no anti-NR2 antibody was detected in the CSF. No anti-Yo, anti-Hu, or anti-Ri antibodies were found in her serum or CSF.

Lupus activity was suspected, because her anti-ds DNA antibody showed continuous elevation from less than 10 IU/ml in 2003 to 31 IU/ml in 2008. A diagnosis of central nervous system (CNS) lupus was made, and 1 mg/kg body weight per day prednisolone (55 mg/day) was started. As a result, she showed gradual improvements in her signs and symptoms, and her dysdiadochokinesia and gait improved markedly. After 6 weeks, she was placed on 45 mg/day prednisolone, and a further spinal fluid examination revealed improvement, a cell count of 2 cells/ml, a protein concentration of 61 mg/dl, an IgG index of 1.22, and an interleukin 6 level of 1.6 pg/ml. Her anti-dsDNA and anti-ssDNA antibody levels were also decreased to 5 IU/ml and 18 AU/ml, respectively. However, a repeat MRI of the brain showed no change in her bilateral cerebellar atrophy (Figure 3). In 2011, about 2 years after the onset of her cerebellar signs, she is currently on low-dose prednisolone (6 mg/day) and remains well. She displays minimal cerebellar signs.

Discussion

The cerebellar ataxia and atrophy in our case can be explained by an autoimmune mechanism associated with the exacerbation of SLE because these symptoms occurred in combination with serological abnormalities compatible with SLE flare and improved after treatment with high-dose prednisolone therapy. The increases in the CSF antineuronal cell antibody level and in the serum anti-ribosomal P antibody level were compatible with CNS lupus.[4] The anti-NR2 antibody level was increased in the serum, but not in the CSF. The significance of the increase in the serum anti-NR2 antibody level is unclear because serum NR2 autoantibodies are detectable in more than a third of patients with SLE, and the correlation between the antibody and neuropsychiatric complications of SLE remains controversial.[5,6] It is also possible that antiphospholipid antibodies directly damage neuronal cells by cross-reacting with epitopes on CNS phospholipids.[7,8] Considering her good response to corticosteroid therapy, it is unlikely that her antiphospholipid antibodies are related to cerebellar atrophy through microvascular occlusion.

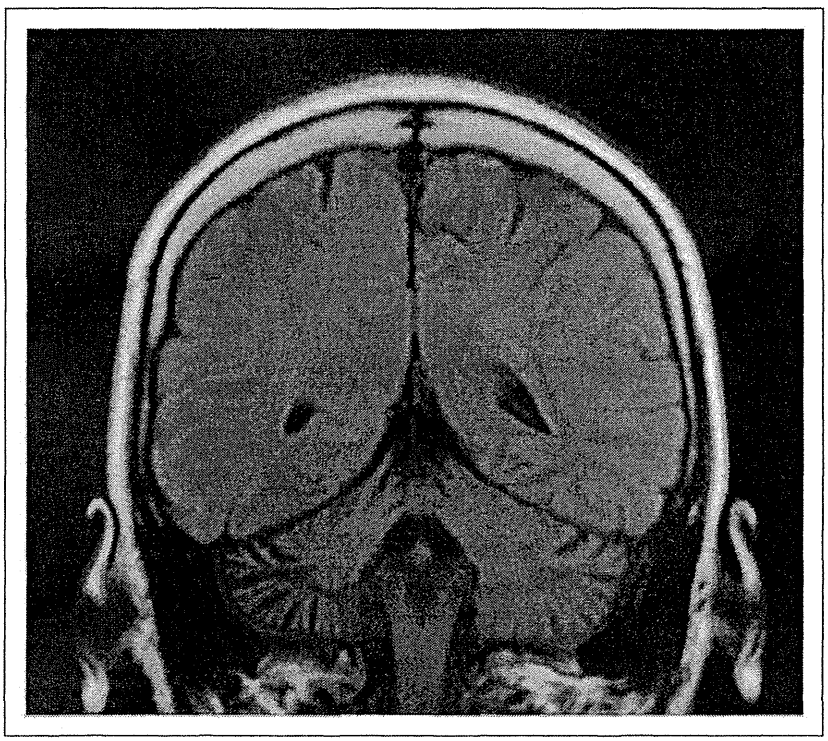

Figure 3 Axial magnetic resonance imaging at follow-up of patient showing no improvement of her atrophic cerebellar hemisphere.

Table 1 Comparison of SLE cases involving both cerebellar ataxia and atrophy

Age	*Sex*	*CT/MRI scan*	*APS*	*Neuronal cell specific antibodies*	*Treatment*	*Treatment effect*	*Ref.*
56	F	Diffuse cerebellar atrophy	NE	NE	no treatment	(improved)	[10]
47	F	No abnormal lesions in the cerebellum at the onset of ataxia	No	Antibody to human Purkinje cells, antibody against a 75 kDa protein in the cerebellar cortex	mPSL pulse therapy followed by 50 mg of oral PSL	Slight improvement in ataxia, progression of cerebellar atrophy	[11]
40	F	Mild cerebellar atrophy in both hemispheres combined with moderately severe atrophy of the vermis	No	NE	1 mg/kg/day oral PSL and 150 mg/day AZA	Improvement of ataxia, unchanged atrophy	[12]
27	F	Pancerebellar atrophy without evidence of demyelination	No	Negative for serum and CSF anti-Yo, anti-Hu, and anti-Ri	mPSL pulse therapy followed by 1 mg/kg/day of oral PSL, 150 mg/day AZA was added when PSL was tapered to 5 mg daily	Improvement of ataxia, unchanged atrophy	[13]

NE, not examined; mPSL, methylprednisolone pulse therapy; AZA, azathioprine; PSL, prednisolone

Although cerebellar ataxia is an uncommon symptom in patients with SLE, cases of cerebellar ataxia presenting with cerebellar atrophy are much rarer. We found only four such cases after searching the PubMed database (Table 1). In these cases, moderate to high doses of corticosteroid therapy including methylprednisolone pulse therapy (intravenous methylprednisolone 1 g daily for 3 days) were the main treatments and obtained a relatively good response. Previous reports revealed that brain MRI of acute cerebellar ataxia SLE patients showed reversible edematous lesions in the brain, suggesting acute blood–brain barrier changes secondary to small-vessel vasculopathy.[9] There might be pathogenetic differences between acute and subacute cerebellar ataxia. In our case, the clinical response to corticosteroid therapy was rapid, although MRI showed no changes in the patient's cerebellar atrophy. We achieved reductions in the patient's CSF IgG index and serum anti-dsDNA and anti-ssDNA levels. It has been suggested that antibody-mediated reactions could explain her cerebellar symptoms. Although cerebellar ataxia may occur secondary to paraneoplastic syndromes associated with anti-Hu and anti-Yo antibodies, the clinical course is usually acute. Our case is characteristic in that autoantibody-mediated subacute cerebellar ataxia was suggested. We could not confirm the binding of her antineuronal cell antibody to cerebellar tissues by immunohistochemical analysis. The autoantigen of her antineuronal cell antibody should be identified to clarify the processes of observed subacute cerebellar ataxia.

Our case highlights the importance of performing MRI for SLE patients with cerebellar signs. Although the significance of specific antineuronal antibodies in manifestations of CNS lupus is still unclear, high-dose corticosteroid therapy is expected to improve the neurological symptoms of patients suffering from this condition, probably by suppressing the production of pathogenic autoantibodies or cytotoxic processes mediated by cellular and humoral immunity.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Late Delirious Behavior With 2009 H1N1 Influenza: Mild Autoimmune-Mediated Encephalitis?

abstract

Delirious behavior associated with influenza usually has an onset within a few days after fever and lasts <24 hours. As we encountered several patients with 2009 H1N1 influenza who presented with late-onset and long-standing delirious behavior, we retrospectively evaluated the clinical, radiologic, and laboratory features to elucidate the possible pathophysiology. This information was collected on 5 previously healthy patients (2 boys and 3 girls, aged 10–15 years) with 2009 H1N1 influenza who presented with late onset (>3 days after fever) and long-standing (>48 hours) delirious behavior. Each exhibited mild to moderate drowsiness between the episodes of delirious behavior. Electroencephalography was normal except for 1 patient with high voltage and slow activity bilaterally in the occipital regions. Brain MRI was normal. The outcome was excellent with no neurologic sequel in 4 of the 5 patients. In all 5 patients, autoantibodies against *N*-methyl-D-aspartate type glutamate receptor were elevated or positive in cerebrospinal fluid or serum; the autoantibody levels normalized in the 3 patients who had follow-up studies. This study indicates that 2009 H1N1 influenza has a tendency to cause late-onset and long-standing delirious behavior, at least in Japanese children. Mild autoimmune-mediated encephalitis should be considered as an underlying cause. *Pediatrics* 2012;129:e1068–e1071

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KEY WORDS

2009 H1N1 influenza, delirium, anti-NMDA-type GluR antibodies

ABBREVIATIONS

CSF—cerebrospinal fluid
GluR—glutamate receptor
MERS—clinically mild encephalitis/encephalopathy with a reversible splenial lesion
NMDA—*N*-methyl-D-aspartate
NR2B—NMDA-type GluRε2
PCR—polymerase chain reaction

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Infection or fever is known to be a common cause of delirium in children. It has been reported that >10% of the patients with influenza during the 2005–2006 season in Japan (299/2846) developed delirious behavior.[1] It usually develops within a few days after onset of fever (91.6% within 2 days, 97.3% within 3 days),[1] and lasts for <24 hours.[2]

Delirious behavior has been reported as the most common neurologic symptom in patients with clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS) (54%, 29/54).[3,4] MRI revealed a reversible splenial lesion with homogenously reduced diffusion in 5 of 11 patients with influenza-associated delirium during the 2007–2008 season.[5] These 11 patients had onset of delirium within 3 days after fever onset and had a duration of <12 hours. This suggested that a reversible splenial lesion may be an associated condition in patients with influenza-associated delirium.

Neurologic complications have recently been reported in children with 2009 H1N1 influenza; these include acute necrotizing encephalopathy, acute encephalopathy with biphasic seizures and late reduced diffusion, and MERS.[6,7] Because we have also encountered several patients with 2009 H1N1 influenza who developed late-onset and long-standing delirious behavior, we evaluated the clinical, radiologic, and laboratory features in an attempt to elucidate the possible causes of the delirium.

PATIENT PRESENTATION

Information on patients with 2009 H1N1 influenza who developed late-onset (>3 days after fever) and long-standing (>48 hours) delirious behavior was collected retrospectively after approval by the institutional review board of the Kameda Medical Center. The diagnosis of 2009 H1N1 influenza was established by polymerase chain reaction (PCR) with a nasopharyngeal swab or, if PCR was not performed, rapid antigen-detection assay for influenza A in the setting of 2009 H1N1 influenza epidemic. In Japan, almost all the cases of influenza A were caused by 2009 H1N1 influenza virus during the 2009–2010 season (21 710 of 21 745, 99.8%). Delirious behavior is divided into the following components: visual hallucinations; sensory misperceptions other than visual ones (such as auditory hallucinations); emotional changes (such as laughter and fear); unresponsiveness; incoherent speech; purposeless movements; and impulsive behavior[2,5]; only patients with some of these components and diagnosis of 2009 H1N1 influenza were enrolled in this study. We reviewed the clinical charts of the patients to accrue information about symptoms, medication, treatments, outcomes, and the results of cerebrospinal fluid (CSF) analysis, MRI and EEG.

Five previously healthy Japanese patients (2 boys and 3 girls, aged from 10–15 years) met the criteria for enrollment in this study, the onset of their disease being during the period from September 2009 to February 2010. A diagnosis of 2009 H1N1 influenza was confirmed by PCR in 2 patients examined (patients 2 and 4); the other 3 were given the diagnosis based on a positive antigen-detection assay result for influenza A in the setting of the 2009 H1N1 influenza epidemic. The clinical records of the 5 patients are summarized in Tables 1 and 2. All were treated with zanamivir hydrate after the diagnosis of influenza A, and the fever was alleviated before the onset of delirious behavior. Zanamivir hydrate was discontinued in 3 patients (patients 1, 2, and 5) before the full period of 5 days because of the onset of delirium. Onset of fluctuating delirium was observed between days 3 and 6 after the fever, with a duration of 6 to 15 days in patients 1 to 4, all of whom showed mild to moderate drowsiness between the episodes of delirious behavior. Patient 5 had episodes of delirium with mild drowsiness between days 5 and 10; occasional subsequent episodes of delirium occurred during the follow-up period of 12 months. No patient developed seizures. The results of neurologic examination were unremarkable except for the delirium or drowsiness. Cerebrospinal fluid (CSF) analysis was normal in the 4 examined patients. EEG was normal except for in patient 1 in whom there was high voltage and slow activity bilaterally in the occipital regions. MRI of the brain was normal in all patients. Methylprednisolone (30 mg/kg per day for 3 days) or dexamethasone (0.4 mg/kg per day for 5 days) was administered to 3 patients (patients 1, 2, and 4) on

TABLE 1 Clinical Data for Infuenza H1N1 Patients With Late-Onset and Longstanding Delirious Behavior

Pt	Age/Gender	Tx	End of Fever	Duration of Delirium	Delirious Behavior Components	Consciousness Between Delirium	Tx for Delirium
1	10/F	ZNV (2–5 d)	5 d	5–12 d	Em (anger, weeping), Inc, Un	Mild drowsiness	DEX (d 6–10)
2	12/M	ZNV (1–3 d)	3 d	3–9 d	Imp, Vis, Mis, Inc	Drowsiness	mPLS (d 4–6)
3	11/M	ZNV (2–6 d)	4 d	6–14 d	Em (laughter, weeping, fear), Imp, Mis	Drowsiness	None
4	15/F	ZNV (2–6 d)	4 d	6–20 d	Vis, Mis, Inc, Un	Mild drowsiness	mPSL (d 17–19)
5	12/F	ZNV (2–4 d)	3 d	5–10 d	Em (anger, weeping), Imp, Inc, Un	Mild drowsiness	None
				11 d 12 mo	Em (anger), Inc	Alert	Resperidone

DEX, dexamethasone; d, day; Em, emotional changes; F, female; Imp, impulsive behavior; Inc, incoherent speech; M, male; Mis, misperception; mPSL, methylprednisolone; Pt, patient; Tx, therapy; Un, unresponsiveness; Vis, visual hallucination; ZNV, Zanamivir.

TABLE 2 Laboratory Data for Influenza H1N1 Patients With Late-Onset and Longstanding Delirious Behavior

Pt	CSF study	MRI results	EEG results	NMDA GluR testing results	Outcome
1	Normal (6 d)	Normal (7 d, 10 d)	Normal (6 d, 13 d), HVS bilateral O (8 d)	Elevated GluRε2 (0.82, serum; 6 d) Normal GluRε2 (0.44, serum; 12 mo)	CR
2	NE	Normal (3 d, 4 d, 7 d)	Normal (6 d, 8 d)	Elevated GluRε2 (0.79, serum; 6 d) Normal GluRε2 (0.31, serum; 12 mo)	CR
3	Normal (11 d)	Normal (11 d, 14 d, 40 d)	Normal (11 d, 14 d, 40 d)	Positive NMDA-GluR (NR1+NR2) (CSF; 11 d)	CR
4	Normal (10 d, 17 d)	Normal (9 d, 20 d)	Normal (10 d, 17 d)	Elevated GluRε2 (0.33, CSF; 10 d) Normal GluRε2 (0.20, CSF; 55 d)	CR
5	Normal (4 mo)	Normal (7 d) Normal (4 mo)	Normal (7 d) Normal (4 mo)	Elevated GluRε2 (0.34, CSF; 4 mo)	Intermittent delirium

CR, complete recovery; GluRε2, anti-NMDA-type GluRε2 antobody; HVS, high voltage slow; NE, not examined; NMDA GluR, anti-NMDA-type glutamate receptor antibody; NMDA-GluR (NR1+NR2), anti-NMDA-type GluR subunit heterocomplex (NR1+NR2) antobody; O, occipital.
Normal values of GluRε2 (ELISA) in serum, 0.43±0.13, GluRε2 in CSF, 0.16±0.05.

the basis of a diagnosis of influenza encephalopathy or limbic encephalitis associated with 2009 H1N1 influenza and was followed by clinical recovery within a few days. The outcome was excellent with no neurologic sequelae in 4 of the 5 patients (as discussed, patient 5 had intermittent delirium). Testing for autoantibodies against *N*-methyl-D-aspartate (NMDA)-type glutamate receptor (GluR) subunit heterocomplex (NR1+NR2; patient 3) or NMDA-type GluRε2 (NR2B; patients 1, 2, 4, and 5) in CSF or serum were positive or elevated in the 5 patients; these values normalized on follow-up studies in 3 patients so examined (1, 2, and 4). We could not compare the antibody levels in serum and CSF because no patient had analysis of both at the same time. Abdominal sonography or CT revealed no abnormality, and specifically excluded an ovarian teratoma.

DISCUSSION

The most important finding in this study is that these 5 patients with 2009 H1N1 influenza and delirious behavior were clinically (late-onset between days 3 and 6 and long-standing for >6 days) and radiologically (no splenial lesion) distinct from previously reported patients with delirious behavior secondary to influenza.[1,2,5] Delirious behavior in influenza usually appears on the day of or the day after the onset of fever, and lasts for <24 hours; it is considered to be a limbic symptom, derived from the temporal lobes.[1,2,8] MRI has revealed a concurrent splenial lesion, of uncertain pathophysiological significance, in approximately half the previously reported patients.[5] Delirious behavior may be observed during the early febrile period of influenza encephalopathy, sometimes before seizures or any disturbance of consciousness.[2] We found reports of only 4 children with influenza (not 2009 H1N1) who exhibited similar clinical manifestations to those observed in our 5 patients.[8,9] All were Japanese children aged from 3 to 12 years, and all were affected during a different calendar year. It is, therefore, reasonable to postulate that 2009 H1N1 influenza is more likely than other influenzas to cause late-onset and long-standing delirious behavior, at least in Japanese children.

The identification of anti-NMDA-type GluR antibodies in these 5 patients may provide a clue as to the pathogenesis of this type of delirious behavior. Anti-NMDA-receptor encephalitis is associated with an anti-NMDA-type GluR subunit heterocomplex (NR1+NR2) antibody and is characterized by neuropsychiatric syndromes, memory problems, seizures, dyskinesias, and movement disorders, with some cases requiring prolonged intensive care support.[10] Anti-NMDA-receptor encephalitis is predominantly observed in young women, because ~60% of the patients have tumors, most commonly ovarian teratomas.[10] Despite the severity, patients often recover after tumor removal and immunotherapy, suggesting an immune-mediated pathogenesis. Anti-NMDA-type GluRε2 antibody, which is homologous to NR2B, has been detected in the CSF of patients with acute limbic encephalitis, both with and without ovarian teratomas,[11] and in Rasmussen syndrome.[12] Of the patients in this small series, all 5 had these antibodies, which longitudinally normalized in 3 patients examined in this manner. Steroid therapy was effective in the 3 patients treated. In addition, positive anti-NMDA-type GluRε2 antibody and effective mPSL administration were previously reported for a 12-year-old Japanese girl with late-onset and long-standing delirious behavior associated with seasonal influenza.[8] These findings suggest that the 5 patients with 2009 H1N1 influenza suffered from a mild autoimmune-mediated encephalitis with anti-NMDA-type GluR antibodies. It would be of interest to compare the levels of anti-NR2B antibodies in our patients with those of patients with MERS, who often present with an acute onset and short duration of delirious behavior; however, no studies with this data have been reported at present, and we do not have this information.

All of the patients evaluated in this study were treated with zanamivir hydrate after being diagnosed with influenza A. This has been a common therapy

in Japan since 2007, when it was recommended that oseltamivir phosphate not be used in patients with influenza aged between 10 and 19 years. Although many Japanese teenagers with seasonal influenza were treated with zanamivir in the past few years, only 1 patient treated with zanamivir hydrate for seasonal influenza has been reported to exhibit late-onset and long-standing delirious behavior similar to that of our 5 patients.[8] Therefore, it seems unlikely that zanamivir is the cause of this type of delirium. To elucidate the precise pathophysiology of this type of delirious behavior associated with 2009 H1N1 influenza, further clinical (for patients with seasonal influenza), laboratory (especially regarding anti-NMDA-type GluR antibodies in patients with MERS), and pathologic studies will be necessary.

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LETTER TO THE EDITORS

Voltage-gated potassium channel complex antibodies in Creutzfeldt-Jakob disease

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Dear Sirs,

Clinical presentations of Creutzfeldt-Jakob disease (CJD) can be mimicked by those of immune-mediated encephalopathies, including limbic encephalitis with anti-voltage-gated potassium channel (VGKC) complex antibodies [1, 2]. To date, anti-VGKC complex antibodies have been reported to be negative in CJD [1], thereby regarded as important to differentiate non-CJD dementia from CJD [3]. Here we report a patient with definite CJD who had serum anti-VGKC complex antibodies.

A 60-year-old seaman acutely presented with blurred vision, disturbance in depth perception and light discrimination, difficulty recalling Chinese characters, and right-left disorientation. An ophthalmologist found no abnormality in his eyes. Two months later, he underwent a brain magnetic resonance imaging including diffusion-weighted imaging, which showed hyperintensity signals in the bilateral occipital and parietal cortices. Neurologically, there were object agnosia, left hemineglect, dressing apraxia, Gerstmann syndrome, ideomotor apraxia, and instant memory disturbance. He also developed visual hallucinations such as flaming fire, and got agitated. Three months after onset, he got confused after taking one tablet of zolpidem and was admitted to a psychiatric department.

Neurological examination on admission demonstrated the following findings: fluctuating levels of consciousness, difficulty in word recall, extinction; visual disturbance, poor pursuit eye movement, mild dysarthria; increased muscle tonus and myoclonus predominantly in the left; Myerson sign, snout reflex, increased jaw jerk and deep tendon reflexes predominantly in the left, bilateral Babinski and Chaddock signs; and he was bedridden. Serum antinuclear, anti-thyroid peroxidase, anti-thyroglobulin antibodies were negative and sodium levels were normal. No malignant tumors were found. Cell count was 2/mm^3, protein level was 27 mg/dl, 14-3-3 protein was positive, and total tau protein level was 3,420 pg/ml (cut-off value, 1,300 pg/ml) in the cerebrospinal fluid. Initial electroencephalography revealed slow waves, and periodic sharp wave complexes were present 23 weeks after onset. The analysis of *PRNP* revealed a substitution of methionine to arginine at codon 232. He received no immunotherapy. He died 8.5 months after onset and underwent necropsy of the left parietotemporal lobe. Neuropathological examination revealed neuronal loss, spongiform change, gemistocytic astrocytosis (Fig. 1a), and synaptic deposition of PrP (Fig. 1b) in the cerebral cortex, confirming the diagnosis of definite CJD. Western-blot analysis showed type 1 PrP^{Sc}. The titer of anti-VGKC complex antibodies, measured with radioimmunoassay using rabbit brain homogenates and

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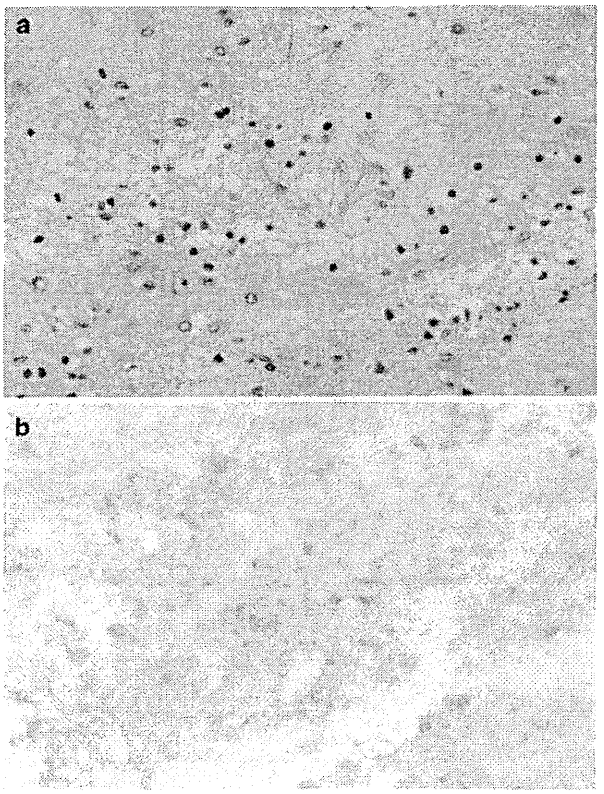

Fig. 1 Neuropathological findings. **a** Hematoxylin and eosin (H&E) staining shows neuronal loss, spongiform change, and gemistocytic astrocytosis in the left parietotemporal cortex. **b** Anti-PrP immunohistochemistry demonstrates synaptic deposition of PrP in the corresponding region

^{125}I-α-dendrotoxin as previously described [4], were 603.5 pM (cut-off values: for limbic encephalitis, 400 pM; for neuromyotonia, 100 pM) in the stored serum obtained 6 months after onset (the earliest time point of sampling). The titer was 0 pM in the serum obtained 8 months after onset. This study was approved by the ethics committee of the Tokushima University Hospital and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

To our knowledge, this is the first report of a CJD patient with anti-VGKC complex antibodies. Rapidly progressive dementia, myoclonus, extrapyramidal dysfunction, visual hallucinations, and psychiatric disturbance can be shared by CJD and anti-VGKC complex limbic encephalitis [1], although we could not specify antibody-related features in the present case. It has been reported that anti-VGKC complex antibodies are true markers of neurologic autoimmunity, because the antibodies were absent in ten patients with histologically confirmed CJD (nine sporadic and one familial) [1]. However, our results showing the antibodies in a pathologically confirmed CJD case indicate that the antibodies could not differentiate autoimmune limbic encephalitis and CJD. The real antigens of anti-VGKC complex antibodies can be leucine-rich, glioma-inactivated 1 (LGI1) or contactin-associated protein-like 2 (caspr2), which form complexes with VGKC [5, 6]. Unfortunately, we have not tested whether the antibodies were directed against LGI1 or caspr2 in the present case. In conclusion, our findings suggest limitation of anti-VGKC complex antibodies test and thus warrant further investigation for the prevalence of the antibodies in CJD patients.

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Conflicts of interest The authors declare that they have no conflicts of interest.

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Anti-glutamate receptor ε2 antibodies in psychiatric patients with anti-thyroid autoantibodies – A prevalence study in Japan

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HIGHLIGHTS

- This is the first report on the prevalence of anti-GluRε2 antibodies in PPATs.
- Anti-GluRε2 antibodies were frequently observed in the CSF of PPATs.
- Anti-GluRε2 antibodies would relate to the neuropsychiatric manifestations of PPATs.

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ABSTRACT

Patients with anti-thyroid antibodies (ATAs) present various kinds of psychiatric conditions. When these psychiatric patients with ATAs (PPATs) show responsiveness to immunotherapy, they are frequently diagnosed with a diffuse progressive type of Hashimoto's encephalopathy (HE). Anti-glutamate receptor ε2 subunit (GluRε2) antibodies have previously been reported in HE patients. However, it is unclear whether there is any relationship between PPATs, including HE patients, and anti-GluRε2 antibodies. We investigated anti-GluRε2 antibodies in the serum and cerebrospinal fluid (CSF) of 15 PPATs, and we compared the results with those of 11 patients with neuropsychiatric systemic lupus erythematosus (NPSLE), an anti-glutamate receptor antibody-related disease. We then compared the neuropsychiatric symptoms between the PPATs with and without anti-GluRε2 antibodies. The prevalence of anti-GluRε2 antibodies was significantly higher in the CSF than in the serum of PPATs (41.7% versus 6.7%; $p = 0.040$). The prevalence of anti-GluRε2 antibodies was slightly higher in the CSF of PPATs than NPSLE patients. PPAT-GluR(+)s showed a significantly higher prevalence of emotional instability (100% versus 33.3%; $p = 0.03$) and also showed a significantly lower prevalence of delusions (0% versus 100%; $p = 0.001$) and hallucinations (17% versus 83%; $p = 0.038$) than PPAT-GluR(−)s. Our results suggest that anti-GluRε2 antibodies may be associated with the neuropsychiatric manifestation of PPATs.

1. Introduction

A number of psychiatric disorders have been reported to have autoimmune factors in their etiologies [23]. Except for the possible effects of thyroid hormonal dysfunction, anti-thyroid antibodies (ATAs) have attracted interest because of their association with psychiatric disorders such as mood disorder [23,32], anxiety disorder [4], schizophrenia [23], and dementia [10]. The ATAs were reported to be found in 9.2% of the admitted psychiatric inpatients. [16] When psychiatric patients with ATAs (PPATs) show responsiveness to immunotherapy, they are frequently diagnosed with a diffuse progressive type of Hashimoto's encephalopathy (HE) [5,12,21,26]. The definition of HE is still unclear, as it involves some unknown autoimmune mechanisms, and the detailed prevalence of HE is still unknown. A diagnosis of HE is usually based on a combination of neuropsychiatric findings and the presence of ATAs and/or responsiveness to immunotherapy. Recently Shindo et al. [27] reported a case of HE with anti-glutamate receptor ε2 subunit (GluRε2) antibodies in the serum and cerebrospinal fluid (CSF). GluRε2 is a subunit of N-methyl-D-aspartate (NMDA) glutamate receptor, which is believed to play an important pathogenic role in schizophrenia and dementia [3,6,24].

Like HE, neuropsychiatric systemic lupus erythematosus (NPSLE) is a well-known autoimmune disease. The overall

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prevalence of neuropsychiatric feature of patients with SLE is varied widely between 37% and 95%. [17] Recently anti-NMDA receptor antibodies in the CSF of NPSLE patients have received particular attention because of their association with psychiatric symptoms [2,9,14,22,25,34]. Among NPSLE patients, the relatively high prevalence of anti-NMDA receptor antibodies in the CSF was reported by Fragoso-Loyo et al. [14] and Arinuma et al. [2] and those were 35% and 69.6%, respectively. Anti-NMDA receptor antibodies in the CSF are believed to represent an important mechanism of cerebral dysfunction in NPSLE. However, it is unclear whether there is any relationship between PPATs, including HE patients, and anti-GluRε2 antibodies.

The purpose of the present study was to clarify the prevalence of anti-GluRε2 antibodies in PPATs. We compared the findings with a control group of patients with NPSLE, an anti-glutamate receptor antibody-associated disease. Additionally, we compared psychiatric features between PPATs with and without anti-GluRε2 antibodies.

2. Materials and methods

2.1. Patients

We evaluated 15 PPATs and 11 NPSLE patients who had been tested for serum and/or CSF anti-GluRε2 antibodies between 2006 and 2011 at Yokohama City University Hospital, Yokohama, Japan. In these cases, some organic psychiatric or autoimmune disease, such as HE or NPSLE, was suspected because of the atypical course of their psychiatric symptoms and/or cognitive dysfunctions, and resistance to psychotropic agents.

All the PPATs visited the Department of Psychiatry at our hospital for treatment of their psychiatric symptoms. They were evaluated for anti-thyroid peroxidase (anti-TPO) antibodies and anti-thyroglobulin (anti-TG) antibodies because of mild thyroid dysfunction found by a screening test or past history of thyroid disease. Some of them had been treated for thyroid dysfunction, and all patients were confirmed to have almost normal thyroid-stimulating hormone (TSH) levels. For this study, "almost normal TSH" was defined by their euthyroid status (TSH: 0.3–5.0 mIU/L) or subclinical hypothyroidism (TSH: 5.1–20.0 mIU/L), which would not account for myxoedema encephalopathy associated with hypothyroidism. Psychiatric diagnoses were based on the Diagnostic and Statistical Manual of Mental Disorders-4th Edition-Text Revision (DSM-4-TR) published by the American Psychiatric Association [1]. Exclusion of SLE was made on the basis of the revised American College of Rheumatology (ACR) criteria of 1997 [19]. Some patients received immunotherapy after anti-GluRε2 antibody evaluation, and their responsiveness was checked.

All NPSLE patients visited the Department of Psychiatry at our hospital for treatment of psychiatric symptoms or for neuropsychological evaluation required by rheumatologists. They had been diagnosed with SLE by rheumatologists, based on the 1997 ACR criteria. They had also been routinely tested for thyroid function, anti-TPO antibodies and anti-TG antibodies in order to check for complications of autoimmune thyroid disease. All patients with NPSLE were confirmed not to have ATAs. The immunotherapy they received after anti-GluRε2 antibody evaluation was checked, and their responsiveness was checked. All NPSLE patients were classified using the ACR consensus document published in 1999 [31].

All PPATs and NPSLE patients were evaluated to exclude any opportunistic infection, other mental disorder, other abnormal metabolic condition, or drug-induced disorder. For this study, the responsiveness to the immunotherapy was evaluated by the psychiatrists, rheumatologists and neurologists, and was defined that the patients show any clinical improvements on the neuropsychiatric symptoms after the immunotherapy. Informed consent was obtained from all patients or their guardians after oral and written explanations according to the ethical principles of the Declaration of Helsinki. The Institutional Ethics Committee of Yokohama City University Hospital approved the study protocol.

2.2. Evaluation for autoantibodies

Anti-GluRε2 antibodies were investigated in the serum and CSF. Serum samples were available for 15 PPATs and 11 NPSLE patients. CSF samples were available for 12 PPATs and 5 NPSLE patients. Anti-GluRε2 antibodies were investigated according to the technique previously reported by Takahashi et al. at the Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan [29]. After establishing stable NIH3T3 transformant cell lines expressing full-length GluRε2 (B18), the supernatants of the cell extracts from B18 and the control cell line (A1) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the gels were transferred to nitrocellulose membranes. Each membrane was cut into strips after blocking with a buffer. The strips of B18 and A1 were reacted with 20-fold-diluted serum or CSF and were stained by alkaline phosphatase-labeled second antibodies. The presence of autoantibodies to GluRε2 was judged by a positively stained band with a molecular size of about 180 kD.

Anti-TPO and anti-TG antibodies were investigated by radioimmunoassay (RIA) with a cut-off value of 0.3 IU/ml. Anti-amino terminal of α-enolase (NAE) antibodies, which have been reported in the serum of HE patients, were investigated in the PPATs according to the technique previously reported by Yoneda et al. [33] and Fujii et al. [15].

2.3. Evaluation of neuropsychiatric symptoms

The psychiatric symptoms of PPATs were evaluated concerning hallucinations, delusions, anxiety, depression, emotional instability, and personality change. These symptoms were examined by psychiatric specialists during routine interviews at our hospital. Hallucinations and delusions were defined as positive if any symptoms were observed. Other psychiatric symptoms such as anxiety, depression, emotional instability or personality change were defined as positive if the symptoms persisted for at least one month and if these symptoms caused clinically significant distress or impairment in social functioning. Cognitive functions were evaluated by using the mini-mental state examination (MMSE).

2.4. Statistical analysis

The prevalence of anti-GluRε2 antibodies in the serum and CSF of PPATs was compared to that of the NPSLE patients by using Fisher's exact test. The prevalence of anti-GluRε2 antibodies in the PPATs and NPSLE patients was compared again between the serum and the CSF by using Fisher's exact test. The PPATs with anti-GluRε2 antibodies in their serum or CSF were classified as PPAT-GluR(+)s, and the PPATs without anti-GluRε2 antibodies in their serum and CSF were classified as PPAT-GluR(−)s. The psychiatric symptoms were compared between the PPAT-GluR(+)s and PPAT-GluR(−)s by using Fisher's exact test. Data were analyzed with the SPSS version 20.0 statistical package. The significance level was set at $p < 0.05$.

3. Results

3.1. Patients and autoantibodies

The clinical profiles of the PPATs and NPSLE patients are shown in Tables 1 and 2, and the demographics are shown in Table 3.

Table 1
Clinical manifestations of psychiatric patients with anti-thyroid antibodies.

Case number	Age/sex	Clinical psychiatric diagnosis (DSM-4-TR)	Duration of disease (years)	MMSE	Anti-thyroid AB		Anti-GluRε2 AB		Anti-NAE AB	Immuno-therapy	Response to immunotherapy
					Anti-TPO	Anti-TG	Serum IgG/IgM	CSF IgG/IgM			
1	61/F	Anxiety disorder Mild cognitive impairment	11.0	26	1.2	5.7	−/−	+/−	+	~	~
2	47/F	Borderline personality disorder Mild cognitive impairment	17.8	25	–	7.3	+/+	−/−	+	PSL, mPSL	Responsive
3	51/M	Dementia NOS	1.9	19	248	7.7	−/−	+/−	–	PSL, mPSL	Responsive
4	38/F	Eating disorder Mild cognitive impairment	29.1	26	133	3.8	−/−	−/+	–	~	~
5	61/F	Mild cognitive impairment	3.5	26	26	>100	−/−	+/−	–	~	~
6	82/M	Mild cognitive impairment	16.8	23	4.2	–	−/−	+/−	–	~	~
7	63/F	Major depression disorder Mild cognitive impairment	18.9	22	–	4.7	−/−	−/−	–	PSL	Non-responsive
8	73/F	Dementia NOS	1.3	22	55.4	>100	−/−	−/−	–	PSL	Responsive
9	71/F	Bipolar disorder Dementia NOS	31.5	20	0.3	0.4	−/−	−/−	–	~	~
10	65/F	Dementia NOS	8.8	ND	–	>100	−/−	−/−	–	PSL	Non-responsive
11	23/M	Schizophrenia Mild cognitive impairment	4.9	23	3.6	9.9	−/−	−/−	–	~	~
12	50/F	Bipolar disorder Mild cognitive impairment	30.0	27	9.1	5.9	−/−	−/−	–	~	~
13	66/F	Bipolar disorder Mild cognitive impairment	38.9	24	–	46.3	−/−	ND	+	~	~
14	50/F	Bipolar disorder	34.5	ND	>60	>100	−/−	ND	–	~	~
15	59/F	Somatoform disorder	0.9	28	–	10.9	−/−	ND	–	~	~

AB, antibodies; anti-TPO AB, anti-thyroperoxidase antibodies; anti-TG AB, ani-thyroglobulin antibodies; anti-GluRε2 AB, anti-glutamate ε2 antibodies; CSF, cerebrospinal fluid; M, male; F, female; NOS, not otherwise specified; ND, not determined; PSL, prednisolone; mPSL, methyl prednisolone

Table 2
Clinical manifestations of neuropsychiatric systemic lupus erythematosus patients.

Case number	Age/sex	Clinical diagnosis (ACR criteria)	Duration of NPSLE (years)	Anti-GluRε2 AB			Immunotherapy	Response to immunotherapy
				MMSE	Serum IgG/IgM	CSF IgG/IgM		
16	24/F	Psychosis Cognitive dysfunction	2.3	26	+/−	+/−	PSL, mPSL	Responsive
17	46/M	Anxiety disorder Cognitive dysfunction	4.5	27	+/−	−/−	PSL	Responsive
18	25/F	Cerebrovascular disease Cognitive dysfunction	0.6	22	+/−	ND	PSL, mPSL, IVIG, IVCY	Responsive
19	20/F	Aseptic meningitis	0.2	29	+/−	ND	PSL, mPSL, IVIG	Responsive
20	69/F	Acute confusional state Cognitive dysfunction	0.7	26	−/+	ND/−	PSL, IVCY	Responsive
21	21/F	Cerebrovascular disease	2.7	30	−/−	−/−	PSL, IVCY	Responsive
22	26/F	Cerebrovascular disease Cognitive dysfunction	0.1	25	−/−	−/−	PSL, IVCY	Responsive
23	32/F	Psychosis	1.7	30	−/−	−/−	PSL, mPSL, IVCY	Responsive
24	44/F	Seizure disorder Cognitive dysfunction	5.9	27	−/−	ND	~	~
25	29/F	Cognitive dysfunction	0.1	26	−/−	ND	PSL, tacrolimus	Responsive
26	43/F	Cerebrovascular disease	0.2	30	−/−	ND	PSL, cyclosporin	Responsive

M, male; F, female; AB, antibodies; anti-GluRε2, anti-glutamate ε2; CSF, cerebrospinal fluid; ND, not determined; PSL, prednisolone; mPSL, methyl prednisolone; IVIG, intravenous immunoglobulin; IVCY, intravenous cyclophosphamide.

3.2. Anti-GluRε2 antibodies

The prevalence of anti-GluRε2 antibodies is summarized in Table 3.

The prevalence of anti-GluRε2 antibodies was significantly higher in the CSF than in the serum of the PPATs (5/12 = 41.7% versus 1/15 = 6.7%; $p = 0.040$). The prevalence of anti-GluRε2 antibodies in the serum of the PPATs was significantly lower than in the serum of the NPSLE patients (1/15 = 6.7% versus 5/11 = 45.5%; $p = 0.032$). No significant difference was found in the prevalence of anti-GluRε2 antibodies in the CSF between the PPATs and NPSLE patients (5/12 = 41.7% versus 1/5 = 20.0%; $p = 0.395$).

Tables 1 and 2 show which patients were found to have anti-GluRε2 antibodies in their serum and/or CSF. Six of the 12 PPATs who underwent CSF analysis (Cases #1–6 in Table 1) had anti-GluRε2 antibodies in their serum or CSF. These patients were classified as PPAT-GluR(+)s. Six PPATs (Cases #7–12 in Table 1) were revealed not to have anti-GluRε2 antibodies in their serum or CSF. These patients were classified as PPAT-GluR(−)s. Table 4 shows the demographics of PPAT-GluR(+)s and PPAT-GluR(−)s.

3.3. Neuropsychiatric symptoms

The neuropsychiatric symptoms of the PPATs-GluR(+)s and the PPAT-GluR(−)s are summarized in Table 4. The PPAT-GluR(+)s showed a significantly higher prevalence of emotional instability (6/6 = 100% versus 2/6 = 40%; p = 0.03) than the PPAT-GluR(−)s. The PPAT-GluR(+)s showed a significantly lower prevalence of delusions (0/6 = 0% versus 5/5 = 100%; $p = 0.002$) and hallucinations (1/6 = 16.7% versus 5/6 = 83.3%; $p = 0.04$).

4. Discussion

The present study is based on small number of cases but is the first report on the prevalence of anti-GluRε2 antibodies in PPATs. The prevalence of anti-GluRε2 antibodies was found to be slightly higher in the CSF of PPATs than in the CSF of NPSLE patients. This suggests that there is some relationship between anti-GluRε2 antibodies and PPATs, as in NPSLE.

De Giorgio et al. [9] reported that anti-DNA antibodies, which are pathologically important in SLE, cross-react with the NMDA receptor subunits NR2(ε2) and cause neuronal apoptosis in the rat hippocampus, with ensuing memory impairment, and also

Table 3
The demographics and prevalence of anti-GluRε2 antibody in PPATs and patients with NPSLE.

	PPATs N = 15	NPSLE patients N = 11
Demographics		
Age (mean year ± SD)	57.3 ± 14.7	34.5 ± 14.8
Sex (female/male)	12/3	10/1
Duration of disease (mean year ± SD)	16.7 ± 13.3	1.7 ± 2.0
MMSE score (mean ± SD)	24.2 ± 2.8	27.3 ± 2.6
The most common clinical diagnosis	Mild cognitive impairment (8) Bipolar disorder (4) Dementia (4)	Cognitive dysfunction (7) Cerebrovascular disease (4)
Prevalence of anti-NAE antibodies	20.0%(3/15)	~
Prevalence of anti-GluRε2 antibodies		
Serum or CSF	50.0% (6/12)	62.5% (5/8)
Serum	6.7% (1/15)*,**	45.5% (5/11)
CSF	41.7% (5/12)	20.0% (1/5)

GluR, glutamate receptor; CSF, cerebrospinal fluid; PPATs, psychiatric patients with anti-thyroid antibodies; NPSLE, neuropsychiatric systemic lupus erythematosus; NAE, amino terminal of α-enolase.
* Significant p values by Fisher's exact test compared to NPSLE patients.
** Significant p values by Fisher's exact test compared to CSF.

Table 4
The demographics and the prevalence of the clinical manifestations of PPATs with or without anti-GluRε2 antibodies.

	PPATs-GluR(+) $N=6$	PPATs-GluR(−) $N=6$
Demographics		
Age (mean year ± SD)	56.7 ± 15.2	57.5 ± 18.7
Sex (female/male)	4/2	5/1
Duration of psychiatric disease (mean year ± SD)	13.4 ± 10.1	15.9 ± 12.9
MMSE score (mean ± SD)	24.2 ± 2.8	23.6 ± 3.2
Clinical psychiatric diagnosis (DSM-4-TR) (number)	Mild cognitive impairment (5) Dementia (1)	Dementia (3) Mild cognitive impairment (3) Bipolar disorder (2)
Neuropsychiatric symptoms (number, %)		
Hallucinations	1, 16.7%*	5, 83.3%
Delusions	0, 0%*	6, 100.0%
Anxiety	4, 66.7%	4, 66.7%
Depression	2, 33.3%	3, 50.0%
Emotional instability	6, 100.0%*	2, 33.3%
Personality change	6, 100.0%	6, 100.0%

PPATs, psychiatric patients with anti-thyroid antibodies.
* Significant p values by Fisher's exact test compared to PPATs-GluR(−).

emotional behavior impairment in mice. More recently, anti-NMDA receptor antibodies have been investigated for their possible association with the neuropsychiatric manifestations of NPSLE patients [2,9,20,22,25,34]. Some studies have indicated that anti-NR2 glutamate receptor antibodies in the CSF are associated with NPSLE [2,34]. Various kinds of assays have been developed to measure anti-NMDA receptor antibodies [7,9,18,28]. We used the anti-GluRε2 antibodies developed by Takahashi et al. [28]. Anti-GluRε2 antibodies were originally used to diagnose idiopathic non-herpetic acute limbic encephalitis (NHALE) [20,28]. NHALE is an immune-mediated encephalitis, which frequently accompanies ovarian teratoma. Although the present study is the first to investigate anti-GluRε2 antibodies in NPSLE patients, the present study also detected anti-GluRε2 antibodies in NPSLE patients in accordance with previous reports [2,9,18,22,25,34].

The present study revealed a significantly higher prevalence of anti-GluRε2 antibodies in the CSF than in the serum of PPATs, in contrast with NPSLE patients. The prevalence of anti-GluRε2 antibodies was significantly lower in the serum of the PPATs than in the serum of the NPSLE patients. The mechanism whereby anti-NMDA receptor antibodies affect the central nervous system in SLE is still unclear; however, some additional factors, such as infection, stress, hypertension – or possibly lupus flares – might disrupt the blood–brain barrier and thus allow serum antibodies into the CSF [14]. Nevertheless, the significantly higher prevalence of anti-GluRε2 antibodies in the CSF of PPATs might suggest a pathological mechanism that is different from that of NPSLE – for example, primary chronic inflammation localized in the central nervous system inside the blood–brain barrier.

In the present study, PPAT-GluR(+)s showed a significantly higher prevalence of emotional instability than PPAT-GluR(−)s. On the other hand, PPAT-GluR(−)s had a significantly higher prevalence of delusions and hallucinations. These differences would suggest that the presence or absence of anti-GluRε2 antibodies plays an important role in the neuropsychiatric symptoms of PPATs. NMDA receptors containing GluRε2 are expressed mainly in the limbic system, including the hippocampus, which is associated with memory and learning, and the amygdala, which is associated with emotions such as fear and depression [3,6,24]. Emmer et al. [11] observed selective damage in the amygdala of SLE patients with anti-NMDA receptor antibodies more frequently than in SLE patients without anti-NMDA receptor antibodies. The present study might suggest that anti-GluRε2 antibodies affect the hippocampus or amygdala via the NMDA receptors.

Five PPATs received immunotherapy, and 3 of these cases showed responsiveness to the therapy; thus, these 3 PPATs could be diagnosed with HE. The diffuse progressive-type HE shows insidious and progressive cognitive impairment or dementia with or without psychiatric symptoms, and these symptoms are similar to those of PPATs. Fujii et al. [15] reported that anti-NAE antibodies are more frequently detected in the serum of HE patients than in the serum of Hashimoto's thyroiditis (HT) patients without neurological symptoms. In the present study, anti-GluRε2 antibodies were more prevalent than anti-NAE antibodies in the CSF of PPATs. These differences may due to the fact that some patients in the present study did not have a diagnosis of HE supported by immunotherapy.

Mild cognitive impairment or dementia was diagnosed in 13 of the 15 PPATs, and two of three patients who showed responsive to immunotherapy had anti-GluRε2 antibodies. Recently, Flanagan et al. [13] advocated the concept of autoimmune dementia. They focused on cognitive impairment and found a broad spectrum of autoimmune dementia characterized by the potential for reversibility by immunotherapy. That characteristic unifies many kinds of immune-mediated neurological diseases, including NHALE, HE, and NPSLE. It is likely that some PPATs in the present study exhibited autoimmune dementia. Further investigation, including an evaluation of anti-GluRε2 antibodies and responsiveness to immunotherapy in a larger number of cases, will be necessary to clarify the relationship between PPATs and patients with HE, autoimmune dementia and other immune-mediated neurological diseases.

The present study has some limitations. Firstly, to investigate the association between anti-GluRε2 antibodies and PPATs, normal controls are needed. In the present study, we did not compare PPATs with normal controls or psychiatric patients without ATAs. This is because such a study would require CSF analysis. Lumbar puncturing is invasive and difficult to justify for even psychiatric patients without organic symptoms or findings. In a prospective study, a comparison between PPATs and normal controls or psychiatric patients without ATAs will be needed.

Secondly, the mean age of the PPATs and NPSLE patients was different. HT is frequent in 45- to 65-year-old women, and SLE is frequent in 15- to 40-year-old women. The results of our comparison of PPATs and NPSLE patients may reflect age effect to some extent. To reduce the effect of age on the results, more data from patients around 40 years old is needed.

Thirdly, we used the anti-GluRε2 antibodies developed by Takahashi et al. NHALE with ovarian teratoma can be detected more specifically by the NR1/NR2B antibodies assayed by Dalmau et al. [8,30] Anti-GluRε2 antibodies are limited in sensitivity and specificity, and further evaluation is needed for PPATs with several other autoantibodies, or – if possible – pathological investigation.

In summary, in the present study, anti-GluRε2 antibodies were frequently observed not in the serum, but in the CSF of PPATs, suggesting that these antibodies affect the neuropsychiatric manifestations of PPATs. Attention should be focused on PPATs with anti-GluRε2 antibodies as a potential clinical phenotype of PPATs. Further investigation in a larger number of cases is needed to clarify their relationship to other PPATs and patients with other autoimmune-mediated neuropsychiatric diseases, and also to establish appropriate therapies for these clinical conditions.

Disclosure statement

None.

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Dysfunction of blood-brain barrier in epileptic patients after acute encephalitis

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SUMMARY

Inroduction. Matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) are key-molecules that play important roles in blood-brain barrier (BBB) integrity. Whether BBB dysfunction persists in postencephalitic epilepsy (PEE) has not been investigated.

Aim. To evaluate BBB integrity in PEE, and the relationship between BBB dysfunction and seizure intractability.

Material and Methods. We investigated BBB integrity in PEE by measuring serum concentrations of MMP-9 and TIMP-1, and examined antibodies against glutamate receptor (GluR) epsilon 2 (NR2B) in 46 patients with PEE.

Results. Mean MMP-9 level was significantly higher in PEE patients having encephalitis at a younger age (0-5 years; 92.7 ± 53.5 ng/ml) than in PEE patients having encephalitis at an older age and controls. Mean serum TIMP-1 level was significantly lower in PEE patients (148.2 ± 38.0 ng/ml) than in controls (180.3 ± 56.7 ng/ml). Mean MMP-9/TIMP-1 ratio was significantly higher in PEE patients (0.553 ± 0.331) than in controls (0.311 ± 0.126), and was particularly high in PEE patients having encephalitis younger than 6 years of age. The ratio was elevated significantly in PEE patients with daily seizures. MMP-9 level and MMP-9/TIMP-1 ratio significantly correlated with the CSF/sera ratio of antibodies against GluR epsilon 2. CSF/sera ratio of antibodies against GluR epsilon 2 also significantly correlated with seizure frequency.

Conclusions. In PEE patients having encephalitis at a younger age, increased MMP-9 levels and/or decreased TIMP-1 levels may contribute to BBB dysfunction as indicated by elevated CSF/sera ratios of antibodies against GluR epsilon 2. BBB hyperpermeability may be causally related to intractability of PEE.

Key words: BBB dysfunction • MMP-9 • TIMP-1• antibodies to GluR epsilon 2 (NR2B)

INTRODUCTION

The blood brain barrier (BBB) is a complex structure designed to maintain a constant neuronal extracellular environment by limiting the penetration of a wide range of hydrophilic molecules, proteins, and cellular elements to the brain (Seiffert et al., 2004). It is composed of the cerebral vascular endothelium with tight junctions but lacking fenestrations. The circumference of the capillary lumen is enclosed by a single layer of endothelial cells. Attached at irregular intervals to the abluminal membrane of the endothelium are pericytes. Pericytes and endothelial cells are ensheathed by the basal lamina, a membrane 30–40 nm in thickness composed of various extracellular

matrix proteins (including collagen type IV, heparin sulfate proteoglycans, laminin, and fibronectin). The basal lamina is contiguous with the plasma membrane of astrocyte end-feet, which ensheathes the cerebral capillaries (Hawkins & Davis, 2005).

BBB dysfunction leads to excessive permeability of blood constituents such as electrolytes, proteins, antibodies, tumor cells and T lymphocytes, and is reported to be causally related to edema and inflammation in several CNS diseases including cerebral ischemia, inflammation, and neurodegenerative disease (Hawkins & Davis, 2005; Persidsky et al., 2006). In these diseases, BBB dysfunction has been studied in relation to matrix metalloproteinase-9 (MMP-9) as a key factor involved in the process (Rosenberg 2009). Matrix metalloproteinases are a major enzyme group involved in extracellular matrix degradation (Murphy & Nagase, 2008). MMP-9 degrades the basal lamina around cerebral vessels. The action of MMP-9 is balanced by the endogenous tissue-inhibitor of metalloproteinase-1 (TIMP-1). When the proteolytic activity is greater than the inhibition by TIMP, extracellular matrix breakdown occurs (Rosenberg 2002). Dysfunction of the extracellular matrix is strongly associated with increased BBB permeability in pathological states (Persidsky et al., 2006).

In infectious CNS diseases, a relationship between BBB dysfunction and MMP-9 has been observed in bacterial meningitis (Leib et al., 2001) and herpes-simplex virus encephalitis (Sellner et al., 2006). In influenza-associated encephalopathy, an imbalance between MMP-9 and TIMP-1 may promote acute febrile seizures through BBB damage (Ichiyama et al., 2007). Although studies using animal models of epilepsy have suggested that BBB leakage may contribute to epileptogenesis and seizure intractability (Seiffert et al., 2004; van Vliet et al., 2007), the effect of impaired BBB function on human epilepsy is unclear. There is also no reports as to whether BBB dysfunction persists after acute encephalitis and contributes subsequently to the pathogenesis of epileptic seizures.

Glutamate receptor epsilon 2 (GluR epsilon 2) (NR2B) is a subunit of N-methyl-D-aspartate (NMDA)-type GluR and plays an essential role in synaptic plasticity and development. The presence of autoantibodies against GluR epsilon 2 suggests an autoimmune mechanism associated with the pathogenesis of epilepsy in Rasmussen syndrome and acute limbic encephalitis (Takahashi et al., 2003; 2005; 2009). Antibodies against GluR epsilon 2 that cross-react with double-stranded DNA caused apoptosis in the rat hippocampus (DeGiorgio et al., 2001) and elicited cognitive impairment in mice (Kowal et al., 2006). Recent studies have reported a causal relationship between antibodies against the N-terminus of NMDA type GluR heterocomplexes and paraneoplastic encephalitis in patients with ovarian teratoma (Dalmau et al., 2007; Takahashi, 2008b). Studies in patients with non-herpetic acute limbic encephalitis suggested that antibodies against GluR epsilon 2 produced in blood after infection infiltrate into the central nervous system (CNS) through the damaged BBB in the acute stage (Takahashi et al., 2003; 2008a; 2008b; 2008c). Partial recovery of BBB function in the chronic stage appears to reduce the levels of antibodies in cerebrospinal fluid (CSF) (Takahashi, 2008a; 2008c). In other words, the extent of excessive BBB permeability can be analyzed indirectly using CSF/sera ratio of antibodies against GluR epsilon 2.

The present study aimed to investigate BBB integrity in PEE using serum MMP-9 levels, serum TIMP-1 levels, and CSF/sera ratios of antibodies against GluR epsilon 2. Furthermore, the relationship between BBB dysfunction and seizure intractability was investigated.

MATERIAL AND METHODS

Participants and control subjects

Almost all hospitalized patients with PEE, treated by the authors at the National Epilepsy Center- Shizuoka from 2004 to 2008, were asked to participate in this study using the manner stipulated by the ethical committee, and patients who provided informed consent were enrolled. Clinical information was collected from medical records, retrospectively. Seizure frequencies were recorded by guardians in seizure diaries. As for the control group, healthy adults and patients having diseases with non-inflammatory etiology were enrolled.

Serum samples were obtained from PEE patients and control subjects for the measurement of MMP-9 and TIMP-1 concentrations, and antibodies against GluR epsilon 2 (NR2B). CSF samples from PEE patients and controls were used for measurement of antibodies against GluR epsilon 2. All samples were collected at 2.5 hours after medication in the morning during interictal stage, independent of latencies from the last seizure.

Determination of MMP-9 and TIMP-1 concentrations

Serum concentrations of MMP-9 were determined using an activity assay kit (Amersham, Buckinghamshire, England) and TIMP-1 was determined by use of a sandwich-

type ELISA kit (Daiichi Fine Chemical Co., Ltd.) according to manufacturer's instructions . The MMP-9 kit measures both the pro- and active forms of MMP-9.

Detection of antibodies against whole molecules of GluR epsilon 2 in sera and CSF

Antibodies against whole molecules of GluR epsilon 2 (NR2B) were detected by immunoblot (Takahashi et al., 2003; 2005) and ELISA (Fujita et al., 2012).

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Data were analyzed based on samples, and differences were analyzed using the Mann-Whitney *U* test.

RESULTS

Forty-six patients with PEE (24 males, 22 females; aged 2 to 45 years), were included in this study (figure1, table 1). The mean (± SD) age at the time of study was 17.5 ± 10.5 years. The causative virus could not be identified in 27 of 46 patients. Localization-related epilepsy is the most common epilepsy syndrome found in PEE patients (41 of 46 patients). Eighteen healthy adults and nine patients having diseases with non-inflammatory etiology served as controls (5 males, 22 females; aged 1 to 58 years, mean age 26.4 ± 19.2 years).

MMP-9 and TIMP-1 concentrations were measured in 59 sera samples from 46 patients. Two of 46 patients provided three samples at different disease stages, and nine provided two samples. Antibodies against GluR epsilon 2 were measured in only 16 PEE patients (due to difficulty in taking agreement from patients' families) and nine disease controls (table 1 and 2).

The mean serum MMP-9 level in PEE patients (82.6 ± 55.3 ng/ml) was not significantly different from that of controls (52.8 ± 18.8 ng/ml). It was possible to classify PEE patients into three subgroups according to serum MMP-9 levels: normal level group, high level group (around 100 ng/ml), and extremely high-level group (above 200 ng/ml) (figure 2A). The mean serum MMP-9 level in patients having encephalitis at a younger age (0-5 years) (92.7 ± 53.5 ng/ml) was significantly higher than that in patients having encephalitis at an older age (6-10 years, $p = 0.010$; 11-20 years, $p = 0.049$) and controls ($p = 0.006$) (figure 2B). There was no significant relationship between the MMP-9 level and interval from encephalitis to the time of study (figure 2C).

The mean serum TIMP-1 level in PEE patients (148.2 ± 38.0 ng/ml) was significantly lower than that in controls (180.3 ± 56.7 ng/ml) ($p = 0.018$), and significant differences were observed in PEE patients having encephalitis between age <1 to 20 years (Figure 2D, 2E). Analysis of the relationship between TIMP-1 levels and interval from encephalitis to TIMP-1 measurement suggested that TIMP-1 levels decreased over time in the first five years after acute encephalitis and thereafter remained at a reduced level (25 percentile of controls; around 140 ng/ml) (figure 2F).

Mean MMP-9/TIMP-1 ratio was significantly higher in PEE patients (0.553 ± 0.331) than in controls (0.311 ± 0.126) ($p = 0.002$), and the ratio was particularly high in PEE patients having encephalitis younger than 6 years of age (Figure 2G, 2H). The ratio tended to increase until 20 years after encephalitis (figure 2I). Mean MMP-9/TIMP-1 ratio in PEE patients with intractable seizures (≥ 28 seizures within four weeks) (0.382 ± 0.539) was significantly higher than that in PEE patients with fewer seizures (< 28 seizures within four weeks) (0.179 ± 0.246) ($p = 0.046$), while MMP-9 and TIMP-1 levels were not significantly different between two groups (figure 3A-C).

The majority of PEE patients had higher levels of antibodies to GluR epsilon 2 (NR2B) in sera and CSF, compared with controls (table 1 and 2). The mean CSF/sera ratio of antibodies against GluR epsilon 2-NT2 was 0.873 ± 0.758 in PEE patients, and 0.417 ± 0.128 in controls ($p = 0.1677$).

Serum MMP-9 levels were not related to antibodies against GluR epsilon 2-NT2, GluR epsilon 2-M3-4, and GluR epsilon 2-CT1 in serum (data not shown). However, serum MMP-9 levels were correlated to the CSF/sera ratio of antibodies against GluR epsilon 2-NT2 (Figure 4A, $r^2=0.456$), GluR epsilon 2-M3-4 and CT1 (data not shown; $r^2=0.299$, 0.283, respectively). Serum MMP-9 levels were correlated to the level of antibody against GluR epsilon 2-NT2 in CSF (Figure 4D, $r^2=0.186$) and GluR epsilon 2-CT1 in CSF (data not shown, $r^2=0.178$). The MMP-9/TIMP-1 ratio was related to the CSF/sera ratio of antibodies against GluR epsilon 2-NT2 (Figure 4C, $r^2=0.136$). The CSF/sera ratio of antibodies against GluR epsilon 2-NT2 and level of antibody against GluR epsilon 2-NT2 in CSF were weakly correlated with seizure frequency, but the albumin level in CSF was not related with seizure frequency (Figure 4G–I).

DISCUSSION

MMP-9 and TIMP-1 are key molecules in maintaining the BBB integrity. Imbalance of the two molecules, as reflected by increased MMP-9/TIMP-1 ratios, is thought to

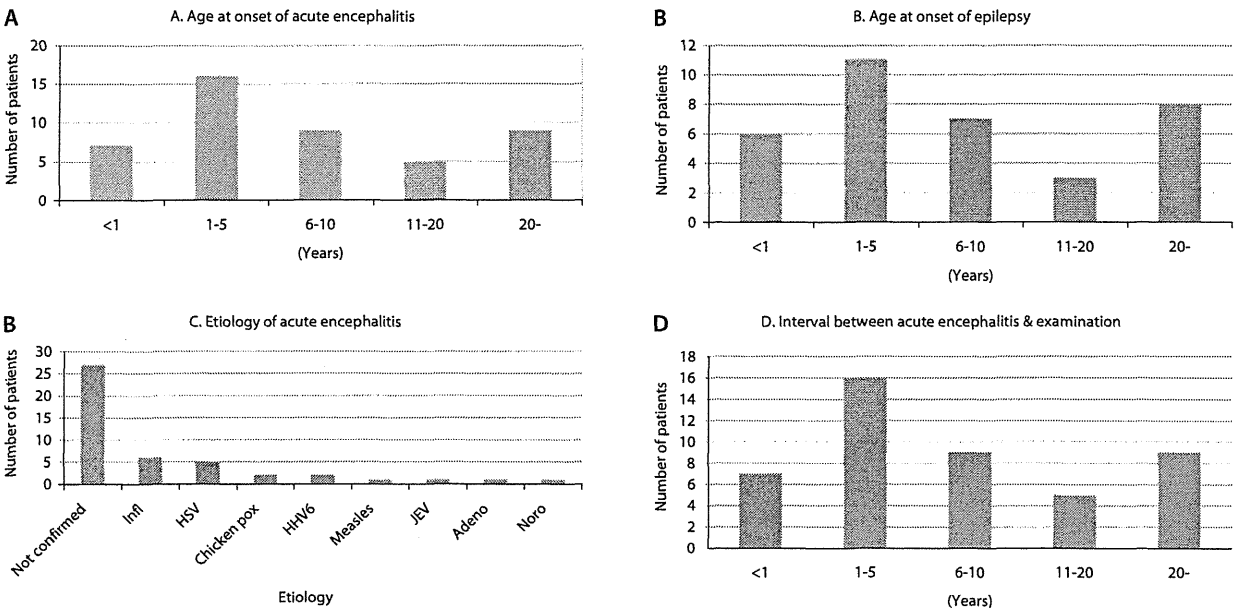

Figure 1. Summary of patients' characteristics.

A – Age distribution at occurrence of acute encephalitis; **B** – Age distribution at onset of epilepsy; **C** – Etiology of acute encephalitis; **D** – Interval between acute encephalitis and time of study.

Inf = Influenza virus; HSV = Herpes-simplex virus; HHV-6 = Human herpes virus-6; JEV = Japanese B encephalitis virus; Adeno = Adenovirus; Noro = Norovirus.

cause BBB dysfunction (Persidsky et al., 2006; Ichiyama et al., 2007; Rosenberg 2009). Our present study demonstrated that increased MMP-9 levels, decreased TIMP-1 levels, and consequently increased MMP-9/TIMP-1 ratios were frequently observed in PEE patients, especially in those having encephalitis at younger ages (figure 2). Our data also shows that MMP-9 levels are correlated with CSF/sera ratios of antibodies against GluR epsilon 2-NT2, suggesting increased permeability of BBB (figure 4). It is estimated that BBB dysfunction causally related to MMP-9 and TIMP-1 exists not only in patients at acute stage of encephalitis (Ichiyama et al., 2009), but also in PEE patients. BBB dysfunction causally related with MMP-9 and TIMP-1 may persist for 20 years after acute encephalitis in some patients (figure 2). The persistence may be causally related with an inhibited production of TIMP-1 (figure 2F). In chronic inflammatory diseases, TIMP-1 down-regulation in astrocytes by transcriptional controls and loss of mRNA stabilization has been reported (Gardner et al., 2006). The low TIMP-1 level in PEE will attenuate BBB repair. Because we could not conduct sufficient longitudinal measurement of MMP-9 levels from the acute stage of encephalitis to PEE, further study is needed to confirm our hypothesis.

The etiological mechanism of BBB disruption in human epileptogenesis has not been elucidated (Oby and Janigro, 2006). In rat epilepsy models, the BBB permeability index correlated with seizure frequency, and mannitol treatment aggravated seizure frequency (van Vliet et al., 2007). Our data shows that MMP-9/TIMP-1 ratios are significantly high in patients with intractable PEE having higher seizure frequency (figure 3C). Therefore, the extent of BBB disruption may determine the seizure frequency in human PEE.

How does BBB dysfunction lead to intractable epileptic seizures? Excessive permeability of BBB may increase the levels of blood constituents such as cytokines, albumin, and autoantibodies in the CNS (van Vliet et al., 2007). Cytokines have been reported recently to play important roles in epileptogenesis (Vezzani et al., 2008; Takahashi et al., 2009). Tumor necrosis factor-alpha (TNF α) is known to modulate AMPA-induced excitotoxicity (Bernardino et al., 2005) and to reduce GABA receptor expression (Stellwagen et al., 2005). Furthermore, transgenic mice of TNF α developed epileptic seizures (Probert et al., 1995). These findings suggest that TNF α may contribute directly to epileptogenesis. On the other hand, interferon-gamma (IFN γ) induces the expression of major histocompatibility complex class I + II and intercellular adhesion molecule-1, and production of TNF α in mi-

Table 1. Patient characteristics and laboratory data

No.	Sex	Age at encephalitis (Y)	Age at epilepsy onset (Y)	Age at time of study (Y)	Epilepsy syndrome	Seizure frequency (times/4 weeks)	MMP-9 (ng/ml)	TIMP-1 (ng/ml)	Ratio of MMP-9/ TIMP-1	S-NT2	S-M3-4	S-CT1	C-NT2	C-M3-4	C-CT1	Albumin
1.	M	1.4	1.4	19	LRE	NA	109.6	208.1	0.53	NA	NA	NA	NA	NA	NA	NA
2.	F	0	0.7	6	LRE	261	123.8	165.4	0.75	0.877	1.015	1.549	0.419	0.404	0.503	NA
3.	M	28	32	32	LRE	NA	203.9	250.7	0.81	0.306	0.368	0.547	0.998	0.853	1.010	NA
4.	F	4.8	5	11	LRE	220	111.7	144.8	0.77	0.442	0.557	0.731	0.986	1.189	1.151	23.1
5.	M	5	5	20	SGE	NA	250.0	159.5	1.57	NA	NA	NA	NA	NA	NA	NA
6.	F	27	28	32	LRE	37	148.0	191.4	0.77	NA	NA	NA	NA	NA	NA	NA
7.	F	21	21	27	LRE	26	40.4	138.9	0.29	1.128	1.216	1.476	0.791	0.672	0.796	NA
8.	M	25	25	28	LRE	21	111.7	147.0	0.76	NA	NA	NA	NA	NA	NA	NA
9.	M	7	7	12	LRE	80	37.8	132.2	0.29	NA	NA	NA	NA	NA	NA	NA
		7	7	12	LRE	80	51.5	127.9	0.40	NA	NA	NA	NA	NA	NA	NA
		7	7	12	LRE	12	53.7	134.0	0.40	NA	NA	NA	NA	NA	NA	NA
10.	M	4	10	11	SGE	84	107.4	148.5	0.72	NA	NA	NA	NA	NA	NA	NA
11.	F	0.7	17	20	LRE	60	64.3	112.5	0.57	NA	NA	NA	NA	NA	NA	19.4
12.	M	1.5	2	7	LRE	460	90.9	93.4	0.97	NA	NA	NA	NA	NA	NA	NA
13.	F	18	NA	36	LRE	NA	24.0	130.9	0.18	NA	NA	NA	NA	NA	NA	NA
14.	M	9	9	15	LRE	9	24.0	113.9	0.21	0.55	0.665	0.610	0.47	0.372	0.273	NA
15.	F	1	NA	45	LRE	NA	66.8	135.3	0.49	NA	NA	NA	NA	NA	NA	NA
16.	M	0.8	1.2	4	LRE	280	94.2	176.4	0.53	NA	NA	NA	NA	NA	NA	NA
		0.8	1.2	6	LRE	112	114.0	156.1	0.73	0.805	0.994	0.969	0.147	0.186	0.146	NA
17.	M	0.3	0.3	2	LRE	NA	222.2	212.9	1.04	NA	NA	NA	NA	NA	NA	NA
18.	F	1	6	9	LRE	280	57.2	100.9	0.57	0.388	0.524	0.614	0.521	0.672	NA	10.2
		1	6	10	LRE	44	38.9	102.0	0.38	NA	NA	NA	NA	NA	NA	NA
		1	6	11	LRE	28	82.2	90.8	0.91	NA	NA	NA	NA	NA	NA	NA
19.	F	5	5	6	LRE	420	38.7	150.7	0.26	0.300	0.388	0.319	0.104	0.102	0.113	19.3
20.	M	2.8	2.8	5	LRE	NA	62.1	163.6	0.38	0.37	NA	NA	NA	NA	NA	29.3
		2.8	2.8	6	LRE	356	79.5	177.8	0.45	NA	NA	NA	NA	NA	NA	NA
21.	M	1.3	1.3	2	LRE	NA	17.6	206.2	0.09	NA	NA	NA	NA	NA	NA	NA
22.	M	1	NA	18	LRE	NA	150.1	214.2	0.70	NA	NA	NA	NA	NA	NA	NA
23.	M	16	NA	19	LRE	NA	373.8	178.4	2.1	NA	NA	NA	NA	NA	NA	NA
24.	F	9	NA	22	LRE	NA	53.7	82.2	0.65	NA	NA	NA	NA	NA	NA	NA
25.	F	10	NA	28	LRE	NA	216.9	204.9	1.06	NA	NA	NA	NA	NA	NA	NA