

CASPR2 NEUROLOGICAL AUTOIMMUNITY IN CHILDREN

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ABSTRACT

Objective

To identify the clinical features of CASPR2 neurological autoimmunity in children and to strengthen the understanding of the disease for developing diagnosis and treatment strategies.

Methods

A multicenter retrospective analysis and prospective observation of CASPR2 autoimmunity in the past 7 years was conducted.

Results

Twenty-six anti-CASPR2-positive patients were enrolled in this study, including 25 with serum positivity and 3 with cerebrospinal fluid (CSF) positivity (2 patients with both serum and CSF positivity); 3 patients were co-positive with anti-NMDAR antibody and 1 was copositive with anti-GABABR antibody. Eleven patients (6 manifesting with refractory epilepsy, 4 manifesting with psychobehavioral abnormalities and 1 accompanied with germinoma) presented with low antibody titers, relatively normal MRI/EEG/CSF examinations, and poor response to immunotherapy and were thus considered false positive (42.3%). Fifteen patients were diagnosed with anti-CASPR2-related neurological autoimmunity: 10 with autoimmune encephalitis (including 2 manifested as Morvan syndrome and 1 whose condition was secondary to Japanese encephalitis), 4 with typical clinical features of autoimmune encephalopathy, and 1 with autoimmune cerebellitis. The most common symptoms included disorders of consciousness (10/15), fever (8/15), psychological symptoms/abnormal behaviors (8/15), sleep disorders (8/15), seizures (7/15), movement disorders (5/15), autonomic symptoms (5/15), peripheral

nerve hyperexcitability/neuromyotonia (5/14) and weakness/hemiplegia (4/15). Brain MRI revealed abnormalities in 10 patients (66.7%). The most common sites of lesions were the cerebral cortex (6/15, widely distributed across the frontal, parietal, occipital and temporal lobes) and thalamus (5/15). Electroencephalography (EEG) recordings revealed a slow wave background in 13 patients (86.7%). Five of 15 patients showed elevated WBCs in the CSF, and 4 of 15 patients showed elevated protein levels in the CSF. Thirteen patients received immunotherapy (rituximab was adopted in 2 cases) and recovered well. Two patients received symptomatic treatment, and the recovery was slow and accompanied by emotional abnormalities and developmental delay.

Conclusions

CASPR2 autoantibody disease is not very rare in children and can occur in infancy. The most common clinical phenotypes of CASPR2-related autoimmunity were encephalitis phenotype, including Morvan syndrome and cerebellar ataxia. We also first reported a case of autoimmune encephalitis secondary to Japanese encephalitis. The prognosis of this disease is good, and rituximab can be used in patients who respond poorly to conventional immunotherapy. The high false-positive rate of anti-CASPR2 in refractory epilepsy and the psychobehavioral abnormalities needs to be explored further.

KEYWORDS: Immunity Pediatrics Neurology

INTRODUCTION

Contactin-associated protein-like 2 (CASPR2) is a transmembrane cell adhesion protein of the neuroligin

family that is expressed in neurons of the central and peripheral nervous systems¹. It plays a role in the localization of voltage-gated potassium channel (VGKC) complex, CASPR2 can promote the aggregation and development of K channels (Kv1.1 and Kv1.2) in the proximal nodal region, which helps to form axons and maintain the stability of axons². CASPR2 antibody targets multiple epitopes of CASPR2 protein, which may lead to brain and peripheral neuropathy by destroying axon potassium current³. CASPR2 autoantibody disease has a variable clinical phenotype that includes cerebellar syndromes, epilepsy, pain syndromes, movement disorders, psychosis, and associations with neoplasms such as thymoma. Middle-aged and elderly men are the most commonly affected by this condition⁴. Qin et al reported 25 patients, the median age of symptom onset was 42 years old, 8 patients met the criteria for limbic encephalitis, epileptic seizure occurred in 6 of these 8 patients; 4 patients were diagnosed as Morvan syndrome⁵. However, data on CASPR2 autoimmunity in children are very limited. Tan et al reported 6 children with CASPR2 autoimmunity, all patients had autoimmune encephalitis, no patients with peripheral nerve involvement. Psychotic disorder was the most common symptom. More than half of the patients manifested altered consciousness, experienced movement disorders, headaches or sleep disturbances. Seizures occurred in only a minority of patients. four patients received first-line therapy alone, and two patients received second-line therapy. All patients had favorable outcomes with no recurrence and no neoplastic disease was found⁶. The Mayo laboratory reported 6 children with CASPR2 antibodies, encephalopathy, neuropsychiatric symptoms, and seizures were common, a neuropathic pain syndrome was the most common finding. All patients received immunotherapy, 2 relapsed and required maintenance immunotherapy⁷. Syrbe et al. describe 8 children with new-onset neuropsychiatric and autonomic symptoms associated with CASPR2 autoantibodies. All the patients had hypertension, six patients received antihypertensive treatment with a minimum of 2 medications, which showed no or limited benefit for control of elevated blood pressure⁸. Therefore, the phenotypes, treatment strategies and outcomes of CASPR2 autoimmunity in children have great heterogeneity. Clinicians must continue to explore and summarize the incidence, clinical features, phenotypes, auxiliary examinations, diagnosis, treatment strategies, and prognosis of CASPR2

autoimmunity in children, for in-depth study and understanding.

METHODS

Patient subjects and ascertainment

Patients under 18 years of age with clinically suspected neurological autoimmunity were identified through neural autoantibody evaluation at Hunan Children's Hospital, Chenzhou Children's Hospital, Longshan People's Hospital, the First Affiliated Hospital of Zhengzhou University and Yiyang Central Hospital. Antibodies against NMDAR-IgG, AMPA1-IgG, AMPA2-IgG, LGI1-IgG, CASPR2-IgG, GAD65-IgG, GABABR-IgG, MOG-IgG, GFAP-IgG and AQP4-IgG were measured in serum and/or cerebrospinal fluid (CSF). Clinical data were recorded, and patients were followed up accordingly. The main collected clinical data included: demographic features, neurological symptoms and signs, laboratory tests, imaging results, treatment strategies, efficacy and prognosis evaluation, the follow-up information. Written informed consent was obtained from the patients and his parents. The study was approved by the Ethics Committees of Hunan Children's Hospital and four other centers.

Since 2020, we have performed retrospective analyses and prospective observations for CASPR2 neurological autoimmunity in multiple centers. The time span of observation was from October 2014 to October 2021. The inclusion criteria: all the enrolled patients were highly clinically suspected of having immune encephalitis, encephalopathy, or epilepsy of unknown cause with Epilepsy and Encephalopathy (AEE) score ≥ 4 ⁹; and the patients with positive CASPR2 antibodies in serum and/or CSF. The exclusion criteria: without neurological symptoms, or negative of CASPR2 antibodies, or with incomplete data or loss of follow-up. Eventually, there were 12 patients enrolled retrospectively, and 14 patients enrolled prospectively.

Autoantibody Testing

Specimens were tested for NMDAR-IgG, AMPA1-IgG, AMPA2-IgG, LGI1-IgG, CASPR2-IgG, GAD65-IgG, GABABR-IgG, MOG-IgG, GFAP-IgG and AQP4-IgG using a cell-based assay (CBA). The CSF was not diluted, while the serum was diluted with 1:10. HEK293T cells were cotransfected with the target antigen and pcDNA3.1-EGFP. Thirty-six hours after transfection, the HEK293T cells were fixed with 4%

paraformaldehyde for 20 min. GFAP-IgG detection required additional permeabilization with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 20 min. Cells were incubated with the specimens for 2 h and then immunolabeled with an AlexaFluor 546 secondary antibody against human IgG (1:1000; ThermoScientific) for 1 h at room temperature. Images were acquired using a Zeiss Axiovert A1 fluorescence microscope. One hundred healthy control serum specimens were provided by normal volunteer donors.

According to the antibody titer, we used the symbols + to ++++ to represent the results of positive antibodies. Representative pictures of each interval are shown in Fig. 1. Due to the limitation of the conditions in China (The testing fee is expensive and no insurance policy), the CBA method used in our cohort is not live cell-based assay, but fixed assay. Therefore, our analysis result of antibody titer is a semi quantitative analysis, according to the strength of the fluorescence signal. The antibody titer is generally lower than that of live cells.

RESULTS

General information of CASPR2-IgG positive patients

Indications for antibody testing for the patients included clinically suspected autoimmune encephalitis, demyelinating encephalopathy, and epilepsy of unknown cause with an Epilepsy and Encephalopathy (AEE) as a model to predict the detection of these Abs based on patients' clinical presentation and initial neurologic evaluation) score ≥ 4 . Over a 7-year period, more than 3000 samples from pediatric patients with clinically suspected neurological autoimmunity were tested. The most common positive antibodies were against NMDAR (128/1358 in CSF), MOG (96/550 in serum) and GFAP (45/278 in serum). Three LGI1 antibody-positive patients (3/2658 in serum) were found among our samples. There was no GAD-65 antibody positive in our study. A total of 26 anti-CASPR2-positive patients (26/2658 in serum, and 3/2016 in CSF) were identified among those examined. The results of the antibody titers for antiCASPR2 were as follows: +++ for 2 patients, ++ for 2 patients, and + for 22 patients; 25 patients showed serum positivity and 3 showed CSF positivity (including 2 patients with both serum and CSF positivity). Three patients were double-antibody positive for anti-NMDAR antibody (2 in CSF, 1 in both serum and CSF) and 1 patient was double-antibody

positive for anti-GABABR antibody in CSF.

The oligoclonal bands were detected in 4 patients, and no positive results were obtained. All the patients had done tumor screen examinations, including chest X-ray or lung CT, color Doppler ultrasound or CT for thyroid, reproductive system, organs or lymph nodes in abdominal and pelvic cavity; and peripheral blood tumor antibody screening in partial patients. No cases with tumors beyond the central nervous system were found.

Among the 26 anti-CASPR2-positive patients, 17 were male and 9 were female. The age of onset ranged from 5 months to 14 years: 4 patients had an age of onset of 0–3 years; 6 patients, 4–6 years; 11 patients, 7–9 years; 4 patients, 10–12 years; and 1 patient had an age of onset of over 12 years. The most common onset age was 4–9 years. The median follow-up period was 22 months (range, 3–62).

Clinical Spectrum Analysis of Caspr2 Autoantibody-related Disease

The key clinical phenotypes included encephalitis phenotype (including autoimmune encephalitis/encephalopathy/cerebellitis), intractable epilepsy, psychobehavioral abnormalities and combined tumors. Considering the possibility of false positive anti-CASPR2 antibodies, we classified the patients according to their main clinical phenotype, auxiliary examination, treatment and prognosis.

CASPR2 autoantibody-related disease mainly manifested as encephalitis phenotype in 15 patients.

Fifteen patients presented with encephalitis phenotype: 10 with autoimmune encephalitis (2 of whom presented with Morvan syndrome), 4 with typical clinical features of autoimmune encephalopathy, and 1 with autoimmune cerebellitis. Three patients presented with autoimmune encephalitis coexisting with antiNMDAR antibody positivity. One patient presented with Morvan syndrome coexisting with anti-GABABR antibody positivity. One patient presented with autoimmune encephalitis secondary to Japanese encephalitis. The results of the antibody titers for anti-CASPR2 were as follows: +++ for 2 patients, ++ for 2 patients, and + for 11 patients; 3 patients showed CSF positivity (including 2 with both serum and CSF positivity).

The age of onset ranged from 5 months to 14 years, the median onset age was 6 years and 5 months. The most common symptoms of those 15 patients included disorders of consciousness (10/15), fevers (8/15), psychological symptoms/abnormal behaviors (8/15), sleep disorders (8/15), seizures (7/15), movement disorder (5/15), autonomic symptoms (5/15), peripheral nerve hyperexcitability/ neuromyotonia (5/14) and weakness/hemiplegia (4/15). The autonomic symptoms (5/15) included 5 cases of hyperhidrosis, 4 cases of hypertension, 4 cases of gastrointestinal dysfunction and 2 cases of paroxysmal arrhythmia. The symptoms such as hyperhidrosis, irritability and tachycardia caused by high-dose of steroid administration were not included. There was no patient with simple visual impairment or paraplegia.

Brain MRI (Fig. 2) revealed abnormalities in 10 patients (66.7%). The most common sites of lesions were the cerebral cortex (6/15, widely distributed across the frontal, parietal, occipital and temporal lobes), thalamus (5/15), caudate nucleus (3/15), cerebral peduncle (3/15), white matter (2/15), hippocampus (2/15), globus pallidus (2/15) and corpus callosum (1/15). Electroencephalography (EEG) recordings revealed a slow wave background in 13 patients (86.7%), 2 (21.43%) of whom had bursts of delta rhythm. Epileptiform discharges, originating from the frontal and temporal lobes, were found in 4 patients (26.7%), and one patient manifested with epilepsy partialis continua (EPC). All 15 patients underwent lumbar puncture examinations, 5/15 showed elevated WBC (range 59–390) in CSF, and 4/15 showed elevated protein (range 0.593–0.72) in CSF. Changes in the CSF were not very common in CASPR2-related patients. The CSF pathogen tests were negative in all patients. For the treatment strategies, 13 patients received immunotherapy, including intravenous immunoglobulin (IVIG) in 3, IVIG plus prednisone in 2, IVIG plus intravenous methylprednisolone (IVMP) plus prednisone in 5, IVMP plus prednisone in 1, IVIG plus IVMP plus prednisone plus rituximab in 1, and IVIG plus IVMP plus prednisone plus plasma exchange (PE) in 1. The modified Rankin Scale (mRS) scores before treatment were 5 in six patients, 4 in two patients, 3 in three patients, and 2 in two patients. After treatment, twelve patients had an mRS score of 0, and one of them had memory loss. One patient had an mRS score of 1, with no motor disorder of left hand; the follow-up time of the patient was only 3

months after immunotherapy, and the patient is in recovery. Two patients received symptomatic therapy (for economic reasons and given the side effects of hormones, the family refused immunotherapy). The mRS scores before treatment were 4 and 5. After 3 years of follow-up, the symptoms of these 2 patients improved gradually, and the mRS scores were 1 and 0, respectively; one patient had emotional agitation, and the other had developmental delay.

Brief Case Descriptions

Patient N.3, a boy aged 6 years, developed neuromyotonia and neuropathic pain, movement disorders, sleep disorders, irritability, eating difficulties and cognitive changes. Both CASPR2 and GABABR antibodies were detected in his serum and CSF. He was treated with intravenous immune globulin (IVIG) (2 g/kg/day for 5 days) and IV methylprednisolone (IVMP) (3 cycles of 20 mg/kg/day for 3 days alternating with 4 days of rest). One month later, his symptoms had not been relieved, his CASPR2 titers had not decreased, and brain MRI (Fig. 2-A1/A2) revealed new lesions. He was treated with rituximab (375 mg/m², once a week, 4 times) and improved remarkably. One month after rituximab treatment, all his symptoms were relieved, his MRI results had become normal, and the CASPR2 antibody test was negative. Moreover, during the treatment, we found that carbamazepine had a significant effect on relieving the symptoms of myotonia.

Patient N.5 was the youngest patient, only 5 months old at symptom onset. His main symptoms included fever, recurrent seizures, disturbance of consciousness, and irritability. Brain MRI (Fig. 2-B1/B2) showed lesions in the globus pallidus, corpus callosum and cerebral peduncle. The WBC, protein, bacterial culture and macrogene examination for virus nucleic acid in CSF were normal. CASPR2 antibody positive was found in the serum. He received symptomatic treatment, including ventilator-assisted ventilation. His symptoms gradually improved, and his development gradually progressed after rehabilitation training. At the age of 2 years and 6 months, he could speak and walk independently.

Patient N.6, a girl aged 2 years, developed fever, recurrent seizures, unconsciousness, sleep and movement disorders, and psychological symptoms. Both CASPR2 and

NMDAR antibodies were detected. Brain MRI (Fig. 2-C1/C2) showed extensive lesions in the cortex and thalamus, with meningeal enhancement of the cortical lesions. The symptoms in the acute stage were very severe but improved greatly after IVIG and half a year of steroid treatment. The patient was able to attend school normally and had normal intelligence.

Patient N.8, a girl aged 5 years, presented with two episodes of fever and seizures within a week. The symptoms were resolved without treatment. Brain MRI showed extensive cortical lesions (Fig. 2D). However, we still used IVIG to prevent aggravation of the clinical symptoms. During the 15-month follow-up, no symptom recurrence was observed.

Patient N.10, a boy aged 6 years, was admitted to the hospital because of fever, ataxia, and slow responses. EEG showed a low wave background, and brain MRI showed lesions in the thalamus and caudate nucleus (Fig. 2-E1/E5), with elevated WBCs in the CSF. Anti-CASPR2 antibody was positive in the CSF and negative in the serum, while NMDAR was initially negative in both the CSF and serum. He was treated with IVIG + IVMP, and the clinical symptoms were improved. One month after he was discharged from the hospital, he presented with hemiplegia and irritability. Brain MRI revealed new lesions in the cerebral peduncle (Fig. 2-E2/E5), and anti-NMDAR antibody was positive in the CSF, but anti-CASPR2 antibody was negative. He was treated with IVMP again and then rituximab. The lesions on brain MRI (Fig. 2; E3/E4/E7/E8, E1/E5, E2/E6, E3/E7, and E4/E8 represent D4, D16, D30, and D50 after onset, respectively) were decreased after immunotherapy. The hemiplegia was improved (at 1 month after discharge) but incompletely, and the patient remains in follow-up.

Patient N.11, a 12-year-old girl, was initially diagnosed with Japanese encephalitis. On 24, July, 2021, she presented high fever, convulsions, disturbance of consciousness and central respiratory failure. Brain MRI showed symmetrical lesions of bilateral thalamus, elevated WBC and positive antibody to Japanese encephalitis in CSF in acute stage. After symptomatic treatment, her clinical symptoms gradually relieved and she was discharged home on August 19. On August 24, she developed convulsions, psychological symptoms and disturbance of consciousness again. Brain MRI shown

lesions in the bilateral thalamus. CASPR2 antibody was positive, while NMDAR and other immune antibodies were negative. She was considered to have CASPR2 antibody-related autoimmune encephalitis secondary to encephalitis B, and the symptoms were cured after immunotherapy.

Patient N.12, a 14-year-old girl, mainly presented with an ataxic gait, elevated WBC and protein in the CSF, without symptoms of encephalitis or encephalopathy. Her EEG and brain MRI were normal. She was diagnosed with autoimmune cerebellitis and recovered to normal after administration of IVIG + IVMP, and oral prednisone for 1 month. She was the only patient with a normal EEG among 15 patients diagnosed with CASPR2-related autoimmune diseases.

CASPR2 autoantibody-related disease mainly manifested as refractory epilepsy in 6 patients.

Six patients presented with refractory epilepsy, which manifested as recurrent focal seizures. The antibody titers for anti-CASPR2 were + in serum for all patients. Brain MRI revealed abnormalities in one patient with tuberous sclerosis (TSC) and another with focal cortical dysplasia (FCD), while the remaining 4 patients had normal MRIs. EEG results were abnormal in all patients: epileptic charges were observed in 5 (83.3%) patients, a slow wave background in 2 (33.3%) patients, bursts of delta rhythm in one patient, and epilepsy partialis continua (EPC) in one patient. WBC, protein concentration and autoimmune antibodies in CSF were examined in four patients and were normal.

Patient N.16 presented with focal seizures. Brain MRI revealed multiple cortical lesions. He was treated with IVIG and antiepileptic drugs (AEDs), but recurrent seizures persisted. Genetic tests showed TSC1 gene pathogenic mutations, which supported a diagnosis of TSC.

Patient N.17 had neuropathic pain and irascibility, with no specific changes on MRI and CSF examination. He was treated with IVIG plus prednisolone and AEDs, but recurrent seizure attacks persisted during the 3-year follow-up.

Patient N.21 also presented with recurrent focal seizures, and EEG and MRI indicated left parietal abnormalities. He

was treated with IVMP plus prednisone and AEDs but responded poorly. Finally, he achieved a seizure-free status after surgery, and pathological examination conformed a diagnosis of FCD.

For the treatment strategies, all patients were treated with antiepileptic drugs (AEDs); 1 patient was treated with IVIG, one with IVIG plus prednisolone, and one with IVMP plus prednisolone. The mRS scores before treatment were 2 in four patients and 1 in two patients. After treatment, the mRS scores were 1 in four patients. One patient achieved a seizure-free status after AED treatment. The FCD patient was seizure free after surgery. The seizure frequency of the other 4 patients decreased. Among these 6 patients, the use of immunotherapy had little correlation with prognosis.

Four patients presented with psychobehavioral abnormalities. Three patients mainly manifested with self-talking, giggling, hallucinations, irritability, social disorders, fear, etc. One patient manifested with personality changes and sleep disorders. The results of the eCASPR2 antibody titers were all positive in serum: ++ for 1 patient and + for 3 patients. Brain MRI and EEG were normal in all patients. WBC, protein concentration and autoimmune antibodies in CSF were examined in two patients, and both were normal.

Patient N.22 was treated with IVIG plus prednisolone. His symptoms improved, with pre-/posttreatment mRS scores of 3/0.

Patients N.23 and N.24 were only given symptomatic treatment; N.24 improved to normal, with mRS scores before/after treatment of 1/0, while the clinical symptoms of N.23 did not improve, with mRS scores before/after treatment of 3/3.

Patient N.25 (with antibody titer ++) was treated with IVIG + IVMP + prednisone + mycophenolate mofetil, but the clinical symptoms did not improve; the mRS scores before/after treatment were 3/3.

CASPR2 autoantibody-related disease accompanied with tumor in 1 patient.

Patient N.26 manifested with hemiplegia of the left limb and headache. Brain MRI showed lesions in the right

globus pallidus and midbrain. EEG showed low voltages in the right central, parietal and temporal areas, with normal WBC and protein concentrations in the CSF. The CASPR2 antibody titer was + in serum.

He was treated with IVMP + prednisone but showed no improvement. Finally, he was pathologically diagnosed with germinoma by brain biopsy.

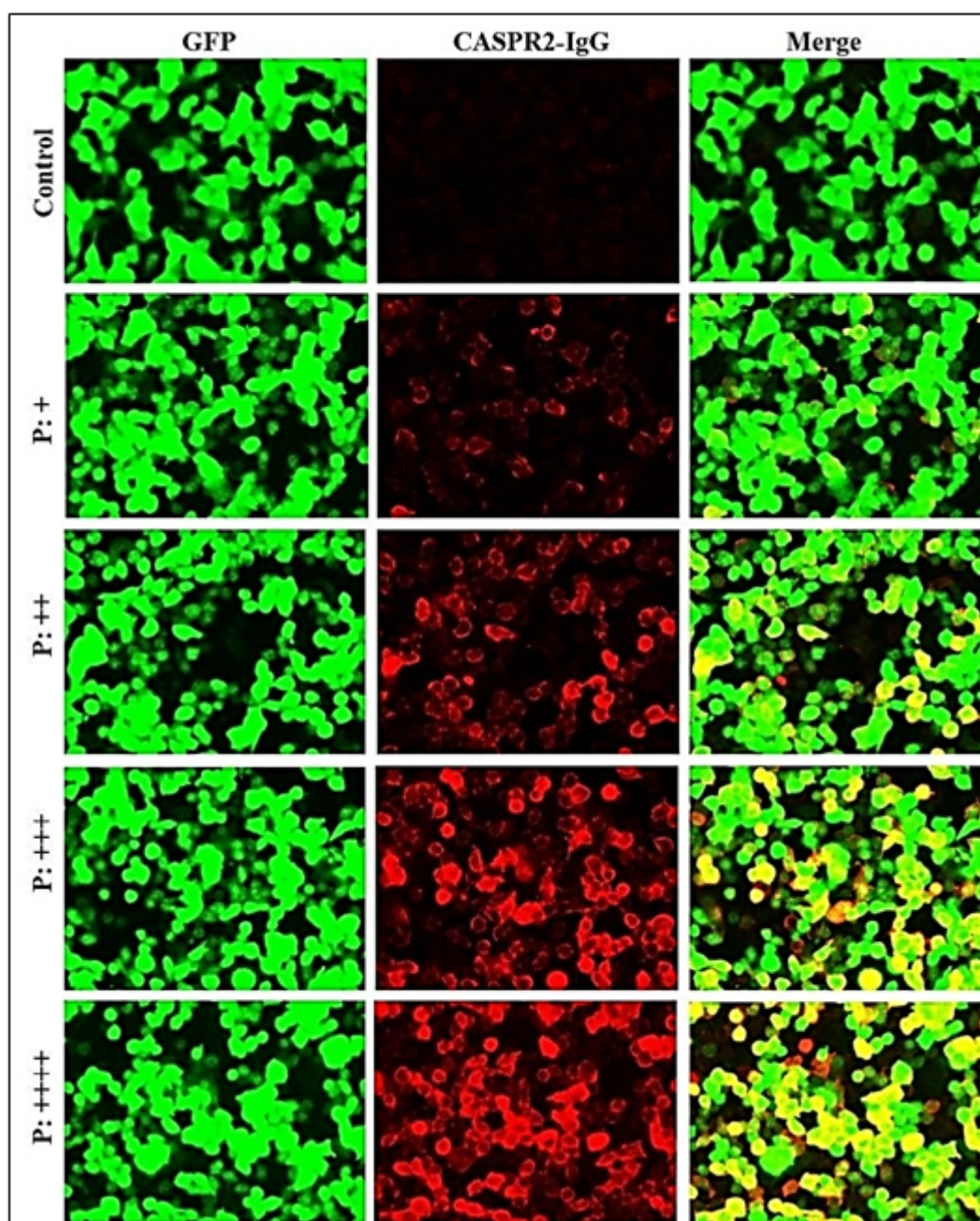


FIGURE 1:

CASPR2-IGG IN SERUM TESTED BY A CELL-BASED ASSAY (CBA) USING HEK293T CELLS TRANSIENTLY COTRANSFECTED WITH FULL-LENGTH HUMAN CASPR2 AND PCDNA3.1-EGFP (SCALE BAR: 20 μ M). THE PATIENT'S IGG BOUND TO CASPR2-TRANSFECTED CELLS AND SHOWED RED UORESCENCE AS A POSITIVE CONTROL.

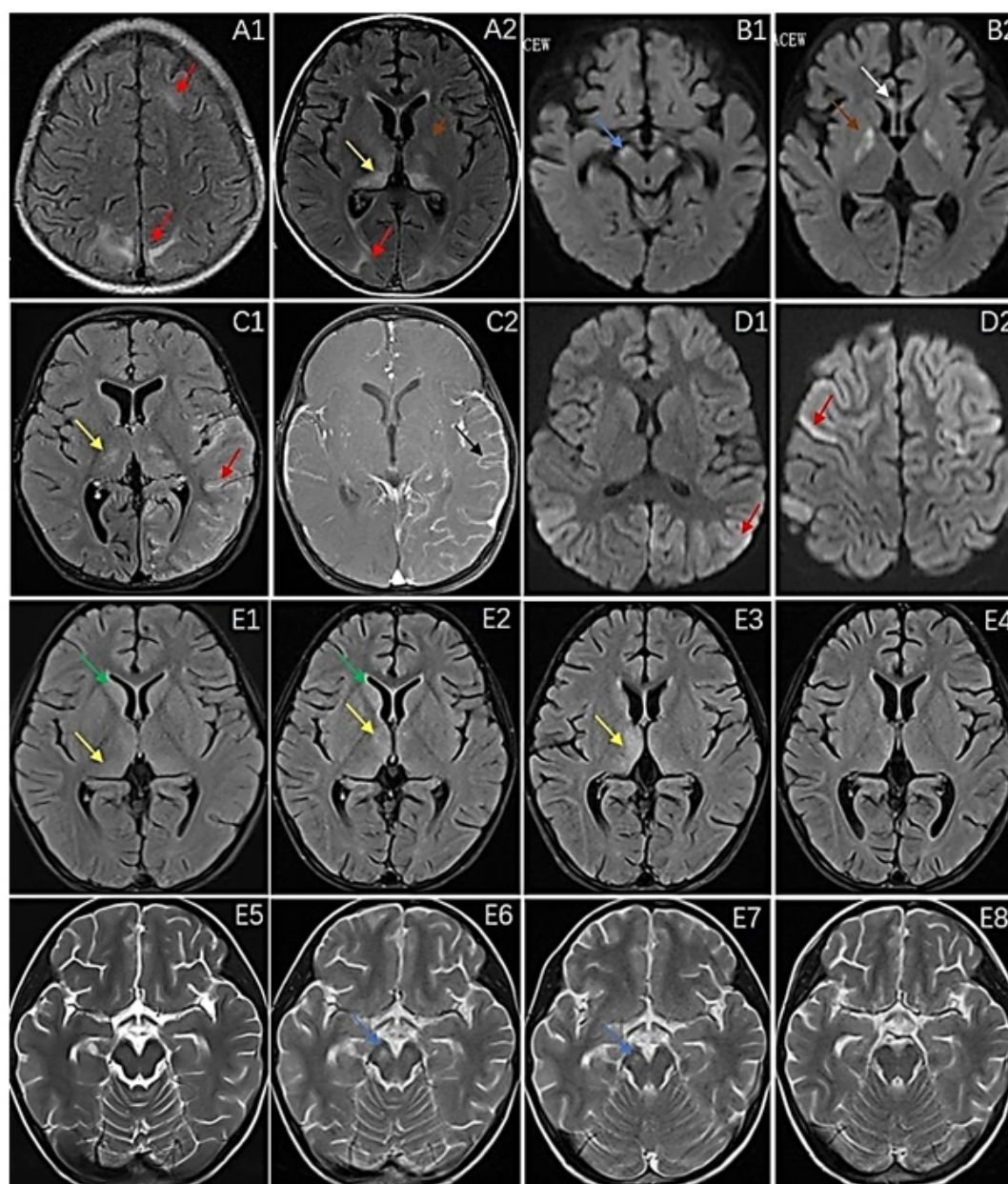


FIGURE 2:

BRAIN MRI FEATURES OF PATIENTS N.3, N.5, N.6, N.8 AND N.10. PATIENT N.3, A 6-YEAR-OLD BOY, HAD A CLINICAL-RADIOLOGICAL PRESENTATION OF MORVAN SYNDROME INCLUDING MULTIFOCAL LESIONS IN THE OCCIPITAL AND PARIETAL CORTEX AND THALAMUS (A1, A2). PATIENT N.5, A 5-MONTH-OLD BOY, PRESENTED WITH ENCEPHALOPATHY AND BILATERAL MRI ABNORMALITIES IN THE GLOBUS PALLIDUS, CORPUS CALLOSUM AND CEREBRAL PEDUNCLE (B1-B2). PATIENT N.6, A 2-YEAR-OLD GIRL, HAD A CLINICAL-RADIOLOGICAL PRESENTATION OF AUTOIMMUNE ENCEPHALITIS, INCLUDING CORTICAL LESIONS IN THE FRONTAL, PARIETAL, OCCIPITAL AND TEMPORAL LOBES AND THALAMUS AND CORTICAL LESIONS SHOWING ENHANCEMENT WITH GADOLINIUM ADMINISTRATION (C1-C2). PATIENT N.8, A 5-YEAR-OLD GIRL, PRESENTED WITH TWO EPISODES OF FEVER AND SEIZURES WITHIN A WEEK. BRAIN MRI SHOWED EXTENSIVE CORTICAL LESIONS IN THE FRONTAL, PARIETAL, OCCIPITAL AND TEMPORAL LOBES (D1-D2). PATIENT N.10, A 6-YEAR-OLD BOY, PRESENTED WITH FEVER, ATAXIA, SLOW RESPONSES. BRAIN MRI (E1-E8) SHOWED A SERIES OF CHANGES INCLUDING LESIONS IN THE THALAMUS, CAUDATE NUCLEUS AND CEREBRAL PEDUNCLE (E1/E5, E2/E6, E3/E7, E4/E8, REPRESENTING D4, D16, D30, AND D50 AFTER ONSET, RESPECTIVELY). FLUID-ATTENUATED INVERSION RECOVERY SEQUENCES WERE USED IN A1, A2, C1, E1-E4, AND C2, WHICH SHOW ENHANCEMENT WITH GADOLINIUM ADMINISTRATION. T2 SEQUENCES WERE USED IN E5-E8. DIFFUSION WEIGHTED IMAGING WAS USED IN B1, B2, D1 AND D2. RED ARROWS REPRESENT MULTIPLE CORTICAL LESIONS, YELLOW ARROWS: THALAMUS, BLUE ARROWS: CEREBRAL PEDUNCLE, GREEN ARROWS: CAUDATE NUCLEUS, BROWN ARROWS: GLOBUS PALLIDUS, WHITE ARROW: CORPUS CALLOSUM, BLACK ARROW: MENINGEAL ENHANCEMENT.

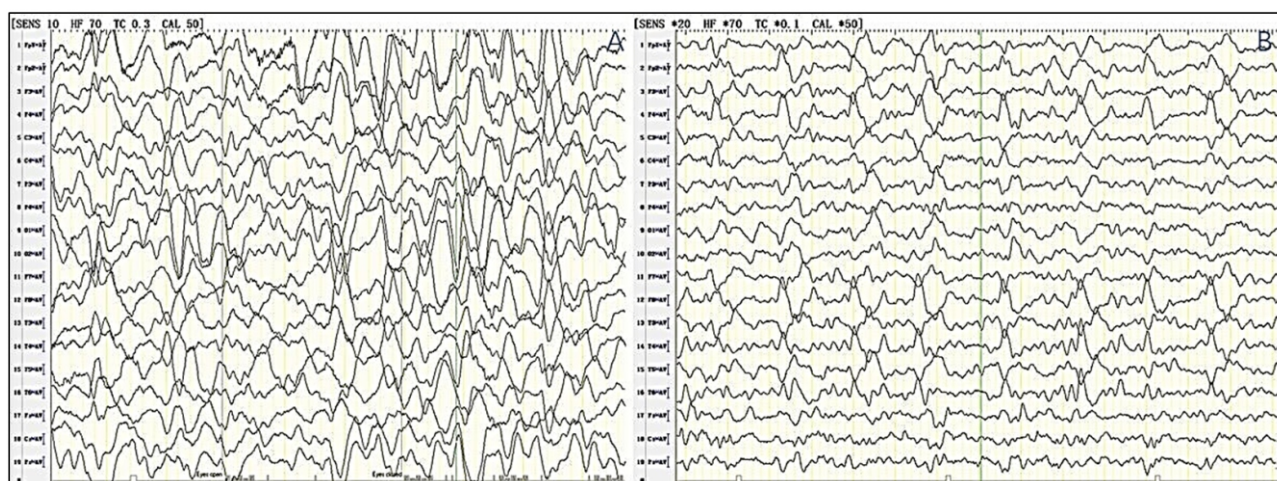


FIGURE 3:

EEG FEATURES OF PATIENT N4, BOY, AGED 6 YEARS, WHO PRESENTED WITH REFRACTORY EPILEPSY.

A: DIFFUSE DELTA RHYTHM DURING AWAKE PERIOD;

B: PERIODIC WAVES FROM RIGHT FRONTAL AND TEMPORAL AREA DURING SLEEP.

COMPREHENSIVE ANALYSIS OF PATIENTS FALSE POSITIVE FOR ANTI-CASPR2 NEUROLOGICAL AUTOIMMUNITY

Based on the clinical symptoms, MRI, EEG, CSF changes, antibody titers, copositivity with other antibodies, response to immunotherapy and other significant findings (such as past history and pathological examinations), we conducted a comprehensive analysis of the 26 patients (Table 2) to distinguish between true- and false-positive cases of CASPR2-related autoimmunity and to explore the correlation between the clinical phenotype and positive anti-CASPR2 antibody.

Among the 26 anti-CASPR2-positive patients, the 15 patients who presented with autoimmune encephalitis/encephalopathy could probably be diagnosed with anti-CASPR2 neurological autoimmunity, as their clinical symptoms and signs and EEG, CSF and MRI changes were consistent with the characteristics of autoimmune diseases. The anti-CASPR2 antibody titers were higher and more frequently accompanied by other autoimmune antibodies than those of the other patients. The responses to immunotherapy were very good: the prognoses of the 13 patients who received immunotherapy were very good, and the 2 patients who did not receive immunotherapy had certain degrees of sequelae.

For the 6 patients who manifested with refractory epilepsy, we could not confirm that their etiology was related to the CASPR2 antibody. The core symptoms of these patients were mainly recurrent epilepsy. Patient N.17 was accompanied by emotional changes such as irritability, but the effect of immunotherapy was poor; the CSF and MRI results showed no specific changes, and the antibody titers were low. The use of immunotherapy had little effect on prognosis.

For the 4 patients who manifested with psychobehavioral abnormalities, we cannot confirm that their etiology was related to the CASPR2 antibody. They showed no solid evidence of encephalopathy and encephalitis except for psychobehavioral abnormalities and no specific changes in the CSF and on MRI. The use of immunotherapy had little effect on prognosis.

Obviously, in the patient diagnosed with germinoma, the positive CASPR2 antibody was likely to be related to the tumor, and immunotherapy was not effective. This suggests that the CASPR2 antibody positivity in this patient may be a paraneoplastic syndrome.

DISCUSSION

The continual discovery of novel forms of encephalitis associated with autoimmune antibodies has changed the paradigms for diagnosing and treating disorders that were

previously unknown or mischaracterized¹⁰. To date, anti-NMDAR encephalitis has been identified as the most common subtype in pediatric patients, but reports of anti-MOG antibody disease are increasingly frequent. There are also an increasing number of reports of patients with multiple antibody positivity⁷. Anti-CASPR2-related encephalitis in children is rarely reported. The phenotypes, treatment strategies and outcomes of CASPR2 autoimmunity in children have great heterogeneity and need to be explored further. Therefore, in this study, we reported 26 children with positive anti-CASPR2 antibody, to analysis their clinical features, MRI changes, treatment strategies and outcomes, aim to provide clues for clinicians to understand CASPR2 autoimmunity in children.

From 2010 to 2019, a total of 667 patients were included in a research review, with a median age of 54 years⁴. Only 0.1% of 13,319 pediatric patients undergoing serological evaluation were identified as positive for LGI1 and CASPR2-IgG¹¹. In a review of LGI1 and CASPR2 autoimmunity in children, 37 pediatric patients with CASPR2 and/or LGI1 autoimmunity were identified in the literature: 37.8% (14/37) of patients had LGI1 antibodies, 37.8% (14/37) had CASPR2 antibodies, and 24.3% (9/37) of patients were double positive for LGI1 and CASPR2 antibodies¹². In our study, we tested for NMDAR-IgG, AMPA1-IgG, AMPA2-IgG, LGI1-IgG, CASPR2-IgG, GABABR-IgG, MOG-IgG, GFAP-IgG, and AQP4-IgG in patients with clinically suspected neurological autoimmunity; NMDAR-IgG and MOG-IgG positivity were most common. The positive rate for CASPR2 was lower than that for NMDAR and MOG but was not very low overall. In the last year, as understanding of the disease has increased, we have been able to identify more than 10 positive patients. Previous literature has suggested that the rarity may be related to the fact that this antibody is not used as part of a routine examination¹³. In total, we screened 26 positive patients out of approximately 3,000 patients. In contrast to NMDARs, which were mainly positive in the CSF, the positive rate for CASPR2 was markedly higher in serum (25/26 vs 3/26 for CSF).

Three patients were co-positive for NMDAR antibody, and one patient was co-positive for GABABR antibody. For the diagnosis of CASPR2 autoantibody disease, this study demonstrated that serum antibody detection is more sensitive than CSF antibody detection and that double

antibody positivity can be existed in CASPR2 autoantibody disease.

False positivity for CASPR2 antibody has been identified in the clinic¹³, and so we, too, considered the possibility of false positives. Indeed, all the enrolled patients were highly clinically suspected of having immune encephalitis, encephalopathy, or epilepsy of unknown cause with Epilepsy and Encephalopathy (AEE) score ≥ 4 ⁹, and the diagnosis was conformed by a comprehensive analysis of multiple factors, such as clinical manifestations, auxiliary examinations, antibody titers and responses to immunotherapy treatment (remission of symptoms, improvement on brain imaging, decrease in or negative conversion of antibody titers). We were unable to rule out the possibility of false positives in 11 patients (6 who manifested with refractory epilepsy, 4 who manifested with psychobehavioral abnormalities, and 1 diagnosed with germinoma) among the CASPR2 antibody-positive patients.

The false positive rate was very high (42.3%, 11/26), mainly due to patients with low antibody titers and a lack of typical encephalitis/encephalopathy symptoms or corresponding changes on EEG and brain MRI or in the CSF. However, CASPR2 antibody positivity was very frequent in patients with refractory epilepsy and psychobehavioral abnormalities, some of whom seemed to respond positively to immunotherapy. This matter should be further investigated with follow-up observation and in depth study to determine whether to strengthen the immunotherapy regimens for patients suspected of immune causes and demonstrating a poor treatment effect.

The most commonly reported presenting symptoms in adults include psychiatric symptoms, seizures, cognitive disturbance/memory impairment, sleep disorders, autonomic disorders and peripheral nerve hyperexcitability/neuromyotonia. The typical symptoms in children have not been characterized in a large-sample study. Among the 15 patients considered to have CASPR2-related autoimmunity in this study, the most common symptoms included disorders of consciousness, fever, psychological symptoms/abnormal behaviors, sleep disorders, seizures, movement disorders, autonomic symptoms, peripheral nerve hyperexcitability/neuromyotonia and weakness/hemiplegia. Fever may be

related to the history of prodromal viral infection and could further induce immune disorders, leading to immune encephalitis. The other common major symptoms were similar to those in adults and are consistent with the literature¹⁴. The most commonly reported clinical syndromes in adults are autoimmune encephalitis, peripheral nerve hyperexcitability/neuromyotonia, and Morvan syndrome. Among our patients, autoimmune encephalitis/encephalopathy was the most common phenotype; among them, only 2 patients manifested with Morvan syndrome, and there was one patient only manifested with cerebellar ataxia. This difference from adults may be a specific characteristic of the disease in pediatric patients.

Anti-NMDAR encephalitis can be secondary to herpes simplex virus encephalitis, Japanese encephalitis or other forms of viral encephalitis⁵. In our study, we also found positive CASPR2 antibody in patients with immune encephalitis secondary to Japanese encephalitis. This is the first report of CASPR2-associated immune encephalitis secondary to viral encephalitis. Carreno et al. reported that CASPR2, GAD, Ma2, LG11 antibodies positive in drug resistant temporal lobe epilepsy¹⁵. Additionally, we found CASPR2 antibody positivity in TSC, FCD and germinoma patients, phenomena that have not been reported in previous studies. Whether there is a correlation between them and whether immune factors are involved in the pathogenesis of these diseases is worthy of in-depth study.

The auxiliary examination of CASPR2-related diseases has low specificity. Among our patients, elevated leukocytes or protein in CSF was observed in only 6. A slow wave background was the most common EEG finding, observed in the 14 patients who presented with autoimmune encephalitis/encephalopathy, 3 of whom had delta rhythms. Only one patient, who manifested with autoimmune cerebellitis, had normal EEG. EEG changes involving a slow wave background with a delta rhythms may thus be a diagnostic indicator of CASPR2 autoantibody disease.

Brain MRI is an important examination in the diagnosis of autoimmune encephalitis. Different types of encephalitis have different MRI features. Previous studies have observed abnormalities in over 50% of adult patients, with the most common sites of lesions in anti-CASPR2-

related encephalitis being the temporal lobe and hippocampus⁴. Due to the insufficient number of large-sample studies, the MRI changes in typical CASPR2 autoantibody disease need to be further explored. We found that 66.7% (10/15) of the patients had abnormalities on brain MRI. The cerebral cortex, including the frontal, parietal, occipital, and temporal lobes, was the most commonly involved lesion site, followed by the thalamus. In addition to the cortex and thalamus, other parts of the brain can be involved, including the basal ganglia, white matter, brain stem, and corpus callosum. Our study shows that the MRI features of CASPR2 autoantibody disease mainly involve lesions of the cerebral cortex, but there is no evidence to indicate which region of the cortex is most commonly involved.

According to the analysis of treatments and prognoses, for most CASPR2-related autoimmunity, immunotherapy strategies can improve the patients' outcomes. This is consistent with the results in another study, Tan reported six patients, all patients had favorable outcomes with recurrence rate at 0%⁶. The intensity of immunotherapy differs depending on the clinical conditions of the patient. Most immunotherapy regimens consist of IVIG and/or steroids; however, two patients were treated with rituximab because IVIG plus IVMP did not lead to a full recovery. The youngest patient, 5 months of age, was treated only with IVIG in the acute stage, resulting in developmental delay. However, at the age of approximately 2.5 years, he was able to communicate with others and walk independently. Among the patients manifesting with refractory epilepsy and psychobehavioral abnormalities accompanied by tumors, some had a good response to immunotherapy, while others did not. We cannot confirm whether these diseases are related to immune disorders, and thus the efficacy of immunotherapy is uncertain.

Relapse of CASPR2-related autoimmunity was reported in adults. The reported relapse rate was between 16–37.5% [5,3]. The Mayo laboratory reported 6 children, two patients relapsed and required maintenance immunotherapy⁷. In our cohort, Patient N.10, who was diagnosed as autoimmune encephalitis firstly with anti-CASPR2 antibody positive in the CSF. He was treated with IVIG + IVMP, and the clinical symptoms were improved. One month later, he was relapsed and brain MRI revealed new lesions with anti-NMDAR antibody positive in the

CSF, but anti-CASPR2 antibody was negative. No other patients relapse.

Relapse in children are rarely reported, may be due to the small sample size and short follow-up time.

In conclusion, CASPR2 autoantibody disease is not very rare in children and can occur in infancy. But this type of encephalitis occur more frequently in older children, this is consistent with Tan's study [6]. The most common symptoms include disorders of consciousness, fever, psychological symptoms/ abnormal behavior, sleep disorders, seizures, movement disorders, autonomic symptoms, peripheral nerve hyperexcitability/ neuromyotonia and weakness/hemiplegia. The most commonly involved part of the brain on MRI examination is the cerebral cortex. EEG changes involving a slow wave background, high antibody titers, co-expression with other antibodies, and well response to immunotherapy may help lead to an accurate diagnosis. The most common clinical phenotypes are encephalitis phenotype (including autoimmune encephalitis/encephalopathy/cerebellitis). Morvan syndrome is a characteristic phenotype of CASPR2-related autoimmunity and can manifest as autoimmune cerebellitis. We also identified patients with CASPR2-related immune encephalitis secondary to Japanese encephalitis and autoimmune encephalitis combined with other antibodies. In addition, we found anti-CASPR2 antibody positivity in many patients manifested as refractory epilepsy and unexplained psychobehavioral abnormalities. These patients have atypical encephalitis symptoms and lack obvious imaging and CSF changes, and the efficacy of immunotherapy remains unconfirmed. The false positive rate is very high, but we still cannot completely rule out whether immune factors are involved in the pathogenesis of the disease in these patients; thus, long-term follow-up and large sample research are needed. We also found anti-CASPR2 antibody positivity in patients with TSC, FCD and germinoma, all of which suggest that CASPR2 antibody positivity is nonspecific. The overall prognosis of these patients is good. Rituximab can be used in patients with a poor response to conventional immunotherapy.

Ethical Approval and Consent to participate

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Hunan Children's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Consent for publication All the authors agreed to publish.

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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