

MINISTRY OF EDUCATION MINISTRY OF DEFENSE
AND TRAINING
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**RESEARCH ON CLINICAL AND SUBCLINICAL
CHARACTERISTICS AND SOME GENE VARIANTS IN
CHILDREN WITH DRUG-RESISTANT EPILEPSY**

Major: Neuroscience

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SUMMARY OF DOCTOR OF MEDICINE THESIS

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ASK THE PROBLEM

Drug-resistant epilepsy accounts for about 30% of the overall epilepsy rate and is considered a major challenge for researchers and clinicians, not only seriously affecting the quality of life of individuals but also causing a great economic burden on families and society. Today, genetic diagnostic methods have opened up a new vision of genetic complexity while improving the effectiveness of epilepsy diagnosis increased from 10% to 30-40%. The combination of clinical characteristics and genetic polymorphism to help identify high-value risk factors for drug resistance is an important issue that clinicians always pay attention to and pose when initially approaching a child with epilepsy.

In Vietnam, in recent years, many studies on drug-resistant epilepsy in children have been published, but genetic analysis studies are still very limited. With the desire to identify risk factors on a clinical, subclinical basis and polymorphism, a number of common genes related to drug-resistant epilepsy include: *SCN1A*, *ABCB1*, *GABRA1* on 6 SNPs by Sanger sequencing, combined with whole-genome coding (WES) sequencing to identify causative variants on a specific group of drug-resistant epilepsy helping to prognosticate and orient the most optimal treatment method for drug-resistant epilepsy, towards further goals in individual medicine in Vietnam. We conducted research on the topic "Research on clinical and subclinical characteristics and some gene variants in children with drug-resistant epilepsy" with 2 objectives:

1. Description of clinical features, some changes on electroencephalography and cranial magnetic resonance in children with drug-resistant epilepsy
2. Identify some gene variants and their association with clinical characteristics in children with resistant epilepsy

NEW CONTRIBUTIONS OF THE THESIS

In pediatric neurology, drug-resistant epilepsy is a top concern for clinicians because of the complexity of classification, etiology, and selection of appropriate treatment regimens. Although there have been many studies on drug-resistant epilepsy risk factors that have been reported, the importance and risk factors vary from study to study. In Vietnam, to the best of our knowledge, there have been a number of studies that have identified a number of risk factors associated with drug-resistant epilepsy, and our research continues to confirm clinical risk factors while analyzing genetic variants in children using Sanger sequencing and using descriptive techniques whole genome coding (WES) on a specific group of children with drug-resistant epilepsy. As a result, we identified 6 independent risk factors including: history of epileptic status, history of neonatal seizures, history of febrile seizures, psycho-motor retardation, abnormal seizures and cranial seizures. In the field of genetics, through Sanger sequencing and the heterozygous genotype *SCN1A* rs2298771AG was recorded as a risk factor for drug-resistant epilepsy. The heterozygous genotype *SCN1A* rs3812718CT serves as a protective factor against the risk of drug resistance. In addition, whole coding genome sequencing (WES) has identified 10 cause/risk variants in 9/11 cases of children with manifestations of early-onset epilepsy. In particular, two variants of *TSC1* c.1888_1891del (p.K630Qfs*22) and *SCN1A* (NM_001165963) c.638C>A (p.S213*) lead to tuberous sclerosis complex and Dravet syndrome in Vietnamese children.

THESIS STRUCTURE

The thesis consists of 137 pages (excluding references and appendices), 4 chapters, 35 tables, 02 charts, 14 figures, 01 diagram; 219 references, including: 05 Vietnamese documents and 214 English documents. Question: 02 pages, document overview: 36 pages, research objects and methods: 19 pages, research results: 45 pages, discussion: 32 pages, conclusion: 02 pages, recommendation: 01 page.

CHAPTER 1 - OVERVIEW

1.1. Overview of epilepsy

1.1.1. Definition of epilepsy

According to the International Anti-Epilepsy Association (ILAE) 2014: Epilepsy is a disease of the brain, diagnosed when one of the following characteristics occurs: There are at least two spontaneous seizures occurring more than 24 hours apart. Having a spontaneous seizure and the chance of recurrence of the next spontaneous seizure is at least 60% in the next 10 years. Has a diagnosis of epilepsy syndrome.

1.1.2. Epilepsy epidemiology

1.1.3. Causes of epilepsy

According to ILAE in 2017, there are 6 groups of causes of epilepsy including: genetic, structural, metabolic, immune, infectious and unknown etiology

1.1.4. Diagnosis of epilepsy

According to ILAE in 2017, the diagnosis of epilepsy consists of 3 steps: Classification of seizures, classification of epilepsy, epilepsy syndrome

1.1.5. Classification of epilepsy

Table 1.1. ILAE 2017 Basic Classification of Epilepsy Types

Local onset		Onset of the body	Unknown onset
Even conscious ness	Decline consciousness	Advocacy Stiffness – seizures Different Inactivity (absenteeism)	Advocacy Stiffness – seizures Different Inactivity
Movement Initiation Inactive onset			
The local attack turned into 2-sided convulsive spasms			Uncategorized

1.1.6. Prognosis and treatment of epilepsy

According to Laxer et al. (2014), the prognostic factors of drug-resistant epilepsy are the high frequency of seizures in the early stages of epilepsy, neurological defects at the time of onset, and structural

pathological causes of epilepsy determined by cranial magnetic resonance (CHT)

Antiepileptic drugs are fundamental in the treatment of epilepsy and achieve seizure control in the majority of epilepsy. Currently, methods used in the treatment of drug-resistant epilepsy such as: combination of new-generation antiepileptic drugs, surgery, ketogenic diet, vagus nerve stimulation, etc.

1.1.7. Electroencephalography in epilepsy

Electroencephalography is a method of recording the electrical activities of the cerebral cortex using electrodes placed on the surface of the scalp (routine EEG) or placed directly on the cerebral cortex in the skull (intracranial EE, infiltrating EE). Epilepsy plays a very important role in: Diagnosis of epilepsy foci location. Diagnosis of epilepsy syndrome, epilepsy (local or general). Detection of subclinical activities (seizures).

1.1.8. Cranial magnetic resonance in epilepsy

Cranial magnetic resonance imaging is the first and most common diagnostic probe in epilepsy diagnosis. However, in clinical practice, there is still a certain percentage of epilepsy for which CHT does not confirm the damage

1.2. Childhood epilepsy

Due to the unique characteristics of the development and differentiation of the central nervous system in children, especially the myelinization of nerve fiber bundles in the brain with age, changes in the branching of dendrites, the maturation of neurons as well as changes in synapses both in quantity and quality, Seizures and epilepsy syndromes in children vary clinically as well as subclinically by age group.

1.3. Overview of drug-resistant epilepsy

1.3.1. Definition and diagnostic criteria for drug-resistant epilepsy

According to ILAE 2010: the diagnosis of drug-resistant epilepsy consists of 2 steps, of which step 1 is to evaluate the response to treatment; Step 2 is to make a diagnosis of drug-resistant epilepsy based on the response classification in step 1. In step 1, the response to

treatment is divided into the following categories: no seizures, treatment failure, and undefined. The concept of "seizure free" refers to all types of seizures, including premillion seizures with a duration of at least three times the interval between the two seizures before starting treatment, or at least 12 months, whichever is greater. The concept of "treatment failure" is when a seizure is still present with the current treatment. Based on the evaluation in step 1, in step 2, drug-resistant epilepsy is identified when it fails with two appropriate antiepileptic drugs and has no side effects (regardless of monotherapy or multitherapy)

1.3.2. Mechanism of drug-resistant epilepsy

Drug-resistant epilepsy by pharmacokinetic mechanisms includes: changes in drug transport proteins, changes in drug metabolism enzymes, changes in the structure of receptors and ion channels

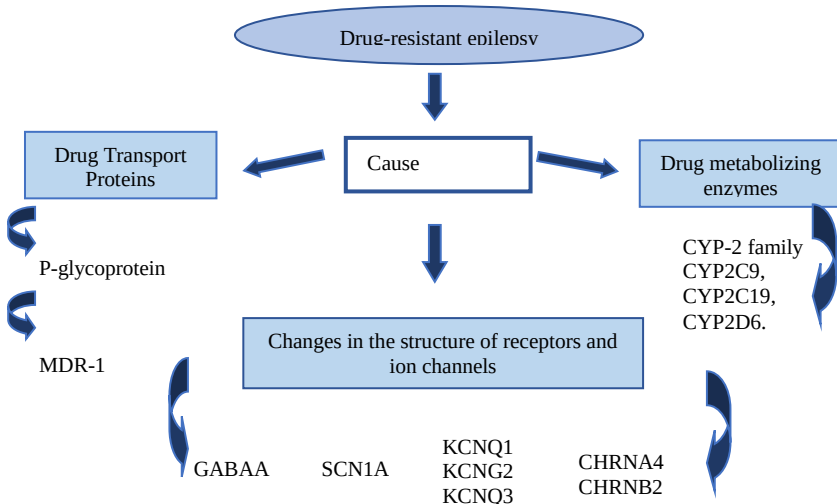


Figure 1.3. Pharmacokinetic mechanisms of drug-resistant epilepsy

1.4. Genetic diagnostic techniques in epilepsy

Genetic diagnostic methods include exome sequencing (WES), genome sequencing (WGS), Gene Panel, Chromosomal microarray

(CMA), Sanger sequencing, and chromosome analysis (NST). In particular, WGS was used for the highest diagnosis rate (48%), followed by WES (24-45%), Gene Panel (19-25%), CMA at 5-18%, Sanger sequencing and NST analysis generally gave a low incidence rate.

1.5. Research on drug-resistant epilepsy in the world and Vietnam

CHAPTER 2- RESEARCH OBJECTS AND METHODS

2.1. Subjects, time and location of the study: All children with epilepsy were examined, diagnosed, treated, and managed at the National Children's Hospital, Nghe An Obstetrics and Pediatrics Hospital.

2.2. Sample Selection Criteria

2.2.1. Study team criteria: All children with epilepsy under 16 years of age diagnosed according to ILAE 2014 criteria had full electroencephalogram and cranial magnetic resonance imaging results

2.2.2. Classification of study groups: Drug resistance and drug response groups according to ILAE 2010 standards

- Epilepsy Response Criteria: When the time interval between all seizures, including presymptomatic symptoms, is reduced by at least three times the time between the two episodes before starting treatment or has no seizures for at least 12 months, whichever is longer.

- Drug-resistant epilepsy group criteria: Persistent recurrence of seizures despite at least 2 changes to appropriately selected antiepileptic drugs (single or multitherapy) during treatment, each treatment for at least 3 months.

2.3. Exclusion Criteria

- Children with epilepsy due to acquired etiologies have been identified:

- Epilepsy due to perinatal brain damage: hypoxic encephalopathy, cerebral hemorrhage, cerebral infarction, obstetric trauma, fetal and perinatal infections, etc.

- Epilepsy after neurological infections: encephalitis, meningitis, cerebral apex...

- Epilepsy due to brain damage after traumatic brain injury or stroke: cerebral infarction, cerebral hemorrhage, etc.
- Epilepsy after autoimmune encephalitis or autoimmune etiology has been identified
- Epilepsy due to identified metabolic disorders.
- Kindergarten people with epilepsy did not agree to participate in the study.

2.4. Research Methodology

2.4.1. Study design: cross-sectional description with controls.

2.4.2. Sample size and sample selection method: The study sample size was taken by a convenient method, including 213 children with epilepsy divided into two groups: 112 children with drug-resistant epilepsy and 101 children with drug-responsive epilepsy according to the diagnostic and classification criteria of ILAE 2010.

2.5. Equipment and chemicals used in research

- Routine computerized EEG, video EEG and cranial magnetic resonance imaging
- Chemicals, machinery and equipment of the Genome Research Institute, Vietnam Academy of Science and Technology

2.6. Contents, research variables and evaluation methods

2.6.1. Objective 1: To describe clinical features, some changes on electroencephalography and cranial magnetic resonance in children with drug-resistant epilepsy

The study variables include:

a. Age: Age will be classified into groups: Infant and infant age (under 1 year old), early childhood age (from 1 year old to under 6 years old), adolescent age (over 6 years old). This is the age classification that is often applied in the diagnosis of childhood epilepsy according to the author Anthony Fine 2020

b. Gender: Male and female

c. Age of onset of first epileptic seizure: Subgroups: Less than 1 month old, from 1 month to less than 6 months, from 6 months to less

than 12 months, from 12 months to less than 18 months, from 18 months to less than 24 months, from 24 months to less than 36 months, from 36 months to less than 72 months, and over 72 months old.

d. Age of early onset of seizures: Early onset of seizures when the age of onset ≤ 12 months

e. Classification of seizures: classification of seizures based on the clinical classification of seizures according to the 2017 ILAE standards

f. Frequency of attacks: including 4 levels according to the classification of Engel and cs in 1993: Daily episodes: over 30 episodes/month, Weekly episodes: from 5 to 30 episodes/month, Monthly episodes: from 1 to 4 episodes/month, Scattered episodes: less than 1 episode/month

g. Psycho-motor development status: Quantified by the psycho-motor development index, also referred to as the development index (DQ) for patients under 5 years old or the intelligence index (IQ) in patients over 5 years old, and then classified by the level of psycho-motor development according to the International Classification of Diseases, ICD-10 version

h. History of epilepsy: according to the new diagnostic criteria of ILAE in 2015, i.e. seizures lasting more than 5 minutes or not waking up between attacks.

i. Epilepsy syndrome

j. History of neonatal seizures: Seizures within 28 days of birth are documented by the doctor in the hospital records or discharge papers.

k. Electroencephalogram: The types of abnormalities on the ND are classified into: localized, localized whole, whole

l. Magnetic Resonance: Listed Descriptions by Vulnerability Characteristics

m. Correlation analysis of drug-resistant epilepsy characteristics

n. Identification of risk factors for drug-resistant epilepsy

independently in multivariate analysis

2.6.2. Objective 2: Identify several gene variants and their association with clinical traits in children with drug-resistant epilepsy

2.6.2.1. SCN1A, ABCB1, GABRA1 Gene Polymorphism Analysis

The process was carried out as follows: At the Institute of Genomics, Vietnam Academy of Science and Technology, Sanger sequencing of 213 blood samples of the research group was carried out. Screening in 112 children with drug-resistant epilepsy was 11 children diagnosed with early-onset epilepsy including the following characteristics: early onset of epilepsy < 12 months), persistent drug resistance with daily frequency of seizures accompanied by nervous system regression manifested by mental retardation – severe level of movement and severely sequencing the whole encoding genome (WES). The analysis process is carried out in the following sequence:

- a) Extraction and determination of total DNA concentration*
- b) PCR for specific amplification of gene fragments containing variants of interest:* Primer design, PCR for specific amplification of *SCN1A*, *ABCB1* and *GABRA1* gene variants
- c) Sanger sequencing:* Sequencing reactions and capillary electrophoresis, analysis of Sanger sequencing results:
- d) Sequencing of the whole coding genome:* Setting up the DNA library, Sequencing the enriched DNA library on the NextSeq 500 machine, Processing the data obtained from sequencing the whole coding genome

2.6.2.2. Research variables

a, SCN1A, GABRA, ABCB1 gene polymorphic characteristics

b. Variable sequencing of the entire coding genome

2.7. Analyze and process data

The study variables were collected according to the uniform study record sample. Entering figures using Excel 2016 software. Data processing using SPSS 2.0 software.

2.8. Research Ethics:

The process of conducting the research has the permission and consent of the Ethics Council of the National Children's Hospital No. 723/BVNTW-HDDD dated April 21, 2022. In addition, before collecting blood samples, the purpose of the study was clearly explained, and the family also confirmed in the volunteer application to participate in the study.

CHAPTER 3 - RESEARCH RESULTS

3.1. General, clinical and subclinical characteristics of the research team

3.1.1. General characteristics

3.1.1.1. General characteristics of the research team

The overall median age was 58.1 ± 45.0 months

3.1.1.2. Research age group: mostly concentrated in the age group from 1 to under 6 years old

3.1.1.3. Distribution by gender: the ratio of males to females is 1.05.

3.1.2. Characteristics of clinical history

3.1.2.1. History of neonatal seizures

Our study found that 20 out of 213 children had a history of neonatal seizures, accounting for 9.38%. In the resistance group, this rate was 15.18%, higher than this rate in the drug response group of 2.97%. This difference was statistically significant with OR = 5.85, $p = 0.006$.

3.1.2.2. History of febrile seizures

Children with a history of febrile seizures in the drug-resistant group were higher than those in the drug-responsive group with rates of 35.71% and 17.82%, respectively. Children without a history of febrile seizures in the drug-resistant group and drug response were 64.29% and 32.18%, respectively. This difference is statistically significant with OR = 2.56, $p = 0.004$

3.1.2.3. History of epilepsy

Children with a history of epilepsy in the drug-resistant group were higher than those in the drug-responsive group with rates of

25.90% and 1.99%, respectively. The children did not have a history of epilepsy in the drug-resistant group and responded to 74.10% and 98.01%, respectively. This difference was statistically significant with $OR = 17.30$, $p = 0.004$.

3.1.2.4. Age of onset of seizures

In the early-onset group (< 12 months), the rate of drug resistance and drug response was 68.27% and 31.73%, respectively. Meanwhile, in the group of children ≥ 72 months, there is a tendency to be the opposite to the rate of drug resistance, responding to 5.88% and 94.12%, respectively. This difference is statistically significant with $p = 0.000$

3.1.3. Clinical characteristics

3.1.3.1. Classification of clinical seizures by the research group

3.1.3.2. Frequency of clinical seizures in the study group

Local onset predominates in the resistance group, whereas global onset predominates in the drug-responsive group, this difference is statistically significant with $p = 0.000$

3.1.3.3. Characteristics of attack frequency and duration of attacks

The drug-resistant group, the weekly attack rate dominated with 50.90%, the drug-responsive group, the monthly attack rate dominated with 59.41%. This difference is statistically significant with $p = 0.001$.

3.1.3.4. Frequency of early-onset group clinical attacks

3.1.3.5. Classification of epilepsy syndrome by the research team

The rate of epilepsy syndrome in the drug-resistant group was 24.14% higher than in the drug-responsive group with a rate of 6.93%. The difference was statistically significant with $OR = 4.27$, $p = 0.001$.

3.1.3.6. Psycho-motor development status

In the drug resistance group, the rate of children with mental and motor retardation dominated with 93.75%. Meanwhile, in the group responding to the drug, most of the children developed normally with a rate of 73.27%. This difference was statistically significant with $OR = 25.96$, $p = 0.000$.

3.1.4. Subclinical characteristics of the research group

3.1.4.1. EEG

100% of the children in the study group were made of epilepsy, 150 children had epilepsy abnormalities on the motherland, accounting for 70.42%. Children had this result in the drug-resistant group higher than the drug-responsive group with rates of 92.68% and 45.55%, respectively. This difference was statistically significant with OR = 15.54, $p = 0.000$. In the resistance group, local paroxysmal waves dominated over global paroxysms with a rate of 59.61% vs. 40.39%. In contrast, in the response group, the overall exacerbation wave was more dominant with a rate of 80.44% compared to 19.56%. This difference was statistically significant (OR = 5.97, $p = 0.000$).

3.1.4.2. Cranial magnetic resonance

In our study, 100% of children received a brain CT scan, recording 38.02% abnormal results. In particular, the drug-resistant epilepsy group with abnormal results on cranial CHT film dominated the drug-response group with a rate of 58.93% compared to 14.86%. In contrast to the abnormal results, the results that did not find lesions in the drug-responsive group were higher with a rate of 85.14% compared to 41.07%. This difference is statistically significant with OR = 8.23, $p = 0.000$.

3.1.5. Risk factors for drug-resistant epilepsy in multivariate analysis

Table 3.20. Risk factors for drug-resistant epilepsy in multivariate analysis

Clinical factors	Beta	SE	OR	95% CI	p
History of neonatal seizures	1,7	0,9	5,7	1,1-31,4	0,04
History of febrile seizures	1,0	0,5	2,6	1,2-6,7	0,04
History of epileptic status	3,2	0,9	24,1	3,9-146,5	0,00
Developmental delay	2,6	0,6	13,8	4,6-41,3	0,00
Abnormal heat stroke	2,1	0,5	7,8	2,7-22,4	0,00
Cranial CHT abnormalities	1,4	0,4	4,2	1,7-10,1	0,00

In multivariate logistic regression analysis, we noted risk factors independent of drug-resistant epilepsy including: history of neonatal seizures (OR = 5.7, $p = 0.04$), history of febrile seizures (OR = 2.6, $p =$

0.04), history of epileptic status (OR = 24.1, $p = 0.00$), psycho-motor delay (OR = 13.8, $p = 0.00$), abnormal heat stroke (OR = 7.8, $p = 0.00$) and cranial CHT abnormalities (OR = 4.2, $p = 0.00$).

3.2. Identification of *SCN1A*, *ABCB1* and *GABRA1* gene polymorphisms

3.2.2. Genotypic and allele distribution of the polymorphisms surveyed in the study sample group

3.2.2.1. *SCN1A* gene variants

A total of three *SCN1A* (NM_001165963.4) gene variants were screened on 213 samples through Sanger sequencing, including: rs2298771 (c.3199G>A/C), rs3812718 (IVS5N+5C>T/A) and rs10188577 (NM_006920.6:c.265-699A>G)

3.2.2.2. *ABCB1* gene variants

Through Sanger sequencing, we investigated two variants *ABCB1* (NG_011513.1) rs1128503 and rs7045642

3.2.2.3. *GABRA1* gene variants

The only *GABRA1* (NG_011548.1) variant screened in this study was rs2279020 (g.53693G>A). Sanger sequencing showed that all three genotypes GG (48/213 samples), GA (122/213 samples) and AA (43/213 samples) were detected

3.3. Investigate the correlation between *SCN1A*, *ABCB1* and *GABRA1* gene variants to antiepileptic drug resistance

3.3.1. Evaluation of the genetic characteristics of the research team

Among the three *SCN1A* gene variants surveyed, the genotypic distribution of rs2298771 and rs3812718 between the two resistance and response groups all exhibited statistically significant differences with p-values of 0.038 and 0.01, respectively. Genotypic distributions, alleles, and dominant/recessive genetic patterns continued to be investigated in two gene variants, *ABCB1* (rs1128503 and rs1045642) and *GABRA1* rs2279020; however, no statistically significant differences were found between the two groups

3.3.3. Evaluation of the association of genetic traits and one/multiple clinical factors associated with drug-resistant epilepsy

3.3.3.1. Relationship between genetic traits and age of onset of epilepsy to drug resistance

In children with epilepsy onset after 1 year of age, the results showed a statistically significant difference in the genotypic distribution of *SCN1A* rs3812718CT (OR = 0.299; $p = 0.042$), *ABCB1* rs1045642TT (OR = 0.275; $p = 0.05$) and two T alleles (OR = 0.522; $p = 0.025$), allele C (OR = 1.914; $p = 0.025$) *ABCB1* variant rs1045642

3.3.3.2. Association of genetic traits and manifestations of convulsive fever to drug resistance

There was a significant difference in the heterozygous CT (OR = 0.276; $p = 0.005$) and allele C (OR = 1.762; $p = 0.018$) genotype distribution of the *SCN1A* rs3812718 variant between the two groups and drug response in the group without a history of febrile seizures

3.3.3.3. Relationship between genetic traits and psycho-motor development status to drug resistance

There was a statistically significant difference between the resistance group and the response group in the *GA* genotypic distribution (OR = 4.373; $p = 0.01$) and both alleles A (OR = 0.246; $p = 0.011$), G (OR = 4.056; $p = 0.011$) of the *SCN1A* rs2298771 variant. The recessive genetic model (GG+GA/AA) of the *RSN1A* rs2298771 gene variant in the drug-resistant group was statistically significantly higher than that in the drug-responsive group (OR = 4.373; $p = 0.01$).

3.3.3.4. Association of genetic traits and history of epilepsy status to drug resistance

In the group with no history of epileptic status, there were statistically significant differences in the *SCN1A* rs3812718CT genotypic distribution (OR = 1.984; $p = 0.01$) and the distribution of both the T (OR = 0.623; $p = 0.032$) and C allele (OR = 1.602; $p = 0.032$) *ABCB1* rs1045642 variant

3.3.3.5. Evaluation of the association of genetic traits combined with multiple clinical factors related to drug resistance

3.4. Results of sequencing of the whole genome encoding 11 drug-resistant epilepsy samples

10 cause/risk variants have been identified in 9/11 cases.

These variants are located on 09 genes, including: *TSC1*, *NOTCH3*, *PHACTR1*, *SCN1A*, *EFHC1*, *CACNA1H*, *SLC12A25*, *DPYD* and *TUBB2B*. Two children with E73 and HANDRE1 encoded drug-resistant epilepsy were cases in which no relevant variant was found to meet the screening criteria

CHAPTER 4 - DISCUSSION

4.1. General characteristics, clinical and subclinical characteristics of the research team

4.1.1. General characteristics

4.1.2. Prehistory characteristics

4.1.2.1. Epileptic state

In our study, the difference in epileptic status rates between the two groups was statistically significant, with OR = 17.3, $p = 0.000$. The results of our study are similar to those of authors Gururaj et al. (2006), Wang Xue-Ping et al. (2019), and Karaoğlu et al. (2021) all show that a history of epileptic status is correlated with drug resistance in epilepsy treatment. Multivariate analysis also noted that epileptic status correlates with drug resistance with OR = 24.1, $p = 0.00$.

4.1.2.2. Neonatal seizures

In the study group, we noted a statistically significant difference in the rate of neonatal seizures between the two groups with OR = 5.85, $p = 0.006$. This result is comparable to the study by Gururaj et al. (2006) with 9.5% of children with a history of neonatal seizures and a significant difference between the two groups with $p = 0.001$. When multivariate regression analysis noted, a history of neonatal seizures was correlated with drug resistance with OR = 5.7, $p = 0.04$. Children with epilepsy who have neonatal-onset seizures have a worse prognosis

in terms of their ability to respond to drug treatment. On the other hand, seizures at this age will increase the amount of time the brain is affected by the seizure and further aggravate brain damage.

4.1.2.3. Febrile seizures

In the resistance group, this rate is 35.71%. The difference in the proportion of children with a history of febrile seizures between the two groups was statistically significant with $OR = 2.54$, $p = 0.004$. Our results are similar to those of Wang Xue-Ping et al. (2019). In the analysis of multivariate regression, we also noted a history of febrile seizures that correlated with drug-resistant epilepsy with $OR = 2.6$, $p = 0.04$. Of the 58 children with a history of febrile seizures, we recorded 7 cases of typical epilepsy syndrome, including 4 Dravet syndrome, which is a syndrome with drug-resistant prognostic factors

4.1.3. Clinical characteristics

4.1.3.1. Classification of clinical seizures

In the study, we used the epilepsy classification according to the 2017 ILAE. Noting the characteristics of seizures in studies describing drug-resistant epilepsy, it has been found that cases with localized seizures at the beginning have a lower response rate to antiepileptic treatment than generalized seizures. In our study, when analyzing local attacks, up to 55.35% of cases were in the drug resistance group, while the drug response group was 32.67%. In contrast, the overall onset of seizures was recorded at 65.34% in the response group which was higher than in the resistance group at 36.60%. The difference is statistically significant with $p = 0.000$. This result is similar to the study of Arafa et al. in 2011 with $p = 0.03$.

4.1.3.2. Classification of epilepsy syndrome

In the study, we recorded 34 cases diagnosed with epilepsy syndrome, accounting for 15.96%, most of which were in the drug-resistant group with 79.41%. Among these syndromes, we pay attention to the syndromes of early-onset epilepsy including: Dravet Syndrome, West Syndrome, Ohtahara Syndrome.

4.1.3.3. Early onset classification

According to ILAE in 2022, early-onset epilepsy is a group of epilepsy that starts before 12 months of age. This group has epilepsy-related epilepsy syndromes, which is one of the prognostic criteria for severe epilepsy. In our study, there were 104/213 (48.82%) children with early-onset epilepsy, of which the drug-resistant epilepsy group was 71/104 children accounting for 68.26% and the response epilepsy group was 33/104 children accounting for 31.73%. This difference was statistically significant with a $p < 0.05$. Our study is similar to that of Berg et al. (1996) and Yilmaz et al. (2013). Clinical classification of early onset we found that movement seizures predominated, there were 2 groups of movement seizures: spasmodic and muscle seizures. These two types of seizures are recorded as having a prognostic role in the treatment of epilepsy. We recorded a much higher frequency of two types of spasms and muscle seizures in the resistance group with rates of 20/112 (17.85%) and 40/112 (35.71%), respectively, compared to 8/101 (7.92%) and 12/101 (11.88%) responses. This difference is statistically significant with $p = 0.001$. The results of our study are similar to those of Chawla and cs (2002).

4.1.3.4. Frequency of seizures

The frequency of daily seizures proves the severity of the disease: the more repeated and prolonged the seizures, the more severe the effects on the development and completion of normal physiological functions of the brain, causing serious consequences for intellectual development in the short and long term, and at the same time participate in the process of causing secondary epilepsy. In the study group, we focused on the proportion of daily frequency of seizures, which is thought to be a factor in the exacerbation of epilepsy. We recorded that the rate of drug resistance in the group accounted for 17.85%, higher than in the epileptic response group of 3.96%. This difference is statistically significant with $p = 0.000$, which is similar to the study of Chawla et al. (2002), Saygi and cs (2014).

4.1.3.5. Psycho-motor retardation

Epilepsy and psycho-motor retardation often coexist and are related to each other, the diagnosis of epilepsy must be based on accompanying pathologies, in which psycho-motor retardation is identified as a disease of the brain caused by epilepsy or the pathology of the brain itself that causes seizures. Thus, psychomotor retardation is considered both a consequence and a cause. In our research group, children with psycho-motor retardation accounted for 66.66%. In the drug resistance group 105/112 (93.75%), the epilepsy group responded 37/101 (37.63%). This difference is statistically significant with $OR = 25.96$, $p = 0.000$, similar to the results of the study of Arafa et al. (2011). When analyzing multivariate regression, the correlation between psycho-motor retardation and drug-resistant epilepsy was determined with $OR = 13.8$, $p = 0.000$.

4.1.4. Subclinical characteristics of the research team

4.1.4.1. EEG

In the study group, abnormal heat stroke in the drug-resistant group dominated 92.86%, the lower response group accounted for 45.55%, the difference was statistically significant with $OR = 15.54$, $p = 0.000$. Our results are similar to those of Sporis et al. (2013), Malik et al. (2008). In the group of PDs with abnormal waves, we recorded that the overall exploding waves accounted for 52.66%, and the local exploding waves accounted for 47.33%. When comparing the characteristics of clinical attacks and EEG, we noted that there was no similarity. Learn about this difference according to author Ajay Gupta and cs at the Cleveland Epilepsy Center, USA, in a study conducted on 176 cases of drug-resistant epilepsy and treated with surgery, confirmed that 10 cases had epilepsy-causing lesions that were localized in only one hemisphere of the brain but the EEG abnormalities were widespread a whole hemisphere like a total epilepsy

4.1.4.2. Geometric characteristics of cranial magnetic resonance

In our study, 82/213 cases of cranial CHT abnormalities accounted for 38.02%, mainly in the drug-resistant group with 66/82 cases accounting for 80.48%; this difference was statistically significant with OR = 8.23, $p = 0.000$. Our results are similar to the studies of Arafa et al. (2015), Gururaj et al. (2006) and Patil et al. (2009). In the group of cranial CHT abnormalities, we paid attention to cases of cortical dysplasia and brain parenchymal atrophy described on the results of cranial CT scans, specifically 21/82 cases of cortical dysplasia with a rate of 25.60% and 20/82 cases of cerebral parenchymal atrophy accounting for 24.39%. These are two typical types of abnormalities that account for a high proportion of the lesions identified on the CHT.

4.1.5. Risk factors for drug-resistant epilepsy

We conducted a multivariate logistic regression analysis to record risk factors associated with drug-resistant epilepsy including: history of neonatal seizures, history of febrile seizures, history of epilepsy, psycho-motor retardation, abnormal manifestations of CND, and abnormal imaging on the cranial CT. When considering the above factors, we found that some cases have more than 1 co-existing factor, for example, when there is a history of epilepsy at the same time, there may also be a history of seizures due to fever, or the onset of neonatal seizures or mental and motor retardation... Therefore, high-risk factors are easy to keep correlated when analyzing multivariate without being lost like other factors such as frequency of seizures, age of early onset, type of seizures. Because of the above limitations, for risk factors that lose significance when analyzing multivariate regression, although there is a correlation when analyzing monovariates, we still consider them as risk factors for drug resistance in the treatment of epilepsy.

4.2. Role and impact of *SCN1A*, *ABCB1* and *GABRA1* gene polymorphisms related to drug resistance in childhood epilepsy

4.2.1. *SCN1A* Gene Polymorphism

The rs2298771 G>A (p.Thr1067Ala) variant is a variant located in the *SCN1A* gene coding region. GA heterozygous genotype may be a

risk factor for drug-resistant epilepsy based on the results of statistical analysis in this study ($p = 0.037$). In addition, the diving model (GG+GA/AA) also showed that children with allele G were at higher risk of drug resistance than other individuals ($OR = 1.963$; $p = 0.042$). The second *SCN1A* variant evaluated in this study is rs3812718 C>T (IVS5N+5C>T). The polymorphism of the rs3812718 variant is considered a risk factor for epilepsy; Notably, a study of epilepsy in Japan published in 2008 showed that the rs3812718 variant may affect antiepileptic resistance when taking carbamazepine (CBZ) and valproic acid (VPA) concomitantly. The rs10188577 A>G variant *SCN1A1* is a common SNP in the intron region of the *SCN1A* gene, which affects transcription factor or methylation. The association of the rs10188577 variant with epilepsy risk has been reported in populations of Indians, Iranians, Taiwan, Malaysians, Hong Kong, China, and some white populations such as Egypt and the Republic of Kosovo.

4.2.2. *ABCB1* gene polymorphism

The rs1128503 (exon 12) and rs1045642 (exon 26) variants are the most common, with a much higher frequency (about 50%, according to gnomAD) and are well-studied variants associated with multidrug resistance. Most studies involving *ABCB1* polymorphism and drug-resistant epilepsy have the presence of these two variants. However, the results were inconsistent between studies. In this study, we also selected these two variants to investigate the genetic correlation to treatment resistance, but the results did not find any association at both the genotypic and allele levels between the *ABCB1* variants rs1128503, rs1045642 and drug-resistant epilepsy. The analysis of the haplotype combining these two variants also did not show any meaningful differences when comparing the two groups of resistance and drug response. These results are similar to the study by Zhao and cs when evaluating 245 Chinese children with epilepsy treated with levetiracetam.

4.2.3. *GABRA1* gene polymorphism

The *GABRA1* rs2279020 variant is a complex polymorphism, which can alter the structural properties of proteins by affecting the natural splicing of mRNA molecules. However, there is still much controversy about the link between *GABRA1* gene variants and drug-resistant epilepsy, including the rs2279020 variant, with inconsistent results reported in studies. For example, in Jordan, people with allele G (GG+GA) tend to develop drug resistance. In our study, we also found no genotypic and allele correlation between the *GABRA1* rs2279020 variant and drug-resistant epilepsy. However, in this study, the sample size is not large enough to confirm this association, and it is necessary to expand the scale of the study to confirm this observation

4.3. Polymorphism of *SCN1A*, *ABCB1* and *GABRA1* gene variants in the group carrying clinical risk factors associated with epilepsy drug resistance

In this study, in addition to the general genetic correlation that has been indicated, we further evaluated the association between the *SCN1A*, *ABCB1* and *GABRA1* gene variants to each of the combinations of clinical risk factors noted above. Notably, the two *SCN1A* rs3812718CT, *ABCB1* rs1045642TT and *ABCB1* allele T RS1045642 has been identified as factors that may help reduce the risk of drug resistance; while allele C *ABCB1* rs1045642 increases the risk of drug resistance in children with onset after 12 months. In this age group, in the general correlation analysis as well as the evaluation of the correlation with age of onset, we noted that the CT genotype of *SCN1A* rs3812718 is at risk of drug resistance. A meaningful association between *SCN1A* rs3812718 and *ABCB1* rs1045642 to drug-resistant epilepsy was also shown in the group that did not record epileptic status in our study. The heterozygous genotype CT *SCN1A* rs3812718 and allele T *ABCB1* rs1045642 are protective factors, while the allele C *ABCB1* rs1045642 is a risk factor that may lead to drug resistance in this group. In addition, we also found an association between the *SCN1A* rs2298771 variant and the risk of drug resistance in children

with psychomotor retardation, the AG genotype, and allele G as high risk factors. However, when conducting a multivariate logistic regression analysis that included all of the above clinical risk factors and the two variants (*SCN1A* rs3812718 and rs2298771) that were assessed as associated with drug-resistant epilepsy through a series of analyses in this study, it was found that these two variants were not the main risk factors for the disease drug resistance.

4.4. Coding genome sequencing helps identify epilepsy-related cause/risk variants and implications in clinical practice toward personalized medicine

In this study, a total of 11 children were sequenced to determine their genetic etiology. Through the analysis process, 9 out of 11 cases have identified causal variants/related risks/possible related to the disease phenotype in children (Table 3.31). Notably, genetic mutations in *the genes TSC1* and *SCN1A* leading to tuberous sclerosis complex and Dravet syndrome were identified in cases of DRE10 and DRE25, respectively. In particular, *TSC1* (NM_000368) c.1888_1891del (p.K630Qfs*22) is a known pathogenic variant (ClinVar ID: [5097](#)); while *SCN1A* (NM_001165963) c.638C>A (p.S213X) is a new variant, which has not been reported in any previous database or scientific publication. Both of these variants are early-terminated coding variants on the polypeptide chain, which in turn can lead to harmful effects on the function of the encoded protein and pathogenic expression. In addition to the two children DRE10 and DRE25 who have identified causative variants, risk variants that follow Mendelian genetics that may be associated with the epileptic phenotype in seven cases DRE17, DRE18, DRE48, DRE49, HANDRE12, HANDRE18 and HANDRE45 have also been indicated. These variants are all missense, mostly known, and only *PHACTR1* (NM_001322313) c.473C>G (p.P158R) is found in the case of DRE18 as the new variant (Table 3.32)

CONCLUDE

1. Clinical features, some changes on EEG and cranial magnetic resonance

- *General characteristics of the research group:* Average age 58.1 ± 45.0 months, male-to-female ratio is 1.05. The distribution of the research age group from 1 to under 6 years old accounted for 57.3%

- *Clinical features:* Prehistoric and clinical features predominate the drug-resistant group with a statistically significant difference of $p < 0.05$ including: Prehistoric features: neonatal seizures, febrile seizures and epileptic status. Clinical features: early onset (≤ 12 months), local motor attacks, intensity of daily seizures, epilepsy syndrome, mental retardation - moderate, severe and severe motor impairment.

- *Characteristics of hypertension and CHT:* 70.4% recorded abnormal hypertension waves. in which, the overall exacerbation waveform accounted for 80.44% of the drug response group dominance, the local exacerbation waveform accounted for 59.61% of the drug resistance group dominance, the difference of statistical significance with $p < 0.05$. Abnormal cerebral CH recorded 5 characteristics of damage, cortical dysplasia accounted for 25.92% of the dominant group of drug resistance, cerebral CH did not find the dominant lesion of the response group, a statistically significant difference with a $p < 0.05$.

- *Risk factors for drug-resistant epilepsy:* 6 independent risk factors including: History of epileptic state OR = 24.1, $p = 0.00$; history of neonatal seizures OR = 5.7, $p = 0.04$; history of febrile seizures OR = 2.6 times, $p = 0.04$; mental and motor retardation OR = 13.8 times, $p = 0.00$; cranial CHT abnormalities OR = 4.2, $p = 0.00$; abnormal OR = 7.8, $p = 0.00$. In addition, we recorded 4 risk factors including: Age of onset of < 12 months, clinical presence of local motor seizures, diagnosis of epilepsy syndrome, frequency of daily seizures with $p < 0.05$.

2. Gene variants SCN1A, ABCB1, GABRA1 and association with clinical features of drug-resistant epilepsy

- Statistically significant differences were found in 2/6 variants of the three genes of interest (*SCN1A*, *ABCB1*, and *GABRA1*) when compared between the drug-responsive and drug-resistant groups. In particular, the heterozygous genotype *SCN1A* rs2298771AG acts as a risk factor for drug-resistant epilepsy (OR = 2.01, p = 0.037). In contrast, the heterozygous genotype *SCN1A* rs3812718CT serves as a protective factor against the risk of antiepileptic resistance (OR = 0.304, p = 0.002).
- No meaningful association was found between the remaining 4 variants *SCN1A* rs10188577, *ABCB1* (rs1128503 and rs1045642) and *GABRA1* rs2279020 with antiepileptic response or resistance.
- Through whole-genome sequencing (WES), 10 cause/risk variants have been identified in 9/11 cases of children with manifestations of early-onset epilepsy. In particular, two variants of *TSC1* c.1888_1891del (p.K630Qfs*22) and *SCN1A* (NM_001165963) c.638C>A (p.S213*) resulting in tuberous sclerosis complex and Dravet syndrome have been identified in two cases (DRE10 and DRE25).

PETITION

From the results of our research, we have some recommendations as follows:

1. Attention should be paid to prognostic factors of drug resistance at the start of epilepsy treatment based on risk factors for drug-resistant epilepsy.
2. It is necessary to investigate *SCN1A* gene polymorphisms in larger sample sizes in future studies, especially related to 2 variants rs2298771 and rs3812718 to further confirm trends related to antiepileptic response and resistance in the Vietnamese population.
3. Children with epilepsy with complex clinical manifestations should undergo molecular diagnosis based on NGS technology, in order to make a definitive diagnosis towards personalized treatment in clinical practice.

LIST OF PUBLISHED WORKS
RESEARCH RESULTS OF THE THESIS

1. **Hai Xuan Tang, Muoi Dang Ho**, Phuong Vu, Hung Vu Cao, Vinh Anh Ngo, Van Thi Nguyen, Thuan Duc Nguyen and Ton Dang Nguyen (2024). Association between genetic polymorphism of *SCN1A*, *GABRA1* and *ABCB1* with drug responsiveness in Vietnamese epilepsy children. *Medicina*, 60(4):637.
2. **Ho Dang Muoi**, Nguyen Dang Ton, Nguyen Duc Thuan (2024). Description of EEG characteristics, magnetic resonance imaging in children with drug-resistant epilepsy. *Vietnam Medical Journal*, 537(2): 253-257.