“VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND PHARMACY TIMIȘOARA

GENERAL MEDICINE

MEDICAL GENETICS DEPARTMENT

ROZA EUGENIA

PHD THESIS

GENETIC BACKGROUND IN EPILEPSY- FROM DIAGNOSIS TO MANAGEMENT IN THE PEDIATRIC POPULATION

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Content table

I. Introduction

Epilepsy is a chronic disorder characterized by the recurrence of convulsive episodes that appear due to spontaneous synchronous electrical discharges of nervous cells from different cerebral areas, resulting in a great variety of clinical manifestations. One cannot establish a diagnosis of an epileptic seizure based on a singular event, and it is required that the patient has a minimum of 2 such events.

The etiology is very important , knowing that different ethiologies and syndromes require different approaches and treatments. Some authors prefer the term „epilepsies" to avoid the exact ethiologcal term whitch imply a different prognosis and management.

It is estimated that in the past, over 70% of epilepsies were considered to be idiopathic , but as genetic testing evolved and became more available, it has been proven that there was an underlying genetic etiology in a significant amount of epilepsis. Presently, the genetic etiology of epilepsy represents a main focus in research, the speed of scientifica advancement in this area being remarkable.

I chose this theme because in Romania, at the present time, there are no clear-cut records of patients with genetic epilepsies, nor are there nataional registries and there is no follow-up or testing protocol for patients that are likely with a genetic etiology.

Medical genetics is an ever evolving field all over the world. Even so, in Romania, patient and even clinician`s access to a genetic diagnosis based on modern genetic techniques is limited due to both the lack of medical genetics specialists and economic reasons.

The present thesis contains a general part which is an overview on the present degree of knowledge in genetic epilesies, historical data, genetic testing options, the importance of establishing a correct diagnosis in epilepsy, as well as a short presentation of the most common phenotypes associated with the most well-known genetic mutations involved in epilepsy at the moment.

The aims of the thesis were :

- To identify the genetic etiology in patients with epilepsy in Dr Victor

Gomoiu Children s Hospital in Bucharest explaining the pathogenesis offers precios informations on management, prognosis and the probability of seizure recurrence.

- Identifying and characterizing the patients that harboured genetic mutations which could explain the epilepsy diagnosis.
- Evaluating the therapeutical attitude and patient management in relation to the genetic result.
- Creating a diagnosis and follow-up protocol for patients with genetic epilepsy.
- Creating a local patient registry that can serve as a starting point for a future national registry.

- Solidifying the collaboration between medical genetics centers and the pediatric neurology department.
- Thus to achieve the aforementioned objectives, I realised a retrospective and prospective longitudinal study on the pediatric neurology department of „Dr. Victor Gomoiu“ Hospital in Bucharest, and the results were detailed in the special part of the thesis.

II. General part

Epilepsy represent one of the most frequent neurological discorders with an overall prevalence of 1% and according to the International League Against Epilepsy the last consensus on the classification of epilepsy shines a special light on genetic epilepsy.

Genetic epilepsy is one of the most frequent neurologic pathologies. It has a prevalence of approximately 1% .

Genetic epilepsy, by definition, is a disorder which appear as a direct result of a known of unknown genetic mutation, and the epileptic seizure is the centerpiece of clinical spectrum. The genetic basis of some forms of epilepsies is hard to demonstrate and the extensive phenotypic variability represents an important obstacle. Since 1995 when the first epilepsy gene was discovered- CHRNA4 , responsible for autosomal dominant nocturnal frontal lobe epilepsy new genes are discovered and the technological advantage in genetics and genomics domains

constantly widens the diagnosis horizon. The progress is considerable because the investigations available only in research settings, are also available now they to the general public. Nevertheless, because of the large variability of epilepsies in which the possibility of a genetic etiology was identified, in most cases, responsible genes were yet unknown. The reasons are variable- starting with phenotypical variability between relatives, or within the same family, in which the inheritance is apparently autosomal dominant, geneti heterogenicity or complex phenotypes requiring detailed evaluations.

III. Special part

Chapter 1. Aim and objectives

The clinical study consisted on the anlysis of pediatric patients diagnosed with epilepsy, admitted in the Pediatric Neurology Department of Dr. Victor Gomoiu Clinical Children s Hospital between October 2016 – June 2020, the study of the genetic etiology in this group of patients, the methods of diagnosis and the impact of a positive result on pacient management.

Aims:

1. Identifying a genetic etiology in pacients with epilepsy admitted in the Pediatric Neurology Department of Dr. Victor Gomoiu Clinical Children s Hospital
2. Identifying and characterizing the clinical picture of patients with genetic mutations that are able to explain epilepsy;
3. Evaluation of the therapeutic attitude and patient management in relation to the genetic diagnostic;
4. Establishing a follow-up and diagnosis protocol of patients with genetic epilepsy.
5. Establishing a pacient registry that may serve as a start point to implementing a national registry in the future.
6. Optimizing the collaboration between medical genetic centers across the country and the pediatric neurology department.

Chapter 2. Methods and materials

Between October 2016 – August 2020 I have realized a prospective and retrospective longitudinal study within the Pediatric Neurology Department of Dr. Victor Gomoiu Clinical Children s Hospital, Bucharest.

a. Inclusion criteria

The present study included patients that fulfilled the following inclusion criteria:

- Patients between the ages of 0-18 years;
- Patients with a suspicion of genetic epilepsy that were genetically tested;
- Patients that have expressed their informed consent for participating in medical education.

a. Exclusion criteria

- Patients that refused participating in medical education.
- Patients lost to follow-up
- Patients with epilepsy otherwise explained:
    - Hypoxic ischaemic encephalopathy patients with seizures as a response to acute injury.
    - Acquired structural epilepsies;
    - Central nervous system infections;

b. Study population:

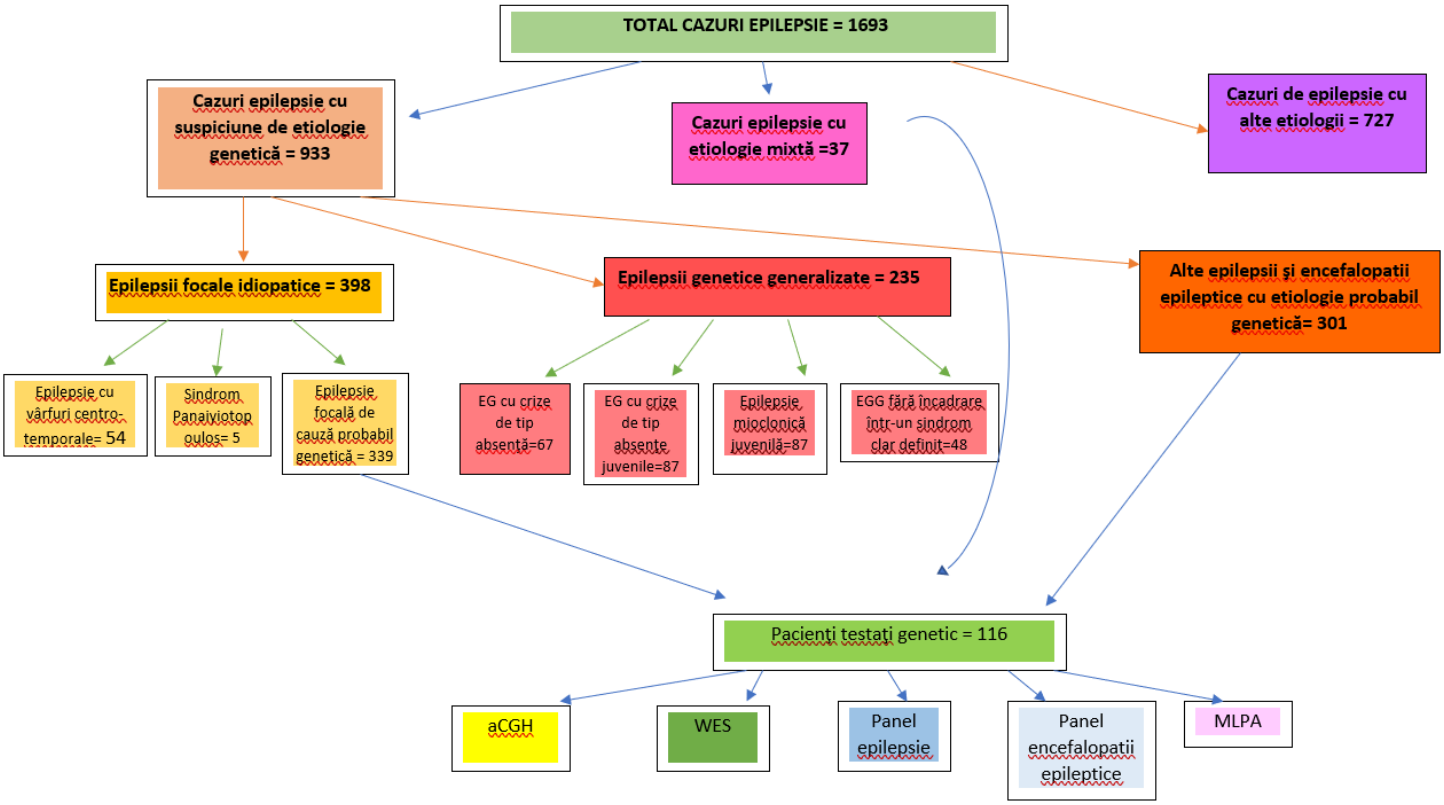

Fig. 1 Distribution of groups and subgroups in the study

1. Patient analysis in the study was performed using the following protocol:

Research protocol	
I. Demographic data	Sex, age, urban/rural area
II. Family history	Mother, father, siblings: - age - personal history - family history
III. Personal history	1. Pregnancy and birth: - Pregnancy monitoring and evolution/ pregnancy patologies - Vaginal birth/ cesarean section - Gestational age - Weight at birth, length, cranial circumferrence

		- Apgar score - Perinatal events - Scor Apgar 2. Developemental milestones 3. Other information‘: vaccination scheme, rickets prophylaxis, nutrition
IV.	***Pathological personal history***	
V.	***Epilepsy history***	Clincal evolution: - Seizure debut - Age - Semiology - Context of appearance - Length - Remission - Frequency - Change of semiology in evolution Treatment: - Antiepilepstic drugs - Chronology of antiepileptic drugs and history of side effects experienced.
VI.	***Physical examination***	
VII.	***Neurological examination***	
VIII.	***Paraclinical investigations***	1. Laboratory exams : - tailored to each case - genetic testing 2. EEG – video EEG with daytime and nightime sleep recordings. Patients were reorded using

		21 electrodes wiht 10-20 system placement. Analysis and acquisition software – NicOne. 3. Imaging studies: - cerebral MRI 1.5/3Tesla / CT - ultrasonography: abdominal, cardiac, bladder , urinary tract.
IX.	***Psychological and psychiatrical evaluation***	1. Psychological evaluation: pregressive Raven scores/ Wechsler's intelligence scale for children (WISC IV)/ A Developemental neuropsychological Assesment (NEPSY), tailored to the age and needs of the patients 2. Psychiatric evaluation

Chapter 3.Results– genetic testing

Testing methods were ArrayCGH (Comparative Genomic Hybridization Array), MLPA (Multiplex Ligation Dependent Probe Amplification), single gene sequencing, epileptic encephalopaties pannel, epilepsy pannel and WES (Whole Exome Sequencing).

ArrayCGH was chosen in patients without clinical characteristics suggestive for a particular syndrome thus targeted testing did not represent an option.

Was opted for in cases of fever-associated epielpsies, suggestive for GEFS+ epilepsies, respectively epilepsies associated with language impairment and a particular EEG trace (ESES or CSWS).

WES testing was used in cases where MLPA and arrayCGH came back negative, but with important arguments backing up the genetic etiology suspicion or as a first-line test in cases where a comprehensive fast result was needed and it was a viable option financially, as the costs are prohibitive for many patients, because it is not covered by the national health insurance system.

62 patients were tested through multiple techniques – the majority of patients were tested thourgh other methods before undergoing WES, with previous negative results. MLPA -tested patients with negative results but with a highly suggestive phenotype for a genetic etiology were retested through single-gene sequencing or epilepsy or epileptic encephalopathy pannels, or WES.

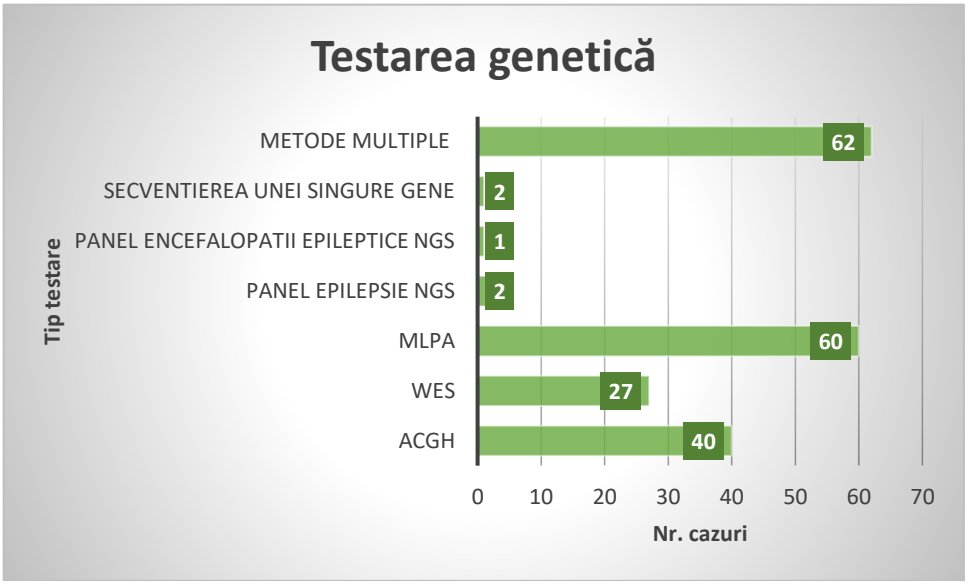

Fig. 2 Distribuţia pacienţilor testaţi în funcţie de metodele de testare genetică folosite

IV. Conclusions, personal contributions and perspectives

The present study allowed for an accurate characterization of patients with genetic epilepsy that were being managed in the pediatric neurology department in “Dr. Victor Gomoiu” Children’s hospital in Bucharest and the creation of an investigation protocol to be used for future suspicions of genetic epilepsies.

Between the months of october 2016 and august 2020, the pediatric neurology department investigated 1693 epilepsy cases between the ages of 1 month and 18 years.Out of all epilepsy cases 55% of patients had a suspicion of a genetic epilepsy, 2% - had a presumable mixed etiology – both structural and metabolic, and 43% were epilepsies with other etiologies.

In genetic epilepsy suspicion patients – 398 case were focal idiopathic epilepsies, 235 cases were generalized genetic epilepsies and 301 cases were epilepsies with other etiologies except genetic.

In the idiopathic epilepsies group, approximately 80% of patients could not be classified as belonging to an epileptic syndrome, with a majority being considered to be focal epilepsies of a probable genetic cause.

In the generalized genetic epilepsies group, 28,5 % were classified into absence seizure epilepsy, 14% juvenile absence epilepsy, 37% - juvenile myoclonic epilepsyand 20,3 % could not be classified under a clearly defined syndrome.

In the mixed etiology group, the case comprised of tuberous sclerosis, focal cortical dysplasia, schizencephaly, polimicrogyria, lyssencephaly, pyridoxin-dependant epilepsy, GAMT deficit and GLUT-1.

Postponing testing was preferred in the following situations:

1. Cases that belonged to the genetic generalized epilepsies group because current literature states that the etiology is most likely polygenic and epigenetic factors have an important contribution, and the diagnosis is established on on the classical semiology of seizures in EGG , correlated with characteristic electroencephalographic findings.
2. Cases with a mixed etiology in which the diagnosis was certain and it totally explained the appearance of epilepsy and genetic testing was performed in cases where the clinical genetic and paraclincal diagnosis were not enough. For instance , in tuberous sclerosis cases only a single patient was tested- he had an incomplete clinical picture at the time of presentation, taking into account the fact that generally the diagnosis of TSC is established on clinical criteria. Also, in pyridoxin-dependant epilepsy, the diagnosis was established by a therapeutical trial with pyridoxin treatment administered under EEG monitoring.
3. Patients with neuronal migration disorders where the structural etiology closely correlated with the epileptic seizures phenotype and EEG aspects, thus establishing a cause-effect relationship, exept one case where genetic testing was performed.

   Finally out of all the patients with genetic etiology suspicion, 12,3% patients were tested.

The 0-3 years group was the largest one, the frequency of genetic testing decreasing with older patients. This can be explained through multilple factors that correlate with current literature like the fact that genetic epilepsies often become evident in younger patients.

In our group MLPA was the most frequent testing method for detecting SCN1A/SCN2A mutations in Dravet syndrome spectrum epilepsies suspicions which usually debut in the first two years of life.

Patients included in the study were evaluated in the department at different stages of evolution – first oppinion or second oppinion cases

The majority of patients had a moderate frequency of seizures closely followed by patients with an increased seizure frequency, thus this represented an important citerion for the decision to test these patients. Most paitents (56%) experienced a change in seizure semiology over time, closely followed by patients with polimorphic seizures. (28%).

In the group of patients tested for a genetic etiology for epilepsy the most frequent anlomalies encountered were: skeletal, ophtalmological, endocrinological and metabolic as well as cardiological and haematological.

Out of the tested patients 27% had mental retardation in varying degrees, 7% had autism and 13% had ADHD and some cases had a mixture of the aforementioned three. Mental retardation was more severe in epileptic encephaopaties cases taking into account that it is duet o both the epileptic acitvity itself as well as other genetically determined mechanisms. 31% did not associate with psychiatric comorbidities or mental retardation and 18% of the patients did not undergo psychiatric evaluation and were only evaluated by a psychologist.

The majority of patients (93%) had were treated with antiepielptic drugs, one patient was treated exclusively with the ketogenic diet and 3 patients were treated with both. 3 patients were on alternative treatments, their families refusing conventional therapies. 44% of the tested patients were pharmacoresistant to the AED used and did not respond to at least 2 AEDs used correctly, thus an important reason to decide and test those patients.

The testing methods were: ArrayCGH (Comparative Genomic Hybridization Array), MLPA (Multiplex Ligation Dependent Probe Amplification),single-gene sequencing, epileptic encephalopathies pannel, epilepsy pannels and WES (Whole Exome Sequencing), whereas 62 patients were tested with multiple methods.

40 patients were tested with arrayCGH with a positive result in 4 cases and a 10% diagnostic yield, 60 patients were tested with MLPA with 6 positive results and a diagnostic yield of 10%.

Gene pannels were used in 3 patients – 2 patients were tested with an epilepsy pannel and one patient was tested with an epileptic encephalopathy pannel. The diagnosis yield was 66% but the main limit was the small number of tested patients and their more specific indication – e.g. in a patient diagnosed with an epileptic encephalopathy with a CDKL5 mutation the epileptic encephalopathy pannel was the first test used because of the highly suggestive clinical phenotype.

2 patients were tested via single gene sequencing, the diagnostic yield being 100%, the limitations being similar to the ones in pannel use. Two cases were diagnosed like this – a case of tuberous sclerosis with an incomplete clinical picture and a GLUT-1 deficite case in which the family already had a sibling with the same genetic diagnostis and clinical phenotype.

WES was used on 27 patients- 59% had positive results and 41% were negative. When it comes to WES, the diagnostic yield is difficult to appreciate because the majority of tested patients which had variants of uncertain significance, could have had their clinical picture explained by those alterations.

Nevertheless, only one patient had a certainly pathogenic mutation identified – a pathogenic mutatuion in the CNKSR2 gene – a very important result because the available data worldwide only revealed male patients with a similar phenotype, females usually presenting a more mild clinical picture on the epilepsy-aphasia spectrum.

In 14 patients the genetic testing helped establish a certain diagnosis. The remaiing 102 pacients had either an uncertaing diagnosis at the time or a anegative result at the end of the study. This can be explained by the fact that the current degree of knowledge and the current discoveries ‘ rate in genetic epilepsy is constantly evolving and new genes are systematically reported, not all patients were tested with all the available diagnostic tests and in some cases even after extensive testing a genetic diagnosis was not reached or the resulting mutations did not correlate with the patient’s clinical phenotype. Also, it is to be kept in mind that genetic epilepsies represent a group of rare disorders.

When it comes to the management of the diagnosed patients, in 10 cases included in the study there is scientific evidence regarding precision medicine, aimed at the identified mutations and the underlying mechanisms, the genetic result actually having an influence over their management. In a patient with KCNQ2 muation sodium channel blockers were recommended - carbamazepine (sodium and potassium channels are co-localized on the neuronal membrane, and their alpha subunits have similar structures), with a favorable outcome for the patient thus genetic testing had implications and benefits for seizure management.

Currently in genetic epileptic encephalopathies there is more and more evidence on the benefits of immunotherapy on epileptic seizures and patient prognosis (de ex: CNKSR2 si CDKL5).

In the Dravet spectrum (SCN1A, SCN9A and PCDH19) tratment was oriented taking into account the well-documented avoidance of sodium channel blockers that can aggravate seizures in this group.

In tuberous sclerosis specific treatment , everolimus , is intensly studied for its therapeutical effects on refractory seizures.

In SLC2A1 mutations responsible for GLUT1 deficiency, antiepileptic drugs have no benefits on seizures, the only tratment consisting of the ketogenic diet – one patient included in the study with a favourable outcome on this particular type of medical diet.

Out of all the patients that were genetically tested, 3 patients underwent pharmacogenomic testing which helped estimat the response tocertain types of medications, based on a individualized genetic profile aiming to optimize therapeutical benefits, identifying adequate treatment and dosage as well as minimizing the risk of side effects. This type of testing can also lead to a personalized emergency protocol in case of an epileptic seizure, paving the way to optimizing individual management.

The duration between clinical symptoms and a genetic diagnosis was quite variable in the studied group with a mean value of 2,5 years and this observations raises particular issues regardin the causes of this delay – lack of access to adequate medical services, prohibitive costs, lack of medical genetics specialists as well as excluding other epilepsy causes before the genetic testing.

Study limits consisted mainly of the fact that geentic epilepsies are a group of rare disorders and worldwide the studies performed are mostly multicentric. Also, the group included in the study may not be representative for the pediatric population with epilepsy in Romania because it took place in a rare neurological disorders center , with a different adressability from other pediatric neurology centers. One must also take into account the fact that the genetic testing methods were heterogenous and even though results were negative after the first line of testing, and the suspicion of a genetic etiology was maintained, to the end of the study, it was not possible to extend the testing of all patients for various reasons.On the one hand WES testing is not readily available , local centers with NGS techniques capabilities being quite few, the rest of the patients were tested in collaboration with private local and external centers and the costs are often prohibitive for the majority of patients.

The patients will continue to have their cases reevaluated and genetic testing for them and their families will be continued for a better characterization of the phenotype-genotype correlation in the cases were mutations were identified.

Therefore , as a result of the study new mutations were identified and after analysing the genotype-phenotype correlations they were included in

international genetic epilepsies databases, by reporting them in published ISI papers. So, from the retrospective study of the genetic etiology suspicion, correlated with the literature, a new epilepsy candidate gene was proposed – NRXN3 as well as two new mutational variants of the KCNQ2 and CNSKR2 genes.

In the specific case of the KNCQ2 mutation identified in the study – it has never been reported as a pathogenic mutation, but ht epatien's phenotype and her evolution consisted a powerful argument in considering it likely pathogenic.

In the identified CNKSR2 mutation the particularity consists of the fact that the patien's phenotype has only been encountered in males, with a much milder phenotype in females, on the benign end of the epilepsy-aphasia spectrum.

Also, the present study made it possible for many families to receive a genetic diagnosis and an estimation of a possible recurrence risk by being promptly redirected to medical genetic specialists and raised a red flag on the large duration between the onset of symptoms and the genetic diagnosis, on the available testing options in Romania and the necessity of interdisciplinary collaboration.