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Correlation between neuronal antibodies and limbic encephalitis in Chinese Han subjects

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ABSTRACT. A variety of anti-neuronal cell membrane antibodies such as voltage-gated potassium channel antibody, *N*-methyl-D-aspartate-2B-antibody, and glutamic acid decarboxylase antibody, are correlated with limbic encephalitis (LE). In this study on patients with LE, the clinical manifestations, psychology Wechsler Adult Intelligence Scale, cerebrospinal fluid, electrophysiology, magnetic resonance imaging, and anti-immune therapy were studied and immunological determination was conducted; it was found that patients of Chinese Han nationality showed 2 types of clinical manifestations: simple and complex. Lesions could also be divided into focal and scalable lesions, and the clinical manifestations and lesions scopes were associated with various antibodies and antibody types. The prognosis may improve if early diagnosis is conducted and early anti-immune therapy is implemented in LE patients.

Key words: Glutamic acid decarboxylase antibody; Limbic encephalitis; *N*-methyl-D-aspartate-2B-antibody;
Voltage-gated potassium channel antibody

INTRODUCTION

Limbic encephalitis (LE) is a rare neurological syndrome that selectively involves the edge structure. Initial cases of paraneoplastic limbic encephalitis (PLE) were often accompanied by small cell lung cancer and breast cancer, among others, with the main clinical manifestations including progressive short-term memory loss, seizures, and varying degrees of involvement of outside-limbic-system tissues such as the cerebellum and brainstem (Urbach et al., 2006; Bien and Elger, 2007).

Further studies revealed that the pathogenesis of PLE was immune-mediated, which was mainly regulated by cytotoxic T cells. This generated antibodies that could act on the neuronal antigen, such as the anti-Hu, Ma2, and amphiphysin antibodies. Treatments include anti-cancer therapy and immunotherapy, but the overall prognosis is typically poor (Bien and Elger, 2007; Dalmau et al., 2008). Studies of LE have progressed in recent years, and some clinical manifestations and the appearance were found to be similar to PLE. However, LE is not always associated with systemic tumors, and its immunological characteristics can be used to generate antibodies affecting neuronal cell membrane antigens to induce an immunotherapy response and improve prognosis. Few immunological studies have been conducted in LE patients of Chinese Han nationality, and thus we summarized 4 cases in this study.

MATERIAL AND METHODS

Clinical data

Four LE patients, including 3 males and 1 female, were hospitalized in the Department of Neurology, The First Affiliated Hospital of Bengbu Medical College, from October 2012 to July 2013. Patients were aged between 37-54 years, with a mean age of 46 years. Two acute cases occurred within 48 h, and the other 2, which were subacute cases, occurred within 3-7 days. The 4 patients experienced serious memory impairment, and could not recall the 5 on-admission designated objects 5 min later. Three cases were accompanied by a 38.8-39°C fever, while 2 cases presented with headache and 2 cases presented generalized tonic-clonic seizures. The female patient appeared to be in a continuous tonic-clonic seizure state, so a tracheotomy was performed. She experienced sweating and had pseudo-intestinal obstruction, autonomic dysfunction, and stiff man syndrome. The serum sodium level of 2 patients was as low as 125-130 mM, and the female patient had intractable hyponatremia. One case was treated with pulse therapy with high-dose gamma globulin 0.4 g/kg x 5 days and high-dose methylprednisolone 1000 mg/kg x 5 days for 2 courses; 1 case was treated for 1 course; 2 cases treated with the pulse therapy with high-dose methylprednisolone 1000 mg/kg x 5 days for 1 course. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Bengbu Medical College. Written informed consent was obtained from all participants.

Wechsler adult intelligence scale (WAIS) determination

A psychiatric doctor performed the test for each patient before and after treatment. Language assays included 6 aspects (knowledge test, comprehension, arithmetic, similarities, digit span, and vocabulary), while the operation test included 5 aspects (number sign, picture

filling, block design, picture arrangement, and object assembly). Scores were determined from the coarse score scale and added to obtain the language intelligence quotient (IQ) score, operation IQ score, and total IQ points.

Enzyme-linked immunosorbent assay

Kits for anti-VGKC-Ab, (E-16152, lot 201305), anti-GAD-Ab (E-10502, lot-201305), and anti-NMDAR-Ab (E-16228, lot-201305) (R&D Systems, Minneapolis, MN, USA) were used. The original densities of the standards were diluted. The blank wells were prepared separately by 40 μL sample dilution and added into the testing sample well, after which 10 μL testing sample was added. The mixture was incubated for 30 min at 37°C, and then washed and air-dried. Washing buffer was added to each well, incubated for 30 s, and then drained 5 times. The wells were dried by patting and 50 μL horseradish-peroxidase-conjugated reagent was added into each well, except for the blank well, incubated for 30 min at 37°C, washed again, and 50 μL chromogen solution A and chromogen solution B were added, respectively. The plate was preserved in the dark for 10 min at 37°C; 50 μL Stop Solution was added into each well to stop the reaction; the blank well was considered to be 0, and the absorbance at 450 nm was read after adding Stop Solution and within 15 min (Synergy 2, BioTek, Winooski, VT, USA).

Immunohistochemistry

Cerebral cortex and cerebellum samples were obtained from neurologically normal individuals within 6 h after death. Six-micrometer-thick tissue sections were sequentially incubated with 0.3% hydrogen peroxide for 10 min, and then 10% normal goat serum was added and incubated for 15 min; patient serum was serially diluted and incubated overnight at 4°C, incubated with biotinylated goat anti-human IgG (Vector Labs, Burlingame, CA, USA) for 1 h, followed by Vectastain avidin-biotin complex (Vector Labs) for 30 min at room temperature. Substrate staining was developed using 0.05% diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) and 0.01% hydrogen peroxide in phosphate-buffered saline.

Western blotting

Western blotting kits (EUROLINE Neruonal Antigens Profile 2 IgG, DL1111-1601-2 g, D-23560, Lubeck, Germany) were used. The film strip was removed and placed in an incubation tank. Next, 1.5 mL sample buffer was added and the sample was incubated in a shaker at room temperature for 5 min, then the liquid was absorbed in the bath; 1.5 mL diluted serum sample was then added to the incubation vessel and shaken at room temperature (18-28°C) for 30 min. The liquid was absorbed in the tank, and the film strip was washed in a shaker with 1.5 mL washing buffer 3 times at 5 min/time. Next, 1.5 mL diluted enzyme conjugate (alkaline phosphatase-labeled anti-human IgG) was added to the greenhouse tank, shaken in the shaker at the greenhouse temperature for 30 min, and 1.5 mL substrate solution was added and incubated at room temperature (18-25°C) for 10 min. The liquid was absorbed in the tank, and the film strip was washed with distilled water 3 times for 1 min each time. The film strip was air-dried and evaluated.

Statistical analysis

The paired *t*-test was performed in the Department of Preventive Medicine to determine WAIS results.

RESULTS

WAIS determination

Before treatment, language IQ scores of the 4 patients were between 53-78 points, operation IQ scores were between 31-70 points, and total IQ scores were between 40-64 points. The patients showed low achievements in arithmetic, picture filling, block design, picture arrangement, and object assembly; after anti-immune therapy, language IQ scores were 93-124 points, operation IQ scores were 90-123 points, and total IQ scores were 106-119 points. IQ scores significantly improved and recovered, with 1 case reaching the middle IQ and 3 cases achieving the normal smart IQ level; there was statistical significance between values before and after treatment ($P < 0.01$, Table 1).

Table 1. Comparison of IQ changes before and after treatment (means ± SD, N = 4).

Observation time	Language (IQ)	Operation (IQ)	Total IQ
Before the treatment	68.75 ± 11.62	47.15 ± 16.54	54.50 ± 10.25
After the treatment	109.25 ± 12.92	109.25 ± 14.43	113.25 ± 5.38
$d \pm S_d$	40.50 ± 12.15	62.00 ± 18.57	58.75 ± 13.40
t	6.67	6.68	8.77
P	<0.01	<0.01	<0.01

Electrophysiology

All 4 cases showed electroencephalography abnormalities, including 3 cases with focal slow waves or sharp slow wave in the bilateral frontotemporal lobes and 1 case with mild abnormalities.

CSF examination

Two cases showed increased intracranial pressure as 200-210 mm H_2O, white blood cell count increased, with 3 cases of 16-610 x 10^6/L, which mainly included lymphocytes. This accounted for more than 70%, and 2 cases showed increased protein to 500-678 mg/L.

Cranial magnetic resonance imaging MRI

MRI showed hyperintensities in bilateral temporal lobes and the hippocampus on both fluid-attenuated inversion recovery and T2-weighted images in 3 cases. One case showed hyperintensities outside the temporal lobe, involving the right occipital lobe and left lateral ventricle rooms, and 1 case showed brain atrophy on computed tomography. MRI 3 months later revealed temporal lobe and hippocampus brain atrophy in 2 cases (Figure 1-4).

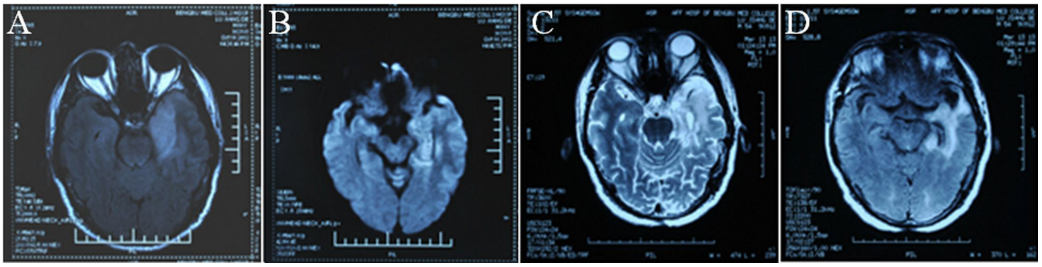

Figure 1. MRI of patient 1. (**A**) MRI showed hyperintensities in the bilateral temporal lobes and hippocampus. (**B**) Fluid-attenuated inversion recovery showed hyperintensities in the bilateral hippocampus. (**C**, **D**) MRI review 3 months later revealed that the left hippocampus and temporal lobe exhibited significant atrophy.

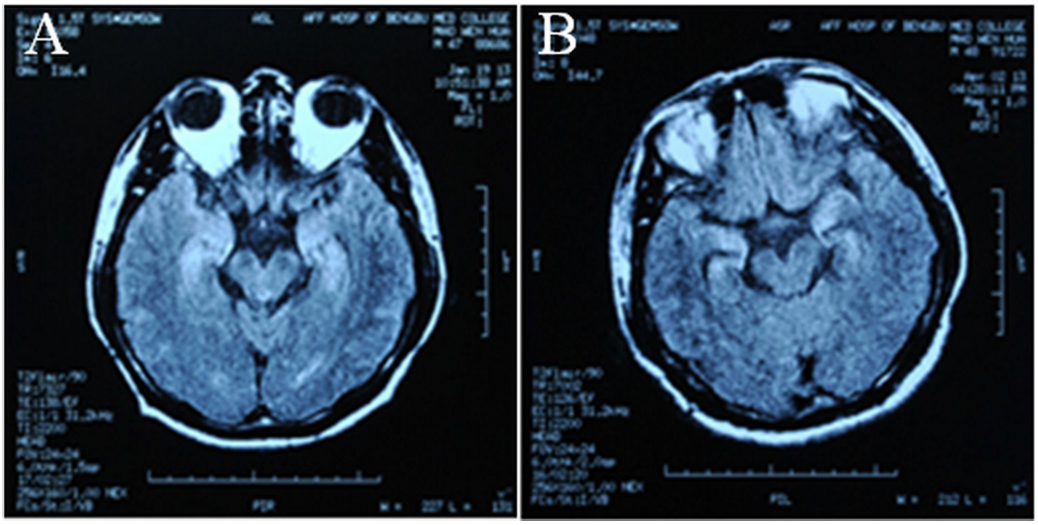

Figure 2. MRI of patient 2. (**A**) MRI showed hyperintensities in the bilateral hippocampal area on fluid-attenuated inversion recovery, (**B**) MRI review 3 months later revealed that the bilateral hippocampus exhibited significant atrophy.

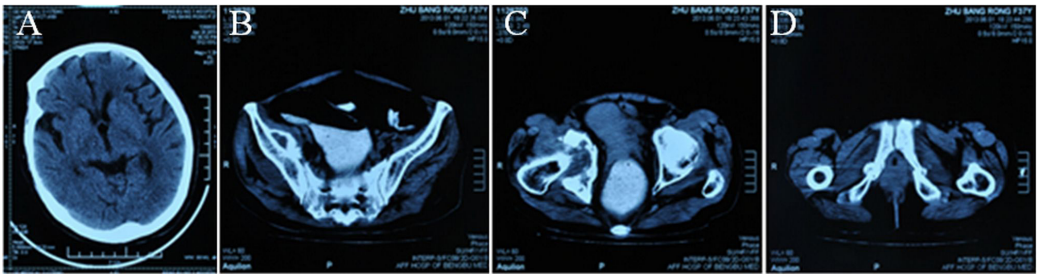

Figure 3. Computed tomography scan of patient 3. (**A**) Cranial computed tomography showed that the bilateral temporal lobe experience significant atrophy 2 months after onset. (**B**, **C**, **D**). Computed tomography scan showed the bone fracture of bilateral ischial ramus of hip joint happened caused by stiff man syndrome, accompanied by mild dislocation, the right acetabulum and femoral neck showed multiple fractures, the soft tissue in the right iliac fossa region swelled, and sclerotin of the sacral bone was destructed.

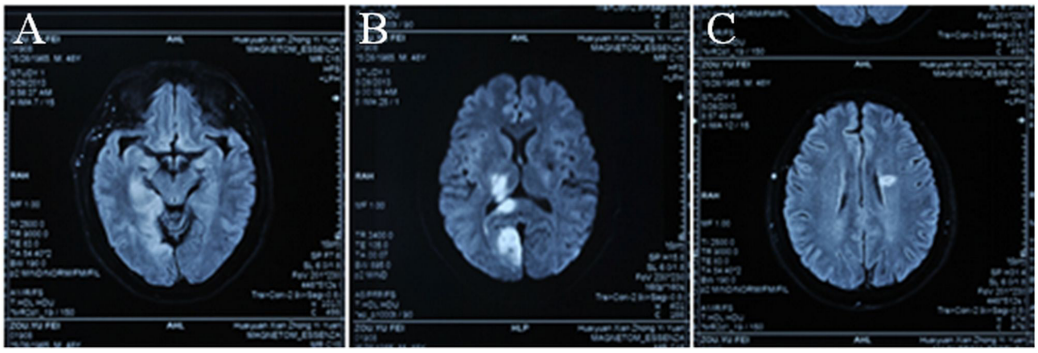

Figure 4. MRI of patient 4. (**A**, **B**) Cranial MRI showed hyperintensities in the bilateral hippocampus, right temporal lobe, thalamus and occipital lobe, (**C**) Fluid-attenuated inversion recovery showed high signal beside the left lateral ventricle.

Enzyme-linked immunosorbent assay

Two patients were positive, including the 37-year-old female, who exhibited positive NMDAR-2B-Ab, VGKC-Ab, and GAD-ab.

Immunohistochemistry

The 1:100 and 1:1000 patient serum dilutions evaluated with anti-Hu, anti-Yo, and anti-Ri were negative.

Western blotting

The paraneoplastic neuronal antibody spectrum examination included 6 specifically certified paraneoplastic neuronal antibodies, including anti-inspection Hu-Ab, anti-Yo-Ab, anti-Ri-Ab, anti-CV2-Ab, anti-PNMA2-Ab (Ma2/Ta), and anti-amphiphysin-Ab, which were all negative, so the possibility of malignancy was excluded.

DISCUSSION

With current progress in immunology, an increasing number of specific neuronal antibodies have been identified, including 2 types of antibodies: antibodies that act on nerve cell antigens, including type I anti-neuronal nuclear antibody (ANNA-1/anti-Hu) and anti-Ma2 protein antibody, among others, and antibodies that act on nervous cell membrane antigens, particularly VGKC-ab, NMDAR-ab, and GAD-ab. We summarized the antigen locations and clinical characteristics of various types of PLE and LE (Table 2).

VGKCs comprise a group of cellular membrane-penetrating proteins that play an important role in regulating resting potential and membrane repolarization (Barajas et al., 2010). VGKC-ab is the most common type of membrane antibody reported in LE patients, and is generally non-tumor-associated (Thieben et al., 2004; Vincent et al., 2004; Graus et al., 2008).

Vicent et al. (2004) retrospectively studied 10 cases of VGKC-ab-associated LE, with early manifestations of memory impairment and orientation; specific clinical features include

Table 2. Antigen locations and clinical characteristics of various types of LE.

Antibody	Antigen location	Relationship with gender	Onset age	Main clinical characteristic of LE	Others	Immunotherapy response
Anti-Hu, Ma2, and CV2	Inside cells	Depends on tumor types	Depends on tumor types	Epilepsy, emotional changes, short-term memory dysfunction	Normally accompanied by small cell lung cancer, thymoma, teratoma or testiculoma, sleeping dysfunction	Poor
VGKC-ab	Cellular membrane	More in male	>40 years old	Epilepsy, forgetting, mental and action disorder	Hyponatremia	Better
NMDAR-ab	Cellular membrane	More in female	Children and adults	Mental disorder and autonomic nerve disorder	Oral or other parts' motion dysfunction or motion abnormality (even could be isolated symptom); may develop into severe situation	Better
GAD-ab	Cellular membrane	No gender difference	<40 years old	Epilepsy, cerebellar ataxia, myoclonus, stiff man syndrome	no	Various

VGKC-ab = voltage-gated potassium channel antibody; NMDAR-ab = *N*-methyl-D-aspartate-2B-antibody; GAD-ab = glutamic acid decarboxylase antibody.

refractory hyponatremia. Compared to PLE, the cerebrospinal fluid examination of VGKC-ab-associated LE primarily showed mild inflammatory changes, and the cerebrospinal fluid of 23% patients was normal (Jarius et al., 2008); the MRI findings may be similar to those for PLE (Thieben et al., 2004; Vincent et al., 2004). Khan et al. (2009) performed the autopsy and observed local inflammatory cell infiltration in the hippocampus and amygdale in 1 case. VGKC-ab-associated LE showed good results towards the immunotherapy response (Vincent et al., 2004, 2010; Barajas et al., 2010). Pertzov et al. (2013) tested a group of VGKC-Ab patients using 2 newly developed visual short-term memory tasks with a sensitive and continuous measurements. These tests can be used to examine the nature of reporting errors. We found that 2 VGKC-ab-positive patients exhibited severe cognitive impairment in the acute phase, with clinical manifestations of memory disorder; IQ scores measured by WAIS were 40-64, which also involved visual-spatial positioning and orientation. One patient could not find the toilet he had just gone to, and exhibited serious obstacles in the tests of picture filling, block design, picture arrangement, and picture operation, suggesting the existence of structural apraxia. One patient could not read his watch, confirming the deletions of object identification and simultanagnosia. One patient could not differentiate between red and green, indicating color agnosia and demonstrating that temporal lobe damage can affect cognitive function in the entire brain, leading to intrahemispheral disconnection syndrome. MRI exhibited lesions in the temporal lobe and hippocampus, while the paraneoplastic antibodies were negative, the anti-immune therapy was effective, and the patients essentially recovered.

NMDAR is an important receptor of excitatory amino acids and plays a key role in the synaptic transmission and synaptic plasticity regulation of the central nervous system. NMDAR is the tetrameric complex among which NR2B is mainly distributed in the hippocampus and forebrain (Dalmau and Rosenfeld, 2008). Dalmau et al. (2007) first reported LE associated with the NMDAR antibodies, and subsequently analyzed the characteristics of 100 patients, often with the psychiatric symptoms as the first symptom; other clinical manifestations also appeared, including epilepsy, movement disorders, ventilation deficiencies, and autonomic nerve dysfunction. MRI showed that the temporal lobe, normally accompanied with the lesions in the frontal or parietal cortex, cerebellum, brain stem, and basal ganglia were involved (Dalmau and Rosenfeld, 2008). In our study, 2 cases were NMDAR-ab positive, including in 1 37-year-old female. Clinical symptoms were complex and the conditions were serious. Because they suffered from epilepticus, we performed tracheotomy. We also observed stiff man syndrome, hyponatremia, autonomic nervous system dysfunction, cardiac faster and pseudo gastrointestinal obstruction, and tonic contraction in the muscle, leading to recurrent pelvic fracture in many pelvic parts. A fracture 3 months ago had formed a callus and some fractures had occurred recently too. These patients were VGKG-ab- and NMDAR-ab-positive, and thus the high titer of antibodies could not be eliminated. This suggests that such complex symptoms, which may include multiple antibodies, can achieve some results with sodium valproate and 300 mg/bid oxcarbazepine to control seizure, and 2 mg/bid clonazepam may be intramuscularly injected, followed by oral administration to control the onset of stiff man syndrome.

GAD is a normal protease in human body that catalyzes glutamate into γ-aminobutyric acid, the most important inhibitory neurotransmitter in the central nervous system. Matà et al. (2008) reported 2 cases of GAD-antibody-associated LE manifested by anterograde amnesia. Marnane et al. (2008) reported 1 case who exhibited partial epilepsy as soft palate myoclonus, and MRI revealed a single lesion in the forehead. Malter et al. (2010) retrospectively studied 9 cases of GAD-ab-associated LE patients, with epilepsy as the first and main symptom. MRI

examination revealed that 3 cases showed hippocampal atrophy, affecting the outer parts of limbic system, such as the hypothalamus, brain stem, and parietal cortex. In our report, 1 case was of GAD-ab-positive, combined with VGKC-ab and NMDAR-ab-positive, causing stiff man syndrome and generalized tonic-clonic seizures. Three patients in our study developed cerebral atrophy rapidly over 2-3 months, including 1 case in which the serum contained 3 types of neuronal cell antibodies and exhibited whole brain atrophy in 2 months. Brain atrophy commonly occurs in LE patients, and patient antibodies may even occur early and severely. One patient, containing 3 types of neuronal cell antibodies in the serum, exhibited a lesion extending to the ipsilateral occipital lobe and right lateral ventricles; however, the mechanism of development remains unknown.

In a correlation study of LE in subjects of Chinese Han nationality, a variety of anti-neuronal cell membrane antibodies such as VGKG-Ab, NMDAR-2B-Ab, and GAD-Ab were found to be related to LE. Based on clinical manifestations, psychological WAIS determination, cerebrospinal fluid, electrophysiology, imaging, immunological examination, and anti-immunotherapy of LE patients, we determined that there were 2 types of clinical manifestations in subjects of Chinese Han nationality with LE, including a simple type and a complex type. The lesions of simple LE are typically limited to the temporal lateral lobe, are antibody-negative and mainly exhibited as cognitive dysfunction, while complex LE lesions are typically involved in multiple sites in the CNS and the clinical symptoms are more severe. The complexity of this disease may lie in the positive detection of a variety of antibodies, and diagnosis and anti-immunotherapy should be performed early to improve prognosis.

Conflicts of interest

The authors declare no conflict of interest.

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