




МЕДИЦИНСКАЯ ГЕНЕТИКА MEDICAL GENETICS

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REVIEW
ОБЗОРНАЯ СТАТЬЯ

Current aspects and approaches to molecular diagnostics of hereditary neuromuscular diseases

Elizaveta A. Fonova  , Irina Zh. Zhalsanova , Nikolay A. Skryabin 

Research Institute of Medical Genetics, Tomsk National Research Medical Center, Tomsk, Russian Federation
 fonova.elizaveta@medgenetics.ru

Abstract. Relevance. The problem of diagnosing hereditary neuromuscular diseases is one of the most difficult in the medical specialists' practice. Molecular genetic diagnostics is one of the fundamental aspects in the classification and subsequent approaches to the treatment and prevention of hereditary diseases. Pathogenic variants identification leads to the formation of separate subtypes and phenotypically identical diseases syndromes. This review examines modern diagnostic methods and algorithmization of patients with neuromuscular diseases. Despite enormous research and clinical efforts, the molecular causes remain unknown for almost half of patients with neuromuscular diseases due to genetic heterogeneity and molecular diagnostics based on a gene-by-gene approach. Next-generation sequencing (NGS) is an effective and cost-effective strategy for accelerating patient diagnosis. However, the diagnostic value of conducting and prescribing whole-exome or whole-genome sequencing is largely dependent on the clinical picture of the disease and the professional competence of the doctor. Hereditary neuromuscular diseases have similar initial symptoms, and molecular genetic diagnostics can pinpoint the cause and pathogenesis of the observed disorders in the patient. **Conclusion.** The molecular diagnostics algorithm is based on sequential analysis, starting with the search for the most common pathogenic variants using inexpensive and rapid methods, and progressing to the search for rare, previously undescribed pathogenic variants using whole-genome/whole-exome studies. The phasing allows science and medicine to uncover previously unknown causes of severe disease in patients with neuromuscular diseases, which often leading to disability or premature death. Earlier genetic diagnosis should provide more effective treatment of the disease and better genetic counseling for families and will also allow access to pathogenetic therapy for neuromuscular diseases.

Keywords: neuromuscular diseases, molecular diagnostics, mutations, sequencing

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Introduction

Hereditary diseases of the nervous system are a severe and widespread form of hereditary pathology that can lead to disability and premature death [1]. Diagnosis of hereditary diseases can often be complicated due to various types of inheritance, genetic heterogeneity, and clinical polymorphism, which can cause delays of several years. Information on the clinical manifestations and diagnostic methods of hereditary diseases has increased significantly in recent years.

One of the largest groups of hereditary diseases affecting the peripheral nervous system is neuromuscular diseases (NMD) [2–4]. These diseases are characterized by damage to the peripheral nervous system, which includes motor and sensory neurons, the muscle itself, or the neuromuscular junction. The OMIM (Online Mendelian Inheritance in Man) database lists 4432 different neurological disorders and 1476 disorders that involve both neurological and muscular symptoms [5].

There are five main nosological groups that can be distinguished according to the degree of damage to the neuromotor apparatus: monogenic myopathies, spinal muscular atrophies, myotonic dystrophies, hereditary myasthenia gravis and hereditary motor-sensory neuropathies [6]. Muscle weakness is a common symptom of all these diseases [7].

Most neuromuscular disorders (NMD) share common features, including muscle weakness, fasciculations, seizures, muscle pain, and bulbar symptoms such as breathing and swallowing difficulties and cranial nerve palsy [8]. In addition, patients may present with non-specific manifestations such as toe walking or musculoskeletal deformities (hollow foot, scoliosis), which are classic secondary pleiotropic manifestations. The term NMD encompasses a wide range of syndromes with a similar clinical presentation but varying pathogenesis and etiology. Therefore, identifying genetic causes and differential diagnosis of NMD is receiving increasing attention. Improving existing classifications and developing effective prevention methods for NMD are also important [9].

Hereditary neuromuscular diseases

Hereditary neuromuscular diseases encompass a wide range of genetically diverse disorders of the nervous system, including progressive muscular dystrophies and disorders of neuromuscular impulse transmission [10–13]. Due to the variable age of disease onset, the pronounced clinical polymorphism, and the different types of inheritance of various forms of NMD, there is an extraordinary variety of classifications that take into account the affected gene or its product.

The topographic principle is still the foundation of modern classifications. It involves grouping diseases based on the location of the lesions in the central and peripheral nervous systems [1, 14–16]. Hereditary neuromuscular diseases can be classified using a variety of methods, including morphological, clinical, and genetic approaches.

Morphological classification of neuromuscular diseases

- I. Associated with lesions of the skeletal muscles.
- II. Associated with lesions of the anterior horns of the spinal cord.
- III. Associated with the lesion of peripheral nerves.
- IV–VII. Associated with lesions not only of nerve and muscle structures, but also of synapses.

Clinical classification

- I. Progressive muscular dystrophies.
 - Ia. Non-progressive muscular dystrophies (myopathies).
 - II. Spinal amyotrophies.
 - III. Neural amyotrophies.
 - IV. Myasthenia gravis.
 - V. Myatonia.
 - Congenital myatonia.
 - Oppenheim's disease.
 - VI. Thomson's myotonia.
 - VII. Paroxysmal myoplegia.

However, recent advances in molecular genetics have fundamentally altered the principles of classification, diagnosis, and treatment of inherited neuromuscular diseases. Molecular biology has demonstrated genetic heterogeneity in a significant number of nosological forms, leading to the establishment of classifications based on the affected gene and/or its product [17].

Hereditary neuromuscular diseases can be caused by various types of mutations, including point pathogenic variants, single-nucleotide insertions and deletions, trinucleotide repeat expansions, copy number variations (CNVs), and large deletions and duplications of entire chromosome regions. Molecular genetic diagnostics employs a range of methods to identify these mutations [18, 19].

Algorithmisation of patients with suspected neuromuscular diseases

The diagnosis of neuromuscular diseases requires collaboration between the attending physician, neurologist, and molecular diagnostics laboratory. Paraclinical investigations, including biochemical tests such as creatine kinase and other enzymes, play a crucial role.

In the diagnosis of NMD, biochemical tests for muscle enzymes, muscle biopsy, and electroneuromyography are the first tests that a physician may order for differential diagnosis [6, 20–22]. Before the advent of molecular genetics, diagnosis was based solely on clinical examination, electroneuromyography, and muscle and nerve biopsies. Verification was performed to determine whether the disease was caused by a primary defect in the muscle (myogenic diseases) or a primary defect in the innervating nerve (neurogenic diseases). If biochemical analyses are the primary and most accessible method of diagnosis for patients with suspected NMD, they should be performed. However, muscle biopsy is also a valuable diagnostic tool, despite the methodological difficulties associated with it. Some patients may not agree to this procedure.

The differential diagnosis of NMD should include a neurological examination followed by electroneuromyography to determine the speed of conduction along the median nerve and the type of neuropathy. At this stage, it may be possible to determine which genetic study should be performed first. Muscle imaging techniques such as magnetic resonance imaging, X-ray computed tomography, or ultrasound are increasingly used to differentiate between clinically similar neuromuscular diseases. A definitive diagnosis can only be accurately made by identifying a specific pathogenic variant in the genes. Therefore, molecular diagnostics is considered the gold standard for the diagnosis of neuromuscular diseases.

The molecular genetic algorithm follows a sequential analysis approach, starting with the search for the most common pathogenic variants, such as duplications or deletions of the *PMP22* gene. This is followed by gene spectrum sequencing using panels and whole-genome or whole-exome studies (Fig. 1).

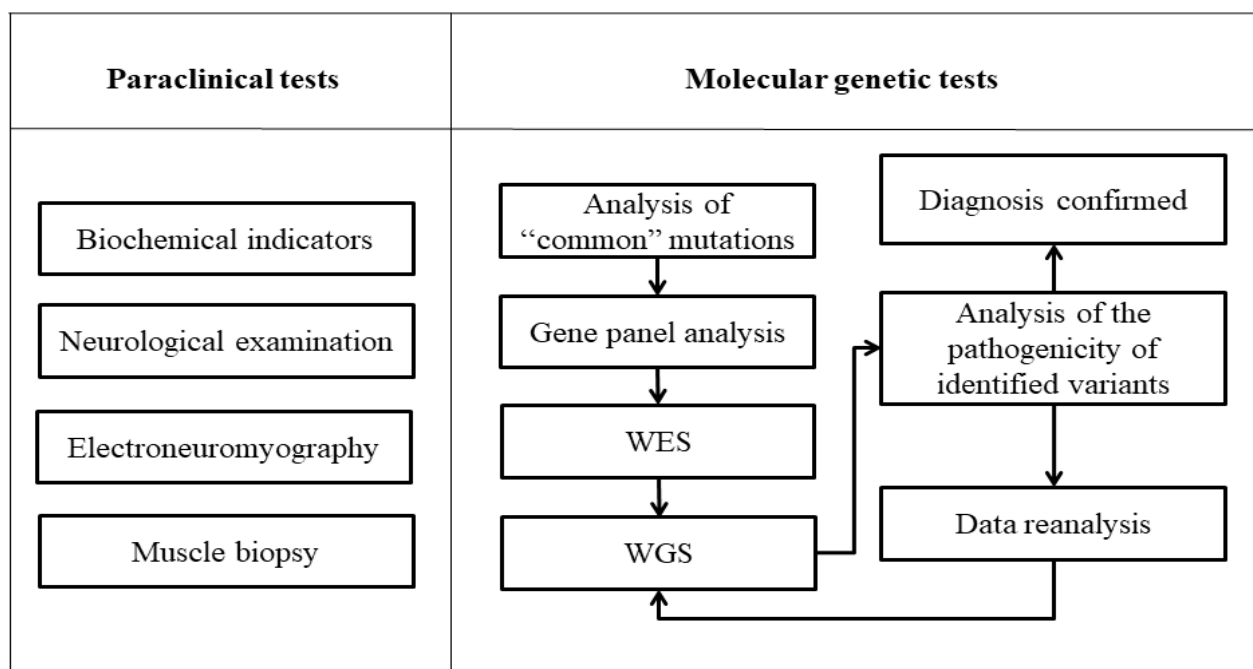


Fig. 1. Algorithm for molecular genetic examination of patients with suspected NMD

Molecular genetic testing has the potential to eliminate the need for invasive and costly diagnostic procedures, such as muscle biopsies. Gene panel sequencing can be used to sequence individual genes that may lead to neuromuscular disorders due to pathogenic variants. Additionally, Whole-Exome Sequencing (WES) and Whole-Genome Sequencing (WGS) can be employed to search for pathogenic variants in protein-coding regions of the genome and the entire genome, respectively [6, 23].

This method enables the screening of multiple genes or genomic regions simultaneously, providing a more cost-effective and efficient means of diagnosis. While next generation sequencing (NGS) methods can aid in molecular diagnosis, they may not be suitable for all types of mutations. In some cases, it may be appropriate to use molecular analysis of a single gene as the primary test, particularly if the majority of pathogenic variants associated with a particular disease are located on that gene and account for more than 50 % of all mutations associated with that form of NMD. For example, in Duchenne-Becker myodystrophy, the search for deletions in the *DMD* gene is carried out

using PCR. The most commonly used technology to search for duplications of the *PMP22* gene is multiplex ligation-dependent probe amplification (MLPA) [24]. Currently, diagnostic molecular neurogenetics aims to detect pathogenic mutations in patients.

The genetic heterogeneity of spinocerebellar ataxia (SCA) can be illustrated by the example provided. In 1993, autosomal dominant cerebellar ataxias (ADCA) were classified into three groups based on clinical features: autosomal dominant cerebellar ataxia types I, II, and III [25–27].

ADCA type I is a mixed cerebellar ataxia where the patient experiences additional neurological symptoms alongside cerebellar ataxia. ADCA type II is characterized by ataxia and retinopathy, while ADCA type III is diagnosed when cerebellar ataxia is the only or predominant neurological manifestation. It is important to note that there is no definitive correlation between phenotype and genotype in SCA, so autosomal dominant SCA is typically classified as mixed or pure SCA by neurologists. Patients with any type of mixed cerebellar ataxia may present only cerebellar signs. However, in the early stages of the disease, additional

neurological abnormalities such as extrapyramidal symptoms, areflexia, seizures, sensory and cognitive impairment may also be noted. The cerebellar ataxias that fall under this category include SCA 1–4, 7, 8, 10, 12–14, 17–21, 23, 25, 27–29, 32, 35, and 36 (Table 1).

Table 1

Spinocerebellar ataxia				
Group	Sca	Localization	Gene	Mutation
mixed SCA	SCA1	6p23	<i>ATXN1</i>	CAG repeat expansion
	SCA2	12q24	<i>ATXN2</i>	CAG repeat expansion
	SCA3	14q24.3-q31	<i>ATXN3</i>	CAG repeat expansion
	SCA4	16q22.1		CAG repeat expansion
	SCA7	3p21.1-p12	<i>ATXN7</i>	CAG repeat expansion
	SCA8	13q21	<i>ATXN8, ATXN8os</i>	Non-coding CTG×CAG repeat
	SCA10	22q13	<i>ATXN10</i>	Non-coding pentanucleotide repeat
	SCA12	5q31-q33	<i>PPP2R2B</i>	Non-coding CAG repeat (5'UTR)
	SCA13	19q13.3-q13.4	<i>KCNC3</i>	Multiple missense mutations
	SCA14	19q13.4	<i>PRKCG</i>	Multiple missense mutations
	SCA17	6q27	<i>TBP</i>	CAG or CAA repeat expansion
	SCA18	7q22-q32	-	-
	SCA19/22	1p21-q21	<i>KCND3</i>	Multiple missense mutations
	SCA20	11p13-q11	-	Multiple missense mutations
	SCA21	7p21.3-p15.1	-	Multiple missense mutations
	SCA23	20p13	<i>PDYN</i>	Multiple missense mutations
	SCA25	2p21-p13	-	-
	SCA27	13q34	<i>FGF14</i>	missense mutations F145S
	SCA28	18p11	<i>AFG3L2</i>	Multiple gene mutations
	SCA29	3p26	-	-
SCA32	7q32-q33	-	-	
SCA35	20p13	<i>TGM6</i>	Multiple gene mutations	
SCA 36	20p13	<i>NOP56</i>	Extension of intronic hexanucleotide repeats	
SCA 37	1p32	-	-	
only SCA	SCA 5	11q13	<i>SPTBN2</i>	missense mutations or in-frame deletion
	SCA 6	19p13	<i>CACNA1A</i>	CAG repeat expansion
	SCA 11	15q15.2	<i>TTBK2</i>	Various mutations
	SCA 15/16	3p26-p25	<i>ITPR1</i>	Large genomic deletions
	SCA 26	19p13.3	<i>eEF2</i>	missense mutations P596H
	SCA 30	4q34.3-q35.1	-	-
	SCA 31	16q21	<i>BEAN</i>	Expansion of intronic pentanucleotide repeat
	SCA 34	6q12.3-q16.2	-	-

The spectrum of mutations observed in 34 types of ADCA includes missense substitutions and expansion of nucleotide repeats. Notably, these repeats are not detected by NGS analysis, raising concerns about the effectiveness of whole-exome sequencing as the primary method of molecular diagnostics.

Polyglutamine diseases are a group of neurodegenerative disorders caused by the expansion of cytosine-adenine-guanine (CAG) repeats encoding the long polyglutamine tract in the corresponding proteins [28]. The expansion of nucleotide repeats is the cause of these diseases. Nine disorders have been described to date, including six types of spinocerebellar ataxias (types 1, 2, 6, 7, 17), Machado-Joseph disease (MJD/SCA3), Huntington's disease, dentatorubral pallidoluysis atrophy (DRPLA or Ho-River syndrome and Naito-Oyanagi disease), and spinal and bulbar muscular atrophy, X-linked 1 (SMA1/SBMA). Polyglutamine diseases are characterized by the pathological expansion of the CAG trinucleotide repeat in the translated region of various genes. The molecular diagnosis of these mutations is based on the MLPA method. This method calculates the number of repeats and determines the patient's status, whether it is the absence of mutation, premutation, or mutation. The phenomenon of anticipatory inheritance, i.e. the progressive deterioration of the clinical features of the disease, must be taken into account. This phenomenon is caused by an increase in the number of repeats in diseases of nucleotide expansion. The necessity of presymptomatic testing for late-onset diseases is driven by anticipation. This is because it is possible to predict earlier and more severe development of the disease in future offspring.

Genetic panels for neuromuscular diseases

After excluding major pathogenic variants, it is recommended to sequence the entire gene to exclude rare pathogenic variants. If no pathogenic variants are found in the target gene, it is recommended to investigate all genes that encode similar proteins, which may lead to the development of neuromuscular diseases. Sequencing a panel of genes provides information on the spectrum of pathogenic variants in multiple genes simultaneously, ensuring sufficient depth of coverage for all exons of the genes of interest. Genetic panels are typically customised by laboratories to meet practical healthcare needs, and can range from single-gene to several-hundred-gene panels. A recent study investigated

the genetic causes of limb-girdle muscular dystrophy in 6473 patients. The study demonstrated the effectiveness of genetic panels and the importance of collaboration between laboratories and clinicians [15]. The cause of the disease was identified in 1266 (19.6 %) patients. Initially, the genetic panel included 105 genes, but during the study, neurologists suggested reducing the number of genes to 66 to focus on the subtypes of lumbosacral muscular dystrophy.

Genetic panels are commonly used in routine diagnostics due to their cost-effectiveness compared to whole-exome and whole-genome sequencing. This is because they target a smaller number of genes, requiring less data processing, analysis and storage. Additionally, the smaller analyzed region allows for deeper coverage, resulting in better detection of certain CNVs and mosaicism when compared to WES [29, 30]. The use of a limited number of target genes reduces the likelihood of detecting incidental findings that are unrelated to the phenotype under investigation, thereby mitigating associated ethical issues [30].

The main challenge in using a disease-specific targeting panel lies in its design. Attention must be paid to which genes to include in order to maximize diagnostic efficiency while minimizing the cost and volume of sequencing data generated. Periodic updates to gene panels are necessary due to the frequent and continuous discovery of new genes that cause inherited diseases.

Whole-exome and whole-genome sequencing

In cases where sequencing of a gene panel is inconclusive, whole exome sequencing is performed. This method covers all coding regions of the genome where an estimated 85 % of disease-causing variants occur [31–33]. Therefore, WES has the potential to identify new disease genes and allows for re-diagnosis at a later time.

Two main approaches are used to verify genes that are causal for certain conditions. Two methods can be used for genetic analysis. The first involves performing WES or WGS analysis on a group of patients with the same clinical features. Variants located in a common

gene for all or some members of the study group are then filtered sequentially. The second method involves analyzing isolated patients along with their parents (trio-analysis) and/or informative family members. Variants are filtered based on different types of inheritance [30, 34]. One limitation of the WES method is its inability to analyze sites located in non-coding regions, such as deep intronic or non-translational regions. Research has shown that 15 % of variants that potentially cause Mendelian traits are located in non-coding regions of

the genome [35]. In contrast, WGS provides uniform coverage of both coding and non-coding regions and can detect CNVs, gross chromosomal abnormalities, and deep intronic variants [32]. WGS is a powerful tool for genomic research. It can verify variants that are not detected by WES in NMD patients.

The more expensive and complex the method, the more data on the mutation spectrum can be obtained (Figure 2). This staging of molecular diagnosis of NMD follows a similar distribution.

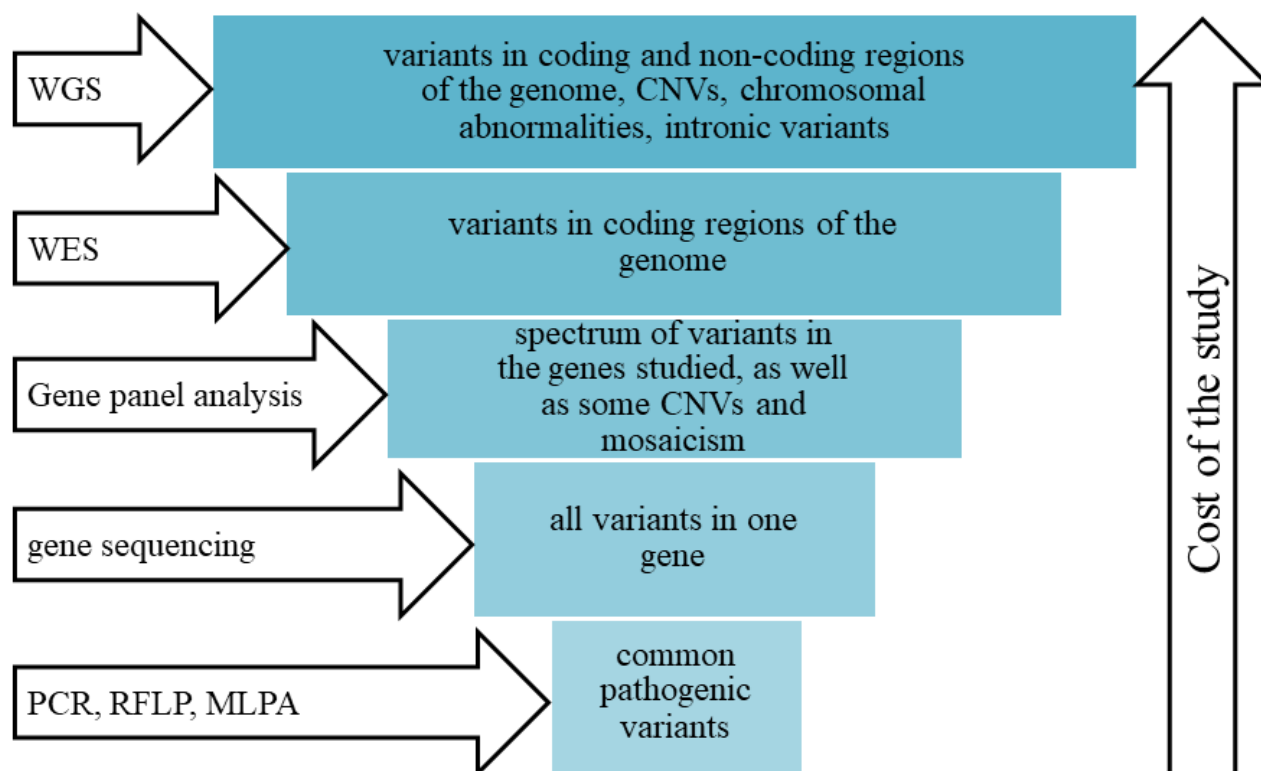


Fig. 2. Methods for studying mutations of monogenic diseases

Efficient and effective use of resources and staff organization are crucial in practical healthcare. The availability of pathogenetic treatment and pre-conceptional or prenatal diagnosis methods directly correlates with the prevention of disabilities in patients or subsequent births of children with inherited forms of NMD. Existing screening programs largely target

diseases that cause severe impairment of quality of life from birth or early childhood, rather than those that manifest in adulthood.

Therefore, the concept of ‘diagnostic cost-effectiveness’ was introduced to compare different types of tests available for the diagnosis of peripheral neuropathies [36].

In the case of NMD diagnostics, this refers to the ratio of diagnostic informativeness to the price and time of the trial. This value enables us to determine the sequence and priority of prescribing tests for genetically heterogeneous pathology of the peripheral nervous system in a particular patient, without evaluating the cost-effectiveness of using different tests in the healthcare system.

Diagnostic cost-effectiveness = diagnostic informativeness / price*timeframe

The diagnostic method for patients with NMD becomes more favorable as the diagnostic cost-effectiveness increases. If the method detects nothing in the study group, the diagnostic cost-effectiveness is assumed to be 0. The diagnostic cost-effectiveness of different tests is not constant and may change with the development of new technologies that enable faster and more affordable genetic testing.

When dealing with well-studied ethnic groups with a high prevalence of pathogenic variants, it is advisable to exclude these variants initially. However, single gene testing is only feasible when minimal locus heterogeneity of a particular nosology is proven. Previous studies have demonstrated that the ‘gene panel first’ strategy is more cost-effective than sequencing a single gene or searching for common pathogenic variants [37, 38]. If gene panels are uninformative, it may be necessary to use a more expensive but informative method such as WES/WGS [39].

Conclusion

Neuromuscular diseases have a highly complex genetic basis. To ensure appropriate genetic testing, clinical judgement is crucial. If one of the molecular genetic analyses yields a negative result, clinicians should proceed to the next analysis of the target gene or consider a broader sequencing approach, such as gene panels, WES, and WGS. The diagnostic performance of neuromuscular disorders is incomplete due to limitations of targeted gene panels and whole-exome sequencing. These methods are unable to detect structural variants, trinucleotide tandem repeats, and non-coding variants.

However, the introduction of whole-genome sequencing, functional tests, and improved analytical methods may enhance the detection and interpretation of pathogenic variants, leading to increased diagnostic outcomes in cohorts of patients with NMD.

The interpretation of variants of uncertain significance or genes of uncertain significance and the identification of complex inheritance patterns are critical factors in the genetic diagnosis of neuromuscular diseases.

The continued development of sequencing technologies, analytical tools, and reference databases will contribute to the understanding and improvement of NMD diagnosis.

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Современные аспекты и подходы к молекулярной диагностике наследственных нервно-мышечных заболеваний

Е.А. Фонова  , И.Ж. Жалсанова , Н.А. Скрябин 

Научно-исследовательский институт медицинской генетики, Томский национальный исследовательский медицинский центр, г. Томск, Российская Федерация
 fonova.elizaveta@medgenetics.ru

Аннотация. *Актуальность.* Проблема диагностики наследственных нервно-мышечных заболеваний является одной из наиболее сложных в практике врачей-специалистов. Одним из основополагающих аспектов классификации нервно-мышечных заболеваний является молекулярно-генетическая диагностика. Выявление патогенных вариантов приводит к формированию отдельных подтипов и синдромов фенотипически идентичных заболеваний. В данном обзоре рассматриваются современные методы диагностики и алгоритмизация пациентов с нервно-мышечными заболеваниями. Несмотря на огромные исследования и клинические работы, молекулярные причины остаются неизвестными почти для половины пациентов с нервно-мышечными заболеваниями из-за генетической гетерогенности и традиционной молекулярной диагностики, основанной на подходе анализа одного гена, наиболее ассоциированного с определенной формой нервно-мышечного заболевания. Секвенирование нового поколения (NGS) представляет эффективную и экономичную стратегию ускорения диагностики пациентов, в том числе и на молекулярно-генетическом уровне. Однако диагностическая рентабельность проведения и назначения в первую очередь полноэкзомного или полногеномного секвенирования во многом зависит от клинической картины заболевания и профессиональной компетенции врача. Начальные симптомы наследственных нервно-мышечных заболеваний во многом идентичны, и именно проведение молекулярно-генетической диагностики может определить причину и патогенез наблюдаемых нарушений у пациента. *Выводы.* Алгоритмизация молекулярной диагностики основана на последовательном анализе от поиска наиболее часто встречаемых патогенных вариантов с помощью недорогих и быстрых методов до поиска редких, ранее неописанных патогенных вариантов с помощью полногеномных полноэкзомных исследований. Благодаря такой этапизации наука

и медицина получает ответы на ранее нераскрытые причины тяжелых заболеваний у пациентов с нервно-мышечными заболеваниями, которые нередко приводят к инвалидизации или преждевременной гибели. Более ранняя генетическая диагностика может позволить обеспечить более эффективное лечение заболевания и более качественное генетическое консультирование для прогноза будущего потомства в семье, а также позволит получить доступ к патогенетической терапии нервно-мышечных заболеваний.

Ключевые слова: нервно-мышечные заболевания, молекулярная диагностика, мутации, секвенирование

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Corresponding author: Fonova Elizaveta Alekseevna — junior researcher at the Research Institute of Medical Genetics, Tomsk National Research Medical Center, 634050, Nab. Ushaiki, 10, Tomsk, Russian Federation. E-mail: fonova.elizaveta@medgenetics.ru
Fonova E.A. ORCID 0000–0002–1338–5451
Zhalsanova I. Zh. ORCID 0000–0001–6848–7749
Skryabin N.A. ORCID 0000–0002–2491–3141

Ответственный за переписку: Фонова Елизавета Алексеевна — младший научный сотрудник лаборатории геномики орфанных болезней НИИ медицинской генетики Томский национальный исследовательский медицинский центр, Российская Федерация, 634050, г. Томск, ул. Набережная реки Ушайки д. 10. E-mail: fonova.elizaveta@medgenetics.ru
Фонова Е.А. SPIN 5198–9456; ORCID 0000–0002–1338–5451
Жалсанова И.Ж. SPIN 9882–3730; ORCID 0000–0001–6848–7749
Скрябин Н.А. SPIN3416–4105; ORCID 0000–0002–2491–3141