Genetic factors and the risk of drug-resistant epilepsy in young children with epilepsy and neurodevelopmental disability: A Prospective Study and Updated Meta-Analysis

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Research article

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Abstract

Background

Drug-resistant epilepsy (DRE) affects 7–20% of children with epilepsy. Although some risk factors for DRE have been identified, the results have not been consistent. Moreover, data regarding the risk factors for epilepsy and its seizure outcome in the first 2 years of life are limited.

Methods

We analyzed data for children aged 0–2 years with epilepsy and neurodevelopmental disability (NDD) from January, 2013, through December, 2017. These patients were followed up to compared the risk of DRE in patients with genetic defect (genetic group) with that without genetic defect (nongenetic group). Additionally, we conducted a meta-analysis to identify the pooled prevalence of genetic factors in children with DRE.

Results

A total of 96 patients were enrolled. A total of 68 patients were enrolled in the nongenetic group, whereas 28 patients were enrolled in the genetic group. The overall DRE risk in the genetic group was 6.5 times (95% confidence interval [CI], 2.15–19.6; \( p = 0.03 \)) higher than that in the nongenetic group. Separately, a total of 1308 DRE patients were participated in the meta-analysis. The pooled prevalence of these patients with genetic factors was 22.8% (95% CI 17.4–29.3).

Conclusions

The genetic defect plays a crucial role in the development of DRE in younger children with epilepsy and NDD. The results can serve as a reference for further studies of epilepsy panel design and may also assist in the development of improved treatments and prevention strategies for DRE.

Introduction

Data regarding epilepsy and its outcome in children aged < 2 years are limited [1]. With proper and adequate treatment, childhood epilepsy can achieve remission in 60–70% of the cases, with nearly 50% being able to discontinue antiepileptic drug (AED) use [2–4]. Unlike epilepsy occurring in later childhood or adulthood, epilepsy during infancy or early childhood could be more clinically complex and etiologically heterogenous. Therefore, the prediction of seizure outcomes during infancy is relatively difficult compared with that beyond infancy. Given the advancement of molecular bioassay technology, an increasing number of genetic etiologies underlying infantile epilepsy have been found.
A meta-analysis of 35 studies on patients with drug-resistant epilepsy (DRE) revealed the pooled prevalence and pooled incidence of DRE across all age groups of epilepsy to be 30% and 15%, respectively [5]. Among adults, the risk factors for DRE included abnormal electroencephalograms (EEGs) (in terms of both epileptiform and slow wave discharges), symptomatic etiologies, febrile seizures, status epilepticus, the presence of developmental delay, and multiple seizure types [6, 7]. However, the risk of DRE in infancy and younger children remain uncertain thus far given the heterogeneous etiologies as well as misdiagnoses [5].

Identifying the causes underlying epilepsy in the first 2 years of life can be challenging for pediatric neurologists. Determining the etiology of epilepsy can aid in predicting the neurodevelopmental outcomes and seizure control in children in addition to the choice of AEDs. Herein, we report a study exploring the incidence of DRE in 0–2-year-old infants and children under a new perspective. To determine DRE incidence, the factors leading to young children epilepsy are classified into genetic and nongenetic factors, with the patients in both groups demonstrating neurodevelopmental disability (NDD). Additionally, a meta-analysis was conducted to serve as a counter-directional comparison for the present study.

**Patients And Methods**

**Patient population**

A comprehensive medical review of patients aged < 2 years diagnosed as having epilepsy and NDD between January 1, 2013 and December 31, 2017 in the China Medical University Children's Hospital was conducted. These children (and their parents) were in contact by our case managers and underwent regular follow-up at our Pediatrics Neurology Clinic until December 31, 2018; recruitment of the children to this study was conducted after approval of the study was obtained from our institutional ethics committee (CMUH108-REC1-023). The patients (if equal to or more than 7 years old) and their parents provided their assent and written informed consent, respectively, before their enrolment. The definition of DRE was referred from the Task Force of the International League Against Epilepsy (ILAE): “The failure of adequate trials of two tolerated, appropriately chosen, and administered AEDs (whether as monotherapy or in combination) to achieve seizure freedom [8].” Moreover, adequate seizure control was defined as the patient remaining seizure-free for either at least 2 months or for two times the length of the usual pretreatment interictal interval, whichever was longer. Poor or partial seizure control in children was defined as the occurrence of more than one seizure per month over a minimum of 6 months, as indicated by Chawla et al [9, 10]. The “Rule of Three” proposed by 2012 ILAE task force was used as the operational definition for seizure freedom: that is, a patient should only be regarded as seizure-free subsequent to an intervention when a seizure-free period that is three times longer than the longest interseizure interval over the previous year before the intervention has elapsed [11]. All patients were followed up for at least 12 months, which included at least three visits to the Pediatrics Neurology Clinic, whereas patients who had intractable seizure due to untreated or incomplete treatment of an underlying disease, demonstrated poor drug compliance, died or were lost to follow-up, or received nonepileptic drug treatment were excluded.
The enrolled patients (n = 96) underwent a series of laboratory tests; they also maintained records and
individual seizure diaries, which contained details about sex, preterm or full-term pregnancy, age at
seizure onset, underlying cause of epilepsy, age at the time when AED therapy was initiated and the
number of AEDs taken, seizure-free status, family history of any seizure disorder, EEG pattern at the time
of the first diagnosis of epilepsy, comorbidities, and neurodevelopmental outcomes. After an assessment
by three independent pediatric neurologists, the study patients were classified into the nongenetic and
genetic groups (Fig. 1). The patients of the genetic group were those in whom epilepsy was because of
pathogenic single-gene mutations or defined structural chromosomal aberrations, such as microdeletion
or microduplication.

**Statistical analysis**

Chi-squared tests were used to analyze the differences in categorical variables between the groups, while
the DRE incidence density rates were also calculated for both groups. In addition, the odds ratios (ORs)
and 95% confidence intervals (CIs) of DRE in the genetic group versus the nongenetic group were
estimated through the application of a logistic regression model. The PASW Statistics software (version
18.0; SPSS Inc., Chicago, IL, United States) was used to perform all the statistical analyses, with a two-
tailed p value of < 0.05 being regarded as statistically significant.

Additionally, we conducted an updated meta-analysis for 10 observational studies exploring genetic
causes of DRE, published between 2014 and 2020 (Fig. 2). To compare the event rates and 95% CIs of
genetic-related DRE between studies, and further transformed them into a common scale. The effect size
estimates of each study were pooled by random effects mode. The standard chi-squared test and I²
statistics were used to evaluate the consistency of the research results. Evidence of publication bias was
assessed with funnel plots

**Results**

**Data analysis**

Between January 1, 2013 and December 31, 2017, 96 children aged < 2 year with epilepsy and NDD were
enrolled in this study (Fig. 1). There are 68 children in nongenetic group and 28 children in genetic group.
Table 1 presents their demographic characteristics: mean (standard deviation [SD]) age at epilepsy
diagnosis and follow-up duration were respectively 7.54 (5.25) months and 2.64 (0.73) years in the
 genetic group and 4.96 (3.99) months and 2.93 (1.04) years in the nongenetic group. The nongenetic
group was divided into four subgroups (Fig. 3): abnormal brain structure (n = 38, 55.8%), infections (n = 7,
10.2%), metabolic disorders (n = 4, 5.8%), and unknown (n = 19, 27.9%). The genetic group was divided
into 2 subgroups, there are 17 children belong to single-gene mutations, and 11 chromosome
abnormalities. Moreover, DRE incidence was 42.8% and 13.2% in the genetic and nongenetic groups,
respectively (Fig. 4).
Table 1
Demographic and clinical characteristics of children with epilepsy and neurodevelopmental disabilities in the genetic and non-genetic groups

| Group                                             | Genetic, n = 28 (%) | Non-genetic, n = 68 (%) | p     |
|---------------------------------------------------|---------------------|-------------------------|-------|
| Gender, Male                                      | 12 (42.9)           | 40 (59.7)               | 0.13  |
| Preterm (%)                                       | 4 (14.2)            | 13 (19.1)               | 0.57  |
| FHx of seizure disorders (%)                      | 2 (7.1)             | 2 (2.9)                 | 0.34  |
| Age of epilepsy Dx (mo) (SD)*                     | 7.54(5.25)          | 4.96 (3.99)             | 0.01  |
| Abnormalities other than the CNS (%)              | 12 (42.8)           | 12 (17.6)               | 0.10  |
| Facial and outward appearance                     | 8 (28.5)            | 4 (5.8)                 | -     |
| Cardiovascular                                    | 5 (17.8)            | 7 (9.8)                 | -     |
| Genitourinary system                              | 4 (14.2)            | 1 (1.4)                 | -     |
| Positive MRI findings (%)                         | 8 (28.5)            | 44 (64.7)               | 0.003 |
| 1st interictal EEG patterns (%)                   |                     |                         | 0.38  |
| Negative                                          | 9 (32.1)            | 11 (16.1)               | -     |
| Epileptogenic discharges                          | 13 (46.4)           | 43 (63.2)               | -     |
| Slow                                              | 3 (10.7)            | 13 (19.1)               | -     |
| Hypsarrhythmia and/or burst suppression           | 3 (10.7)            | 1 (1.4)                 | -     |
| F/U year (SD)*                                    | 2.64(0.73)          | 2.93 (1.04)             | 0.19  |
| Prenatal, perinatal or postnatal risk factors for epilepsy |          |                         | <0.001|
| Genetic                                           | 28 (100)            | 0                       | -     |

*t test

AEDs, Anti-epileptic drugs; CNS, central nervous system; Dx, diagnosis; DRE, drug-resistant epilepsy; FHx, family history; F/U, follow-up; mo, month; MRI, magnetic resonance imaging
Difference In DRE Incidence Between Genetic And Nongenetic Groups

Table 2 compares DRE relative risks and incidence rates for the nongenetic and genetic patient groups. The overall risk of DRE for the genetic group was greater than that for the nongenetic group (adjusted OR, 6.50; 95% CI, 2.15–19.6; \( p = 0.03 \)). Furthermore, DRE risk in female patients in the genetic group was higher than that in those in the nongenetic group (adjusted OR, 8.88; 95% CI, 1.38–57.1; \( p = 0.03 \)). In addition, full-term genetic group had a 7.1 times (95% CI, 2.02–24.9; \( p = 0.001 \)) higher DRE risk than did full-term nongenetic group. Excluding those with unknown causes of epilepsy in the nongenetic group, the DRE risk in the genetic group was 4.3 times (95% CI, 1.18–9.43, \( p = 0.03 \)) higher than the nongenetic group.
Table 2
Incidence rates and relative risks of drug-resistant epilepsy (DRE) for the infants with genetic-risk factor group and infants with non-genetic risk factor group and those stratified by sex and term and preterm neonates by using a logistic regression model.

| Group                  | DRE | Event (No.) | IR(%) | OR (95% CI) | Adj. OR (95% CI) |
|------------------------|-----|-------------|-------|-------------|------------------|
| **Non-Genetic**†       |     | 9           | 13.2  | Reference†  | Reference†       |
| (n = 68)               |     |             |       |             |                  |
| **Non-Genetic**‡       |     |             |       | Reference‡  | Reference‡       |
| (n = 49)               |     |             |       |             |                  |
| **Sex**                |     |             |       |             |                  |
| M (n = 40)             | 6   | 8.8         |       | Reference†  | Reference†       |
| F (n = 28)             | 3   | 4.4         |       | Reference†  | Reference†       |
| **Gestation**          |     |             |       |             |                  |
| Full term (n = 55)     | 7   | 10.3        |       | Reference†  | Reference†       |
| Preterm (n = 13)       | 2   | 2.9         |       | Reference†  | Reference†       |
| Genetic (n = 28)       | 12  | 42.8        | 4.91 (1.76, 13.7) * | 6.50 (2.15, 19.6) * |
|                        |     |             | 3.33 (1.18, 9.43) * | 4.38 (1.41, 13.6) * |
| **Sex**                |     |             |       |             |                  |
| M (n = 12)             | 6   | 21.4        | 5.66 (1.36, 23.5) * | 5.66 (1.35, 23.6) * |

† Whole non-genetic group (n = 68) and serves as corresponding comparative references for those of genetic group; ‡ Non-Genetic group but exclude those with unknown causes (n = 49) and serves as corresponding comparative references for those of genetic group

Adj OR, adjusted odds ratios; IR, Incidence rate; CI, Confidence interval; M, male; F, female

Model adjusted by gestational age, sex, days of hospitalization

*p < 0.05

**p < 0.01
| Group          | DRE | Gestation |
|---------------|-----|-----------|
| F (n = 16)    | 6   | 21.4      |
|               |     | 7.50 (1.29, 43.6)* | 8.88(1.38,57.1)* |
| Full term (n = 24) | 10  | 35.7      |
|               |     | 4.89(1.57,15.2)**  | 7.09(2.02,24.9)** |
| Preterm (n = 4) | 2   | 7.1       |
|               |     | 5.50(0.46,65.0)  | 5.48(0.46,65.0)  |

† Whole non-genetic group (n = 68) and serves as corresponding comparative references for those of genetic group; ‡ Non-Genetic group but exclude those with unknown causes (n = 49) and serves as corresponding comparative references for those of genetic group

Adj OR, adjusted odds ratios; IR, Incidence rate; CI, Confidence interval; M, male; F, female

Model adjusted by gestational age, sex, days of hospitalization

*p < 0.05

**p < 0.01

Systematic Review And Updated Meta-analyses

After a rigorous screening, 10 relevant published studies for the genetic characteristics in DRE were included (6 with epilepsy next generation sequencing (NGS) panel; 1 with only whole exome sequencing (WES); 3 with WES plus array-based comparative genomic hybridization (aCGH) or NGS panel). Study algorithm is provided in (Fig. 2). Five studies were based from western countries (USA, Denmark, Italy and UK) and the other five were from Asia (China, Taiwan, south Korea, Hong Kong). Most participants of those studies were children (age < 18 years), and since the molecular diagnostic tool (WES, NGS panel) become blooming over the past 6 years, the first included study was published in 2014. A total of 1308 DRE patients were participated in the meta-analysis and revealed that the pooled prevalence of in DRE patients of genetic factors was 22.8% (95% CI 17.4–29.3) (Fig. 5). The result showed a significant heterogeneity across all studies ($I^2 = 81.6%$; $Q = 49$, df = 9, $P < 0.001$). The pooled prevalence was based on the random effect model due to the observed heterogeneity across the studies.

Genetic Characteristics In Drug-resistant Epilepsy Of Published Studies

Table 3 provides a summary of previous studies about genetic characteristics in DRE[12–21]. Regardless of varied molecular diagnostic tool used between studies, some gene mutations were found to appear
repeatedly, such as *SCN1A* (5.6%, n = 74/1308), followed by *SCN8A* (1.37%, n = 18/1308), *TSC2* (1.22%, n = 16/1308), *SCN2A* (1.07%, n = 14/1308) and *KCNQ2* (0.99%, n = 13/1308)
Table 3
Genetic characteristics in drug-resistant epilepsy of published research (2014–2020)

| Study names, year | Research areas | Research objects with DRE, (n) | Research tools | The detected genetic or cytogenetics abnormalities (n) |
|-------------------|----------------|-------------------------------|----------------|-----------------------------------------------|
| Ream, 2014 (12)   | USA            | Children (25)                 | karyotype, aCGH, single gene sequencing, Epilepsy NGS panels (38/40/53 genes) and WES | PCDH19(1), SCN1A(3), SPTAN(1), SLC2A1 (1), CDKL5 (1), SLC9A6 (1), EFHC1 (1), 69,XXX{28}/46,X X{22} (1), arr 2p25.3p25.1(2,772 – 10,840,014)x3 dn (1), 6q27(165,143,532 – 170,824,447)x1 dn (1) |
| Segal, 2016 (13)  | USA            | Children (49)                 | Epilepsy NGS panels | SCN1A (3), PCDH19 (2), DLG3 (1), MECP2 (1) |
| Parrini, 2016 (14)| Italy          | Children (349)                | Epilepsy NGS panels (30 genes/ 95 genes) | SCN2A(9), SCN1A(8), KCNQ2(6), STXBP1(6), SCN8A(5), CDKL5(4), MECP2(4) and others† |
| Tsang, 2018 (15)  | Hong Kong      | Children (50)                 | aCGH plus WES     | SCN8A (1), SCN1A(1), MECP2 (1), CDKL5 (1), DEPDC5 (1), CHD2 (1) |

*aCGH, array Comparative Genomic Hybridization; MLPA, Multiplex Ligation-dependent Probe Amplification; NGS, Next generation sequencing; WES, Whole exome sequencing
† The authors did not list all the mutated genes in the paper
‡ The genes shown in bold are common gene mutation amid studies
| Study names, year | Research areas | Research objects with DRE, (n) | Research tools | The detected genetic or cytogenetics abnormalities (n) |
|------------------|----------------|-------------------------------|----------------|--------------------------------------------------|
| Peng, 2018 (16)  | China          | Children (273)                | WES, MES,     | SCN1A (21), SCN2A (1), SCN8A (5), GABRG2 (1), KCNQ2 (3), DOLK (1), KCNT1 (2), TSC1 (4), TSC2 (7), PNPPO (1), PCDH19 (3), TRPM6 (1), DNM1 (1), SLC35A2 (1), ALDH7A1 (1), GNAO1 (1), HCN1 (2), KCNMA1 (4), SLC6A1 (1), SPTAN1 (1) |
| Liu, 2018 (17)   | China          | Children < 14 years (172)     | Epilepsy NGS panels (153 genes) | SCN1A (16), TSC2 (5), STXBPI (2), SCN8A (2), TSC1 (1), MECP2 (1), CHD2 (1), PCDH19 (1), GABRA7 (1), GABRB3 (1), SLC2A1 (1), SLC9A6 (1), IQSEC2 (1), KCNQ2 (1), SCN2A (1), CACNA1A (1), KCNT1 (1), SYNGAP1 (1), ATP1A2 (1), CDKL5 (1), ADSL (1), VRK2 (1) |

*aCGH, array Comparative Genomic Hybridization; MLPA, Multiplex Ligation-dependent Probe Amplification; NGS, Next generation sequencing; WES, Whole exome sequencing

† The authors did not list all the mutated genes in the paper

‡ The genes shown in bold are common gene mutation amid studies
| Study names, year | Research areas | Research objects with DRE, (n) | Research tools | The detected genetic or cytogenetics abnormalities (n) |
|-------------------|----------------|-------------------------------|----------------|-----------------------------------------------------|
| Oates, 2018 (18)  | Denmark        | Children (96)                 | Epilepsy NGS panels (46/76/85/102 genes) | SCN8A (4), SCN2A (3), SCN1A (2), KCNQ2 (2), HNRNPU (1), GRIN2A (1), SYNGAP1 (1), STXBPI (1), STX1B (1), CDKL5 (1), CHRNA4 (1), PCDH19 (1), PIGT (1). |
| Kang, 2019 (19)   | South Korea    | Adults (122)                  | WES            | GABRG2 (2), KCNT1 (1), SCN1A (3), SCN9A (1), DEPDC5 (1), TSC1 (2), TSC2 (4), ADGRV1 (1), CNTNAP2 (2), PRICKLE1 (1), RELN (3). |
| Symonds, 2019 (20)| UK             | Children under 36 months (76) | Epilepsy NGS panels (104 genes) and MLPA for relevant genes | SCN1A (12), CDKL5 (4), PCDH19 (4) and other 56 genes † |
| Wu, 2020 (21)     | Taiwan         | Children (96)                 | Epilepsy NGS panels (24/122 genes) | SCN1A (5), TBC1D24 (1), KCNT7 (1), KCNQ2 (1), GRIN2A (1), ARX (1), ADSL (1), CHD2 (1), SCN8A (1) |

*aCGH, array Comparative Genomic Hybridization; MLPA, Multiplex Ligation-dependent Probe Amplification; NGS, Next generation sequencing; WES, Whole exome sequencing

† The authors did not list all the mutated genes in the paper

‡ The genes shown in bold are common gene mutation amid studies

Discussion
Childhood DRE risk is serious and can have catastrophic effects on neurodevelopment [22, 23]. The brain undergoes an extended period of growth and maturation during the first 2 years of life. Thus, intractable seizures are refractory during this critical time, particularly in the early infancy, can negatively affect the cognitive and motor development, consider by affecting the children's cognitive, behavioral, and psychiatric function [24, 27]. Here, we investigated the risk of DRE in infants and younger children in a tertiary hospital over 5 years and noted a relatively higher risk of DRE combined with NDD caused by genetic factors (6.5 times (95% CI, 2.15–19.6). By contrast, the risk of DRE caused by nongenetic factors was relatively low, even in those with severe NDD.

Our results also demonstrated heterogenous genetic causes of epilepsy which range from neonate to late infancy (29.1%, n = 28/96). Single-gene mutations and cytogenetic abnormalities accounted for genetic causes of specific epilepsy phenotypes or selected recognizable syndromes with a high prevalence of seizures. Seizure control in this group was relatively difficult, and therefore, this group demonstrated a high DRE incidence rate (42.8%, n = 12/28), and the high risk of NDD.

To our knowledge, we carried out the first meta-analysis with regards to genetic causes of DRE. From the meta-analysis, we found high incidences of gene related DRE in children and infancy (pooled prevalence 22.8%, 95% CI 17.4–29.3). The result is consistent with our study although with different methodology. It goes without saying that we could not encompass all epilepsy genes in a single research and then estimate the incidence of DRE, and besides, the detected gene mutations vary between studies. Even so, after combining the two, bidirectional comparison as a result, we demonstrate that genetic factors play a crucial role in childhood and infantile epilepsy and otherwise provided a reliable evidence to make believe a high probability of DRE existing in patients with genetic factors during childhood and infancy.

Additionally, we discovered some common genetic mutations among childhood DRE through our study (n = 96) and the meta-analysis (n = 1308). Namely, SCN1A, which presented in almost every study including ours, and the others were PCDH19, SCN8A, SCN2A, MECP2, KCNQ2, CDKL5, TSC1 and TSC2 (Table 3). Even now that molecular diagnostic tools are cutting prices for competition, the cost factor is still a major concern for most clinician and patients of DRE who sought for an accurate diagnosis for treatment [28]. Hopefully, this result could serve as an informative reference for future DRE panel design to make it cost effective and more efficient, especially in which WES or comprehensive epilepsy panels are not easily available or affordable.

Perinatal and prenatal insults are major risk factors for infantile epilepsy [29]. The proposed pathophysiological mechanism is that hypoxia–ischemia that can have deleterious effects on the vulnerable regions of the developing brain lead to substantive injuries that could affect not only seizure threshold but also cognition [30]. Studies have also explored the mechanisms underlying neuronal injury, which could be a cause of epilepsy; first, hypoxic–ischemic encephalopathy (HIE) initially affects various processes that potentially contribute to energy failure and loss of mitochondrial function, including brain edema, membrane depolarization, increased levels of neurotransmitter release and uptake inhibition, and increased levels of intracellular calcium (which can cause the initiation of further pathological cascades)
Second, excitotoxic cellular injury occurring through excess activation of the four glutamate receptors (N-methyl-d-aspartate, alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid, kainate, and metabotropic glutamate receptors), which leads to several forms of cell death, is another possible seizure mechanism associated with HIE [32–34].

Traumatic brain injury (TBI), whether accidental or inflicted, is another common cause of epilepsy development in infancy [35]. Notably, TBI often coexists with HIE, which worsens the condition of the already fragile brain through molecular injury mechanisms similar to those of HIE [36, 37], including excitotoxicity mediated by neurotransmitters that results in glutamate, free-radical injury to cell membranes, mitochondrial dysfunction, electrolyte imbalance, inflammatory response, focal microvascular occlusion, secondary ischemia from vasospasm, apoptosis, and vascular injury. These mechanisms result, in turn, in neuronal cell death concomitant with cerebral edema and an elevated risk of epilepsy [38–40].

Gene-related epilepsy involves various heterogenous seizure mechanisms that depend on the role of the genes. There are over 970 genes associated with epilepsy, and the number is increasing year by year [41, 42]. Although the seizure mechanisms are complex and diverse between the causative genes, we could roughly categorize them into ion and nonion channel genes. Given the role played by epigenetics in neuronal function from the time of embryogenesis and early brain development, as well as in tissuespecific gene expression, epigenetic regulation also contributes to neurodevelopment through gene–environment interaction influencing epilepsy occurrence. The same principle is likewise applicable to localized multiple loci in which susceptibility genes to epilepsy are harbored and can explain epilepsy cases with cytogenetic abnormalities [43].

Drug resistance mechanisms in epilepsy remain unclear. Margineanu DG et al proposed two current hypotheses that underscore the roles played by changes in the targets of medications that render them drug-insensitive and by the elevated actions of blood-brain barrier multidrug transporter proteins. However, the hypotheses in question do not seem to adequately account for the complicated nature of the brain alterations that occur in DRE [44]. The current consensus on is that DRE mechanism is multifactorial, including factors relating to the environment and genetics, in addition to both disease-related and drug-related factors [45, 46]. Relatively, the occurrence of at least two of these factors in combination may be of value in identifying those patients who are unlikely to be responsive to medical therapy [3, 47–51]. In our study, we proposed two explanations for genetic factors increasing DRE risk during infancy with epilepsy and NDD: First, during the neonatal or infancy period, early infantile epileptic encephalopathy accounts for a major part of genetic epilepsy, which are difficult to treat and often medically refractory [52]. Second, on the basis of several hypotheses proposed on DRE, including hypotheses regarding pharmacokinetics, intrinsic severity, neural networks, gene variants, transporters, and targets [53], we assumed that a sustained “neuroimmunoinflammatory” status can be implicated as not only epileptogenic but also indicative of a drug-resistant profile [54]. Additional relevant cell line- and animal model–based studies are thus warranted.
The present study had several limitations. First, while a risk of DRE in cases of genetic epilepsy was determined through this observational study, it is possible that the results may have been impacted by various confounding factors, such as the health status of the mother prior to and during pregnancy, socioeconomic status, pharmacological therapy of the children for conditions other than neurological conditions, poor nutrition (including malnutrition), or other environmental factors. Second, the size of the study sample was insufficient. We could not include patients of all types of genetic epilepsy in one study; moreover, one gene could have many different genotypes. Third, although our DRE definition was explicit, the seizure control results may have varied among physicians; that is, a DRE case for one physician could have attained seizure control with another. Fourth, although a comprehensive investigation was performed in all of the enrolled patients, an exact cause for epilepsy and NDD could not be determined in 19 patients; and thus the possibility of genetic factors’ involvement in those cases could not be completely dismissed, which was an inevitable confounder in the study. As such, further investigations of a thorough nature will be needed going forward in order to ascertain the related risks and pinpoint their underlying mechanisms in genetic and nongenetic epilepsy.

In summary, our study reveals that genetic factors act crucial role in younger children with epilepsy and NDD. Initiation of a genetic-based AED-development model, based on our current results, is warranted. In addition, the study can serve as a reference for further studies of epilepsy panel design and may also assist in the development of improved treatments and prevention strategies for DRE, particularly for drug control in more extensive and diverse genetic epilepsy, which has received insufficient attention thus far. That said, more data from relevant patients, as well as and more comprehensive studies of those data, will be needed in order to identify possible maternal, prenatal, perinatal, and postnatal confounders that could in turn help to clarify the effects of both genetic and nongenetic factors, as well as their associations with DRE.

Abbreviations

aCGH: array-based comparative genomic hybridization; AED: antiepileptic drug; DRE: Drug-resistant epilepsy; EEG: electroencephalograms (EEGs); HIE: hypoxic–ischemic encephalopathy; NDD: neurodevelopmental disability; NGS: next generation sequencing; TBI: Traumatic brain injury; WES: whole exome sequencing

Declarations

Availability of data and materials:

Datasets are available on request.

Competing interest:

The authors declare that they have no competing interests.
ETHICS STATEMENT

Written informed consent regarding participation in the study was obtained from the legal guardians of the patients after they were provided with a full description of the study. Approval of the protocol of the study was obtained from the Ethics Review Board of the China Medical University ethics committee (Approval #CMUH108-REC1-023 and #DMR-108-199 and # DMR-109-042).

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Author contributions

S-Y H collected the data, analyzed the data, and prepared the initial draft of the manuscript. C-H Lin took part in designing the study and wrote the final draft of the manuscript. The statistics for the study were compiled by I-C C, who also took part in the editing process and the revision of the tables. All of the authors read and approved of the final manuscript.

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References

1. Bui TT, Delgado CA, Simon HK. Infant seizures not so infantile: first-time seizures in children under six months of age presenting to the ED. Am. J Emerg Med. 2002;20:518–20.
2. Fangsaad T, Assawabumrungkul S, Damrongphol P, Desudchit T. Etiology, clinical course and outcome of infant epilepsy: Experience of a tertiary center in Thailand. J Clin Neurosci. 2019;59:119–23.
3. Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med. 2000;342:314–9.
4. Brorson LO, Wranne L. Long-term prognosis in childhood epilepsy: survival and seizure prognosis. Epilepsia. 1987;28:324–30.
5. Kalilani L, Sun X, Pelgrims B, Noack-Rink M, Villanueva V. The epidemiology of drug-resistant epilepsy: A systematic review and meta-analysis. Epilepsia. 2018;59:2179–93.
6. Xue-Ping W, Hai-Jiao W, Li-Na Z, Xu D, Ling L. Risk factors for drug-resistant epilepsy: A systematic review and meta-analysis. Med (Baltim). 2019;98:e16402.
7. Voll A, Hernández-Ronquillo L, Buckley S, Téllez-Zenteno JF. Predicting drug resistance in adult patients with generalized epilepsy: A case-control study. Epilepsy Behav. 2015;53:126–30.
8. Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia. 2010;51:1069–77.
9. Chawla S, Aneja S, Kashyap R, Mallika V. Etiology and clinical predictors of intractable epilepsy. Pediatr Neurol. 2002;27:186–91.
10. Poudel P, Chitlangia M, Pokharel R. Predictors of Poor Seizure Control in Children Managed at a Tertiary Care Hospital of Eastern Nepal. Iran J Child Neurol. 2016;10:48–56.
11. Westover MB, Cormier J, Bianchi MT, Shafi M, Kilbride R, Cole AJ, et al. Revising the "Rule of Three" for inferring seizure freedom. Epilepsia. 2012;53:368–76.
12. Ream MA, Mikati MA. Clinical utility of genetic testing in pediatric drug-resistant epilepsy: a pilot study. Epilepsy Behav. 2014;37:241–8.
13. Segal E, Pedro H, Valdez-Gonzalez K, Parisotto S, Gliksman F, Thompson S, et al. Diagnostic Yield of Epilepsy Panels in Children With Medication-Refractory Epilepsy. Pediatr Neurol. 2016;64:66–71.
14. Parrini E, Marini C, Mei D, Galuppi A, Cellini E, Pucatti D, et al. Diagnostic Targeted Resequencing in 349 Patients with Drug-Resistant Pediatric Epilepsies Identifies Causative Mutations in 30 Different Genes. Hum Mutat. 2017;38:216–25.
15. Tsang MH, Leung GK, Ho AC, Yeung KS, Mak CC, Pei SL, et al. Exome sequencing identifies molecular diagnosis in children with drug-resistant epilepsy. Epilepsia Open. 2018;4:63–72.
16. Peng J, Pang N, Wang Y, Wang XL, Chen J, Xiong J, et al. Next-generation sequencing improves treatment efficacy and reduces hospitalization in children with drug-resistant epilepsy. CNS Neurosci Ther. 2019;25:14–20.
17. Liu J, Tong L, Song S, Niu Y, Li J, Wu X, et al. Novel and de novo mutations in pediatric refractory epilepsy. Mol Brain. 2018;11:48.
18. Oates S, Tang S, Rosch R, Lear R, Hughes EF, Williams RE, et al. Incorporating epilepsy genetics into clinical practice: a 360°evaluation. NPJ Genom Med. 2018;3:13.
19. Kang KW, Kim W, Cho YW, Lee SK, Jung KY, Shin W, et al. Genetic characteristics of non-familial epilepsy. PeerJ. 2019;7:e8278.
20. Symonds JD, Zuberi SM, Stewart K, McLellan A, O'Regan M, MacLeod S, et al. Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort. Brain. 2019;142:2303–18.
21. Wu CC, Tsai MH, Chu YJ, Weng WC, Fan PC, Lee WT. The role of targeted gene panel in pediatric drug-resistant epilepsy. Epilepsy Behav. 2020;106:107003.
22. Raimalwalla T, Udani V, Mhatre D. A Retrospective Analysis of the Long-Term Outcome of Drug-Resistant Epilepsy in Children Treated in Urban India. Child Neurol Open. 2018;5:2329048 × 18795277.
23. Marsh ED, Brooks-Kayal AR, Porter BE. Seizures and antiepileptic drugs: does exposure alter normal brain development? Epilepsia. 2006;47:1999–2010.

24. Berg AT, Zelko FA, Levy SR, Testa FM. Age at onset of epilepsy, pharmacoresistance, and cognitive outcomes: a prospective cohort study. Neurology. 2012;79:1384–91.

25. Vasconcellos E, Wyllie E, Sullivan S, Stanford L, Bulacio J, Kotagal P, et al. Mental retardation in pediatric candidates for epilepsy surgery: the role of early seizure onset. Epilepsia. 2001;42:268–74.

26. Cormack F, Cross JH, Isaacs E, Harkness W, Wright I, Vargha-Khadem F, et al. The development of intellectual abilities in pediatric temporal lobe epilepsy. Epilepsia. 2007;48:201–4.

27. Hermann BP, Seidenberg M, Dow C, Jones J, Rutecki P, Bhattacharya A, et al. Cognitive prognosis in chronic temporal lobe epilepsy. Ann Neurol. 2006;60:80–7.

28. Howell KB, Eggers S, Dalziel K, Riseley J, Mandelstam S, Myers CT, et al. A population-based cost-effectiveness study of early genetic testing in severe epilepsies of infancy. Epilepsia. 2018;59:1177–87.

29. Scheidegger S, Held U, Grass B, Latal B, Hagmann C, Brotschi B, et al. Association of perinatal risk factors with neurological outcome in neonates with hypoxic ischemic encephalopathy. J Matern Fetal Neonatal Med. 2019;1–8.

30. Nalivaeva NN, Turner AJ, Zhuravin IA. Role of Prenatal Hypoxia in Brain Development, Cognitive Functions, and Neurodegeneration. Front Neurosci. 2018;12:825.

31. Volpe JJ. Perinatal brain injury: from pathogenesis to neuroprotection. Ment Retard Dev Disabil Res Rev. 2001;7:56–64.

32. Monaghan DT, Holets VR, Toy DW, Cotman CW. Anatomical distributions of four pharmacologically distinct 3H-L-glutamate binding sites. Nature. 1983;306:176–9.

33. Ambrogini P, Torquato P, Bartolini D, Albertini MC, Lattanzi D, Di Palma M, et al. Excitotoxicity, neuroinflammation and oxidant stress as molecular bases of epileptogenesis and epilepsy-derived neurodegeneration: The role of vitamin E. Biochim Biophys Acta Mol Basis Dis. 2019;1865:1098–112.

34. Levite M. Glutamate receptor antibodies in neurological diseases: anti-AMPA-GluR3 antibodies, anti-NMDA-NR1 antibodies, anti-NMDA-NR2A/B antibodies, anti-mGluR1 antibodies or anti-mGluR5 antibodies are present in subpopulations of patients with either: epilepsy, encephalitis, cerebellar ataxia, systemic lupus erythematosus (SLE) and neuropsychiatric SLE, Sjogren's syndrome, schizophrenia, mania or stroke. These autoimmune anti-glutamate receptor antibodies can bind neurons in few brain regions, activate glutamate receptors, decrease glutamate receptor's expression, impair glutamate-induced signaling and function, activate blood brain barrier endothelial cells, kill neurons, damage the brain, induce behavioral/psychiatric/cognitive abnormalities and ataxia in animal models, and can be removed or silenced in some patients by immunotherapy. J Neural Transm (Vienna). 2014;121:1029–75.

35. Reece RM, Sege R. Childhood head injuries: accidental or inflicted? Arch Pediatr Adolesc Med. 2000;154:11–5.
36. Glass HC, Hong KJ, Rogers EE, Jeremy RJ, Bonifacio SL, Sullivan JE, et al. Risk factors for epilepsy in children with neonatal encephalopathy. Pediatr Res. 2011;70:535–40.
37. Singh R, Turner RC, Nguyen L, Motwani K, Swatke M, Lucke-Wold BP. Pediatric Traumatic Brain Injury and Autism: Elucidating Shared Mechanisms. Behav Neurol. 2016;2016:8781725.
38. Dash HH, Chavali S. Management of traumatic brain injury patients. Korean J Anesthesiol. 2018;71:12–21.
39. Fujikawa DG. The role of excitotoxic programmed necrosis in acute brain injury. Comput Struct Biotechnol J. 2015;13:212–21.
40. Lucke-Wold BP, Nguyen L, Turner RC, Logsdon AF, Chen YW, Smith KE, et al. Traumatic brain injury and epilepsy: Underlying mechanisms leading to seizure. Seizure. 2015;33:13–23.
41. Wang J, Lin ZJ, Liu L, Xu HQ, Shi YW, Yi YH, et al. Epilepsy-associated genes. Seizure. 2017;44:11–20.
42. Myers KA, Johnstone DL, Dyment DA. Epilepsy genetics: Current knowledge, applications, and future directions. Clin Genet. 2019;95:95–111.
43. Chen T, Giri M, Xia Z, Subedi YN, Li Y. Genetic and epigenetic mechanisms of epilepsy: a review. Neuropsychiatr Dis Treat. 2017;13:1841–59.
44. Margineanu DG, Klitgaard H. Mechanisms of drug resistance in epilepsy: relevance for antiepileptic drug discovery. Expert Opin Drug Discov. 2009;4:23–32.
45. Sisodiya SM, Lin WR, Harding BN, Squier MV, Thorn M. Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. Brain. 2002;125:22–31.
46. Depondt C. The potential of pharmacogenetics in the treatment of epilepsy. Eur J Paediatr Neurol. 2006;10:57–65.
47. Hughes DM, Bonnett LJ, Czanner G, Komárek A, Marson AG, García-Fiñana M. Identification of patients who will not achieve seizure remission within 5 years on AEDs. Neurology. 2018;91:e2035–44.
48. Dlugos DJ, Sammel MD, Strom BL, Farrar JT. Response to first drug trial predicts outcome in childhood temporal lobe epilepsy. Neurology. 2001;57:2259–64.
49. Berg AT, Shinnar S, Levy SR, Testa FM, Smith-Rapaport S, Beckerman B. Early development of intractable epilepsy in children: a prospective study. Neurology. 2001;56:1445–52.
50. Callaghan BC, Anand K, Hesdorffer D, Hauser WA, French JA. Likelihood of seizure remission in an adult population with refractory epilepsy. Ann Neurol. 2007;62:382–9.
51. Kwan P, Brodie MJ. Drug treatment of epilepsy: when does it fail and how to optimize its use? CNS Spectr. 2004;9:110–9.
52. Stafstrom CE, Kossoff EM. Epileptic Encephalopathy in Infants and Children. Epilepsy Curr. 2016;16:273–9.
53. Tang F, Hartz AMS, Bauer B. Drug-Resistant Epilepsy: Multiple Hypotheses, Few Answers. Front Neurol. 2017;8:301.
Study flowchart. aCGH, array comparative genomic hybridization; MRI, magnetic resonance imaging; WES, whole-exome sequencing. * The investigation was comprehensive and included analyses of and the performance of, respectively, the following factors and procedures: arterial blood gas, pH, lactate, and pyruvate levels; complete serum biochemistry testing; analysis of cerebrospinal fluid; and toxicology screening. † Causes of epilepsy were determined by three independent pediatric neurologists, in case of inconsistent conclusions, patients were classified as unknown in the nongenetic group.
Figure 2

Flow diagram of the search process and search results.
**Figure 3**

Composition of nongenetic risk factors group. E.coli, Escherichia coli; GBS, group-B streptococcus; HIE, hypoxic–ischemic encephalopathy; HSV, herpes simplex virus; Inf, infections; IVH, intraventricular hemorrhage; M, metabolic disorders; PVL, periventricular leukomalacia; STRU, structural abnormalities in brain; TBI, traumatic brain injury; U, unknown

**Figure 4**

Seizure control status and their proportions in genetic and nongenetic groups. Inf, infections; M, metabolic disorders; STRU, structural abnormalities in brain; U, unknown
Figure 5

The forest plot of the prevalence of genetic causes of DRE in children

Keys: I-square=31.6%, Q=49, def=9; P=0.001; Based on random effect analysis