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SPINAL MUSCULAR ATROPHY: NEWS AND PERSPECTIVES

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REZUMAT

ATROFIA MUSCULARĂ SPINALĂ: NOUTĂȚI ȘI PERSPECTIVE

Introducere. Atrofia musculară spinală (SMA) este o boală neuromusculară progresivă moștenită recesiv autozomal. Boala este cauzată de mutații care apar în gena *SMN1* (Survival Motor Neuron) și se consideră că severitatea sa este modulată de gene precum *SMN2, NAIP* (Proteina Inhibitoare Neoponală) sau *GTF2H2* (General Transcription Factor IIH Subunitate 2)

Material și metode. Diagnosticul pentru maladia SMA este disponibil prin diferite metode. Diagnosticul genetic molecular este efectuat prin PCR-RFLP, dar mai detaliat și considerat ca fiind standard de aur este tehnica MLPA. Diagnosticul se efectuează la persoanele care prezintă simptome, dar și înainte de apariția lor. Astfel, diagnosticul poate fi pus cât mai curând posibil, de exemplu, prenatal pentru familiile în care s-au născut deja copii cu SMA, pentru nou-născuți (screeningul nou-născutului) și chiar în procesul de planificare familială (screening al purtătorilor). Diagnosticul diferențial al SMA este complicat și necesită luarea în considerare a multor boli cu simptome similare.

Rezultate. În baza de date a Laboratorului sunt înregistrați 131 de pacienți diagnosticați cu SMA. Până în 2016, tratamentul pacienților a inclus doar îngrijire curativă îmbunătățind simptomele bolii. După 2016, Food and Drug Administration (FDA) a fost aprobat singurul tratament pentru SMA. În prezent, la nivel mondial, se fac eforturi pentru implementarea screeningului nou-născutului.

Concluzii. Odată cu reevaluarea criteriilor necesare pentru screening și aprobarea tratamentului, screeningul pentru această boală devine esențial. Diagnosticul precoce poate îmbunătăți evoluția stării pacienților cu SMA.

Cuvinte-cheie: genetică moleculară, simptome, diagnostic, screening, tratament.

РЕЗЮМЕ

СПИНАЛЬНАЯ МЫШЕЧНАЯ АТРОФИЯ: НОВОСТИ И ПЕРСПЕКТИВЫ

Введение: Спинальная мышечная атрофия (СМА) является прогрессирующим нервно-мышечным заболеванием с аутосомно-рецессивным типом наследования. Заболевание вызывается мутациями в гене *SMN1* (Survival Motor Neuron) и его тяжесть модулируется такими генами, как SMN2, NAIP (белок, ингибирующий апоптоз нейронов) или GTF2H2 (субъединица 2H общего транскрипционного фактора IIH).

Материалы и методы: Диагностика проводиться различными методами. Молекулярно-генетическая диагностика проводится методом ПЦР-РФЛП, но золотым стандартом является метод MLPA. Диагностика может проводиться у людей, у которых уже есть симптомы, но и до их появления. Диагноз СМА может быть поставлен пренатально в семьях, где уже имеется случаи СМА и постнатально у новорожденных (скрининг новорожденных) и у больных, а также в процессе планирования семьи (скрининг носителей). Дифференциальная диагностика СМА сложна и требует рассмотрения многих заболеваний с похожими симптомами.

Результаты: В базе данных лаборатории зарегистрировано 131 пациент с диагнозом СМА. До 2016 года лечение пациентов включало только лечебную помощь для улучшения симптомов заболевания. С 2016 года Управление по контролю за продуктами и лекарствами (FDA) одобрило единственное лечение СМА (Spinraza). В настоящее время во всем мире предпринимаются усилия по внедрению скрининга новорожденных на СМА.

Заключение: После переоценки необходимых критериев скрининга и утверждения лечения скрининг на это заболевание становится необходимым. Ранняя диагностика может улучшить состояние пациентов с СМА.

Ключевые слова: молекулярная генетика, симптомы, диагностика, скрининг, лечение.

Introduction.

Spinal muscular atrophy (SMA) is a progressive neuromuscular disease with autosomal recessive transmission, characterized by muscle weakness and atrophy caused by degeneration of motor neurons in the spinal cord and brainstem nuclei [15]. This hereditary condition has an incidence of about 1 in 8,43±0,15:100 000 of R. Moldova population [24]. In over 95% of cases, this disease is caused by abnormalities of the *SMN1* gene (Survival Motor Neuron 1), which results in a major deficiency of the SMN1 protein.

Cause of SMA. In 1995, the gene involved in the onset of spinal muscular atrophy was also described. It encodes a specific neuronal protein, the neuronal survival protein (SMN). The disease is caused by mutations that occur in the *SMN1* (Survival Motor Neuron) gene, and its severity is thought to be modulated by genes such as *SMN2*, *NAIP* (Neuronal Apoptosis Inhibitory Protein) or *GTF2H2* (General Transcription Factor IIH Subunit 2). Expression of the *SMN1* gene produces full-length SMN protein, in contrast, *SMN2* expression results in a truncated version of the polypeptide that lacks 16 amino acids at the carboxy-terminal end [20].

People with SMA are either homozygous for the deletion of exon 7 from *SMN1* (Δ7 *SMN1*), or heterozygous compounds for Δ7 *SMN1* and an intragenic mutation of *SMN1*. The deletion of the telomeric copy of the *SMN*

Table 1. Disorders to Consider in the Differential Diagnosis of Spinal Muscular Atrophy (SMA)[14]

Age of Onset	Disorder	Gene(s) or Region	MOI	Clinical Features of Differential Diagnosis Disorder	
				Overlapping w/SMA	Distinguishing from SMA
Congenital to <6 months	X-linked infantile spinal	UBA1	XL	Hypotonia, weakness, areflexia	Multiple congenital contractures, intrauterine fractures
	SMARD1 1 (OMIM 604320)	IGHMBP2	AR	Weakness, respiratory failure, hypo- or areflexia	Distal predominant weakness, diaphragmatic paralysis
	Prader-Willi syndrome	15q11.2-q13	Many factors	Hypotonia, feeding difficulties	Poor respiratory effort is rare.
	Myotonic dystrophy type 1	DMPK	AD	Hypotonia, muscle weakness	Marked facial weakness
	Congenital muscular dystrophy	Many genes	AR AD	Hypotonia, muscle weakness	CNS, eye involvement, possible increased tone
	Zellweger spectrum disorder	PEX family of genes	AR	Hypotonia	Hepatosplenomegaly, CNS
	Congenital myasthenic syndromes	CHAT, CHRNE COLQ, DOK7 GFPT1,RAPSN	AR AD	Hypotonia	Ophthalmoplegia, ptosis, episodic respiratory failure
	Pompe disease	GAA	AR	Hypotonia	Cardiomegaly
	Other: congenital myopathies, 4 metabolic/mitochondrial myopathies, 5 peripheral neuropathies 6				
>6 months	Botulism	NA	NA	Proximal muscle weakness, decreased reflexes	Prominent cranial nerve palsies, acute onset
Later childhood	Guillain-Barré syndrome	NA	NA	Muscle weakness	Subacute onset, sensory involvement
	Duchenne muscular dystrophy	DMD	XL	Muscle weakness, motor regression	Serum creatine kinase concentration 10-20x > normal
	Hexosami-nidase A deficiency	HEXA	AR	Lower motor neuron disease	Slow progression, progressive dystonia, spinocerebellar degeneration, cognitive/psychiatric involvement
	Fazio-Londe syndrome	SLC52A2 SLC52A3	AR	Progressive bulbar palsy	Limited to lower cranial nerves; progresses to death in 1-5 years
	Monomelic amyotrophy (OMIM 602440)	Unknown		Muscle weakness	Predominantly cervical; tongue may be affected (rare); other cranial nerves spared
	Other: peripheral neuropathies, 6 muscular dystrophies 7				
Adulthood	Kennedy disease	AR	XL	Proximal muscle weakness, muscle	Gradually progressive; gynecomastia, testicular
	Amyotrophic lateral sclerosis	Many genes 8	AD AR XL	May begin w/pure lower motor neuron signs	Progressive neurodegeneration; involves both upper & lower motor neurons

(*SMN1*) is directly involved in SMA because the absence of exon 7 or exon 7 and 8 is detectable in more than 95% of the affected persons regardless of the clinical manifestation. In patients with mutations, approximately 70-80% of the SMN gene product is in the form of the truncated protein [21].
Also, in 1995, the *NAIP* gene that encodes the *NAIP* (apoptosis neuronal inhibitory protein) protein was identified. The deletion of this gene, which is close to the SMN gene, is associated with SMA, a fact proven by the presence of homozygous mutations of the NAIP gene in 45% of patients with SMA type I and in 18% of patients with SMA type II or III [8].
GTF2H2 (General Transcription Factor IIH Subunit 2) is a Protein Coding gene. Diseases associated with GTF2H2 include Spinal Muscular Atrophy and Cockayne Syndrome.
Types of SMA.
Genetically factors that influence the SMA phenotype are the number of SMN2 gene copies and a deletion in the NAIP gene. A higher number of SMN2 copies makes the clinical symptoms more benign, and the NAIP gene deletion is associated with a more severe phenotype. Clinically (*Table 1*), depending on the age of onset, life expectancy, distribution of muscular hypotonia and stage motor development of patients, several phenotypes of SMA have been described of which the most important are: acute infant (type SMA) I or Werdnig-Hoffman's disease), chronic infant (SMA type II), chronic juvenile (SMA type III or Kugelberg-Welander disease) and adult form (SMA type IV) [22]. Alongside these are described: prenatal form (SMA 0), congenital axonal neuropathy and spinal muscular atrophy associated with congenital arthroplasty [14].

AD = autosomal dominant; AR = autosomal recessive; MOI = mode of inheritance; SMARD = spinal muscular atrophy with respiratory distress; XL = X-linked

Treatment. Until 2016, treatment of patients included only curative care improving the symptoms of the disease. However, since 2016 new therapies have advanced in clinical trials for diagnosed patients and the Food and Drug Administration (FDA) has approved the only treatment for SMA which is called Spinraza (Nusinersen) that later in 2019 a second treatment called Zolgensma will be approved [5].
Spinraza Nusinersen is an antisense oligonucleotide that binds to the pre-mRNA of survival motor neuron 2 (SMN2). It is used for the management of spinal muscular atrophy for a full range of patients with spinal muscular atrophy (from disease with infant onset until later onset) [11].
Zolgensma (AveXis, Inc.) designed to provide a normal copy of the gene that encodes SMN protein is an adeno-associated virus (AAV) based on vector gene therapy indicated for the treatment of patients younger than 2 years of age with spinal muscular atrophy (SMA) caused by bialelic mutations in the motor neuron 1 survival gene (*SMN1*), type I SMA.
According to the recent reevaluation of the Wilson-Jungner criteria (2008), SMA may be included in screening studies [4].
Thus, starting in 2018, pilot studies have been launched in some European countries (Belgium, England, Germany), Taiwan, Russia, America, as well as Australia, in some cases with the implementation of neonatal screening and heterozygous carriers atrophy to prevent the loss of motor neurons, just before the onset of symptoms. It should allow maximum benefit for the people who will develop SMA.

Materials and Methods.
*Molecular genetics diagnosis***.** There is a number of methods available to screen DNA for SMN1 deletion or SMN2 copy number. The standard molecular diagnosis of SMA is based on a PCR-RFLP test [6], able to detect homozygous *SMN1* loss [2; 9].
At present, in Moldova, the genetic molecular diagnosis is performed in the Laboratory of Human Molecular Genetics (LGMU) of the IMSP of the Mother and Child Institute. This involves the study of ADN extracted from biological samples (blood on anticoagulant) that were collected from the patients who were consulted. Thus, the presence or absence of exons 7 and 8 in the SMN1/SMN2 genes is performed by PCR-RFLP method [24].
For families at-risk (where the birth of a child with SMA has already been registered) is available prenatal diagnosis.
Now, in our Laboratory, the optimization of the genetic molecular diagnosis method through qPCR is being considered. The parameterization and design of the test to identify the deletions associated with the SMA will include the establishment of optimal conditions with the elaboration of the working algorithm [18]. This test will be possible to use in the NBS diagnosis.

Results and Discussions.
In the database of laboratory 131 patients diagnosed with SMA are registered. Respectively for Nr = 40 (30.5%) deletion of only exon 7 *SMN1,* for Nr = 23 (17.5%) deletion of only exon 8 *SMN1* and for Nr = 68 (52%) deletion was registered for both exons.
Is very important to quantify the number of copies of genes *SMN1* and *SMN2*. PCR -RFLP test does not detect heterozygous *SMN1* loss, and cannot be used for identifying healthy carriers, which can be checked by quantitative analysis of *SMN1* copy number.
Considering that, is required other methods, like Quantitative PCR and MLPA for diagnosis of SMA. Multiplex ligation-dependent probe amplification (MLPA) is a modern quantitative molecular method. MLPA [19] is considered the gold standard for diagnosis of SMA. It improves diagnostics by simultaneously identifying several target sequences in the SMN1 gene and in nearby genes, the copy number of both *SMN1* and *SMN2* genes. Thus, trough MLPA both homozygous and heterozygous *SMN1* deletions

or conversions to *SMN2,* can be detected, allowing the diagnosis of affected patients or healthy carriers.

Quantitative PCR (qPCR), also known as real-time PCR, has become a powerful tool for the amplification, identification, and quantification of nucleic acids. Its ability to quantitatively and specifically detect genes has been invaluable for both research and diagnostic applications [17].

Disease pathogenesis all type of disease is influenced by *NAIP* and *GTF2H2* gene deletion. High frequency of *NAIP* deletion in SMA type I, is associated to be a modifier factor in the severity of the disease, that is why these genes need to be added to the diagnostic routine [6].

The new test based on qPCR that is elaborated in our institution will facilitate the identification of the causes of the disease onset and the establishment of heterozygous carriers in the population.

With the advent of effective pharmacological treatment for SMA, there is a worldwide discussion about strategies to identify patients as early as possible. This is especially so for children with an expected severe form of SMA who in our view should be treated immediately. This fact is achievable only with the implementation of neonatal screening (NBS) for SMA. Since the lack of SMN leads to an irreversible loss of motor neurons, the timing of treatment before the onset of symptoms is crucial for a good outcome. In patients with SMA type 1, about 95% of motor neurons are lost within the first 6 months of life. At present, only two of the patients for which a molecular diagnosis has been made confirming the deletion of exons 7 and 8 *SMN1* benefit from Spinraza treatment in Romania. They addressed for diagnosis at the end of 2019, after confirming the diagnosis within the LGMU, they counted the number of copies of the *SMN2* gene by the MLPA method in another institution. Quantification of the number of copies of *SMN2* is essential for treatment with the use of Spinraza. Consequently, there is a need for newborn screening, similar to other treatable inborn diseases. NBS is possible at a low cost and with a high predictive value [1], is available by molecular-genetic real-time PCR testing of DBS (dried blood spot), specific for the homozygous deletion of exon 7, *SMN1* and by MLPA molecular-genetic testing. NBS assumed to identify 95% of SMA cases, but this method will omit approx. 5% of SMA cases that do not have the homozygous deletion of exon 7, *SMN1*. To reach a sensitivity of 99.9%, it is necessary: testing and monitoring for carriers [3], elaboration carrying out level 2 test to exclude a second pathogenic mutation.

Conclusions. Given the progressive nature of motor neuron loss, early intervention to prevent motor neuron loss, even before the onset of symptoms, should allow maximum benefit for people who will develop SMA. Results of Germany NBS study pilot [23] show that newborn screening for SMA, resulting in pre-symptomatic treatment, can prevent the disease and partially rescue motor neuron function. Spinal muscular atrophy can cause tremendous suffering – physical, financial, and emotional – to the patient and the family of the affected individual. Testing of individuals at high risk, such as those with a family history of SMA, is prudent [13]. This fact will subsequently provide informational, social-economic and descriptive population support for at-risk families and those adopting a family planning process.

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