS: Carbamazepine, Stevens-Johnson syndrome, HLA class I, HLA-B*5901, Cutaneous adverse drug reactions.

Background

Skin rash is a well-known complication of antiepileptic drug (AED) treatment. The risk of cutaneous adverse drug reactions (cADRs) of AED treatment is reported to be higher compared to drugs other than AEDs (Roujeau et al., 1995). In particular, carbamazepine (CBZ), which is commonly used to treat partial epilepsy, frequently causes a wide spectrum of cADRs including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug-induced hypersensitivity syndrome (DIHS), macropapular eruption, and mild skin rash. CBZ induces cADRs in 2.9% of Japanese patients (http://www.info.pmda.go.jp/). Recently, a strong association has been reported between a genetic marker, the human leukocyte antigen HLA-B*1502 and SJS induced by CBZ in Han Chinese (Chung et al., 2004; Hung et al., 2006). However, HLA-B*1502 is rare in the Japanese population and is not found in patients with SJS induced by CBZ (Kashiwagi et al., 2008). The genetic markers for SJS seem to be heterogeneous in each race (Ueta et al., 2008). In the present study, we try to identify the HLA class I genetic markers in the Japanese population that may predict patients at high risk of cADRs induced by CBZ.

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Patients and Methods

Patients

We classified cADRs into two categories: group A (10 patients) with mild cADRs such as exanthema and rash with or without fever, and group B (five patients) with severe cADRs such as SJS, TEN, and DIHS. The diagnosis of each cADR was based on the clinical criteria provided by Pharmaceuticals and Medical

Devices Agency (PMDA) (http://www.info.pmda.go.jp/juutoku/juutoku_index.html). TEN and SJS are both defined as mucocutaneous disorders characterized by extensive erythema, blisters, epidermal detachment, erosions, enanthema, and high fever. However, SJS is defined as skin detachment of 10% or less of the body surface area, whereas TEN is defined as skin detachment of more than 10%, excluding staphylococcal scalded skin syndrome (Roujeau et al., 1995). DIHS is characterized by multiorgan involvement such as hepatitis and nephritis accompanied by systemic manifestations, including fever, eosinophilia, and lymphadenopathy, in addition to skin rashes. The five patients with severe cADR were diagnosed as SJS by the above-mentioned clinical criteria, patients B-2 and B-4 were reported in a previous report (Kashiwagi et al., 2008), and patient B-5 was included in a previous paper (Kaniwa et al., 2008). The characteristics of the patients are shown in Table 1. We conducted HLA genotyping after obtaining informed consent from each patient by the methods approved by the ethical committee of our hospital.

HLA genotype

High-resolution typing of HLA class I loci was performed by the sequence-based method using the SeCore sequencing kits (Invitrogen Corp., Brown Deer, WI, U.S.A.) and the ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.). Using the kits for HLA-A, -B, and -Cw, exons 2–4 of each gene were amplified and sequenced to identify the genetic polymorphisms. HLA-A, -B, and -Cw alleles were estimated using the ASSIGN SBT software version 3.2.7b (Conexio Genomics, Freemantle, Western Australia, Australia).

Statistical analysis

The HLA-A and -B allele frequencies obtained from 493 Japanese healthy subjects were used as the frequencies for Japanese general population (Table 2). The HLA-C allele frequencies obtained from 114 Japanese healthy subjects were used as the frequencies for Japanese general population (Table 2). Relative risks were calculated according to the reference (Marsh et al., 2000). Relative risk in this study is defined as hK/Hk, where h is the allele frequency in patients with the antigen, k is the allele frequency in the patients without the antigen, H is the allele frequency in healthy controls with the antigen, and K is the allele frequency in controls without the antigen.

RESULTS

HLA-A

Ten patients in group A and five patients in group B were analyzed (Table 2). In group A, relative risk was the highest for HLA-A*2603 allele (5.37), and zero for HLA-A*0201 and HLA-A*2601 alleles. In group B, the highest relative risk was 2.03, and zero risk was observed for HLA-A*1101 and HLA-A*3303 alleles among alleles with relatively high frequencies in the Japanese population.

Table 1. Clinical characteristics of patients in groups A and B

Type and Pt number	Age	Sex	Epilepsy	Associated disease	Dose of CBZ (mg)	Latency to cADRs (days)	Concurrent AEDs	History of cADRs
A-1	12	M	PE	Brain tumor	?	11	–	–
A-2	7	F	PE	Hyperthyroidism	?	30	–	CLB, ZNS
A-3	11	M	RS	–	?	4	–	
A-4	1	F	PE	Tuberous sclerosis	60	12	–	
A-5	8	M	PE	Sequelae of encephalitis	100	35	VPA	PB
A-6	7	F	PE	Mental retardation	150	17	VPA+ZNS	CBZ, PHT
A-7	6	F	PE	Sequelae of encephalitis by influenza vaccine	110	9	–	
A-8	9	M	PE	Autism	180	12	VPA	
A-9	14	F	PE	Mental retardation	200	30	–	
A-10	7	F	PE	–	100	14	VPA	ZNS, PHT
B-1	60	F	PE	–	100	1	–	CBZ, PHT
B-2	33	M	PE	Operated AVM	400	7	–	
B-3	38	F	PE	SLE	?	?	–	PHT
B-4	24	F	PE	Sequelae of Influenza encephalitis	?	7	–	
B-5	52	F	PE	SLE	100	11	ZNS	ABPC

M, male; F, female; PE, localization-related epilepsy; RS, Rasmussen syndrome; AVM, arteriovenous malformation; SLE, systemic lupus erythematosus; cADRs, cutaneous adverse drug reaction; Concurrent AEDs, concurrently used AEDs on cADRs; CLB, clobazam; ZNS, zonisamide; PB, phenobarbital; CBZ, carbamazepine; PHT, phenytoin; ABPC, aminobenzylpenicillin.

Epilepsia, 51(2):297–300, 2010
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Table 2. HLA-A, -B, and -C alleles in patients with cADRs

HLA allele	Japanese population allele frequency[a] (%)	Group A (mild cADR) Allele frequency	Group A (mild cADR) Relative risk	Group B (severe cADR) Allele frequency	Group B (severe cADR) Relative risk
A*0201	10.9	0/20	0.00	2/10	2.03
A*0206	10.4	1/20	0.46	1/10	0.96
A*0207	3.4	2/20	3.11	0/10	0.00
A*1101	8.1	2/20	1.26	0/10	0.00
A*2402	35.6	10/20	1.80	5/10	1.80
A*2601	9.8	0/20	0.00	1/10	1.03
A*2603	2.1	2/20	5.37	0/10	0.00
A*3101	7.7	2/20	1.33	1/10	1.33
A*3303	7.9	1/20	0.61	0/10	0.00
B*1302	0.1	0/20	0.00	0/10	0.00
B*1501	7.2	2/20	1.45	0/10	0.00
B*1518	0.9	0/20	0.00	1/10	13.58
B*3501	8.6	2/20	1.19	1/10	1.19
B*4001	5.1	1/20	0.99	0/10	0.00
B*4002	8.2	1/20	0.60	1/10	1.26
B*4006	5.3	1/20	0.95	0/10	0.00
B*4403	6.9	0/20	0.00	0/10	0.00
B*4601	3.8	3/20	4.40	2/10	6.24
B*4801	2.7	1/20	1.94	1/10	4.10
B*5101	7.9	3/20	2.05	0/10	0.00
B*5201	13.7	3/20	1.10	0/10	0.00
B*5401	6.5	2/20	1.60	1/10	1.60
B*5502	3.2	1/20	1.57	1/10	3.31
B*5901	1.7	0/20	0.00	2/10	15.16
B*6701	1.0	0/20	0.00	0/10	0.00
C*0102	17.0	5/18	1.87	4/10	3.23
C*0103	r	1/18		0/10	
C*0303	7.8	1/18	0.71	2/10	3.00
C*0304	11.3	3/18	1.60	1/10	0.89
C*0401	6.5	1/18	0.92	0/10	0.00
C*0602	1.7	0/18	0.00	0/10	0.00
C*0702	11.3	1/18	0.47	0/10	0.00
C*0704	0.9	0/18	0.00	1/10	12.89
C*0801	10.9	2/18	1.00	1/10	0.89
C*1202	10.4	2/18	1.09	0/10	0.00
C*1402	5.7	2/18	1.96	0/10	0.00
C*1502	1.7	0/18	0.00	1/10	6.39

HLA, human leucocyte antigen.
[a]HLA*A & B, Tanaka et al. Clinical Transplants 1996; 139–144; HLA*C, Tokunaga et al. Immunogenetics 1997, 46: 199–205.

HLA-B

Ten patients in group A and five patients in group B were analyzed. In group A, relative risk was highest for HLA-B*4601 allele (4.40), and zero in HLA-B*4403 allele among alleles with relatively high frequencies in the Japanese population. In group B, relative risks were high for HLA-B*1518 (13.58) and HLA-B*5901 alleles (15.16), and zero in HLA-B*1501, HLA-B*4403, HLA-B*5101, and HLA-B*5201 alleles among alleles with relatively high frequencies in the Japanese population.

HLA-C

Nine patients in group A and five patients in group B were analyzed. In group A, relative risks that can be calculated were lower than 2.0. Although C*0103 was detected in one patient only in group A, the relative risk could not be calculated because of rare frequency in the Japanese population. In group B, relative risks were high for HLA-C*0704 allele (12.89) and HLA-C*1502 allele (6.39), and zero for HLA-C*0702 and HLA-C*1202 alleles among alleles with relatively high frequencies in the Japanese population.

HLA A-B-C haplotype

Relative risks of the A-B-C haplotype were calculated depending on data of the Japanese population. The haplotype HLA-A*2402-B*5901-C*0102 and the haplotype HLA-A*0201-B*1518-C*0704 were found in two and one patient of group B, respectively. The relative risks of the former haplotype and the latter haplotype for severe cADRs are 16.09 and 28.94, respectively.

Discussion

We have experienced many patients manifesting cADR at various levels of severity induced by AEDs. A total of 21,655 patients with epilepsy visited our epilepsy center up until 2006, and 166 patients (0.767%) experienced cADRs. Among them, 139 patients experienced cADRs causally related to AEDs, with 118 patients (0.545%) having mild cADRs (group A) and 21 patients (0.097%) having severe cADRs (group B) (Takahashi, 2007). Various attempts have been made to identify individuals at high risk of developing severe cADRs with intolerable sequelae. In the Han Chinese (Chung et al., 2004; Hung et al., 2005) and the Thai population (Locharernkul et al., 2008), a strong association between HLA-B*1502 and CBZ-induced SJS has been found. Our previous studies found that HLA-B*1502 is a rare allele in Japanese, and that no Japanese patients with CBZ-induced SJS have HLA-B*1502 (Kaniwa et al., 2008; Kashiwagi et al., 2008). Following these studies, we found that HLA-B*1518, HLA-B*5901, and HLA-C*0704 alleles showed higher relative risks above 10.0 in the extended group of patients with severe cADRs (group B). Although HLA-B*1518 and HLA-C*0704 alleles were found in only one patient, HLA-B*5901 was found in two patients in group B (B-2 and B-5). The haplotype HLA-A*2402-B*5901-C*0102 has a prevalence of 1.530% in the Japanese population. The relative risk of this haplotype for severe cADRs is 16.09. These data may suggest that HLA-B*5901 is a candidate marker of CBZ-induced SJS. The relative risk of HLA-B*5901 for CBZ-induced SJS (15.16) is higher than that for pemphigus vulgaris (DR4, relative risk = 14), acute anterior uveitis

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(B27, 10), and systemic lupus erythematosus (DR3, 6), but lower than that for ankylosing spondylitis (B27, 87) and Goodpasture syndrome (DR2, 16) (Marsh et al., 2000). HLA-B*5901 has been reported to be weakly associated with SJS/TEN with ocular complications in a Japanese study (Ueta et al., 2008), although this study included patients with SJS/TEN independent of offending drugs. Further studies will reveal the significance of HLA-B*5901 in SJS/TEN induced by CBZ.

HLA-B*5801 is reported to be strongly associated with severe cADRs caused by allopurinol in Han Chinese (Hung et al., 2005). HLA-B*5701 is reported to be strongly associated with a hypersensitivity reaction caused by abacavir in U.S. white and black patients (Saag et al., 2008). Because the amino acid sequence of HLA-B*5901 shares 93.9% homology with that of HLA-B*5701, and 95.0% homology with that of HLA-B*5801, the homology of amino acid residues among these HLA subtypes seems to be high. Therefore, HLA-B*5901 may be causally related to severe cADRs induced by CBZ. Further investigations with a larger number of patients with CBZ-induced SJS are required to confirm the involvement of HLA-B*5901 in CBZ-induced SJS in the Japanese population. We expect that this report would encourage HLA examinations in Japanese patients, leading to the development of prevention methods for CBZ-induced SJS.

Our data suggest that possible HLA class I markers for mild cADRs induced by CBZ are completely different from those for SJS. In the Thai population also, HLA-B*1502 had a strong association only with CBZ-induced SJS, but not with CBZ-induced maculopapular eruptions (Locharernkul et al., 2008). Therefore, differentiation between severe cADRs and mild cADRs in each patient is very important to confirm the true markers for CBZ-induced SJS. In patients with severe cADRs, frequencies of HLA-A*1101, HLA-A*3303, HLA-B*1501, HLA-B*4403, HLA-B*5101, HLA-B*5201, HLA-C*0702, and HLA-C*1202 alleles are relatively lower than in the Japanese population. Whether these HLA class I markers are inhibitory for SJS may be answered in the future through precise immunologic studies.

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Original article

Nationwide survey (incidence, clinical course, prognosis) of Rasmussen's encephalitis

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Abstract

Purpose: Rasmussen's encephalitis (RE) is a progressive and catastrophic epileptic disorder caused by chronic localized encephalitis. We performed a nationwide survey of RE to assess the clinical picture, treatment effect, and prognosis of Japanese RE patients. *Subjects & methods:* The subjects were 27 patients (male:12; female:15) from 13 medical facilities. All of them satisfied the clinical and neuroimaging criteria for RE, including 14 pathologically proven cases. *Results:* They were divided into the childhood-onset rapidly progressive type (CORP, $n = 19$), and late-onset slowly progressive type (LOSP, $n = 8$). The mean age at epilepsy onset was 4 years and 4 months in CORP, and 16 years in LOSP. The mean period between the onset age of epilepsy and development of frequent seizures was 1 year and 4 months in the former, and 3 years and 4 months in the latter. The immunomodulatory treatment including high-dose steroid ($n = 14$) and high-dose intravenous immunoglobulin therapies (IVIgG, $n = 12$) achieved more than a 50% reduction in the seizure frequency in 5 (36%) and 4 (33%) patients, respectively. Eight and seven patients underwent focal cortical resection and functional hemispherectomy, leading to significant improvement in 5 of the 8 patients and excellent seizure control in all 7 patients, respectively. *Conclusion:* Although the high-dose steroid and IVIG therapies may have alleviated the exacerbation of seizures in those with RE, they could not halt the disease progression. Functional hemispherectomy is still the only curative therapy for RE, despite the fact that the early introduction of this procedure remains controversial.

Keywords: Rasmussen's encephalitis; Chronic localized encephalitis; Intractable epilepsy; Epilepsia partialis continua; Immunomodulatory therapy; Nationwide survey

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1. Introduction

Rasmussen's encephalitis (RE) is characterized by intractable focal epilepsy and slowly progressive unilateral neurological deficits due to chronic localized encephalitis, primarily affecting children [1,2]. Although RE was fundamentally viewed as a clinico-pathological entity requiring pathological confirmation for a definite diagnosis, recent progress in neuroimaging techniques, excluding tumors or vascular abnormalities and visualization of slowly progressive unilateral cortical atrophy, now allows us to make a clinical diagnosis of RE with a relatively high accuracy [3–6]. The seizure and intellectual outcomes of this disorder are almost always poor, with few exceptions, often requiring radical epilepsy surgery, that is, functional hemispherectomy [7,8]. Attention has progressively been paid to immunomodulatory treatment, since evidence for the immune-mediated inflammatory process of this disorder has been accumulated since the 1990's [9–13]. Thus, recognition of this rare catastrophic disorder is important in order to predict poor responses to antiepileptic drugs, the appearance of epilepsia partialis continua (EPC) as well as progressive neurological deterioration, and to consider the early introduction of immunomodulatory treatment and surgical therapy. The various studies on RE have been conducted in North American as well as European countries, and exceptionally in Asian countries including Japan. However, there have been a few case reports of pathologically-proven RE from Japan, and the concept of RE is increasingly being recognized along with growing interest in epilepsy surgery in Japan [14–17]. Recently, we conducted a nationwide survey of RE to reveal the clinical picture, treatment effect, and prognosis of Japanese RE patients. We herein report the results of this first nationwide survey of RE in Japan.

2. Subjects and methods

We distributed a questionnaire, assessing whether physicians had ever encountered the cases fulfilling the criteria of RE, to 139 facilities all over Japan, including university hospitals, special epilepsy centers, and general as well as children's hospitals, where members of Japanese epilepsy and child neurology societies were practicing. The diagnostic criteria for RE proposed by Bien et al. [18] were employed in this survey. If we received a positive response, we then asked them to answer detailed questions with respect to the clinical pictures, neuroradiological findings, immunological examinations and their results, immunomodulatory as well as surgical treatment and the response, and neurological outcomes.

The responses to immunomodulatory and surgical treatments were evaluated primarily based on the seizure frequency and intensity before and after treatment. The effect of the treatment was defined as a more than 80% reduction in the seizure frequency or intensity as an excellent response, 50–80% reduction as a good response, less than 50% reduction as a poor response, more than 50% transient reduction as a transient response, and more than 50% increase in seizure frequency as aggravation at the latest follow-up. In addition, we objectively estimated progressive brain atrophy using the method described previously, assessing the volume of the affected hemisphere in relation to the unaffected one on the axial slice at the level including the Sylvian fissure by T1-weighted imaging (Hemispheric ratio: HR) [2]. The study protocol was approved by the ethical committee of Tokyo Women's Medical University. Informed consent was obtained from all parents or their caregivers.

Chi-square tests were used for comparisons between the two groups. $P < 0.05$ was considered to indicate a significant difference.

3. Results

We obtained responses from 62 of the 139 medical facilities, in which 13 including our hospital reported 27 patients (male:12; female:15) who fulfilled the criteria for RE. The diagnosis of RE was made clinically in 13 patients, and both clinically and pathologically in 14 patients. The clinical data of the 27 patients were shown in the Table 1. The details of 4 patients were also described elsewhere [14–17].

3.1. Clinical characteristics

The 27 patients were classified into 19 with the childhood-onset rapidly progressive type (CORP) and 8 patients with late-onset slowly progressive type (LOSP), based primarily on the interval between the onset age of epilepsy and that of frequent seizure recurrences (Table 2). They all showed normal development before the onset of epilepsy. Preceding infectious episodes within 6 months prior to the onset of epilepsy were confirmed in 8 cases (30%), including 2 with uveitis (Table 1). The ages at the onset of epilepsy ranged from 2 months to 9 years (mean: 4 years and 4 months) in those with CORP and from 6 years and 6 months to 28 years (mean: 16 years) in those with LOSP (Table 2). The initial epileptic seizures comprised focal motor seizures in 17 patients (3 with status epilepticus), complex partial seizures in 7, and generalized tonic–clonic seizures in 3. Epilepsia partialis continua (EPC) developed in 11 patients (58%) with CORP and 3 patients (38%) with LOSP. EPC involved the left side of the body in 9 patients (64%) and right side in 5 patients (36%). It started either from upper or lower extremity, and gradually affected both extremities (Table 1). The mean

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Table 1
Clinical data of 27 patients with Rasmussen's encephalitis.

		Clinical features							Examinations					
Patient No.	Sex	Type	Preceding infectious episodes	Age at epi. onset	Epilepsia partialis continua (EPC)	Period between epi. onset and the development of frequent seizures	Period between epi. onset and the development of hemiparesis	Neurological impairment	Glu-R antibodies	CSF finding	EEG findings (epileptic EEG foci)	Brain MRI finding	Intelligence test at first examination	Intelligence test at last examination
1	F	CORP	N	2 m	–	0 d	2 y	Left hemiplegia	CSF&S:+	WNL	Right hemisphere	Right hemispheric swelling	NP	DQ = 53
2	F	CORP	N	1 y 6 m	+	2 y 10 m	4 y 6 m	Right hemiplegia, retardation	NP	WNL	Left hemisphere	Progressive left atrophy	IQ = 84	IQ = 52
3	M	CORP	N	2 y	–	12 m	N	N	CSF&S:+	Increased lactate and pyruvate	Right F ~ T	T2 high spot of right T,O, and P regions	NP	FIQ = 72 VIQ = 87 PIQ = 61
4	F	CORP	2 y 5 m varicella 5 y ITP	2 y	–	3 y	6 y	Right hemiplegia	S:R2 – R3+	NP	Left hemisphere	Mild atrophy and T2 and FLAIR high signal of left area	NP	IQ = 29(9 y)
5	M	CORP	N	2 y 7 m	–	7 y	N	N	S:+	NP	Right F	No lesion	IQ = 85	FIQ = 82 VIQ = 80 PIQ = 87
6	F	CORP	Impatigo	3 y 6 m	+	1 y 6 m	4 y 6 m	Right hemiplegia	NP	WNL	Left hemisphere	Progressive left atrophy	IQ = 55	IQ = 47
7	M	CORP	Common cold and vomiting	3 y 7 m	+	3 m	N	N	CSF&S:+	WNL	Right F-C-P	FLAIR lesion of right F area	IQ = 95	IQ = 71
8	M	CORP	N	3 y 10 m	+	4 y	1 y 7 m	Left hemiplegia	S:+	NP	Right C-T	Atrophy of right F area	DQ = 73	IQ = 56
9	F	CORP	N	3 y 10 m	+	0 d	2 m	Right hemiplegia mental retardation		NP	Left hemisphere	Minimal progressive left atrophy	NP	TIQ = 40] VIQ = 51 PIQ = 46
10	M	CORP	Influenza	3 y11 m	+	1 y 5 m	1 y 6 m	Left hemiplegia	CSF&S:+	WNL	Right FP-F	FLAIR lesion of right F, lright atrophy	IQ = 98	IQ = 94
11	M	CORP	N	5 y 0 m		12 m	N	Mental retardation	S:+	WNL	Left O F C	Repetition of atrophy and swelling of left T ~ O area	NP	IQ = 53
12	F	CORP	N	5 y 3 m	+	11 m	1 y 5 m	Left hemiplegia	CSF&S:+	WNL	Right hemisphere	Progressive right atrophy, FLAIR high spots	TIQ = 85	TIQ = 89
13	M	CORP	N	5 y 4 m	+	1 m	7 y	Left hemiplegia	NP	Hyper NSE	Right F ~ FP	Progressive right atrophy	TIQ = 86	TIQ = 57
14	M	CORP	N	5 y 5 m	+	1 m	4 y	Left hemiplegia	NP	WNL	Right hemisphere	Mild progressive atrophy of right F area	TIQ = 77	TIQ = 66
15	M	CORP	N	5 y 10 m	–	8 m	4 y	Right hemiparesis	NP	Hyper NSE	Left F ~ C	Very minimal atrophy of left O area	TIQ = 73	NP
16	F	CORP	N	5 y 10 m	–	1 y 8 m	2 y 1 m	Right hemiplegia mental retardation	NP	Pleocytosis, Oligoclonal band(+)	Left C	Atrophy of left F, P,and T areas, LAIR high spots at insula	PIQ = 110	IQ 60
17	F	LOSP	Left uveitis	6 y 6 m	Only angle of mouth	7 y 5 m	5 y	Right hemiplegia	S:+	WNL	Left hemisphere	Progressive left atrophy	TIQ = 78	TIQ = 60
18	F	CORP	Influenzae	6 y 8 m	+	3 m	3 y	Left hemiplegia	CSF&S:+	WNL	Right T ~ O, P	Atrophy of right area	IQ = 105	NP
19	F	CORP	N	7 y 5 m	–	9 m	N	N	S:R2IgG(±), IgM(–) CSF:-	WNL	Left F,O low wave	Very minimal atrophy of left T area and caudate nucleus and DWI high signal of left T area	WISC-III (8 y 1 m) VIQ66, PEQ61, FIQ60	WISC-III (8 y 7 m) VIQ68, PIQ67, FIQ64
20	F	CORP	N	9 y 0 m	+	0 m	2 m	Left hemiplegia	IgG δ2(+) CSF&S: IgG ε2(–)	WNL	Right hemisphere	Atrophy of whole cortex, FLAIR high signal of right T ~ O area	VIQ = 103 PIQ = 51 TIQ = 76	VIQ = 76 PIQ = 57 TIQ = 99
21	F	LOSP	Left uveitis	11 y 6 m	–	9 m	N	Right hemiparesis mental retardation	NP	Oligoclonal band(+)	Left F ~ C	Mild atrophy of left F, T2 high signal of F and P areas	IQ = 87	IQ = 50
22	M	LOSP	N	12 y 2 m	–	1 y 10 m	1 y 1 m	Minimal right hemiparesis, mental retardation	NP	WNL	Left F	Repetition of appearance and disappearance of T2 high signal in left F area	TIQ = 67 VIQ = 62 PIQ = 79	NP
23	F	LOSP	Right uveitis	13 y 0 m		8 y 9 m	N	N	Glu R3:( )	Increased protein, increased IgG	Right C-T	Minimal progressive atrophy of right FC area	IQ = 101	TIQ = 80
24	M	LOSP	Vaccination for Japanese encephalitis	15 y	+	6 m	N	N	S:+	WNL	Left F-Fz	T2 high signal of left F area	FIQ = 82 VIQ = 93 PIQ = 75	FIQ = 80 VIQ = 84 PIQ = 78
25	M	LOSP	N	18 y	+	5 y	10 y	Left hemiplegia	CSF:–	WNL	Right F-aT	T2 and FLAIR high signal of right C area, minimal atrophy of right area	IQ = 86	IQ = 103
26	F	LOSP	N	25 y	–	1y6m	N	N	S:+	NP	Bilateral F - c	No lesion	FIQ = 84 VIQ = 76 PIQ = 98	FIQ = 92 VIQ = 79 PIQ = 112
27	F	LOSP	N	28 y	–	1y	N	Aphasia	S:+	NP	Right F-Fz	FLAIR lesion of right F ~ P	FIQ = 64 VIQ = 62 PIQ = 75	PIQ = 74

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Patient No.	High-dose steroid therapy	Treatment: Period between epi. onset and treatment (m)	Treatment: Treatment effect	Treatment: IVIG and other immunomodulatory agent	Treatment: Treatment effect	Surgical treatment	Treatment response	Pathological findings	Follow-up period	Age at last seizure	Outcome
1	NP	–	–	IVIG	Good	Right FH	Excellent	Chronic encephalitis	2 y 6 m	2 y 10 m	No seizure
2	ACTH	55	Transient improvement	NP	–	NP	–	–	3 y 7 m	7 y 9 m	Unknown
3	MP pulse Prednisolone	72	No improvement	NP	–	Focal resection	Difficult to judge	No specific finding	4 y 4 m	11 y 11 m	Frequent seizures persisted
4	NP	–	–	NP	–	NP	–	–	7 y	9 y	Seizures persisted
5	NP	–	–	NP	–	Focal resection	Excellent	Chronic encephalitis	7 y	14 y 2 m	No seizure
6	MP pulse	29	No improvement	INF-α	Aggravation	NP	–	–	1 y 8 m	7 y 4 m	Died
7	NP	–	–	NP	–	Right FH	Excellent	Chronic encephalitis	3 y	4 y 10 m	No seizure
8	NP	–	–	IVIG	Transient improvement	Right FH	Excellent	Chronic encephalitis	1 y 2 m	9 y	No seizure
9	NP	–	–	NP	–	Focal resection	Excellent	Chronic encephalitis	6 y	24 y 7 m	Tonic seizure of right arm 1×/month
10	NP	–	–	NP	–	Right FH	Excellent	Chronic encephalitis	3 y	5 y 9 m	No seizure
11	Dexamethasone Prednisolone MP pulse	72	Transient improvement Excellent	IVIG CyA	No improvement Good No improvement	NP	–	–	11 y	17 y	Clustering on right side 1×/month
12	MP pulse	14	No improvement	NP	–	Right FH	Excellent	Chronic encephalitis	2 y 3 m	8 y 6 m	No seizure
13	MP pulse	61	Transient improvement	IVIG	No improvement	NP	–	–	8 y 6 m	14 y 3 m	EPC every day
14	MP pulse prednisolone	19	Aggravation	NP	–	Focal resection + MST	Excellent	No specific finding	13 y 8 m	19 y 8 m	EPC every day
15	NP	–	–	IVIG	Exellent	NP	–	–	6 y 5 m	13 y 3 m	Seizure only during sleep
16	NP	–	–	IVIG	Excellent	NP	–	–	4 y 11 m	10 y 9 m	Rare seizure persisted
17	NP	–	–	IVIG	No improvement	NP	–	–	9 y 6 m	22 y 1 m	Clustering of seizures 3-4×/month
18	Prednisolone	4	Excellent	IVIG tacrolimus	Good improvement	Right FH	Excellent	Chronic encephalitis	5 y	10 y	No seizure
19	MP pulse	9	Transient improvement	tacrolimus	Good	NP	–	–	15 m	9 y 2 m	Seizures 2×/month
20	MP pulse prednisolone	8	Good	IVIG	Transient improvement	Right FH	Excellent	Chronic encephalitis	1 y 2 m	10 y	no seizure
21	MP pulse	24	No improvement	IVIG	No improvement	NP	–	–	5 y 3 m	17 y 11 m	Died
22	Steroid	53	Excellent	IVIG ganciclovir	Transient improvement	Focal resection after brain biopsy	Excellent	Chronic encephalitis	9 y 8 m	21 y 10 m	GTCS 1×/month, atonic seizure 1×8/day
23	MP pulse	87	Aggravation	AZT	No improvement	Focal resection + MST (2 times)	Excellent	Chronic encephalitis	19 y 7 m	29 y 5 m	Mild seizures 1×/month
24	NP	–	–	NP	–	Focal resection	Difficult to judge	No specific finding	16 y	31 y	Seizures persisted
25	MP pulse prednisolone	96	Good	IVIG	No improvement	Brain biopsy	–	Chronic encephalitis	2 y	25 y 4 m	No seizure
26	NP	–	–	NP	–	Brain biopsy	–	Suggestion of chronic encephalitis	7 y	34 y	Right-sided seizures persisted
27.	NP	–	–	NP	–	Focal resection	Difficult to judge	Chronic encephalitis	12 y	40 y	Seizures persisted

Abbreviations: S, serum; WNL, within normal limit; NP, not performed; NSE, neuron-specific enolase; VIQ, verbal intelligence quotient; PIQ, performance intelligence quotient; FIQ, full intelligence quotient; TIQ, total intelligence quotient; DQ, development quotient; MP, methyl prednisolone; IVIG, intravenous immunoglobulin; AZT, azathioprine; CyA, cyclosporine; FH, functional hemispherectomy.

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Table 2
Clinical characteristics of 27 patients.

	Childhood-onset rapidly progressive type (CORP)	Late-onset slowly progressive type (LOSP)
N (M:F)	19 (9:10)	8 (3:5)
Age at epilepsy onset (mean)	2 m~9 y (4 y 4 m)	6 y 6 m ~ 28 y (16 y)
Period between epilepsy onset and development of frequent seizures (mean)	0 d ~ 7 y (1 y 4 m)	6 m ~ 8 y 9 m (3 y 4 m)
Period between epilepsy onset and development of hemiplegia (mean)	2 m~7 y (3 y)	1 yl m ~ 10 y (5 y 4 m)
EPC	11	3
Hemiplegia	14	4
Surgical treatment	11	4
Hemispherectomy	7	0
Death	1	1

interval between the onset age of epilepsy and that of frequent seizure recurrences (daily seizures developed) were 1 year and 4 months in the former and 3 years and 4 months in the latter. The seizures affected the left side of the body in 14 patients (52%) and right side in the remaining 13 patients (48%). Fourteen of the 19 patients with CORP (74%) and 4 of the 8 patients with LOSP (50%) developed hemiplegia, either at the latest follow-up period or before epilepsy surgery. The average time from the onset age of epilepsy to the development of hemiplegia was 3 years and 5 years and 4 months, respectively. Thus, patients with CORP showed more frequent complications with EPC and hemiplegia than those with LOSP. Three patients with LOSP were accompanied by uveitis, ipsilateral to the involved hemisphere at 2 years before, at the period of, and at 6 years after epilepsy onset, respectively. One patient each with CORP and LOSP died of status epilepticus caused by infection at 7 years of age and of a sudden unknown cause at 17 years of age, respectively.

3.2. Immunological examinations

Serum or CSF autoantibodies against the *N*-methyl-D-aspartate glutamate receptor (NMDA-type GluR) ε2 subunit and its epitopes [11] were positive in 13 of the 18 patients (72%) and 6 of the 8 patients (67%), respectively (Table 1). CSF oligoclonal IgG banding was positive in one patient each. Serum cytokine levels were within the normal range in two patients with CORP, but were only measured during steroid therapy. CSF IL-6 was measured to be normal in one patient with LOSP.

3.3. Effect of immunomodulatory treatment

Immunomodulatory treatments including high-dose steroid therapy, high-dose intravenous immunoglobulin (IVIG) administration, and other immunomodulatory agents were tried in a total of 19 patients. They were initiated 4–96 months after the onset of epilepsy (Table 1). The high-dose steroid therapy in most cases was started with the intravenous administration of methyl-prednisolone (MP) for 3 consecutive days (MP pulse therapy) given twice or three times every other week, followed by oral prednisolone (1–2 mg/kg) over a period of a few to several months depending on the response. The high-dose IVIG therapy consisted largely of an initial administration of 200–400 mg/kg consecutively for 3 days, followed by the same single dose once a month for a few months depending on the response.

The high-dose steroid and high-dose IVIG therapies were tried in 14 and 12 patients, respectively. The duration of the one treatment course ranged from 1 to 4 months, depending on the response to treatment. The high-dose steroid therapy achieved more than a good response in 5 patients (36%), and transient response in 3 cases. The IVIG therapy achieved a more than good response in 4 cases (33%) and transient response in 3 cases (Fig. 1). The high-dose steroid and IVIG therapy appeared better in response for those with CORP and LOSP, respectively despite no statistical significance ($P > 0.05$). Azathioprine, INF-α, cyclosporine, and ganciclovir were tried in a few patients without appreciable effects. Three patients have now been placed on tacrolimus, but one of them recently underwent hemispherectomy because of neurological deterioration and continuous EPC, leading the patient to be confined to a wheelchair.

3.4. Neuroimaging characteristics

MRI demonstrated progressive atrophy of the left hemisphere in 11 patients and of the right hemisphere in 14, although 2 pathologically-proven RE patients showed no apparent hemispheric MRI lesions. SPECT and PET studies all supported the lateralization of the MRI and EEG findings. The evolutionary changes in the HR were evaluated in 9 patients (CORP: 6 cases;

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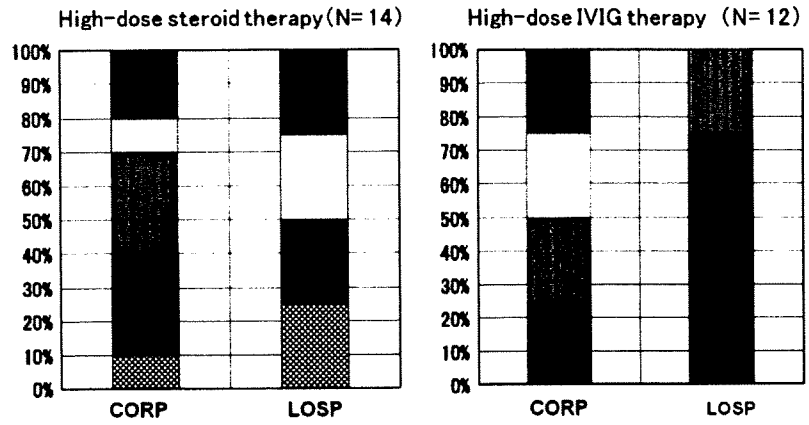

Fig. 1. Effectiveness of immunomodulatory therapy. Excellent, good, no improvement: more than 80% reduction, 50% to 80% reduction, and less than 50% reduction of the seizures, respectively. Transient improvement: more than 50% transient reduction of the seizures. Excellent ■, good response [ ], transient response ■, poor response ■, aggravation ▩.

LOSP: 3 cases). The HR changes in the former showed a more rapid decline from the first examination than that in the latter, indicating a more rapidly progressive shrinking of the affected hemisphere in the former (Fig. 2).

3.5. Response to epilepsy surgery

Focal resection with or without multiple subpial transection and functional hemispherectomy was undertaken in 8 and 7 patients, respectively. The former procedure achieved a more than good response in 5 (63%) patients, although none of them became seizure-free. All 7 patients undergoing functional hemispherectomy have remained seizure-free.

4. Discussion

This study is the first Japanese nationwide cohort study involving 27 patients with RE. There have been a large number of investigations regarding various aspects of RE, all of which involved Caucasian patients. The clinical pictures, as well as progressive nature of the disorder in our patients recruited based on the proposed RE criteria, are consistent with those from previous studies. We can subclassify them into those with a childhood-onset rapidly progressive clinical course (CORP) compatible with classical RE, and those with a late-onset slowly progressive clinical course (LOSP) compatible with adult type RE [1,2,18,19]. The average time between the onset age of epilepsy and that of frequent seizure recurrence, and the mean period between the onset age of epilepsy and development of hemiplegia in our series were also similar to those of previous reports.

Bien et al. measured the hemispheric ratio (HR), in which the axial cross-section of the affected hemisphere is expressed in relation to the unaffected one, and quantitatively showed the progressive destruction of the affected hemisphere over time [2]. This method applied to 9 of our patients demonstrated the difference in the HR between those with CORP and those with LOSP, and seemed to be useful for the evaluation of treatment.

Regarding the pathogenesis of RE, since Rogers et al. [9] identified the autoantibody against the ionotropic glutamate receptor GluR3 in the serum of RE patients, the autoimmune process underlying RE has received growing attention. Subsequently, autoantibodies against GluRε2, munc-18, and glial cells have been demonstrated in the serum of RE patients [10,11,13]. We also found GluRε2 antibody in the serum or CSF of roughly 2/3 of our patients at either the onset of epilepsy or in the middle of the clinical course [20]. However, the specificity of these autoantibodies as a primary etiology remains unclear because they were also found in other noninflammatory focal epilepsies or nonspecific encephalitis. Recently, interest is growing toward cell-mediated rather than humoral immunity, with the speculation that cytotoxic T cells destroy neurons through the release of granzyme-B, leading to the progressive destruction of the hemisphere [12,20]. Thus, the notion of a previous infection or vaccination prior to the onset of RE triggering the autoimmune process of the disorder has been challenged, although we could identify these episodes in only one third of our series [21]. However, among them, there were 3 patients with LOSP experiencing uveitis ipsilateral to the affected hemisphere, either long after or before the onset of epilepsy. Uveitis is caused mostly either by viral infections or an autoimmune process in those with systemic autoimmune disorders. Together with previous case reports, the combination of uveitis and RE is not a coincidental event but indicates the same autoimmune process involving both the uveal tract and ipsilateral hemisphere with other sys-

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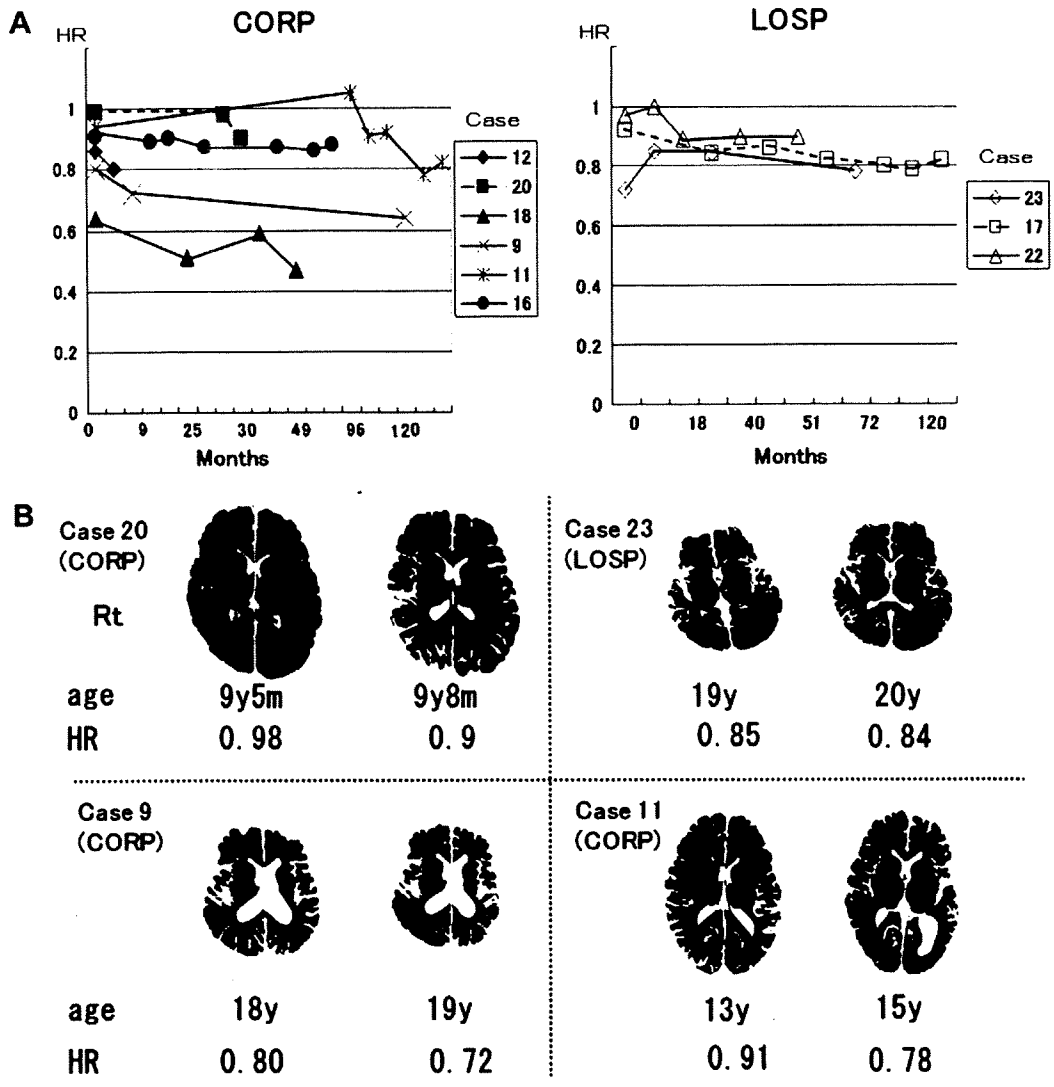

Fig. 2. Serial changes in the hemispheric ratio (HR). (A) Serial HR changes depicted as a sequential line graph for each RE type. (B) Typical examples of HR. Case letters correspond to those of Fig. 2A.

temic autoimmune disorders [14,22]. There must be various factors contributing to the onset of RE, in which the mutation of *SCN1A* causing severe myoclonic epilepsy in infants or generalized epilepsy with febrile seizure plus may be a candidate [17].

Thus, various immunomodulatory treatments have been attempted in patients with RE based on the autoimmune hypothesis. Promising results of high-dose intravenous immunoglobulin (IVIG) treatment have recently been demonstrated in patients with adult-onset RE [23,24]. Leach et al. demonstrated sustained improvement in patients following the long-term usage of high-dose IVIG, despite resistance to steroid treatment in these patients [23]. High-dose steroid treatment has been attempted more frequently than IVIG therapy, with inconsistent results [25–27]. In our results, the high-dose steroid and IVIG therapies brought a better than good response in approximately one third of cases, respectively. The high-dose steroid therapy was more effective for those with LOSP than the IVIG therapy, while the latter therapy appeared to be more beneficial in those with CORP than the former therapy. However, these two therapies could not fully control the seizures nor halt the neurological deterioration, even in responding cases. Tacrolimus has been shown to have a beneficial effect on reducing the progression of hemiatrophy, but does not improve the seizure outcome [28]. In our series, there were 3 patients taking tacrolimus, although one of them finally underwent hemispherectomy due to neurological deterioration and daily EPC up to the level of becoming unable to walk. The effect of tacrolimus will remain undetermined until long-term follow-up data

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from a larger number of patients are provided. The effect of immunomodulatory therapy for RE remains equivocal in part due to a limited number of case trials, the differences in treatment regimens, and the slowly progressing and relapsing-remitting clinical course of this disorder itself, hampering a precise assessment of efficacy [1]. In the meantime, these immunomodulatory treatments should be initiated cautiously and stopped when no meaningful improvement is recognized.

Regarding surgical treatment, functional hemispherectomy has been shown to be the final and best option for condition, although this procedure is only possible when hemiplegia has stabilized [7]. In addition, this procedure should be cautiously considered when RE affects the dominant hemisphere. In our series, the hemispherectomy was performed on only non-dominant right hemisphere in all 7 patients. If dominant left hemisphere is affected, we have to wait for this radical procedure until the language center transfer to the non-dominant hemisphere, which can be ascertained by Wada test or fMRI study [29]. Although early hemispherectomy is recommended to reduce the involvement of the unaffected hemisphere by some, a consensus regarding when to introduce hemispherectomy has not been determined globally [8,30]. The hemispheric ratio may become one of the objective markers to introduce hemispherectomy.

In this study, we were able to identify a significant number of patients with RE in Japan, who showed a similar clinical course as well as neuroimaging findings with those reported from Western countries, and have received appropriate immunomodulatory as well as surgical treatment.

Disclosure of conflict of interest

We have no conflicts of interest.

Acknowledgement

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CORRESPONDENCE

Correspondence: a further case of opsoclonus–myoclonus syndrome associated with *Mycoplasma pneumoniae* infection

Takashi Shiihara · Yukitoshi Takahashi

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Sir:

Huber et al. have reported the cases of three adolescents with opsoclonus–myoclonus syndrome (OMS) after *Mycoplasma pneumoniae* infection [2]. We report another such patient with OMS, who showed autoantibodies against glutamate receptors (GluR).

A 12-year-old girl, otherwise healthy except for bronchial asthma, presented with a 5-day history of jerky movements of her extremities and eyes and inability to walk. One week earlier, she had suffered from a respiratory disease, and at that time, the particle agglutination test for *M. pneumoniae* antibody was strongly positive (>1:10,240; titers of ≥1:40 are regarded positive). Thorough examinations such as hematology, blood chemistry, electroencephalography, brain imaging, and cerebrospinal fluid (CSF) examination revealed normal findings; no signs of neuroblastoma were observed.

Initially, the symptoms of OMS gradually improved. However, these symptoms worsened on day 28; therefore, intravenous immunoglobulin (IVIG) was administered at 2.0 g/kg. The symptoms of OMS began to improve within several days; however, they worsened around day 50. Therefore, IVIG was added at 1.0 g/kg, and intravenous methylprednisolone pulse therapy (30 mg/kg/day for three consecutive days) was administered three times at intervals of 1 week. The symptoms of OMS gradually disappeared by day 150 with no apparent sequelae.

GluR δ2 is predominantly expressed in cerebellar Purkinje cells; it plays a crucial role in cerebellar functions and is reportedly associated with cerebellitis [3]. On day 30, the serum was positive, but CSF was negative for anti-GluR IgG-δ2 and IgM-δ2 antibodies. These findings indicate that the etiological role of these antibodies is uncertain; however, they may be a surrogate marker of autoimmunity. Autoimmunity may play a role in OMS. Further studies are required to detect specific autoantibodies in cases of *M. pneumoniae*-related OMS [1].

Conflict of interest There was no conflict of interest.

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Genomic copy number variations at 17p13.3 and epileptogenesis

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17p13.3;
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LIS1;
YWHAE;
Fluorescent in situ hybridization (FISH);
Epileptogenesis

Summary Deletion of the terminal end of 17p is responsible for Miller-Dieker syndrome (MDS), which is characterized by lissencephaly, distinctive facial features, growth deficiency, and intractable seizures. Using microarray-based comparative genomic hybridization, 3 patients with epilepsy were revealed to have genomic copy number aberrations at 17p13.3: a partial LIS1 deletion in a patient with isolated lissencephaly and epilepsy, a triplication of LIS1 in a patient with symptomatic West syndrome, and a terminal deletion of 17p including YWHAE and CRK but not LIS1 in a patient with intractable epilepsy associated with distinctive facial features and growth retardation. In this study, it was suggested that the identified gain or loss of genomic copy numbers within 17p13.3 result in epileptogenesis and that triplication of LIS1 can cause symptomatic West syndrome.

Introduction

The terminal end of the short arm of chromosome 17 is crucial for neurodevelopment and deletion of this region is associated with Miller-Dieker syndrome (MDS), a congenital malformation syndrome consisting of typical lissencephaly and distinctive facial features. Patients with MDS also show growth deficiency, severe developmental delays, and intractable seizures (Dobyns et al., 1991). MDS results from chromosomal disruption, including cytogenetically visible or submicroscopic deletions of the 17p13.3 region, which includes LIS1, a key indicator of MDS (Dobyns et al., 1993; Reiner et al., 1993). LIS1 encodes PAFAH1B1 and participates in neural migration, disruption of which is responsible for lissencephaly. Indepen-

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dent LIS1 deletions or nucleotide alterations in its coding exons cause isolated lissencephaly without growth deficiency or distinctive facial features (Cardoso et al., 2000). This finding indicates that the clinical manifestations associated with MDS patients, such as growth deficiency and dysmorphic features, are likely derived from other genes included in the 17p13.3 region. Genotype–phenotype correlation studies in patients with deletions in the terminal region of 17p revealed that LIS1 deletion is responsible for lissencephaly and that combined deletion of LIS1 and YWHAE results in severer lissencephaly and a distinctive facial appearance, the hallmarks of MDS (Cardoso et al., 2003).

Recent revolutionary technological advances in molecular cytogenetics have enabled us to identify submicroscopic chromosomal abnormalities including gain or loss of genomic copy numbers (Emanuel and Saitta, 2007). Such genomic copy number variations (CNV) have only recently been identified using microarray-based comparative genomic hybridization (aCGH), and the incidence of such abnormalities seems to be more frequent than was thought prior to the human genome project (Shaffer et al., 2007). Genomic duplications are of particular interest because many submicroscopic duplications have been shown to be related to neurological disorders, including developmental delay and epilepsy (Lee and Lupski, 2006). Small genomic deletions and duplications have also been reported in 17p13.3 (Bi et al., 2009; Haverfield et al., 2009; Mei et al., 2008; Mignon-Ravix et al., 2009; Roos et al., 2009; Sreenath Nagamani et al., 2009).

In this study, we identified three types of genomic CNVs in the chromosome 17p13.3 region in 3 patients with epilepsy. This result implicates the dose effects of the genes in the 17p terminal region in epilepsy.

Materials

After obtaining informed consents from the patients' families based on the permission approved by the ethical committee of the institution, peripheral blood samples were obtained from 300 patients with psychomotor developmental delay and/or epilepsy, which included 10 patients with early infantile epileptic encephalopathy, 43 patients with West syndrome, 2 patients with Lennox-Gastaut syndrome, 12 patients with symptomatic generalized epilepsy, 14 patients with symptomatic partial epilepsy, and 5 patients with other types of epilepsy.

Methods

aCGH analysis was performed using the Human Genome CGH Microarray 105A chip (Agilent Technologies, Palo Alto, CA, USA), according to the manufacturer's protocol (Shimojima et al., 2009). Genomic DNAs were extracted from peripheral blood using a QIAquick DNA extraction kit (Qiagen, Hilden, Germany), and genomic copy numbers were determined using CGH Analytics software version 3.5 (Agilent Technologies).

To confirm the genomic copy number variations identified by aCGH, FISH analysis was performed as described (Shimojima et al., 2009). To confirm whether the identified genomic copy number variations were de novo or not, parental samples were also obtained and analyzed.

The wild-type genomic sequence of LIS1 exons 9–11 was amplified by long PCR using LA-Taq (Takara, Otsu, Japan), a forward primer designed to anneal within intron 8 (5′-CAGTGCTGTGCTATAACTGCACTATC-3′), and a reverse primer designed to anneal within exon 9 (5′-CACTGGCAGGTGTATACTATCAGATAC-3′), according to the manual provided by the manufacture. Then, the 2867-bp amplicon was cloned into the p-GEM T-vector® (Promega, Madison, WI, USA), and the resulting plasmid was used as a probe for FISH analysis. The bacterial artificial chromosome (BAC) clones mapped to chromosome 17p13.3 (Table 1) were selected from an in silico library (UCSC Human Genome Browser, March 2006, http://genome.ucsc.edu).

Fiber-FISH analysis was performed to determine the directionality of the repeated segments as described elsewhere (Shimojima et al., 2009).

Results

Molecular and cytogenetic analysis

In patient 1, a loss of genomic copy number was identified by aCGH. The deletion was comprised of a 294-kb region of chr17 (2,522,672–2,816,939), including the last 5 exons of LIS1 (exons 7–11) and the neighboring KIAA0664 and GARNL (Fig. 1). FISH analysis using an originally cloned plasmid probe containing the predicted deletion sequence confirmed the deletion of one copy of LIS1 (Fig. 2A). The fact that neither parent had the LIS1 deletion (data not shown) confirmed it as a de novo deletion in patient 1.

Table 1 Summary of FISH analyses.

Clone	Band	Start[a]	End[a]	Patient 1	Patient 2	Patient 3	Coverage genes
RP11-629C16	17p13.3	373,082	560,333	NT	NT	Deletion	
RP11-356I18	17p13.3	707,755	880,135	NT	NT	Deletion	
RP11-294J5	17p13.3	1,146,211	1,299,309	NT	NT	Deletion	*YWHAE, CRK*
RP11-380H7	17p13.3	2,026,967	2,250,500	NT	Duplication	NT	
RP11-135N5	17p13.3	2,312,022	2,492,178	NT	Triplication	NT	*LIS1*
CTD-2576K4	17p13.3	2,492,176	2,643,505	NT	Triplication	NT	*LIS1*
Plasmid[b]	17p13.3	2,528,949	2,530,730	Deletion	NT	NT	*LIS1*
RP11-1D5	17p13.1	7,918,567	8,082,208	Marker	Marker	NT	
RP11-153A23	17q25.3	73,516,547	73,694,284	NT	NT	Marker	

NT: not tested.
[a] Genomic position is according to the May 2006 human reference sequence (Build 2006).
[b] Originally constructed plasmid probe.

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