Genes & Diseases

FULL LENGTH ARTICLE

Genetic diagnosis of neonatal-onset seizures

Xueling Ma [a,b,e], Fengzhu Yang [a,b,e], Ziyu Hua [a,b,c,d,*]

[a] The Department of Neonatology, Children's Hospital of Chongqing Medical University, Chongqing, 400014, China
[b] Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing, 400014, China
[c] Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Chongqing, 400014, China
[d] Chongqing Key Laboratory of Child Infection and Immunity, Chongqing, 400014, China
[e] National Demonstration Base of Standardized Training Base for Resident Physicians, Chongqing, 400014, China

Abstract Many seizures in neonates are due to early-onset epilepsy, which is often difficult to diagnose, especially to explore the causes. Recently, the development of next-generation sequencing (NGS) has led to the discovery of a large number of genes involved in epilepsy. This may improve prompt detection of early-onset epilepsy in neonates. This study aimed at analyzing the genotype-phenotype correlations in neonates with seizures in a bid to improve the understanding of genetic diagnosis of early-onset epilepsy. Clinical features and prognosis of 15 children who underwent genetic testing having had unexplained seizures from February 2016 to May 2018 in Children's Hospital of Chongqing Medical University were analyzed retrospectively. The salient findings were: poor response to stimulus and abnormal electroencephalogram (EEG) in the initial period were observed in the group with concomitant genetic abnormalities. Despite the recent progress in genetic technology, molecular diagnosis for neonatal-onset epilepsy can be challenging due to genetic and phenotypic heterogeneities. However, some genotypes are associated with specific clinical manifestations and EEG patterns. Therefore, in-depth understanding of genotype-phenotype correlations would be useful to clinicians managing neonates with early-onset seizures.

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* Corresponding author. The Department of Neonatology, Children's Hospital of Chongqing Medical University, Chongqing, 400014, China.
E-mail address: h_ziyu@126.com (Z. Hua).

Introduction

Seizures are a common phenomenon in the neonatal period, which are attended by abnormal over-discharges from a group of neurons, resulting in sudden and temporary brain dysfunction. The causes are diverse and the clinical manifestations variable. Epilepsy is a common paroxysmal disease in the nervous system that presents with seizures. In children, it is often injurious to the brain and it may cause irreversible mental retardation or even threaten children's lives. Approximately 13% of neonatal-onset seizures are due to epilepsy.[1] The clinical manifestations of neonatal epilepsy are usually not specific which makes it difficult to diagnose early. In addition, when compared to other childhood epilepsy syndromes, epilepsy in the neonatal period is usually difficult to control and has a poor prognosis. Common neonatal-onset epilepsy syndromes recognized by the International League Against Epilepsy (ILAE) include: benign familial neonatal epilepsy (BFNE) which often has good outcomes. Other examples are early-onset epileptic encephalopathies (EOEEs) and early-onset myoclonic encephalopathy (EME), which have poor outcomes.[2]

In recent years, with the development of NGS, a large number of genes involved in early-onset epilepsy have been discovered. These have been shown to lead to cortical dysplasia, metabolic abnormalities, ion channel dysfunctions and so on.[3] In a recent report, 83% of newborns with early-onset epilepsy had genetic aetiologies.[1] The study by Yang et al also confirmed that about 28% seizures had pathogenic genes, and some of them had comorbidities such as developmental delay.[4]

However, molecular diagnosis and precise treatment for neonatal epilepsy is still challenging due to genetic and phenotypic heterogeneities. For example, mutations in KCNQ2 are associated with self-limited early-onset epilepsies and neonatal-onset epileptic encephalopathies as well.[5] As a result, when talking about the correlation between genotypes and phenotypes, the locus heterogeneity and variable expressivity are needed to be considered. This implies that the same clinical syndrome may be caused by variants in different genes. Meanwhile, pathogenic variants in the same gene may lead to different epileptic phenotypes in different individuals. Although genotypes do not have a one-to-one correspondence with phenotypes, we can anticipate that certain genotypes are usually associated with some specific clinical manifestations and EEG patterns. In term infants, the most common type of gene-induced seizures are sequential seizures, followed closely by tonic seizures, while in preterm infants, electrographic seizures only are commonly observed. Additionally, metabolic diseases often manifest as myoclonic seizures.[6,7]

The aim of this study is to analyze the genotype-phenotype correlations in neonates with unexplained seizures and compare the clinical differences between the group with gene abnormalities and those without. This is done in a bid to improve clinicians' understanding of genetic diagnosis for early-onset epilepsy.

Subjects and methods

Subjects

Patients who underwent genetic testing for unexplained seizures within 28 days after birth from February 2016 to May 2018 at Neonatal Care Unit in Children's Hospital of Chongqing Medical University were recruited. Some neonates were excluded if they had a diagnosis of a central nervous system infection, hypoxic-ischemic encephalopathy or other definite causes leading to symptomatic convulsions. This study was approved by the Institutional Review Board.

Methods

All blood samples of these children and their parents were collected in the neonatal department of Children's hospital and sent to one of the four professional gene companies in Beijing (Beijing Mackinaw, Joy Orient, Beijing Fuyou Longhui and Deyi Oriental) depending on the preference of the family. Medical records were reviewed to evaluate the initial condition of the children. Follow up data related to disease progression and outcomes were obtained from their families via phone. Basic information including gestational age (GA), birth weight, and family history were recorded. Initial clinical manifestations, laboratory test and image results and follow-up clinical details after discharge were also recorded. All patients were followed up until October 2018 except for those who died before then.

The patients were then divided into two groups based on whether pathogenic genes with close correlation with epilepsy were detected. The differences between the two groups were analyzed. Statistical analysis was performed with SPSS software 23.0. Categorical variables were assessed using the *Chi-square* test and continuous variables were assessed by the *Student-t* test, with a two-tailed *P*-value of less than 0.05 considered significant.

Results

Patient characteristics

This study included 15 children with unexplained seizures hospitalized in the neonatal care unit from February 2016 to May 2018. All samples obtained from these patients underwent epilepsy-related gene test (full exon sequencing or epilepsy gene package testing). Gestational age, birth weight, seizures onset time, and age of genetic testing are shown in Table 1.

Genes and clinical features

In the current study, 12/15 (80.0%) children were detected to carry mutated genes closely associated with epilepsy. Four of these children died within 1 month after discharge from hospital. Two of them had uncontrollable seizures

Table 1 Basic information of the samples included.

	Range	Median	Mean ± SD
Gestational age (weeks)	35^{+4}–41^{+3}	39^{+3}	39.320 ± 1.320
Birth weight (grams)	2580–4000	3500	3486.670 ± 419.279
Seizures onset age (days)	1–16	2	5.200 ± 5.583
Gene test age (days)	5–51	24	23.267 ± 12.464

before death. Thirteen children were diagnosed with epilepsy. Among them, 7 (58.3%) were considered to have intractable epilepsy. Three variants in KCNQ2 were detected, 2 of them were retrospectively diagnosed with BFNE, since they had a similar clinical presentation in the neonatal period. They both had frequent stereotyped generalized seizures (more than 10 times/day) initially and relieved after the neonatal period. The seizures were well controlled with one antiepileptic drug. No significant developmental delay existed compared to the children of same age. Another patient carrying KCNQ2 gene mutation also had frequent seizures at presentation. The initial EEG showed obvious burst-suppression. Levetiracetam combined with Topiramate was used to control seizures. Though there were no clinically identifiable seizures in the past one year, the patient showed significant developmental delay. As a result, Ohtahara syndrome was diagnosed retrospectively.

The other gene mutations identified in those children were SCN8A, TSC2, TSC1, AIFM1, NFIX, IFIH1, GABRG2, RPGRIP1L, PCCA and SCN9A. The diseases that related to those genes are demonstrated in Table 2, and some important clinical features are shown in Table 3.

Children with SCN8A and SCN9A gene mutations both started having seizures within 3 days after birth and had 3 attacks in the first day of the disease. There were significant changes in their consciousness and the initial EEG showed obvious epileptic discharges. Due to the nature of their seizures and the difficulty experienced trying to control them, their conditions deteriorated and succumbed to the illness within a month after discharge. Retrospectively, it was highly likely that they had EOEEs. Two children in the study had gene mutations associated with tuberous sclerosis, TSC1 and TSC2. Both presented with

myoclonic seizures. The patient with the TSC1 gene mutation was delivered preterm at 35^{+4} weeks gestational age. Her initial seizures were stereotypical and video-EEG showed multi-focal spike/slow waves during sleep with discontinuous discharges. After administering Levetiracetam, seizures persisted and the child's condition worsened. The child succumbed to the illness within 1 month after discharge from hospital. By contrast, the child with TSC2 had seizures without obvious stereotype and EEG showed suspicious epileptic waves. After hospitalization, the child did not experience any clinically identifiable seizures nor abnormality in the EEG. Hence a diagnosis of epilepsy was unlikely. The initial seizures were probably due to acute phase of brain injury.

For the child with AIFM1 gene mutation, the initial attacks were frequent, but the seizures relieved spontaneously after the neonatal period. In child with GABRG2 gene mutation, frequent focal myoclonic seizures combined with generalized tonic-clonic seizures were observed initially, and the EEG showed a whole brain burst-suppression. However, it spontaneously relieved after 4 months old without long term antiepileptic drugs (AEDs). The one with RPGRIP1L gene mutation developed seizures 15 days after birth, exhibited as generalized tonic-spasm, after the use of levetiracetam, seizures were controlled well. Not so lucky is the child with IFIH1 gene, frequent seizures since the 1st day of life, and the general condition was poor. The initial brain MRI showed a wide range of symmetrical abnormalities in the white matter. The patient, now more than 1 year old, has multiple AEDs, continues to have frequent seizures and also has severe developmental delay. A diagnosis of EOEE and white matter dysplasia was made. The child with PCCA gene mutation had a significant

Table 2 Epilepsy-related mutated genes found in the study.

Gene name	Inheritance mode	OMIM number	Related diseases
KCNQ2	AD	602235	Early infantile epileptic encephalopathy-7; benign familial neonatal seizures-1
SCN8A	AD	614558	Infantile epileptic encephalopathy-13
TSC2	AD	613254	Tuberous sclerosis-2
TSC1	AD	191100	Tuberous sclerosis-1
AIFM1	XR	300816	Combined oxidative phosphorylation deficiency-6
IFIH1	AD	615846	Aicardi-Goutieres syndrome-7
GABRG2	AD	607681	Childhood absence epilepsy-2
		611277	Generalized epilepsy with febrile seizures plus, type 3; familial febrile seizures-8
RPGRIP1L	AR	216360	COACH syndrome
PCCA	AR	606054	Propionic acidemia; ketosis hyperglycinemia
SCN9A	AD	613863	Epilepsy, generalized, with febrile seizures plus, type 7; febrile seizures, familial, 3B

AD: autosomal dominant inheritance; XR: X-linked recessive inheritance; AR: autosomal recessive inheritance.

Table 3 Some clinical features of enrolled patients.

Num	Related gene	Mutant site[a]	Onset age(d)	Seizure type	Seizure control	Development delay[b]	Etiology diagnosis for seizures
1	KCNQ2	chr20,c.998G > A(exon7)	1	generalized	well	without	epilepsy; BFNE
2	KCNQ2	chr20,c.1678C > T(exon15)	16	asymmetric	badly	with	epilepsy; Ohtahara syndrome
3	SCN9A	chr2,c.2132T > C(exon14)	1	generalized	badly	—	epilepsy; EOEEs
4	SCN8A	chr12,c.5257T > G(exon27)	2	generalized	badly	—	epilepsy; EOEEs
5	KCNQ2	chr20,c.916G > C(exon6)	2	generalized	well	without	epilepsy; BFNE
6	TSC2	chr16,c.169C > T(exon3)	1	generalized	well	with	symptomatic seizure
7	TSC1	chr9,c.3266G > C(exon23)	7	generalized	badly	—	epilepsy; EOEEs
8	AIFM1	chrX,c.1030C > T(exon10)	1	focal	well	with	epilepsy
9	IFIH1	chr2,c.2020_c.2023(exon10) delAGAT	1	focal	badly	with	epilepsy; EOEEs
10	GABRG2	chr5,c.406C > T(exon4)	14	focal	well	without	epilepsy
11	RPGRIP1L	chr16,c.910G > A(exon8)	15	generalized	well	with	epilepsy
12	PCCA	chr13,c.524G > A(exon7)	7	focal	badly	—	epilepsy; EOEEs
13	—		7	generalized	well	without	low calcium convulsions
14	—		2	generalized	well	without	epilepsy
15	—		1	generalized	badly	without	epilepsy; cortical dysplasia

[a] Mutant site: point mutation such as chr20,c.998G > A(exon7) means that the gene is located on chromosome 20, and the base G of the coding sequence at position 998 is mutated to A, and the mutation is located in the 7th exon; while deletion mutation such as chr2,c.2020_c.2023(exon10)delAGAT means the bases AGAT are deleted, whose location is from the position of coding sequence 2020 to 2023 (at the 10th exon).

[b] Development delay: children with SCN9A, SCN8A, TSC1 and PCCA gene mutations died 21 days, 40 days, 3 months, and 10 days after birth respectively, so follow-up for developmental delay is unachievable.

increase in blood ammonia (1717umol/L) and poor response to stimulus was noticed after admission. Dialysis was performed twice to correct metabolic disorders but was not successful. Eventually, the patient succumbed on the day of discharge.

For three patients in whom no genetic mutations were identified, the causes of seizures were thought to be due to: low-calcium levels, symptomatic epilepsy (due to intracranial hemorrhage), and secondary epilepsy (due to cerebral cortical dysplasia). As for the third one, during the course of the disease, atypical spikes on both sides of the frontal areas were noted on this patient's EEG. Initially, levetiracetam was administered but there was no relief in the seizure frequency. Re-examination of EEG showed multifocal spikes and fast waves. Therefore, oxcarbazepine was chosen as an alternative, but no relief either till now.

From the results obtained on genetic testing, two group were divided based on whether pathogenic genes with close correlation with epilepsy were detected. Group 1 included those with pathogenic genes while group 2 included those with no related gene mutation detected. The initial clinical manifestations, initial laboratory test and imaging results as well as outcomes of the two groups of children were compared. Since the sample size is smaller than 40, categorical variable is analyzed using *Fisher's* exact test. For continuous variables, variance equivalence test showed no heterogeneity ($P = 0.878$) hence the *Student-t* test was used.

Group 1 had a significantly higher incidence of poor response in the initial stages of the disease ($P = 0.009$). The rate of abnormal EEG was also higher ($P = 0.027$) in group 1 (Table 4). There were no significant differences in other initial clinical manifestations or laboratory results. These included: age of onset (less than 3 days), focal seizure type, single duration more than 1min, seizures more

than twice a day, abnormal manifestations during quiescent stage, stereotype, hypermyotonia, NBNA score, metabolic/ electrolyte abnormality and initial MRI abnormality. In the follow-up phase, there was no significant difference in the incidence of mortality, epilepsy control state, oral long-term AEDs, multiple drug in combination, developmental delay or retrospective diagnosis as epilepsy.

Discussion

The clinical manifestations of neonatal seizures are atypical and the etiology is complex. This study demonstrates that most of seizures with unexplained causes in the neonatal period were due to epilepsy (13/15). Epileptic seizures are common 3 days after birth with frequent episodes at the beginning of the disease. In addition, seizures in the neonatal period, when infections and HIE were excluded, are likely due to an underlying genetic condition (12/15). Since it is often difficult to ascertain the underlying etiology, current medical therapy for epilepsy is not based on the etiology, but clinical manifestations. The main aim of treatment is to prevent secondary brain injury.[8,9] In practice, we found that although some patients had a reduction in seizure frequency, they still have significant developmental delays. The neonatal brain is sensitive to various injurious factors such as hypoxia. Repeated long-lasting seizures may cause brain damage, which in turn aggravates the seizures leading to a vicious circle. In addition, once the diagnosis is confirmed as epilepsy, AEDs were necessary, which might affect cognitive development. Therefore, differentiating neonatal-onset epilepsies from acute symptomatic seizures is crucial for optimal management. Moreover, recognition of underlying genetic causes

Table 4 Comparison of clinical features of patients in whom genetic mutations were identified and whom weren't.

Variable	group 1 ($N_1 = 12$)	group 2 ($N_2 = 3$)	
	n_1 (%)	n_2 (%)	P-value
Initial clinical manifestations			
Onset age <3d	7 (58.3)	2 (66.7)	1.000
Seizure type:focal	5 (41.7)	0 (0.0)	0.505
Single seizure duration>1min	4 (66.7)	3 (100.0)	0.500
Seizure frequency > twice a day	10 (83.3)	2 (66.7)	0.516
Quiescent stage abnormal	7 (58.3)	2 (66.7)	1.000
Stereotype	7 (58.3)	2 (66.7)	1.000
Poor response to stimulus	11 (91.7)	0 (0.0)	0.009
Hypermyotonia	3 (25.0)	0 (0.0)	1.000
NBNA score	33.170 ± 1.722	34.330 ± 1.528	0.356
Initial laboratory test and image results			
Metabolic abnormal	4 (33.3)	1 (33.3)	1.000
Electrolyte abnormal	3 (25.0)	1 (33.3)	1.000
Initial EEG[a] abnormal	9 (81.8)	0 (0.0)	0.027
Initial MRI abnormal	7 (63.6)	1 (33.3)	0.538
Follow-up after discharge			
Death	4 (36.4)	0 (0.0)	0.505
Poor seizure control	6 (50.0)	1 (33.3)	1.000
Initial long-term AEDS	5 (41.7)	2 (66.7)	0.569
Multiple AEDs now	3 (37.5)	0 (0.0)	0.491
Development delay	5 (62.5)	0 (0.0)	0.182
Diagnosed as epilepsy	11 (91.7)	2 (66.7)	0.371

[a] Initial EEG:If video-EG was done in the neonatal period, the video-EEG results were preferred. If not, other kinds of EEG results including common EEG and amplitude integrated encephalogram (aEEG) would be considered.

enables the practice of precision medicine achievable leading to better outcomes.[10,11]

The development of NGS has led to the discovery of a large number of genes involved in epilepsy.[3] The use of genetic diagnosis is a valuable tool in the practice of modern medicine. The data obtained may be used for family genetic counseling as well as guiding the treatment approaches and predicting the prognosis. With increasing availability of genetic diagnosis, precision therapy will also be an option of management for neonatal-onset epilepsy.[12] For example, encoding a voltage-gated potassium channel, electro-physiological analysis showed that the majority of KCNQ2 mutations lead to an increase in potassium current, which may be treated by retigabine to reduce the current amplitude or to depolarize shift of the activation curve.[13,14]

However, not all the pathophysiological results gene mutations are known. For instance, some KCNQ2 mutations may not only change the function of the channel, but also its location in the neurons.[15] We propose that in-depth studies of the clinical manifestations of children with specific genetic abnormalities may in turn be helpful in exploring the mechanism of disease progress.

In this study, poor control of seizures was noticed in most of the patients at the beginning of the disease, especially in patients with gene abnormality. This was probably due to neurological function inhibition attended by brain damage caused by the pathogenic gene expression. Consequently, the rate of abnormal EEG was also higher in the gene abnormality group. It reflects that the gene mutations may lead to more significant abnormal discharges of neurons. It can also be inferred that early EEGs in neonates with EOEEs usually have specific characteristics. These include: burst-suppression and multi-focal seizures. Children with these manifestations often have a high mortality rate in the infantile period and severe mental retardation later on.

The study by Yang et al showed that the most common genotype identified in Chinese children affected by seizures was KCNQ2 (23.3%) followed by STXBP.[4] In our study, we found 3 children with unexplained convulsions carrying mutated KCNQ2 genes (20.0%), including 1 case eventually diagnosed as Ohtahara syndrome and 2 cases as BFNE. The KCNQ2 gene encodes a potassium channel that is expressed in the brain, especially in cortex, amygdala, caudate nucleus and hippocampus.[16] KCNQ2-related BFNE is characterized by early onset between two and eight days after birth and spontaneously disappearing in the first year of life. Normal physical examination and laboratory test results between and after seizures are also noted with no residual developmental delays. Meanwhile, KCNQ2-related EOEE is characterized by early-onset seizures in the first week of life with frequent seizures in the first few months of life. Seizures present as tonic seizures, sometimes associated with focal motor and autonomic deficits. Encephalopathy presents from birth and persists even after cessation of seizures. Subsequently, developmental impairment persists. In these patients, brain MRI frequently shows symmetrical hyperintensities in the basal ganglia and uncommonly in the thalamus, which may resolve over time.[2,13,17]

The other mutated genes identified in the children included in the study were: SCN8A, TSC2, TSC1, AIFM1, NFIX, IFIH1, GABRG2, RPGRIP1L, PCCA and SCN9A. Although reports of involvement of these genes are still rare, predictions based on protein structure suggest that these genes may be harmful and associated with epilepsy. In the study, the retrospective diagnosis of children carrying the mutated genes SCN8A, SCN9A, TSC1 and IFIH1 were EOEE. There were few reports in previous studies of the same. Children with mutated SCN9A gene in previous studies showed hyperthermia-related epilepsy, such as Dravet syndrome. In this study, no febrile-related seizures were noted. Hence phenotypic diversity needs to be considered.[18-21] It is worth noting that one of the children with the nodular sclerosis-related gene TSC2 had no seizures after discharge and no signs of nodular sclerosis. The baby's mother also carried the mutated gene but had no abnormal clinical manifestations. It is unclear whether the initial convulsions in the neonatal period are related to the gene mutation. Another interesting case was a child carrying mutated PCCA gene. This gene is closely related to a congenital amino acid metabolism abnormality, propionic acidemia or ketosis hyperglycinemia, which can be present with seizures.[22] In the acute phase, there was severe high blood ammonia (1717umol/L). This presented in combination with significant EEG abnormalities thus epilepsy was considered as a probable diagnosis. However, hyperammonemia may also cause seizures. Unfortunately, due to early death of the child, it cannot be confirmed.

The causes of the neonatal-onset seizures are variable. Exclusion of intracranial infections, HIE or vascular causes, as in the current study, point to most of them being genetic in origin. Currently, several dozens of genes are known to be involved in these diseases.[3,23] Those with genetic abnormalities are different from those of non-genetic abnormalities in brain electrophysiology due to genotype-phenotype connections. Hur et al analyzed the results of epilepsy due to genetic and non-genetic causes and suggested that gene abnormality induced epilepsy had higher rates of epileptic recurrences and much significant developmental delays than non-gene abnormality group. Furthermore, diffuse slow focal/multifocal epileptiform discharges in the initial EEGs were more common in the gene abnormality group.[24] In our study, the seizures in the gene-positive group showed more focal episodes. The ratio of intractable epilepsy and developmental delay were higher than that of the gene-negative group, but the difference between the two groups was not statistically significant. This may be due to the small sample size used. Therefore, clinical studies with more participants are needed to provide a comprehensive analysis of neonatal seizures and epilepsies with different phenotypes and genotypes.

Conclusion

Despite the recent progress in genetic technology, molecular diagnosis for neonatal-onset epilepsy can pose numerous challenges due to genetic and phenotypic heterogeneities. However, some genotypes are known to be associated with specific clinical manifestations and EEG activities. Early genetic diagnosis is helpful in providing optimal management and potentially improving outcomes of children with early-onset epilepsy.

Conflict of interest

The authors declare that there are no financial and personal relationships with other people or organizations that could inappropriately influence (bias) the present work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2019.02.002.

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