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Gene variations of glutamate metabolism pathway and epilepsy

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Abstract

Background: Epilepsy is a paroxysmal disorder of the brain, caused by an imbalance of neuronal excitation and inhibition. Glutamate is the most important excitatory neurotransmitter in the brain and plays an important role in epileptogenesis. Mutations in genes at any step/component of the glutamate metabolic pathway may lead to the development of epilepsy or epileptic encephalopathy.

Methods: Clinical history of 3 epilepsy patients with genetic variations of the glutamate metabolism pathway was collected. Electroencephalogram recording and magnetic resonance imaging were performed in each patient. We also reviewed recent literature for a variety of the genetic variations involved in epilepsy.

Results: Case 1 was a *SLC1A2* mutation-carrier diagnosed with developmental and epileptic encephalopathy (DEE) 41, whose seizures decreased after start of the ketogenic diet. Case 2 carried a *GRIN2A* gene mutation and was seizure-free for three years after taking levetiracetam and vitamin B6. Case 3 was a *GRIN2B* mutation-carrier diagnosed with DEE 27, who seizures diminished after taking oxcarbazepine.

Conclusions: Preclinical and clinical evidence supports the therapeutic potential of glutamatergic signaling-targeting treatments for epilepsy. More studies are needed to discover novel DEE-related genetic mutations in the glutamate metabolic pathway.

Keywords: Glutamate, Epilepsy, Developmental and epileptic encephalopathy

Background

Epilepsy is a common chronic neurological disorder characterized by excessive neuronal firing in the brain leading to recurrent, episodic, and transient dysfunction of the central nervous system. Epilepsy affects nearly 10 million people in China, with an incidence of 7 in 1000 [1]. Seizures are the result of an imbalance between neuronal excitation and inhibition, and glutamate plays a very important role in this process. The Japanese chemist Ikeda Kikunae purified a crystalline substance, called sodium glutamate, from the kelp at the end of the 19th century [2]. Krebs in 1935 demonstrated that glutamate is linked to the tricarboxylic acid cycle [3]. Glutamate and gama-aminobutyric acid (GABA) are excitatory and inhibitory neurotransmitters, respectively, in the brain. In the cerebral cortex, about 70%-80% of neurons are glutamatergic, and the rest are GABAergic [4]. The metabolic pathway of glutamate is as follows (Fig. 1): (1) upon a nerve impulse, glutamate is released from the vesicles in axon terminals into the synaptic cleft; (2) glutamate binds to the postsynaptic glutamate receptors; (3) part of glutamate diffuses rapidly from synapses and is taken up into astrocytes and neurons by excitatory amino acid transporters (EAATs); (4) glutamate in astrocytes is converted to glutamine by the enzyme glutamine synthetase; (5) the glutamine is then shuttled from astrocytes to neurons via glutamine transporters; and (6)

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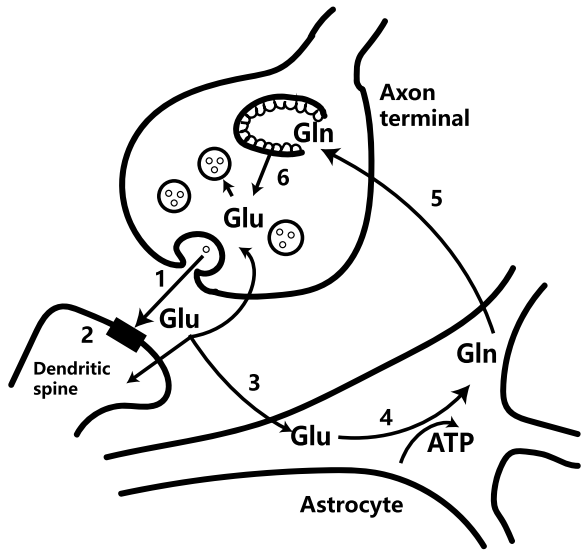

Fig. 1 Illustration of the glutamate–glutamine cycle. (1) When receiving a nerve impulse, glutamate is released from vesicles in the axon terminals into the synaptic cleft. (2) Glutamate binds to postsynaptic glutamate receptors. (3) Part of the glutamate diffuses rapidly from synapses and is taken up into astrocytes and neurons by excitatory amino acid transporters (EAATs). (4) Glutamate in astrocytes is converted to glutamine via the enzyme glutamine synthetase (GS). (5) Glutamine is then shuttled from astrocytes to neurons via glutamine transporters. (6) After entering neurons, glutamine is converted to glutamate by glutaminase (GLS), and glutamate is concentrated in synaptic vesicles via vesicular glutamate transporters, completing the cycle

after entering neurons, the glutamine is converted to glutamate by glutaminase (GLS), and glutamate is concentrated in synaptic vesicles via vesicular glutamate transporters, completing the Glutamate–Glutamine cycle [5]. Olney first proposed in 1986 that epilepsy may be caused by increased neuronal excitability due to altered glutamate function, and the persistently high levels of excitatory neurotransmitters in the brain can produce excitotoxicity that breaks the balance between neuronal excitation and inhibition [6]. Therefore, abnormalities of any step/component of the glutamate metabolism pathway may lead to the development of epilepsy or epileptic encephalopathy. The developmental and epileptic encephalopathies (DEEs) are the most severe type of epilepsy. DEE occurs when the underlying genetic etiology and frequent epileptic activity are both associated with regression or slowing of development. DEE usually begins in infancy or childhood with drug-resistant seizures, epileptiform electroencephalogram (EEG) patterns, developmental slowing or regression, and cognitive impairment. In this article, we present 3 cases with different genetic mutations in the glutamate metabolism pathway, in order to provide a basis for precision diagnosis and treatment of some DEE patients. We also review progresses on the association of genetic variants in the glutamate metabolism pathway with epilepsy.

Methods

Detailed clinical information of the three epileptic patients with genetic variation in the glutamate metabolic pathway was described in this study. Each patient also received EEG recording and magnetic resonance imaging (MRI). Genetic variation in any step of the glutamate metabolic pathway will lead to a loss of balance between excitation and inhibition of neurons, resulting in epilepsy and other abnormalities in the nervous system. Therefore, we systematically reviewed the related literature to better understand the characteristics, types, and mechanisms of this disorder. This study was approved by the Institutional Ethics Committee of the Fourth Military Medical University First Hospital. Informed consent was obtained from all patients in this report.

Results

Patient 1 was a baby girl with DEE41. At the full-term natural delivery, the baby weighted 2.65 kg, with no history of hypoxia at birth and no history of jaundice after birth, but she had hypoglycemia 40 h after birth, with lowest blood glucose level of 1.5 mmol/l. The patient did not respond to external stimuli at that time, and was discharged with a diagnosis of "neonatal hypoglycemia, neonatal intracranial hemorrhage, neonatal septicemia, neonatal hypoglycemic encephalopathy?". The growth and development of the patient lagged slightly behind the age. Under healthy conditions, babies of 2–3 months old can chase the voice and people, look up at 3 months, sit at 6 months, turn over at 6–7 months, climb at 10–11 months, walk alone at 13 months, and speak "Mom and Dad" at 1 year. At age 2, the patient could walk steadily but not run steadily or jump, could eat by herself with a spoon, and say duplicated words. The patient suffered the first attack without any induction at the age of 1 year and 4 months. The patient nodded involuntarily during the awakening period, once or twice a day. One week later, the patient had severe seizures, more than 5–50 times in each group, and 2–3 groups per day, such as nodding, hugging and paroxysmal falls. MRI axial T2 flair images showed abnormal signals around the bilateral lateral ventricles, multiple small polygyri in bilateral occipital lobes, obviously on the posterior horn of the right lateral ventricle. The gray and white matter boundary in the left parietal and temporal lobes was unclear in the sagittal position (Fig. 2a, b). EEG showed medium- to high-amplitude, irregular sharp slow waves and spike slow waves at each electrode on the bilateral

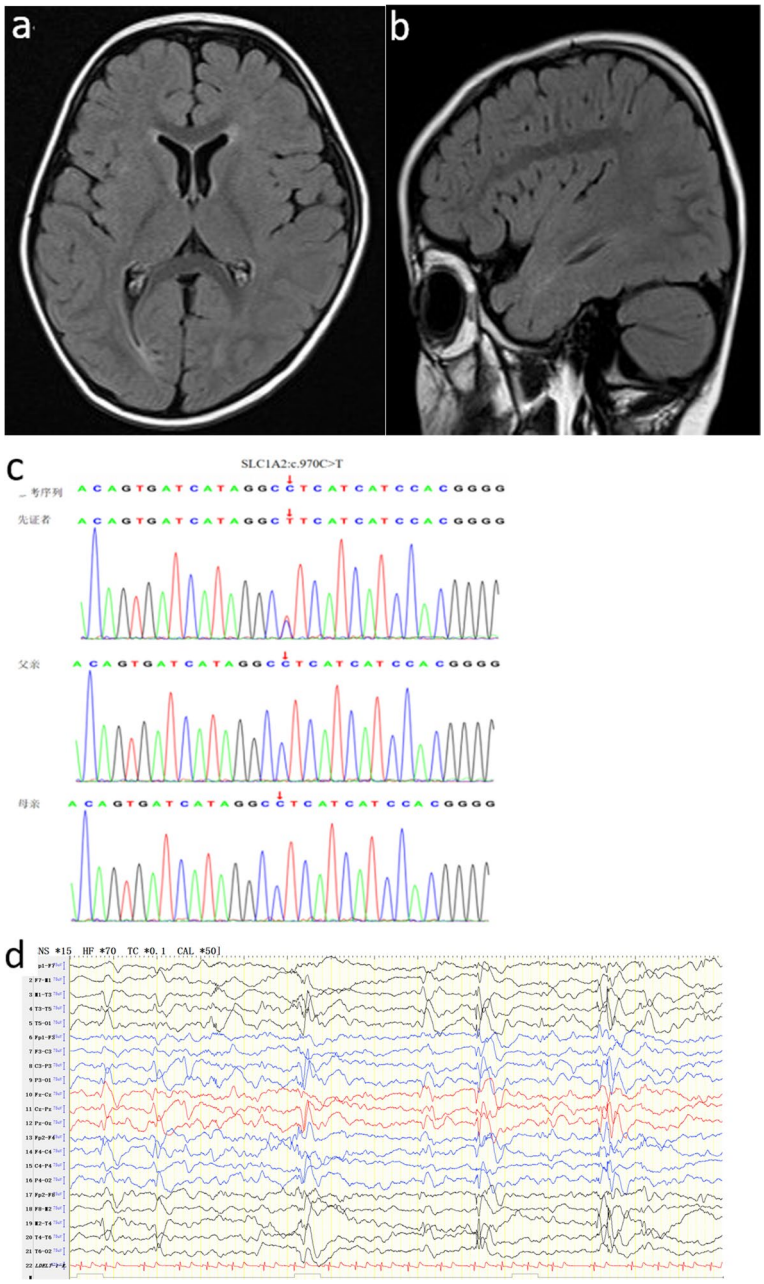

Fig. 2 Brain MRI, EEG and Sanger sequencing of patient 1. **a** Axial T2 flair images showed abnormal signals around bilateral lateral ventricles, multiple small polygyrus in double occipital lobes and obviously on the posterior horn of right lateral ventricle. **b** The boundary of he gray and white matter of the left parietal and temple lobe is unclear in sagittal position. **c** Scalp EEG. Medium- to high-amplitude, irregular sharp slow waves and spike slow waves can be seen in each lead of bilateral hemispheres during the interictal period. **d** Sanger sequencing verified c970C > T de novo mutation of *SLC1A2*, while her parents were wild type at this site

hemispheres during the interictal period (Fig. 2d). Sanger sequencing verified de novo c970C > T mutation of *SLC1A2*, as her parents were wild-type at this site (Fig. 2c). Topiramate effectively controlled the attacks for 20 days, but there were adverse reactions such as blocking of perspiration and loss of appetite. The number of seizures decreased after the start of ketogenic diet, and the current seizures were as follows: nodding, more than 20 times a day; paroxysmal falls, 2–3 times a day.

Patient 2 was a 5-year-old boy carrying *GRIN2A* gene mutation. He was delivered by full-term cesarean section, with no history of birth defects, febrile

convulsions, poisoning, craniocerebral trauma, or central nervous system infection, and no family history of developmental delay. Nervous system physical examination: finger-to-nose test, alternating movement test and heel-knee-tibia test did not show coordination, Romberg sign (+), and the other tests showed no obvious positive signs. Three years ago, the patient suffered transient involuntary tremor of both lower limbs without induction, accompanied by dumping of the body to the right with clear consciousness, and the attacks occurred about 10 times a day. With aggravation of the disease, the tremor extended to both upper limbs and the torso, accompanied by unstable walking and falling. Sanger sequencing verified c3074G > C (pSer1025*) de novo trunked mutation of *GRIN2A* gene, while his parents were wild-type at this site (Fig. 3f). Brain MRI only showed vascular spaces in the centrum semiovale of both hemispheres, with no other structural leisions (Fig. 3e). During the treatment, 4 ml of valproic acid (VPA) oral solution was given twice a day, and there was no relapse thereafter. A year later, EEG recording still showed a large number of interictal epileptic discharges, showing a persistent state of electricity (Fig. 3a–d), and his intelligence was slightly lower than that of the peers, so VPA was changed to LEV and he did not have any attack thereafter. The present prescription is LEV 0.5 g in the morning and 0.75 g in the evening, and vitamin B6 10 mg 2 times a day. The patient had remained attack-free for three years, and the abnormal discharges were significantly reduced.

Patient 3 was a 3-year-old boy with *GRIN2B* gene mutation. At full-term natural delivery, he did not have any birth defects, or a history of febrile convulsion, poisoning, craniocerebral trauma, or central nervous system infection, and did not have a family history. Nervous system physical examination: language growth retardation, motor coordination slightly poor, no cooperation in physical examination. At the age of 2 years and 3 months, the patient presented with generalized seizures and tonic clonus during sleep, which lasted for about 10 min, accompanied by vomiting and incontinence. Nine months later, the patient again suffered a seizure attack during sleep. He learnt to speak and walk later than children of the same age. MRI T2-weighted images showed widening of the septum pellucidum with formation of the fifth and sixth ventricles, a slightly wider median cleft of the brain, and bifrontal gyral atrophy (Fig. 4a). Sanger sequencing verified *GRIN2B* c.870(exon3)del mutation (Fig. 4b). This was a de novo mutation, as the parents were wild-type at this site. EEG showed interictal single or continuous spike slow waves in F7 and T5 (Fig. 4c-d). After OXC treatment, there was no attack again.

Discussion

Glutamate synthesis

Glutamate is generated from hydrolysis of glutamine by glutaminase to remove one ammonia. Glutaminase is specific to the brain and kidney and is enriched in neurons [7]. Glutaminase is encoded by the *GLS* gene, which is located at 2q32.2 and widely expressed in all brain regions since early development, especially in the cerebral cortex and cerebellum [8, 9]. Because neurons cannot re-synthesize glutamate from glucose, they primarily rely on the cycling of glutamine and glutamate between neurons and astrocytes for neural transmission. *GLS* gene mutations can lead to high levels of glutamine and various nervous system dysfunctions, including DEE71, infant cataract, skin abnormalities, psychomotor retardation, and progressive ataxia [10]. Lynne Rumping reported in 2018 that four infants from two unrelated families suffered from early neonatal epileptic encephalopathy with glutaminase deficiency [11]. All of the four infants had intractable early neonatal seizures, status epilepticus, and fatal respiratory failure. The seizures displayed as asymmetric catatonic movement, irregular eye movement, eyelid clonus, upper and lower limb clonus, and myoclonus. EEG recording indicated a continuous burst-inhibition pattern, which can be superimposed with rhythmic α /β activity. MRI showed smaller brainstem, thinning of corpus callosum, cerebral cortical dysplasia, and brain edema. In terms of treatment, there is no known effective treatment, most of the children with this condition die within 1-2 years after birth, and the rest of the surviving children would be in a persistent vegetative state. The researchers found one homozygous truncated mutation, one heterozygous truncated mutation and one missense mutation in the two families. The *GLS* gene deletion mutations cause activity-dependent defects in glutamatergic synaptic transmission, resulting in respiratory dysfunction in affected children. Glutamate promotes signal transduction in the brainstem respiratory center, which regulates the respiratory tidal volume, frequency, and rhythm after birth. As a support for this observation, the *GLS* knockout mice develop respiratory dysfunctions. *GLS* deficiency leads to reduced glutamate release, decreased chemical sensitivity to carbon dioxide, hypoventilation, and a decreased tidal volume. This is consistent with the respiratory phenotype of the affected children, which is characterized by hypoventilation, apnea, and Cheyne-Stokes respiration. The Arg272 plays a key role in stabilizing protein folding, and heterozygous variant of p.Arg272Lys reduces the amount

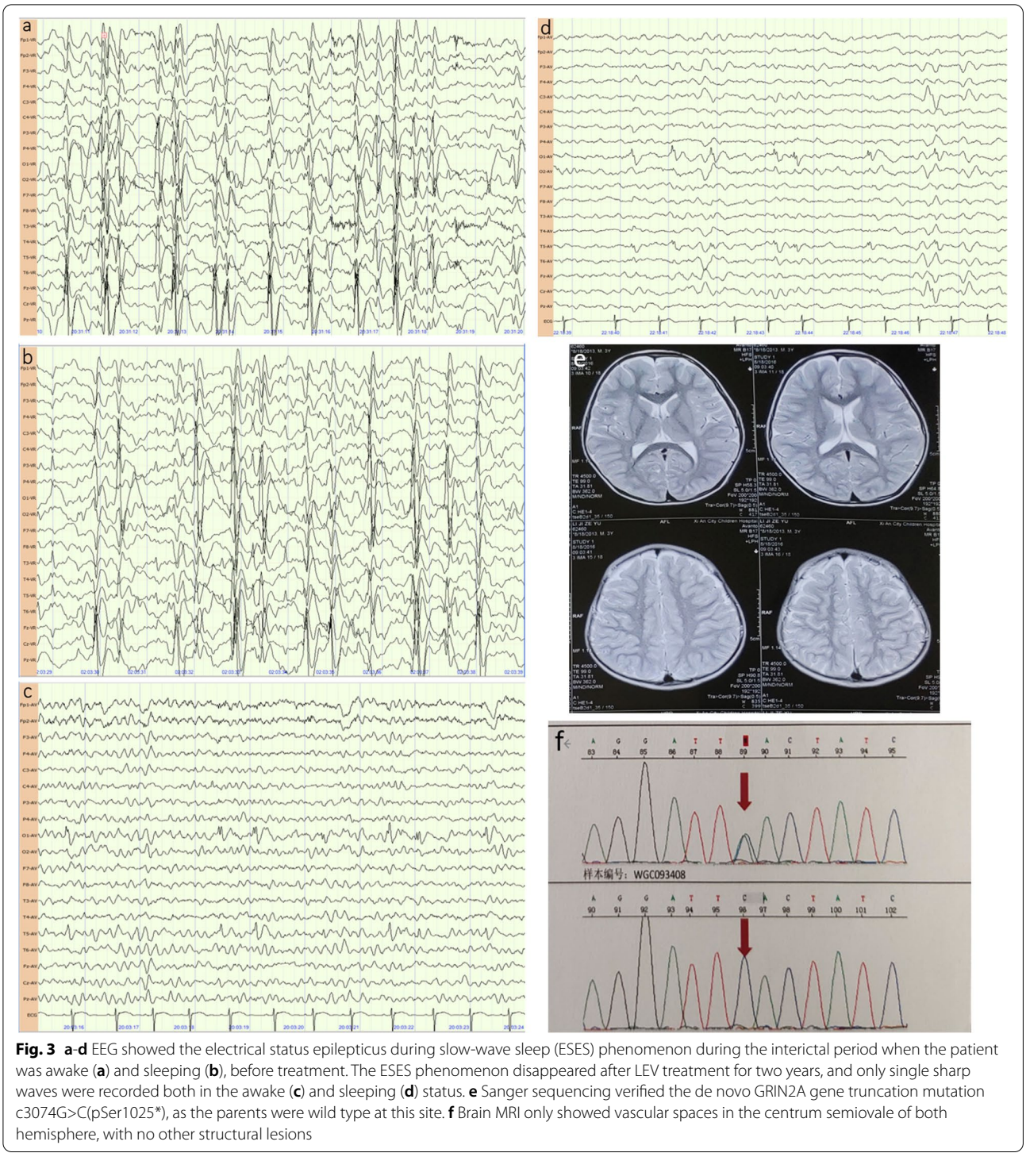

Fig. 3 **a**-**d** EEG showed the electrical status epilepticus during slow-wave sleep (ESES) phenomenon during the interictal period when the patient was awake (**a**) and sleeping (**b**), before treatment. The ESES phenomenon disappeared after LEV treatment for two years, and only single sharp waves were recorded both in the awake (**c**) and sleeping (**d**) status. **e** Sanger sequencing verified the de novo GRIN2A gene truncation mutation c3074G>C(pSer1025*), as the parents were wild type at this site. **f** Brain MRI only showed vascular spaces in the centrum semiovale of both hemisphere, with no other structural lesions

of active glutaminase, resulting in loss-of-function of the glutaminase enzyme [12, 13].

Glutamate can also be synthesized from α-ketoglutaric acid and ammonia by glutamate dehydrogenase (GDH), with NAD and/or NADP working as coenzymes (Fig. 5). This reaction is reversible with consumption of certain amount of ATP [14]. GDH is a crucial regulator of amino acid metabolism in the liver and participates in ammonia metabolism [15]. GDHis encoded by *GLUD1* gene, which is located at 10q23.2, about 45 kb in length and contains

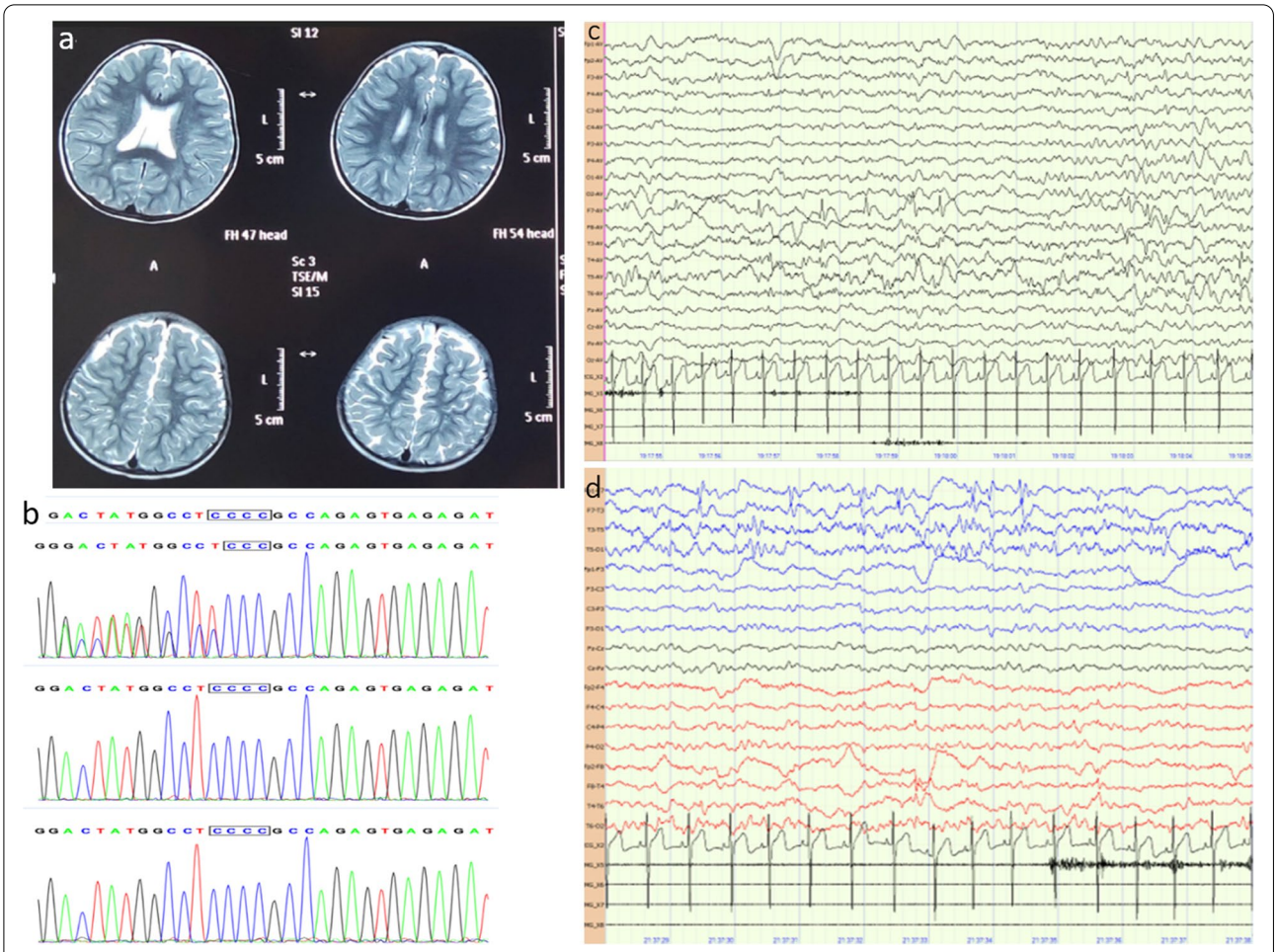

Fig. 4 **a** Brain MRI. Cranial magnetic resonance T2-weighted images showed widening of the septum pellucidum with formation of the fifth and sixth ventricles, a slightly wider median cleft of the brain, and bifrontal gyral atrophy. **b** Sanger sequencing verified the *de novo* GRIN2B c.870(exon3) del, as the parents were wild type at this site. **c**, **d** Scalp EEG. Interictal single or continuous spike slow waves were recorded at F7 and T5 both on average lead (**c**) and bipolar (**d**)

13 exons. The phenotypes of *GLUD1* mutation include hyperinsulinemia-hyperammonemia syndrome and seizures, which show an autosomal dominant inheritance [16–18]. Su et al. reported 26 Chinese cases of congenital hyperinsulinemia in 2018 [19]. The cases had onset age from 1 day to 3 years old, and showed hypoglycemia after protein diet, suggesting increased blood ammonia concentration. Eleven children had psychomotor retardation, and most of them had seizures (23/26) in the forms of myoclonic absence seizures, focal motor seizures, and general tonic-clonic seizures. Diazoxide could raise the blood sugar level, relieve hypoglycemia and other symptoms, and diazoxide dosage could be reduced by limitation of protein intake [20]. The combination of levetiracetam and zonisamide was effective in some patients [16]. Among them, 24 cases were found to carry a new *GLUD1* gene mutation, 2 cases showed dominant inheritance, and all had heterozygous mutations. The speculated causes for epilepsy are recurrent acute cerebral hypoglycemia or chronic hyperammonemia injury. Another possible reason may be that the concentration of neurotransmitters such as glutamine and GABA in the brain decreases due to the increased GDH activity [17]. Increased levels of extracellular glutamate are a characteristic of hippocampal epileptic foci in temporal lobe epilepsy. The activity of GDH in the temporal lobe cortex and hippocampus of patients with refractory temporal lobe epilepsy is significantly decreased, and the activity of GDH is negatively correlated with the time from the first intractable seizure. Significant changes of GDH activity may be one of the reasons for the decrease of glutamate metabolism and extracellular glutamate accumulation [21].

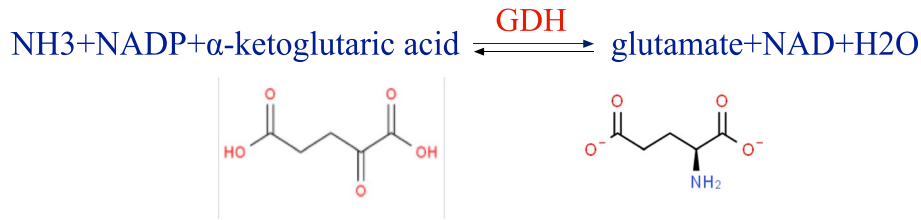

Fig. 5 Reversible reaction of glutamate and a-ketoglutaric acid

Glutamate transport

Glutamate transportation includes synaptic release, exocytosis and the release of cystine-glutamate antiporter. To terminate the action of glutamate and maintain its extracellular concentration below excitotoxic levels, Na^+-dependent high-affinity glutamate transporters (excitatory amino acid transporters, EAATs) located on the plasma membrane of neurons and glial cells rapidly remove glutamate from the extracellular space (Fig. 6). All glutamate transporters are transmembrane proteins belonging to the solute carrier (SLC) family 1, SLC7, SLC17, or SLC25. The SLC1 family consists of seven members, with five members being EAATs and two members being alanine-serine-cystine transporters [22]. Up to now, five glutamate transporters have been found. EAAT1 is highly expressed in the central nervous system. The lack of EAAT1 does not lead to spontaneous seizures, but may increase the duration and severity of seizures. Mutations of the *SLC1A3* gene that encodes EAAT1 are associated with episodic ataxia [23]. EAAT2, the first glutamate transporter isolated in a functional form, is an essential glutamate transporter [24]. EAAT3 is selectively expressed in neurons throughout the central nervous system, with highest concentrations in the hippocampus, followed by the neocortex [25]. EAAT4 is mainly expressed in dendrites and somas of cerebellar Purkinje cells [26]. EAAT5 is mainly expressed in the retina [27]. EAAT1 and EAAT2 are primary transporters for synaptic glutamate uptake to maintain their optimal extracellular level, thereby preventing glutamate accumulation in the synaptic cleft and excitotoxicity, respectively.

The excitatory amino acid transporter EAAT2, encoded by the *SLC1A2* gene located at 11p13, is mainly expressed in astrocytes and responsible for 95% of the glutamate uptake in the mammalian brain. *SLC1A2* gene mutations cause DEE41 with autosomal dominant inheritance [28]. A *SLC1A2* knockout mouse model shows fatal spontaneous seizures and progressive neuronal death due to excitotoxicity [29, 30]. Guella et al. reported in 2017 two cases with *SLC1A2* mutations [31], with phenotypes

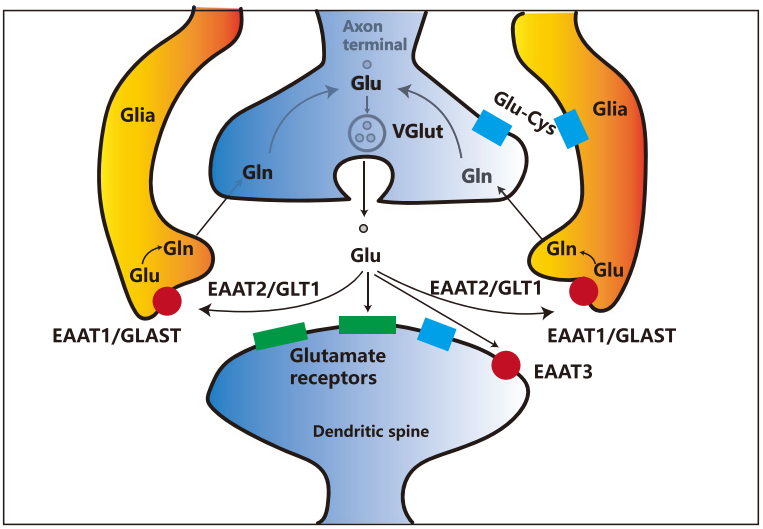

Fig. 6 Role of glutamate transporters at glutamatergic synapases. The glutamate transportation includes synaptic release, extrasynaptically by exocytosis and the release of cystine-glutamate antiporter.To terminate the action of glutamate and maintain its extracellular concentration below excitotoxic levels, Na + -dependent high affinity glutamate transporters (EAATs)located on the plasma membrane of neurons and glial cells rapidly remove glutamate from the extracellular space. *Gln* Glutamine, *Glu* Glutamate, *VGlut* Vesicular glutamate transporter

including early-onset epilepsy and severe developmental delay. Patients mainly present with seizures in the first week of life, including tonic and myoclonic seizures. EEG showed frequent bilateral parietal median spikes and sharp waves, mixed generalized sharp waves, a background of slow rhythm disturbances, and multifocal epileptiform discharges. MRI showed atrophy and abnormalities of the cerebral white matter and basal ganglia, and the atrophy may be partly due to the glutamate-induced excitotoxicity. In treatment, if it is determined that the mutation leads to haploinsufficiency, earlier initiation of SLC1A2-modulating agents may be efficacious to overcome the cumulative dominant negative effects of the variant SLC1A2 allele [32]. Ketogenic diet can reduce the attacks in patients. In addition, overexpression of *SLC1A2* prevents neuronal loss, decreases astrocyte proliferation, and reduces inflammation in the hippocampus and the extent of seizures [33].

Glutamate receptors

Glutamate receptors can be divided into the ionic type and the metabolic type (Fig. 7). The ionic type of glutamate receptor includes *N*-methyl-*D*-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptors, and kainite (KA) receptors. The metabolic glutamate receptors (mGluRs) function through G-protein coupling and are divided into three categories according to their coupled secondary messenger system and pharmacological action: the first category of metabolic glutamate receptors (mGluR1 and mGluR5) that activate phospholipase C, the second category of metabolic glutamate receptors (mGluR2 and mGluR3) that inhibit adenylate cyclase, and the third category of metabolic glutamate receptors (mGluR4, mGluR6, mGluR7 and mGluR8) [34].

NMDA receptors

Soto discovered many disease-related glutamate receptor mutations in 2014, of which more than 80% were found in the NMDA receptor subfamily [35]. The NMDA-selective glutamate receptor is a tetrameric complex composed of two glycine-bound GluN1 and two glutamate-bound GluN2 subunits. One gene encoding the GluN1 subunit (*GRIN1*), four genes encoding the GluN2 subunit (*GRIN2A-D*), and two genes encoding the GluN3 subunit (*GRIN3A-B*) endow the receptor with multiple types of subunits with distinct functional and pharmacological properties [36]. NMDA receptor mediates a slow calcium-permeable synaptic current. Due to the blockade of the extracellular Mg^{2+} channel, the current is voltage-dependent and participates in the development of the central nervous system. Overactivation of the NMDA receptor can promote seizures and cause cell death [37].

The *GRIN1* gene is located at 9q34.3, which encodes the GluN1 subunit of NMDA receptor. The *GRIN1* gene mutation-related diseases are autosomal dominant or recessive inherited. Lemke et al. identified *GRIN1* heterozygous mutations in 14 individuals in 2014 and reviewed the phenotypes of all nine previously reported patients [38]. These 23 patients showed different phenotypes such as general psychomotor retardation, epilepsy, hypodystonia, hyperactivity disorder, and extensive brain atrophy. Various types of seizures were shown by these patients, including infantile spasm, tonic seizures, ADHD seizures, focal seizures, febrile seizures, generalized seizures, and status epilepticus. EEG indicates

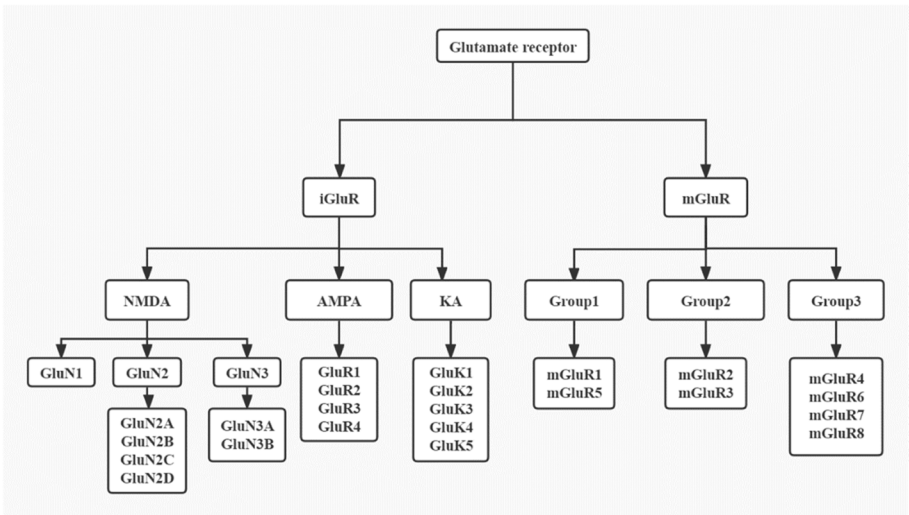

Fig. 7 Classification of glutamate receptors

focal, multifocal, and systemic spikes. MRI showed brain atrophy, enlargement of lateral ventricle, and thinning of the corpus callosum. Five patients (31%) had refractory epilepsy. Two patients had complete remission of seizures based on sodium valproate (VPA), and two additional patients responded well on a combination of topiramate, levetiracetam, and clobazam or the introduction of vigabatrin and clonazepam in addition to VPA. All the newly reported mutations were missense mutations. *GRIN1* deletions as well as truncation or splice-site mutations are seen in the control database, which indicates that the *GRIN1* gene is haploinsufficiency, and the loss of NMDA receptor function may be a potential disease mechanism. Ohba et al. speculated that the *GRIN1* mutations cause hyperkinesia and dyskinesia via disorders of monoamine neurotransmitters [39]. Extrapyramidal symptoms common in patients overlap with the clinical features of monoamine metabolic disorders, and it is speculated that the carriers have Rett syndrome, displaying typical stereotyped hand movements, and other features such as bruxism, sudden crying and laughter, hyperventilation, and sleep disorders. These findings suggest that the clinical features caused by *GRIN1* mutations overlap with epileptic encephalopathy, monoamine neurotransmitter disorders, and Rett syndrome. Therefore, *GRIN1* mutations may affect the function of NMDA receptors and D1 receptors, and lead to abnormalities. Based on this hypothesis, the extrapyramidal symptoms caused by *GRIN1* mutation may be alleviated by drugs for Parkinson's disease, and the impairment of NMDA receptor function may also be involved in the pathogenesis of Rett syndrome.

The *GRIN2A* gene is located at 16q13.2 and encodes the GluN2 subunit of the NMDA receptor, which contains 14 exons. The *GRIN2A* gene mutation-related diseases are autosomal-dominant inherited. The first potentially pathogenic mutation in NMDA receptors was described in 2010 by Endele in *GRIN2A* (N615k) [40], a mutation that abolishes the voltage-dependent block of Mg^{2+}, thereby increasing the current flow of NMDA receptors upon activation at standard resting membrane potentials. This may lead to aberrant excitation and possibly neuronal loss, resulting in the clinical symptoms such as epilepsy. *GRIN2A* mutant-related epilepsy phenotypes include slow-wave sleep syndrome, epileptic encephalopathy, Landau Kleffner syndrome (LKS), and Rolandic epilepsy. *GRIN2A* mutations are highly associated with epileptic aphasia disorders, including LKS, epilepsy with continuous spikes and waves during slow-wave sleep, and intermediate epileptic aphasia disorder (IEAD) [41, 42]. Therapeutically, in 2014, Pierson reported a patient, in whom the frequency of seizures was reduced from over 11 per week to 3 per week after addition of the NMDA receptor antagonist memantine to VPA treatment, accompanied by improvement in EEG and motor function, suggesting that the cause of seizures involves excessive NMDA receptor excitation [43]. Venkateswaran et al. reported in 2014 a patient who showed significantly decreased duration and frequency of attacks within one month after addition of topiramate, from a dozen attacks to 1-2 attacks per day [44]. Although the exact mechanism for the antiepileptic properties is unknown, it is speculated that topiramate may enhance GABA-induced currents and inhibit sodium and calcium channels. Felbamate, levetiracetam and clobazam all target the glutamate pathway and improve seizures [45].

GRIN2B gene is located on chromosome 12p13.1 and encodes the GluN2 subunit of the NMDA receptor, which contains 13 exons. The phenotypes of *GRIN2B* mutation are DEE27 and mental retardation, which show autosomal dominant inheritance. Platzer et al. reported 58 patients with *GIRN2B* mutations [46], who suffered epilepsy, neurodevelopmental disorders and a spectrum of hypotonia, movement disorder, cortical visual impairment, cerebral volume loss and malformation of cortical development. About half of the patients had seizures, ranging in age from birth to 9 years old, and the frequency of seizures ranged from multiple seizures a day to several times a year. The patients showed generalized seizures, mostly ankylotic or tonic clonus, focal seizures, and/or epileptic spasms. The EEG patterns included hypsarrhythmia, as well as focal, multifocal and/or generalized epileptiform activity. All of the patients had developmental delay, most of them developed severe intellectual disability, and one-fourth of the patients had autism. Less common findings in DEE27 include generalized cerebral volume loss, cortical visual impairment, hyperkinetic movement disorders (dystonia, dyskinesia, chorea) and developmental regression. These characteristics have been repeatedly observed in other epileptic encephalopathies [38, 47], suggesting a common phenotypic spectrum, and these differences may reflect different categories, locations, and roles of different NMDA receptor subunits. MRI of 6 patients showed cortical dysplasia, hypoplastic corpus callosum of varying degrees, enlarged and mildly dysplastic basal ganglia, hippocampal dysplasia, and brain atrophy. Genetic mutations enhance the function of NMDA receptors, which may lead to excitotoxic cell death, changes in neuronal migration, or a persistent increase in excitatory synapses and non-synaptic drives through surface receptors, resulting in clinical symptoms [48]. In terms of treatment, memantine can reduce the NMDA receptor overactivity caused by these mutations, improve consciousness, behavior and sleep, with individual differences in the control of frequency of seizures.

The *GRIN2D* gene is located at 19q13.33 and encodes the GluN2 subunit of the NMDA receptor [49]. The phenotype is DEE46, which shows autosomal dominant inheritance. XiangWei reported six new *GRIN2D* variants and one previously-described disease-associated *GRIN2D* variant in DEE patients [50]. Of the 9 cases with available epilepsy data, 44% (4/9) patients had epilepsy as their initial symptom, and most of the patients had seizures within one-year-old. All patients had general growth retardation, some had low intraocular pressure and dyskinesia, three had autistic behavior, and one showed ADHD symptoms. The results of EEG could be divided into two groups, focal spike-wave in 56% (5/9) and high-amplitude dysrhythmia in 44% (4/9). The types of seizure include focal seizures, atypical absence seizures, and tonic or catatonic seizures. MRI revealed cortical atrophy, white matter volume reduction, and lateral ventricle enlargement. In terms of treatment, most patients were medically refractory, but the seizures disappeared or were reduced after multiple antiepileptic drugs and/or NMDAR-targeting combination therapy (memantine, intravenous immunoglobulin, oral steroids, and magnesium) [51, 52].

The structure of GluN3 is most similar to that of GluN1. Both have an extracellular domain containing a glycine- or *D*-serine-binding site, and their carboxyl termini are also shorter than that of GluN2 subunits [53]. According to the location and temporal expression, GluN3 can be divided into Glu3A and Glu3B. The temporal expression of GluN3B in the mouse forebrain is almost the opposite to that of GluN3A. The GluN3B expression is at a low level in the early stage of development and increases steadily from 7 to 14 days after birth to adulthood. GluN3A and GluN3B have 1115 and 901 amino acids, respectively. GluN3A is widely distributed in the central nervous system, including neocortex, hippocampal CA1 region, olfactory bulb, cerebellum, amygdala, thalamus, hypothalamus and brainstem nuclei [54]. GluN3B is mainly expressed in motor neurons of brainstem and spinal cord, and also at a low level in hippocampus, cerebral cortex, striatum, nucleus accumbens and cerebellum [55]. GluN3 plays a role in regulating synaptic maturation and stabilization, regulating neuronal activity, participating in presynaptic NMDA receptor-mediated glutamate release, and affecting oligodendrocyte maturation.

AMPA receptors

AMPA-selective glutamate receptor is a tetramer composed of GluA1-4 subunits encoded by GRIA1-4 genes. It interacts with various helper proteins, such as transmembrane AMPA receptor regulatory protein, and locates at the postsynaptic site. AMPA receptor-mediated conductance is the basis for most excitatory synaptic signals in the central nervous system, usually within milliseconds, as glutamate rapidly dissociates from AMPA receptors and is removed from synaptic spaces through diffusion and active transport [56].

GRIA2 gene is located on chromosome 4q32.1 and encodes the GluA2 subunit of the AMPA receptor. The phenotype of *GRIA2* mutation is the impaired neurodevelopment with language disorders and behavioral abnormalities, showing an autosomal dominant inheritance. Salpietro reported heterozygous mutations in *GRIA2* in 28 patients with mental disability and neurodevelopmental disorders (including autistic spectrum disorders, Rett syndrome features, and unrelated seizures or developmental epileptic encephalopathy) in 2019 [57, 58]. Among them, 12 patients usually began seizures or DEE within six months after birth, including infantile spasm, tonic-clonus, myoclonus, and focal seizures. EEG showed multi-spike waves, slow peak slow-waves, and bilateral temporal lobe asynchronous epileptic activity. MRI showed progressive (mainly) cerebellar atrophy and partial white matter abnormalities in some patients. A total of 20 different *GRIA2* mutations were found, including 15 missense mutations, two splice-site mutations, one frame deletion, one nonsense mutation, and one frameshift mutation. Mutations lead to a decrease in the inhibitory current mediated by the mutant subunit, most *GRIA2* mutations will decrease the current amplitude, and some will affect the voltage rectifiers. *GRIA2* de novo mutation and microdeletion of *GRIA2* are the causes of neurodevelopmental disorders and DEE. GluA2 subunits regulate Ca^{2+} penetration and voltage rectifier of AMPA receptors, thus playing an essential role in synaptic plasticity as well as brain development and function. Due to the decrease of AMPA receptor function and the increase of Ca^{2+} permeability, it is worth studying whether drugs targeting AMPA receptors would improve clinical outcomes in patients [59].

GRIA4 gene is located on chromosome 11q22.3 and encodes the GluA4 subunit of the AMPA receptor. The phenotype of *GRIA4* mutation is the neurodevelopmental disorder with or without epilepsy and gait abnormality, which shows autosomal dominant inheritance. In 2017, Martin et al. found a novel heterozygous variant in *GRIA4* in 5 unrelated individuals with mental disability and other symptoms: c.1915A > T (p.Thr639Ser), c.1921A > G (p.Asn641Asp), c.1928C > G (p.Ala643Gly), c.1931C > T (p.Ala644Val), and c.2090G > C (p.Arg697Pro) [60]. Four of the five affected individuals had seizures or abnormal EEG, with different symptoms including high muscle tension, severe spastic quadriplegia, and high tension with contracture. Brain MRI showed extensive

symmetrical atrophy of the bilateral frontal lobes, mild ventricle enlargement, and optic nerve hypoplasia of the corpus callosum. Four variants are located in the highly conserved motif of transmembrane protein M3, and the fifth variant is located in the extracellular domain. Molecular simulation of the changed protein shows that the three variants in the motif are located in the center of the pore region, which is likely to lead to the disorder of the gating mechanism. The fourth variant in the motif is most likely to lead to a decrease in permeability. The variant in the extracellular region may interfere with the binding between monomers, resulting in clinical symptoms such as epilepsy. Beyer et al. found that mice with *GRIA4* mutations resulting in decreased or complete loss of function show persistent spike discharges on EEG, which may be related to absence epilepsy [61].

KA receptors

KA receptor is a tetramer ionic glutamate receptor composed of five subunits GluK1-GluK5. KA receptors are structurally related to AMPA receptors, but they regulate the activity of presynaptic and postsynaptic circuits through ionization or metabolism, thus playing different functions. Kainic acid is a potent neurotoxin that can induce acute seizures by activating KA receptors. The subunits GluK2/GluK5 may be associated with recurrent seizures of temporal lobe epilepsy [62].

Metabotropic glutamate receptors

According to the coupling and pharmacological characteristics of the second messenger system, the metabotropic glutamate receptors can be divided into three groups. The first group of mGluR includes mGluR1 and mGluR5, which are Gq-coupled receptors. They are mainly located on postsynaptic neurons and astrocytes. Intracellular Ca^{2+} release is thought to cause glutamate release from astrocytes and induce excitatory postsynaptic potentials of neurons. The second group of mGluR includes mGluR2 and mGluR3, which are coupled to Gi/o. They are usually located presynaptically, and the activation of these receptors leads to the inhibition of neurotransmitter release. The third group of mGluR includes mGluR4, 6, 7, and 8, and they are located on neurons, also coupled to Gi/o, and are essentially inhibitory [63, 64].

mGluR5 is overexpressed in mouse epileptic models. Selective knockout of astrocytic mGluR5 during epilepsy slows down glutamate transporter clearance, indicating that mGluR5 plays an important role in regulating these transporters during epilepsy. The level of mGluR5 in patients with temporal lobe epilepsy is increased, and the expression of mGluR5 is correlated with the frequency of seizures. The low expression of mGluR5 is negatively correlated with the frequency and duration of seizures [65].

The *GRM7* gene encoding mGluR7 is located on chromosome 3p26.1, containing nine exons. The phenotype of *GRM7* mutation is the neurodevelopmental disorder with epilepsy, low intraocular pressure and brain abnormality, and shows autosomal recessive inheritance [66, 67]. mGluR7 is a highly conserved type of mGluR, expressed only in the central nervous system. mGluR7 plays a key role in synaptic transmission by inhibiting the further release of excitatory neurotransmitter glutamate. Spontaneous irritant seizures occur in mGluR7 knockout mice, suggesting that the interruption of *GRM7* expression may lead to epilepsy. Marafi reported 11 *GRM7* gene mutations from 6 unrelated families in 2020. The new pathogenic mutations identified included two homozygous missense mutations (c.2671G > A:p.Glu891Lys and c.1973G > A:p.Arg685Gln) and one homozygous nonsense mutation (c.1975C > T:p.Arg659Ter) [68]. The patients were characterized by growth retardation, seizures in newborns or infants, and microcephaly. Three patients had hypothalamus-pituitary axis dysfunction, and five patients died in childhood. MRI showed brain atrophy and myelin reduction in most cases. In terms of treatment, the selective positive allosteric modulator (PAM) of type III mGluR may represent a new targeted and individualized therapy. PAM is a non-competitive agonist that binds to sites other than the ligand-binding site to enhance its effect. N, N ’-diphenylethane-1, 2-diamine dihydrochloride (AMN082) is the first found selective PAM for mGluR7 [69].

Pathway of glutamate inactivation

Glutamate is utilized in the formation of many products in the body, such as glutamine catalyzed by glutamine synthetase, synthesis of GABA, combined deamination to α-ketoglutaric acid, synthesis of proteins, cyclic metabolism of tricarboxylic acid, and gluconeogenesis to produce glucose. Glutamine synthetase, encoded by the *GLUL* gene, catalyzes the connection of ATP-dependent glutamate and ammonia to glutamine and is the only source of endogenous glutamine that is necessary for many critical metabolic and developmental pathways [70]. Glutamine is involved in cell proliferation, apoptosis inhibition, and cell signal transduction. *GLUL* gene is located on chromosome 1q25.3 and contains six exons with a molecular weight of 42 kb. The phenotype of *GLUL* mutation is glutamine synthetase deficiency, characterized by systemic glutamine deficiency, persistent moderate hyperammonemia, violent clinical convulsions, and multiple organ failure shortly after birth, and shows autosomal recessive inheritance.

In 2005, Haberle et al. reported three unrelated patients of congenital systemic glutamine synthetase deficiency caused by *GLUL* mutations [71]. All patients showed brain malformations and epilepsy, including 1 with multifocal seizures, 2 with generalized tonic clonic seizures, and two with multiple organ failure, resulting in death within the first month after birth. One patient survived with low intraocular pressure, lower limb hyperreflexia, clonus, generalized seizures, gaze, and generalized tonic–clonic seizures with severe growth retardation, and necrotizing erythema at the age of three years and two months. Biochemical examinations showed that the levels of glutamine in serum and cerebrospinal fluid decreased, the glutamate level was slightly decreased, and hyperammonemia appeared. EEG showed multifocal sharp waves. MRI suggests severe brain atrophy caused by leukopenia, which leads to thinning of the corpus callosum. All three known mutations affect the active site of *GLUL*, and it can be speculated that early death is caused by protein instability and degradation caused by the mutations. In 2012, Haberle conducted a therapeutic trial of enteral and parenteral glutamine supplementation in surviving patients [72], which increased the plasma and cerebrospinal fluid levels of glutamine. It is suggested that glutamine supplementation can counteract the imbalance between glutamate and GABA that occurs in some forms of epilepsy, successfully increase the supply of glutamine in the whole body, and stimulate cell metabolism and maintain normal functions of cell metabolism. Glutamine supplementation should be started as early as possible, and maternal glutamine supplementation during pregnancy may be beneficial for a child with glutamine synthetase deficiency and should be considered [73]. Ünal et al. reported the fourth case of congenital glutamine synthetase deficiency in 2019 [74]. The patient was seizure free within five months of VPA and aminohexenoic acid treatment, and her biochemical indexes were improved at 6-month follow-up with *L*-glutamine and nicotinamide treatment.

Conclusions

Glutamate is a very important excitatory amino acid neurotransmitter. Genetic variations in any step of the glutamate metabolic pathway will lead to a loss of balance between neuronal excitation and inhibition, resulting in epilepsy and other abnormalities in the nervous system. Identification of DEEs caused by these single gene mutations can provide clues for further precision diagnosis and treatment (Table1), such as diazoxide for *GLUD1* mutation-related DEE, memantine

Table 1 Genetic variations in the pathway of glutamate metabolism and related phenotypes

Pathway	Gene name	Phenotype	Inheritance	Treatment	Prognosis
Glutamate synthesis	*GLS*	DEE71, DD, ataxia	AR, AD	Unknown	DRE, DD
	GLUD1	Hyperinsulinism-hyperammonemia syndrome	AD	diazoxide, ASM (LEV, ZNS)	DRE, DD
Glutamate transport	*SLC1A2*	DEE41	AD	EAAT2 translation, ketogenic diet	Unknown
	EAAT1, 3, 4, 5				
Glutamate receptors	*GRIN1*	Neurodevelopmental disorder with or without hyperkinetic movements and seizures	AD, AR	ASM (VGB, VPA, TPM, LEV, CBZ, CZP)	DRE, DD
	GRIN2A	Focal epilepsy with speech disorder, intellectual disability	AD	MAT, ASM (TPM, CBZ, FBM, LEV)	DRE, DD
	GRIN2B	DEE27, intellectual disability	AD	MAT, OXC	DRE, DD
	GRIN2D	DEE46	AD	MAT, intravenous immunoglobulin, oral steroids, and magnesium	DRE, DD
	GRIA2	Neurodevelopmental disorder with language impairment and behavioral abnormalities	AD	AMPA receptor inhibitors (perampanel)	Unknown
	GRIA4	Neurodevelopmental disorder with or without seizures and gait abnormalities	AD	Unknown	Unknown
	GRM7	Neurodevelopmental disorder with seizures, hypotonia, and brain abnormalities	AR	PAM	DRE, DD
Glutamate metabolism	*GLUL*	Glutamine deficiency	AR	Glutamine	Effective drug control

Abbreviations: *ASM* antiseizure medications, *AR* autosomal recessive, *AD* autosomal dominant, *CBZ* carbamazepine, *CZP* clonazepam, *CBZ* carbamazepine, *DD* developmental delay, *DRE* medically refractory epilepsy, *DEE* developmental epileptic encephalopathy, *EAATs* excitatory amino acid transporters, *FBM* felbamate, *KA* kainite, *LEV* levetiracetam, *MAT* memantine, *OXC* oxcarbazepine, *PAM* positive allosteric modulator, *TPM* Topamax, *VPA* valproic acid, *VGB* vigabatrin, *ZNS* zonisamide

for NMDA receptor mutation-related DEE, AMPA receptor antagonists (pyropanide, amparkin, etc.) for AMPA receptor mutation-related DEE, and glutamine for *GLUL* mutation-related DEE. Much preclinical and clinical evidence supports the potential of glutamatergic signaling as the therapeutic target for epilepsy, leading to the precise treatment of DEE related with *SLC1A2, GLS, GRM7* and other genetic mutations. There are also novel genetic mutations related to DEE that are waiting to be discovered in the glutamate metabolic pathway.

Abbreviations

AMPA: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic; ASM: Antiseizure medications; AR: Autosomal recessive; AD: Autosomal dominant; CBZ: Carbamazepine; CZP: Clonazepam; CBZ: Carbamazepine; DD: Developmental delay; DRE: Medically refractory epilepsy; DEE: Developmental epileptic encephalopathy; EAATs: Excitatory amino acid transporters; FBM: Felbamate; ID: Mild mental retardation; KA: Kainate; GABA: Gamma-aminobutyric acid; Gln: Glutamine; Glu: Glutamate; GS: Glutamine synthetase; GLS: Glutaminase; GLUL: Glutamate ammonia ligase; GDH: Glutamate dehydrogenase; LEV: Levetiracetam; MRI: Magnetic resonance imaging; MAT: Memantine; NMDA: N-methyl-D-aspartate; OXC: Oxcarbazepine; PAM: Positive allosteric modulator; TGB: Tiagabine; TPM: Topamax; VPA: Valproic acid; VGB: Vigabatrin; ZNS: Zonisamide.

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Authors' contributions

YF and YD conceived the article and wrote the manuscript. ZW, CL, GL, YG and CZ reviewed and edited the manuscript. CZ collected information of some of the cases. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of Fourth Military Medical University First Hospital (KY20182057-F-1). Informed consent was obtained from all patients in this report.

Consent for publication

All authors and patients agreed on publishing of the study.

Competing interests

Corresponding author YD is the editorial board member for ***Acta Epileptologica.*** YD was not involved in the journal's review of, or decisions related to this manuscript.

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