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GENOME ANALYSIS

SIGNIFICANCE OF TARGETED EXOME SEQUENCING AND METHODS OF DATA ANALYSIS IN THE DIAGNOSIS OF GENETIC DISORDERS LEADING TO THE DEVELOPMENT OF EPILEPTIC ENCEPHALOPATHY

Conflict of Interest

None declared.

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Abstract

Epilepsy is the most common serious neurological disorder, and there is a genetic basis in almost 50% of people with epilepsy. The diagnosis of genetic epilepsies makes to estimate reasons of seizures in the patient. Last decade has shown tremendous growth in gene sequencing technologies, which have made genetic tests available. The aim is to show significance of targeted exome sequencing and methods of data analysis in the diagnosis of hereditary syndromes leading to the development of epileptic encephalopathy. We examined 27 patients with c early EE (resistant to antiepileptic drugs), psychomotor and speech development delay in the psycho-neurological department. Targeted exome sequencing was performed for patients without a previously identified molecular diagnosis using 454 Sequencing GS Junior sequencer (Roche) and IlluminaNextSeq 500 platform. As a result of the analysis, specific epilepsy genetic variants were diagnosed in 27 patients. The greatest number of cases was due to mutations in the SCN1A gene (7/27). The structure of mutations for other genes (mutations with a minor allele frequency of less than 0,5% are presented): ALDH7A1 (n=1), CACNA1C (n=1), CDKL5 (n=1), CNTNAP2 (n=2), DLGAP2 (n=2), DOCK7 (n=2), GRIN2B (n=2), HCN1 (n=1), NRXN1 (n=3), PCDH19 (n=1), RNASEH2B (n=2), SLC2A1 (n=1), UBE3A (n=1). The use of the exome sequencing in the genetic practice allows to significantly improve the effectiveness of medical genetic counseling, as it made possible to diagnose certain variants of genetically heterogeneous groups of diseases with similar of clinical manifestations.

Keywords: *genetic disorders, epileptic encephalopathy, targeted exome sequencing*

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1 Introduction

Epilepsy is the most common serious neurological disorder, affecting around 65 million people in the world (Moshé SL et al, 2015). Up to one-third of epilepsies are refractory to medical therapy, and a significant proportion of childhood intractable epilepsies have significant neurodevelopment morbidities, such as developmental delay and/or regression (Banerjee PN et al, 2009). These conditions, in which the epileptiform abnormalities are thought to significantly contribute to the overall brain disturbance, are referred to as epileptic encephalopathy (EE). This term is widely accepted by the epilepsy community to encompass a range of clinical syndromes characterized by severe epilepsies of childhood. The cause of seizures is not known in more than 50% of people with epilepsy (Pal DK et al, 2010). These types of epilepsies were called as “idiopathic” in the 1989 (ILAE, 1989). However, our understanding of idiopathic epilepsies has improved exponentially with rapid development of the molecular genetics techniques. More than half of all epilepsies are now known to have a genetic basis. This led the International League Against Epilepsy (ILAE) to replace the term idiopathic epilepsies with «genetic generalized epilepsies» (Berg AT et al, 2010).

As a result of scientific studies, a significant number of genetics syndromes leading to the development of EE have been identified in the last decade. The term «epileptic encephalopathy» is used to refer to a heterogeneous group of diseases characterized by frequent polymorphic seizures resistant to anticonvulsant therapy and "aggressive", between seizures epileptic activity associated with a pronounced of psychomotor development delay. It should be noted that both idiopathic EE and symptomatic epilepsy can have genetic causes - congenital malformations of the brain, hereditary metabolic defects (Moshé SL et al, 2015).

These rapid developments in the molecular genetics of epilepsy have changed thinking about the causes of epilepsies, and it is bound to have an impact on the diagnosis and management of patients with epilepsy (Mirza N et al 2015). Whole and targeted exome sequencing is considered to be a highly effective method in identifying a causative gene in the clinical setting (Veeramah KR eta l, 2013). In the present times, everyone involved in the management of epilepsy patients, especially the clinician should have some grounding information about epilepsy genetics.

It is very important to note that the clinical genetic testing must be done in a certified genetic laboratory equipped with quality control standards, accurate methods to interpret results, and the facility to offer genetic counseling to the patient and family members.

The genetic tests available for a clinician in patients with epilepsy are array comparative

genomic hybridization (aCGH) for detecting copy number variants, sanger sequencing for screening candidate genes and next generation sequencing (NGS), or massively parallel sequencing for gene panels (Mefford HC et al, 2015). aCGH is usually a first-line investigation in patients with an epilepsy syndrome, for example, seizures associated with facial dysmorphism, congenital malformations, intellectual disabilities, or psychiatric problems (Ottman R et al, 2010, Helbig I et al, 2009). NGS technologies allow screening of as many genes as possible in clinical setting in patients with epilepsy, particularly in patients with highly heterogeneous epileptic syndromes (Lemke JR et al, 2012).

Genetic testing has important psychological, social, and financial implications (Ottman R et al, 2010, Helbig I et al, 2009). Knowing the genetic basis of their disease makes the patient relieved about the diagnosis and prevents them from undergoing unnecessary, laborious, invasive, and often expensive investigations (Pal DK et al, 2010). Thus, it is important to have a pre- and post-genetic test counseling by a genetic counselor (Pal DK et al, 2010). Another important clinical implication is in marriage and reproductive decisions. Genetic testing is usually advised in people where there is family history of genetic epilepsies, or parents share a common genetic pool (consanguineous marriage) (Ottman R et al, 2010). The chances of developing a recessive disorder are higher if both parents share a common ancestry. Therefore, it is important to get genetic counseling and testing done in such couples before marriage and conception (Ottman R et al, 2010). Another aspect of genetic testing is the cost-effectiveness (Leu C et al, 2010). The high cost of genetic testing has some limitations in less developed countries, but with rapid progress in gene sequencing technologies and wider availability, the cost of genetic testing is going to reduce in the coming years (Jiang T et al 2014).

The aim is to show significance of targeted exome sequencing and methods of data analysis in the diagnosis of hereditary syndromes leading to the development of epileptic encephalopathy.

2 Patients and methods

We examined 27 patients with c early EE (resistant to antiepileptic drugs), psychomotor and speech development delay in the psycho-neurological department of the St.Luka Scientific and Practical Center of Specialized Medical Care for Children. Resistant epilepsy is called epilepsy in which severity and frequency of seizures, neurological and psychological disorders or side effects of antiepileptic drugs are not corrected.

We performed detailed phenotypic assessment of seizures, cognitive and behavioral disorders, EEG and MRI of the brain. Informed consent for targeted exome sequencing of the gene panel associated with epilepsy was received from all 27 patients. Targeted exome sequencing was performed for patients without a previously identified molecular diagnosis.

The isolated genomic DNA was used to prepare genomic libraries for massively parallel sequencing. Only DNA parts which correspond to gene exons and splice sites were selected from the obtained libraries by hybridization method (SeqCap EZ Library, Roche). Next, their nucleotide sequence was determined on 454 Sequencing GS Junior sequencer (Roche) using NimbleGen oligonucleotide probes and IlluminaNextSeq500 platform using targeted DNA technique TruSightOne V1.1. The received reads were mapped to the coding regions of the human genome.

All stages of sample preparation and sequencing were carried out in accordance with the protocols of the manufacturer of equipment and reagents.

Sequencing data was processed using an automated algorithm that included alignment of reads to reference sequence of the human genome (hg19), post-processing of the alignment, identification of variants and filtering of variants in according with quality and annotation of the identified variants for all known transcripts of each gene from RefSeq database using methods pathogenicity predictions (SIFT, PolyPhen2-HDIV, PolyPhen2-HVAR, MutationTaster, LRT), as well as methods for calculating evolutionary conservatism of positions (PhyloP, PhastCons). The identified variants were classified into 6 classes based on the ACMG standards and recommendations of interpretation of polymorphisms: Class 1 – pathogenic, Class 2 - likely pathogenic, Class 3 - variants with uncertain clinical significance, Class 4 - likely benign, Class 5 – benign, Class 6 - variants associated with the disease.

The databases «1000 genomes», ESP6500, Exome Aggregation Consortium and Genome Aggregation Database were used to estimate population frequencies of the identified variants. To assess the clinical relevance of the identified variants, OMIM database, Orphanet database, specialized databases for selected diseases (if available) and literature data were used. Also, we were guided by the existing protocols for interpreting the NGS results - ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing; ACMG clinical laboratory standards for next-generation Sequencing; Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Robert C et al, 2013, Sue R et al, 2015, Rehm L et al, 2013).

Limitation of methods: Only known and described genes in the scientific literature are studied. Some coding sequences can be read with insufficient coverage. The method does not provide determination of cis or trans-position of heterozygous mutations pairs, as well as detection of mutations in introns outside splice sites. The method does not allow detecting insertions and deletions longer than 50 bp; studying variations of repeat length (including expansion of triplets); assessing level of methylation; identifying chromosome rearrangements, polyploidy; to detect mutations in a state of mosaicism.

3 Results

The long-term medical support and phenotype analysis of patients with epileptic encephalopathy resistant to antiepileptic drugs did not allow making diagnosis of frequent monogenic forms. Based on this, there were indications for targeted exome sequencing of the gene panel. As a result of the analysis, specific epilepsy genetic variants were diagnosed in 27 patients. The greatest number of cases were due to mutations in the SCN1A gene (7/27), responsible for EE type 6, of which 2 - nonsense mutations, 4 - missense mutations.

The structure of mutations for other genes (mutations with a minor allele frequency of less than 0,5% are presented): ALDH7A1 (n=1), CACNA1C (n=1), CDKL5 (n=1), CNTNAP2 (n=2), DLGAP2 (n=2), DOCK7 (n=2), GRIN2B (n=2), HCN1 (n=1), NRXN1 (n=3), PCDH19 (n=1), RNASEH2B (n=2), SLC2A1 (n=1), UBE3A (n=1).

Clinical and molecular genetic data are given in Table 1.

Table 1 – The spectrum of mutations detected in patients with epilepsy

№	Gene	Mutation	Allele frequency*	Diagnosis
1	***ALDH7A1***	p.Arg82X p.Glu399Gln	N\A** N\A	Epilepsy, pyridoxine-dependent Superrefractory status epilepticus
2	***CACNA1C***	p.Val1596Met	0,0067	Timothy syndrome
3	***CDKL5***	p. Tyr286X		Epileptic encephalopathy, early infantile, 2
4	***CNTNAP2***	p.Gly285Ala	0,005	Cryptogenic focal epilepsy
5	***CNTNAP2***	p.Ile1331X	N\A	Symptomatic multifocal epilepsy. Tetraparesis. Psycho-speech development delay
6	***DLGAP2***	p.Thr293Met	0,003	Idiopathic generalized epilepsy syndrome of infantile spasms
7	***DLGAP2***	p.Arg134Lys	0,003	Rett-like syndrome
8	***DOCK7***	c.1872-8G>T	N\A	Idiopathic generalized epilepsy with myoclonic-astatic seizures
9	***DOCK7***	p.Pro2074Leu	0,009	Congenital malformation of brain. Symptomatic focal epilepsy
10	***GRIN2B***	p.Arg1138Gln	0,0002	Idiopathic generalized epilepsy with febrile seizures plus
11	***GRIN2B***	p.Arg1241Trp	N\A	Early epileptic encephalopathy type 27
12	***HCN1***	p.Ser403Leu	N\A	Cryptogenic focal epilepsy
13	***NRXN1***	p. Ile649Val	0,0006	Symptomatic multifocal epilepsy
14	***NRXN1***	p.Leu748Ile	0,003	Symptomatic multifocal epilepsy
15	***NRXN1***	p.Asp5His	N\A	Idiopathic generalized epilepsy with febrile seizures plus
16	***PCDH19***	p.Ala517Thr	0,000011	Cryptogenic generalized epilepsy Syndrome West
17	***RNASEH2B***	p.Ala287Ser	0,006	Symptomatic focal epilepsy. Aicardi-Goutieres syndrome 2
18	***RNASEH2B***	p.Leu138Phe	0,00025	Cryptogenic generalized epilepsy. Aicardi-Goutieres syndrome 2
19	***SCN1A***	p.Trp153X	N\A	Epileptic encephalopathy, early infantile, 6 (Dravet syndrome)
20	***SCN1A***	p.Ser723Pro	N\A	Epileptic encephalopathy, early infantile, 6 (Dravet syndrome)
21	***SCN1A***	p.Lys41X	N\A	Idiopathic generalized epilepsy with myoclonic seizures
22	***SCN1A***	p.Leu215Ser	N\A	Cryptogenic focal epilepsy
23	***SCN1A***	p.Ile1754Leu	N\A	Idiopathic generalized epilepsy with febrile seizures plus
24	***SCN1A***	p.Asp382Tyr	N\A	Symptomatic focal epilepsy Cerebral palsy, atonic-astatic form
25	***SCN2A***	p.Val1287leu	N\A	Idiopathic generalized epilepsy with febrile seizures plus
26	***SLC2A1***	p.Val435fs	N\A	Cryptogenic focal epilepsy GLUT1 deficiency syndrome
27	***UBE3A***	p.Asp754Gly	N\A	Symptomatic focal epilepsy Cerebral palsy, atonic-astatic form. Psycho-speech development delay.

*Exome Aggregation Consortium Database (ExAc) и gnomAD; ** N\A – not available

The different phenotypic features were described in patients with functionally different mutations in genes. So, patients with functionally different mutations in DOCK7 gene had idiopathic generalized epilepsy with myoclonic-astatic seizures (splicing site mutation), focal symptomatic epilepsy with congenital brain defect of the left frontal-parietal region (missense mutation); in CNTNAP2 gene - symptomatic multifocal epilepsy with tetraparesis and psycho-speech development delay (nonsense-mutation) and focal epilepsy with remission (missense mutation). Patients with functionally similar mutations in GRIN2B gene had different clinical manifestations - generalized febrile seizures and myoclonic seizures, single serial epileptic spasms; in DLGAP2 gene - generalized seizures and tonic spasms.

Sever myoclonic early infantile epileptic encephalopathy, type 6 (Dravet syndrome) was diagnosed in patients with mutations in the SCN1A gene, which resulted in stop codon (nonsense-mutation). Clinical manifestations in patients with missense mutations differed from those in patients with nonsense-mutation in the SCN1A gene and were characterized by generalized epilepsy with febrile seizures plus.

In the case of mutation detection in the other genes, clinical manifestations included: HCN1 gene - generalized seizures and severe development speech and psychomotor delay; PCDH19 gene - generalized seizures, axial tonic spasms; SCN2A gene - generalized epilepsy with febrile seizures plus; NRXN1 - symptomatic focal epilepsy and generalized seizures; CDKL5 - infantile spasms, West syndrome.

Sever clinical manifestation was observed in patient with pyridoxine-dependent epilepsy, associated with mutations in ALDH7A1 gene. The clinical features included superrefractory status epilepticus, hypoxic-ischemic encephalopathy, muscular dystonia, sever psychomotor and speech development delay.

Aicardi-Goutieres syndrome (OMIM # 610181) was diagnosed in patients with following clinical manifestations: focal epilepsy, infantile spasms, development delay, severe cerebral atrophy, leukodystrophy, intracranial calcifications, chronic cerebrospinal fluid (CSF) lymphocytosis, increased CSF alpha-interferon and negative serologic investigations of common prenatal infections; and was confirmed by detection mutation in RNASEH2B gene.

Glucose transporter deficiency syndrome type 1 (GLUT1 deficiency syndrome OMIM #606777) was diagnosed in one patient with early encephalopathy, symptomatic epilepsy (generalized seizure with drug resistance), microcephaly, psychomotor development delay with spasticity, ataxia, dysarthria and alternating hemiplegia and was confirmed detection mutation in SLC2A1 gene.

4 Discussion

The results of the study correspond to those obtained in a number of the largest laboratories in the world, and indicate the effectiveness of the gene panel for the diagnosis of genetic variants of EE (Lemke JR et al, 2012).

A significant number of genetics syndromes leading to the development of EE have been identified in the last decade. The term «epileptic encephalopathy» is used to refer to a heterogeneous group of diseases characterized by frequent polymorphic seizures resistant to anticonvulsant therapy and "aggressive", between seizures epileptic activity associated with a pronounced of psychomotor development delay. (Moshé SL et al, 2015).

The genetic heterogeneity of clinically similar conditions (progressive myoclonic epilepsy, early EE, mitochondrial epilepsies) and significant clinical polymorphism of individual genetic variants (high variability of seizure, different progression of neurological symptoms) cause the verification complexity of the etiologic factor of the disease. To date, 56 types of early EE in children have been described (Staley K et al, 2015).

The clinical manifestations and severity of genetics epilepsy are variable. The types of epilepsy with benign and malignant course are described. The most severe course, characterized by the drug-resistance to anticonvulsive therapy, is observed in the patients with EE. These facts determine necessity carrying out targeted exome sequencing of gene panels for the diagnosis of individual genetic variants of EE (Thomas RH et al, 2014).

The ILAE Commission on Classification and Terminology (2005–2010) defined genetic epilepsies as in which seizures occur as a result of a known or presumed genetic defects (Berg AT et al, 2010).

The history of gene discovery in EE is intertwined with the overall dynamics of gene discovery in epilepsies in general, which occurred largely in 3 stages: a stage of gene discovery in familial epilepsies (SCN1A, SCN1B, KCNQ2, KCNQ3 and GABRG2, a period of relative stagnation, and the current era of massive parallel sequencing that was ushered in by large-scale studies for copy number variants (Ingo Helbiga et al, 2016).

The genetic epilepsies seen during the 1 year of life are benign familial neonatal seizures (KCNQ2 and KCNQ3), benign familial neonatal-infantile seizures (SCN2A), and benign familial infantile seizures (Zara F et al, 2010). These are autosomal dominant epilepsy syndromes characterized by onset of seizures before first birthday and have a strong positive family history (Zara F et al, 2010, Muntoni F et al, 2015). Other epilepsies with more complex pattern of inheritance in this age group include Ohtahara syndrome (STXBPI and ARX), West syndrome and Dravet syndrome (SCN1A) (Veeramah KR et al, 2013, Lemke JR et al, 2012). Febrile seizures affect 3% of children between age group of 6 months and 6 years (Nabbout R et al, 2002). Genetic epilepsy with febrile seizure plus (SCN1A, SCN1B and GABRG2) and Dravet syndrome (SCN1A) form an important differential diagnosis, especially in a child with febrile seizures and developmental delay (Veeramah KR et al, 2013, Lemke JR et al, 2012).

In childhood, classic generalized epilepsies, for example, childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy and epilepsy with generalized tonic–clonic seizures, which form about 20% of all epilepsies, are more common (Guerrini R, 2006). Although the genetic basis of most of these epilepsies remains elusive, early onset absence epilepsy (SLC2A1) and juvenile myoclonic epilepsy (GABRA1 and EFHC1) have a stronger genetic basis (Guerrini R, 2006). Partial epilepsies have long been associated with focal structural lesions. The genetic linkage analysis in multiplex families with partial epilepsies has identified various causative genes, for example, autosomal dominant nocturnal frontal lobe epilepsy (CHRNA4, CHRNB2 and CHRNA2) (Becchetti A et al, 2015), autosomal dominant epilepsy with auditory features (LGI1) (Michelucci R et al, 2009), familial temporal lobe epilepsies and familial focal epilepsy with variable foci (DEPDC5) (Picard F et al, 2014). Progressive epileptic syndromes such as progressive myoclonus epilepsies (Unverricht–Lundborg disease Lafora body disease) are also now known to have a strong genetic

basis (Kälviäinen R et al, 2008). In the past few decades, genetics have also been implicated in various nonsyndromic epilepsies like those caused by structural and/or metabolic reasons, for example, malformations of cortical development (lissencephaly), neurocutaneous syndromes (tuberous sclerosis, TSC1 and TSC2), tumors, infections, brain trauma, and perinatal insults (Cardoso C et al, 2002, Guerrini R et al, 2006).

It is important to take the advancements in epilepsy genetics from bench to bedside. As stated previously, the basis of most of the common genetic epilepsies is multifactorial with both genetic and environmental factors playing major roles, but nevertheless, there are multiple genetic tests available in the market to make diagnosis of specific type of epilepsy syndromes. Genetic testing in patient is done mainly in two scenarios, first, where the patient is already known or suspected to have epilepsy and second, to predict the occurrence of epilepsy in a person with a family history of epilepsy (Ottman R, 2010). The latter situation is commonly encountered, and the knowledge of percentage risk of manifesting diseases in different modes of genetic inheritance would be useful for clinicians in day-to-day practice.

5 Conclusion

Genetic variation is a major contribution to the etiology of epilepsy. The genetic heterogeneity of early EE determines the practicability and effectiveness of targeted exome sequencing for the diagnosis of genetic variants using the gene panels responsible for the diseases associated with seizures. Early identification of pathogenic genetic variation in patients with epilepsy is important for providing accurate prognosis and for optimizing management, including the application of precision medicine as targeted therapeutics emerges. Fortunately, the ability to identify genetic causes of epilepsy is improving as testing approaches are becoming more sophisticated and more widely available.

The use of the exome sequencing in the genetic practice allows to significantly improve the effectiveness of medical genetic counseling, as it made possible to diagnose certain variants of genetically heterogeneous groups of diseases with similar of clinical manifestations. However, interpreting the results of exome sequencing by a geneticist increases a number of problems that require discussion. It is shown that the main problems are the following:

1. There are several allelic variants which differ in terms of severity of clinical manifestations, caused by mutations in one gene;
2. Incomplete penetrance and variable expressivity of the gene responsible for autosomal dominant disease, leading to the absence or presence of minimal clinical manifestations;
3. There is gonadal mosaicism, the level of which is not known;
4. Detection of previously not described nucleotide substitutions, the functional significance of which is not known;
5. Detection of heterozygous mutations in gene responsible for an autosomal recessive disease, the clinical manifestations of which are similar to those of a patient. In this case, it can be assumed that the second mutation is deep in the intron or there is a deletion of the chromosome region.

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ТАРГЕТНОЕ ЭКЗОМНОЕ СЕКВЕНИРОВАНИЕ В ДИАГНОСТИКЕ ГЕНЕТИЧЕСКИХ НАРУШЕНИЙ, ПРИВОДЯЩИХ К РАЗВИТИЮ ЭПИЛЕПТИЧЕСКОЙ ЭНЦЕФАЛОПАТИИ

Конфликт интересов

Не указан.

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Аннотация

Эпилепсия является наиболее распространенным неврологическим расстройством, а генетическая основа данной патологии установлена у 50% людей с судорожным синдромом. Диагностика генетической эпилепсии позволяет установить причины приступов у пациента. В последнее десятилетие произошел огромный скачок в развитие новых технологий секвенирования генов, которые сделали генетические тесты более доступными. Цель исследования - показать значимость таргетного экзомного секвенирования и методов анализа данных в диагностике наследственных синдромов, ведущих к развитию эпилептической энцефалопатии (ЭЭ). В исследовании представлены клинические наблюдения 27 пациентов с ранним ЭЭ (резистентными к противоэпилептическим препаратам), задержкой психомоторного и речевого развития. Таргетное экзомное секвенирование было выполнено с использованием анализатора 454 GS Junior sequencer (Roche) и платформы IlluminaNextSeq500. В результате анализа у 27 пациентов был диагностирован определенный генетический вариант эпилепсии. Наибольшее число случаев было связано с мутациями в гене SCN1A (7/27). Выявленные мутации в других генов (мутации с малой частотой аллелей менее 0,5%): ALDH7A1 (n = 1), CACNA1C (n = 1), CDKL5 (n = 1), CNTNAP2 (n = 2), DLGAP2 (n = 2), DOCK7 (n = 2), GRIN2B (n = 2), HCN1 (n = 1), NRXN1 (n = 3), PCDH19 (n = 1), RNASEH2B (n = 2), SLC2A1 (n = 1), UBE3A (n = 1). Использование таргетного экзомного секвенирования в практике врача-генетика позволяет значительно повысить эффективность медико-генетической консультации, поскольку это позволит диагностировать определенные варианты генетически гетерогенных групп заболеваний с аналогичными клиническими проявлениями.

Ключевые слова: *генетические синдромы, эпилептическая энцефалопатия, таргетное экзомное секвенирование.*