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METHODS OF GENETIC RESEARCH FOR THE DIAGNOSISOF EPILEPSY

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Abstract:

Epilepsy is a chronic brain disease characterized by repeated unprovoked attacks of motor, sensitive, vegetative, mental or psychological disturbances caused by excessive neuronal discharges in the gray matter of the cerebral cortex.

In most cases, epilepsy is genetically determined (hereditary or sporadic). Genetic forms of epilepsy fall into two broad categories: primary (idiopathic), when mutations in genes / loci trigger the onset of epilepsy, and secondary (symptomatic), when mutations in genes / loci cause disorders in the brain structure that trigger the onset of epilepsy. To date, genetic studies are necessary, both to clarify the diagnosis of a specific form of epilepsy, and to reveal new forms of epilepsy. Molecular genetic analysis becomes a routine method due to its increasing availability. At the same time, the spectrum of genetic research methods is very large, and to determine the diagnosis it is necessary to choose the most optimal one. The creation of an algorithm for diagnostic search for suspected hereditary forms of epilepsy is an important and urgent task, especially since carrying out these studies is a laborious and costly process.

The article discussed modern methods of genetic analysis used in the diagnosis of various forms of epilepsy.

Keywords: *genetic research, molecular genetic examination, genetic panels, high-performance sequencing techniques, exome sequencing, genome sequencing, FISH method.*

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INTRODUCTION:

Epilepsy is a chronic, frequently occurring, in most cases disabling neurological disease [Gamirova 2018]. New technologies and the detection of specific genetic causes of epilepsy influence the depth of research on the genetically determined forms of epilepsy. In recent decades, the number of genes which role is recognized as a causative factor in various forms of epilepsy has increased dramatically. Identified genes are components of a neural connection, including voltage-dependent channels, receptors of neurotransmitters, protein-associated ion channels, and synaptic proteins. The discovery of these genes also provided useful information on the molecular basis of epileptogenesis. It became clear that in some cases the cause of an epileptic syndrome is a mutation not in one particular gene but in a group of genes – and each of them (or their interaction) can cause the development of epilepsy. Although most family epilepsies demonstrate a simple path of inheritance (autosomal dominant, autosomal recessive), cases of complex inheritance (multifactorial inheritance, mitochondrial type of inheritance, imprinting, transmission of chromosomal abnormalities, etc.) should not be excluded from the data analysis [Belousova 2014]. In the latest classification of epilepsy ILAE (2017) it is recommended to use the term "genetic epilepsy" instead of the definition of "idiopathic" (presumably genetic) epilepsy, and to confirm the etiology by molecular genetic methods or clinical diagnostic methods (such as "twin studies" and "family studies").

MATERIALS AND METHODS:

The materials for research were processed in the laboratory "Clinical Linguistics" (Kazan Federal University).The theoretical basis of the study is presented by materials collected as a result of own research and by the method of continuous sampling from scientific databases: PubMed(https://www.ncbi.nlm.nih.gov/pubmed, April 2018), Scopus (https://www.scopus.com, April 2018), Web of Science (https://www.webofknowledge.com, April 2018), Elibrary (https://elibrary.ru, April 2018), Academia.edu (https://www.academia.edu/, April 2018).The purpose and objectives of this research have determined the methodological basis for the study. The results were received with the help of the experimental and descriptive methods
and can be used in clinical practice.

RESULTS AND DISCUSSION:

1. The "family studies" method.

The "family studies" method combined with genetic analysis is currently the most effective method of investigating family epilepsy, which is necessary to minimize the problem of genetic heterogeneity.

The method includes:

–detailed inquiry of all available family members and close relatives to collect complete clinical and genealogical information, and analyze the patterns of phenotypic manifestations. It is necessary to mention that some gene mutations that cause epilepsy can occur de novo and, in this case, there will be no inheritance scheme that could be traced through the bloodline;

–electroencephalographic (EEG) study of each member of the family, comparing EEG results with the clinic;

– Neurological examination of all family members to identify possible abnormalities in psychomotor development (in childhood) and changes in neurological status [Marini 2002];

–selection of the most "suitable" epileptic syndromes on the basis of the data obtained, the targeting of molecular genetic analysis for the presence of mutations specific for the syndrome in known genes.

2. Methods used for the genetic analysis of epilepsy.

There are several methods that can be used for genetic analysis of epilepsy. It is important to note that there is no single method for determining all types of genetic mutations.

2.1. Sanger direct sequencing method.

Until now, it is the "gold standard" of sequencing due to its accuracy. It allows detecting a suspected (for example, clinical manifestation) mutation in a previously known gene, confirming the already available high-throughput sequencing results for the proband and revealing the presence of the same mutation in the parents.

The direct sequencing method (i.e., determining the nucleotide sequence) of the amplified sites (Sanger method) is based on the use of dideoxynucleotides labeled with a fluorescent dye. In short, the Sanger protocol sequencing protocol is as follows:

–A sample of deoxyribonucleic acid (DNA) is added to the reaction mixture from DNA polymerase, ddNTP of all 4 types (labeled with different fluorescent dyes) and oligonucleotide;

–During the reaction of enzymatic DNA synthesis, the labeled ddNTP is included in the chain instead of dNTP, which leads to the stoppage of the synthesis;

– Electrophoresis is performed in a thin capillary filled with gel;

– the fluorescence spectrum obtained is registered.

Currently, all these steps are automatically produced using sequencers [Rebrikov 2015].

2.2. High-performance sequencing techniques

New sequencing technologies have improved the Sanger method, the main disadvantage of which is low throughput [Sanger 1977]. NGS (Next Generation Sequencing) technologies allow the simultaneous sequencing of thousands of DNA molecules, thereby increasing the research speed and increasing the amount of data obtained.

The principle of NGS is based on a mass parallel sequencing of pre-prepared single-stranded libraries of DNA fragments. Currently, the following commercial platforms are used for high-performance sequencing: HiSeq, MiSeq and NextSeq 500 (Illumina), Ion torrent (Thermo Fisher Scientific) and SOLiD (Applied Biosystems) [Ross 2011; Rizzo 2012]. Most high-performance sequencing technologies include the following steps:

–preparation of libraries;

–sequencing;

–signal detection;

–analysis of the data obtained.

The preparation of libraries includes:

– DNA fragmentation up to 300-500 bp;

–ligation of sequencers (synthesized oligonucleotides with a known sequence) with the ends of DNA fragments;

–amplification of the received libraries.

The amplification method can be different for different platforms. For example, the Ion torrent platform from Thermo Fisher Scientific uses the emulsion polymerase chain reaction (PCR) method to amplify single-stranded fragments in microspheres, while Illumina technology is based on the amplification of fragments by bridging PCR and the formation of clusters on a flow cell [Berglund 2011; Quail 2012].

Sequencing is performed by synthesizing new DNA fragments on single-stranded DNA libraries (matrices). Nucleotides are inserted into a new chain in a certain order, corresponding to the matrix chain. After the inclusion of each subsequent nucleotide in the chain, the device registers the signal.

Different platforms for NGS use different methods of detecting nucleotides embedded in a new chain:

–Detection of the fluorescent signal after inclusion in the chain of the complementary matrix of the nucleotide (Illumina);

–a change in the pH of the solution in the microreactor associated with the release of hydrogen ions into the medium during the synthesis of DNA (Thermo Fisher Scientific);

–recording of the light signal after the release of pyrophosphate activating the cascade of chemical reactions (Roche) [Quail 2012].

After sequencing, the received data are processed using special software. Data passes through several processing steps:

– excluding reads with poor quality of reading;

–alignment of data relative to the reference sequence or de novo sequence assembly;

–analysis of the results of sequencing, allowing to determine the type of genetic variants, including their hereditary character, to estimate the level of gene expression, to identify new genes and regulatory elements.

2.3. Genetic panels

Genetic panels are designed to analyze sequence variants, complete or partial deletion and duplication in various genes responsible for the occurrence of epilepsy according to international genetic databases. They are created by laboratories to simplify the diagnosis of genetic diseases by combining genes specific for a particular syndrome, disease or group of diseases (i.e. the panel "Hereditary epilepsies" usually includes all genes which mutations cause seizures, and also candidate genes which role is possible). Genes included into the panel are selected after the analysis of common genetic databases (OMIM, CCDS, GENCODE, miRBase, VEGA, etc.) and specialized (eg. epileptic –GenEpi, epiGAD, etc.) genetic databases.

Genetic panels are pre-prepared matrices with known nucleotide sequences of selected genes applied to them. This method is an intermediate link that combines the "sighting" of direct sequencing with Sanger and the mass and speed of full genomic sequencing (since a large number of genes are analyzed by high-throughput sequencing).

2.4. Full exome sequencing

It is the strategy of sequencing all protein-coding genes in the genome (exome), suggesting the selection of only those sections of DNA that encode proteins (exones) using high-performance sequencing (NGS) technologies. This method is used when it was not possible to identify the cause by other, more targeted genetic analyzes, but the genetic etiology of the disease is still expected. In such cases, full exome sequencing proves to be a very useful diagnostic tool,

especially if the "Trio" approach is used (the analysis is conducted by the proband and both parents to identify the inherited and / or de novo mutations).

2.5. Full genome sequencing

It gives the possibility to analyze changes in the full genome including non-coding regions that are "skipped" by the method of complete exome sequencing. This method has recently been applied in clinical practice, due to the complexity and still high cost of the process [El Achkar 2015]. In most cases, it is used for research purposes. Conduction of full genome sequencing is necessary if there is a suspicion of the multigenic nature of the disease, as well as in the case of negative results of targeted analyzes. Currently, full genome sequencing is also performed using NGS technologies and needs to be confirmed by direct sequencing with Sanger.

2.6. Determination of pathogenic mutations of mtDNA

MtDNA mutations (including mitochondrial tRNA and rRNA genes) can lead to a disturbance in the production of energy by mitochondria, defects in the oxidative phosphorylation system, which in these cases is the basis for the appearance of various pathological conditions. Pathogenic mutations of mtDNA are divided into 3 groups:

1) Mutations of structural genes;
2) Mutations of rRNA and tRNA genes;
3) Structural rearrangements affecting large segments of mtDNA.

To establish the correct diagnosis, the prognosis for the sick and the degree of risk to healthy carriers, their posterity, the use of molecular analysis of mtDNA is necessary. Currently, a relative set of diagnostic methods is used for this purpose (fluorescent PCR, quantitative real-time PCR, denaturing high-resolution liquid chromatography, Southern blot hybridization, mini-sequencing (SNaPshot), Biplex Invader, pyrosequencing, chip technology, etc.) [Mazunin 2010].

2.7. Karyotyping

Karyotyping is a cytogenetic study of a person's chromosome set, which allows specialists to reveal abnormalities in the structure and / or number of chromosomes.

Karyotype (karyogram) is a graphic representation of all chromosomes of a diploid set of one cell arranged in pairs in accordance with the size and distribution of bands.

This analysis helps to diagnose chromosomal diseases caused by a violation of the number of chromosomes and / or chromosomal structure disorder, as well as the multiplicity of cellular karyotypes in the body (mosaicism). Earlier karyotyping was carried out to all patients with signs of dysmorphism, multiple congenital anomalies, with suspicion of trisomy or monosomy of a particular chromosome. At present, chromosome microarray analysis is the more preferred method, since it has a greater resolution.

However, karyotyping is still used in diagnosis (including epilepsy) if there is a suspicion of complex chromosomal rearrangement (eg, ring chromosome) [El Achkar2015].

2.8. FISH method

Fluorescent in situ hybridization (FISH) is a cytogenetic method that involves the use of fluorescently labeled probes to identify chromosome rearrangements. The analysis can detect aneuploidy, deletions and duplications of certain chromosome regions, low-level mosaicism, but it is necessary to determine in advance the area to be investigated.

This method does not allow full-genomic scanning and has a relatively low resolution, so it is inferior to the method of chromosomal micromatrix analysis in routine diagnosis of chromosomal abnormalities [El Achkar 2015].

2.9. Chromosomal micromatrix analysis

The use of chromosome microarray analysis (XMA) provides a full genomic coating for detecting chromosomal imbalance, detection of microdeletions and microdilutions.

Unlike routine karyotyping, XMA has a higher resolution. The possibility of studying the genome at this high resolution led to the discovery of widespread variations in the number of copies in the human genome, such as polymorphic changes in healthy people and new pathogenic imbalances in the number of copies of genes, which are often the cause of epilepsy [Genetzkaya 2013].

This method involves either the use of special matrices for single nucleotide polymorphisms, or matrices for comparative genomic hybridization with oligonucleotide probes [El Achkar 2015].

XMA is recommended as a first-line test if epilepsy combines with autism spectrum disorders, multiple small developmental anomalies, congenital malformations and mental retardation [Gnetetskaya 2013]. This method is characterized by a relative speed of implementation (results within a few weeks).

2.10. Target sequencing of variations in the number of copies

Target sequencing of copy number variations (CNV-seq) in a particular gene is necessary when mutations (in the Sanger analysis or high-throughput sequencing method) have not been detected, but there are still suspicions that the cause of the disease lies in the anomaly of the gene. In this case, targeted sequencing of copy number variations is a more sensitive method than chromosome micromatrix analysis [El Achkar2015, Xie2009].

SUMMARY:

Summing up the analysis of the diagnostic methods used for the diagnosis of the genetic form of epilepsy, it is necessary to distinguish the following stages of the patient management algorithm.

In patients with epileptic seizures (especially generalized: myoclonic, typical and atypical absences, atonic, generalized tonic-clonic) signs of mental retardation, small anomalies, congenital malformations, autistic spectrum disorders, the most informative will be chromosome microarray analysis.

In the absence of the above-mentioned signs in patients with epileptic seizures, the molecular genetic method of the first choice will be the most optimal epileptic genetic panel.

If there is a suspicion of the multigenic nature of epilepsy and the negative results of XMA and the genetic panel, the choice method may be full exome or full-genome sequencing.

With catastrophic progression of epilepsy symptoms and signs of an organic degenerative process in patients with a favorable perinatal history, favorable development before the debut of the disease, suspected mitochondrial disease and negative results of XMA and targeted NGS (panel) analysis, mtDNA sequencing is recommended.

Absolute indications for genetic testing are hereditary family burden of epilepsy.
The results obtained by the NGS method should be confirmed by direct Sanger sequencing with the proband and biological parents to identify the inherited or de novo mutation.

CONCLUSIONS:

The analysis of the diagnostic significance and cost-effectiveness of various methods used in molecular genetic examination revealed that at the present the epileptic panel is the most optimal for many forms of genetic epilepsy.Molecular genetic analysis becomes a routine method in medicine, but the spectrum of genetic research methods is very wide.

The diagnostic algorithm developed by the authors helps to choose the most optimal variant of genetic analysis in different clinical situations.

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