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PERSPECTIVE OF NEONATAL SCREENING OF SPINAL MUSCULAR ATROPHY

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REZUMAT

PERSPECTIVA SCREENING-ULUI NEONATAL PENTRU ATROFIA MUSCULARĂ SPINALĂ

Cuvinte cheie: atrofie musculară spinală, nou-născut, screening, DBS, rtPCR, mutație, MLPA, fezabilitate, perspectivă.

Atrofia musculară spinală (SMA) este cea mai frecventă patologie neuromusculară ereditară și principala cauză genetică a mortalității infantile cauzată de mutații în gena SMN1. Detectarea precoce a SMA prin screening-ul nou-născutului (NBS) este esențială pentru selectarea tratamentului pre-simptomatic și pentru asigurarea unui follow-up optim. Scopul lucrării este de a evalua perspectiva implementării screening-ului nou-născutului pentru SMA în Republica Moldova.
Materiale și metode. În scopul evaluării perspectivei, în cadrul LGMU al IMșiC am dezvoltat un algoritm pentru implementarea screening-ului mutațiilor asociate SMA, care include metode bazate pe genetica moleculară cum ar fi PCR in timp real și MLPA, cu utilizarea petelor de sânge pe carduri din hârtie de filtru colectate de la nou-născuți.
Rezultate. În scopul implementării metodei molecular-genetice de detecție a mutațiilor asociate SMA au fost elaborate sonde și matrițe specifice pentru metoda PCR in timp real, de asemenea a fost implementată metoda MLPA care are ca scop confirmarea și aprecierea numărului copiilor genelor SMN1/SMN2. Totodată, la momentul actual au fost aprobate designul și protocolul de cercetare pentru această inițiativă. De asemenea au fost elaborate consimțământul si formularul de acceptare pentru confirmarea acceptării participării la studiu, care a fost aprobat de către Comitetul de Etică a Cercetării al USMF „N.Testemițanu". Totodată suntem parte a CLSI în scopul elaborării unui protocol internațional pentru screening neonatal al SMA.
Concluzii. Screening-ul nou-născutului poate detecta pacienții afectați de SMA înainte de apariția simptomelor și poate permite intervenția terapeutică precoce. Inițiativa de implementare al algoritmului pentru screening, în cadrul LGMU, IMșiC, vine în suportul evaluării perspectivei acestuia prin aprecierea fezabilității și rentabilității sale pentru Republica Moldova.

РЕЗЮМЕ

ПЕРСПЕКТИВА НЕОНАТАЛЬНОГО СКРИНИНГА СПИНАЛЬНОЙ МЫШЕЧНОЙ АТРОФИИ

Ключевые слова: спинальная мышечная атрофия, новорожденный, скрининг, DBS, rtPCR, мутация, MLPA, перспектива.

Спинальная мышечная атрофия (CMA) является наиболее распространенным нейромышечным наследственным заболеванием, вызванным мутациями в гене SMN1, которые приводят к младенческой смертности. Раннее выявление CMA с помощью скрининга новорожденных (NBS) имеет важное значение для выбора пре-симптоматического лечения и обеспечения оптимального последующего наблюдения. Цель статьи – оценить перспективы внедрения скрининга новорожденных на CMA в Республике Молдова.
Материалы и методы. Для выполнения поставленной цели, в рамках Лаборатории Молекулярной Генетики Человека, Институт Матери и Ребенка мы разработали алгоритм проведения скрининговой диагностики мутаций, ассоциированных со CMA. Алгоритм включает молекулярно-генетические методы, такие как ПЦР в реальном времени и MLPA, с использованием пятна крови на фильтровальную бумагу, собранных у новорожденных.

Полученные результаты. Для реализации молекулярно-генетического метода выявления мутаций, ассоциированных со СМА, были разработаны специфические зонды и праймеры для метода ПЦР в реальном времени, а также внедрен метод MLPA, целью которого является подтверждение и оценка количества копий генов SMN1/SMN2. В то же время, дизайн исследования и протокол этой инициативы уже утверждены в ИМиР. Для получения согласия на участие в исследовании также были разработаны информационное согласие, которые были одобрены Комитетом по Этике Исследований ГУМФ «Н. Тестемицану». Мы работаем вместе с CLSI над разработкой международного протокола неонатального скрининга на СМА.
Выводы. Скрининг новорожденных может выявить пациентов, страдающих СМА, до появления симптомов и позволить раннее терапевтическое вмешательство. Инициатива по внедрению разработанного алгоритма проведения скрининга на мутации, в рамках ЛМГЧ, Институт Матери и Ребенка, даст оценку перспективы ее осуществимости и экономической эффективности для Республики Молдова.

Key words: spinal muscular atrophy, newborn, screening, DBS, rtPCR, mutation, MLPA, feasibility, perspective.

INTRODUCERE

SMA is an inherited neuromuscular disease historically associated with high morbidity and mortality. Spinal muscular atrophy (SMA) arises from homozygous loss of the Survival Motor Neuron1(SMN1) gene, caused by pathogenic variant in both alleles of SMN1[1].This causes degeneration of lower motor neurons and muscle atrophy, as well as an array of systemic defects[3], [4]. The past decade has brought tremendous therapeutic advances for treating SMA [4]. With the emergence of these novel therapeutics for SMA, all traditional criteria from Wilson and Junger's World Health Organization principles of early detection have been satisfied, prompting the recent successful nomination and inclusion of newborn screening for this condition[5][6]. Newborn bloodspot screening (NBS) continues to be one of the most successful population health programs, yielding greatly improved health outcomes for identified cases achieved by a combination of very early diagnosis and expedient initiation of treatment and management [7].
At European Union level, three treatments for spinal muscular atrophy are approved through the centralized approval procedure: Spinraza (Nusinersen), developed by the pharmaceutical company Biogen, Everysdi (Risdisplam) developed by the pharmaceutical company Roche and Zolgesma (onasemnogene abeparvovec-xioi) developed by the pharmaceutical company Novartis[8].
Direct treatment of the disease is recommended for newborns with a defective SMN1 gene in combination with a maximum of four SMN2 copies. SMN2 is a pseudogene presenting more than 99% of sequence homology with the SMN1 gene. The severity of the SMA phenotype is inversely correlated to the SMN2 copy number [8]. Early commencement of treatment appears to offer the best prognosis for survival, reduction in need for permanent ventilation, and progress in motor development in symptomatic patients. The greatest benefit across all these parameters has been observed in infants treated within the pre-symptomatic period[5]. NBS is likely to be key for the delivery of early treatment, but many countries who have the therapies available do not yet include SMA in their NBS programs [4]. As well as increasing therapeutic efficacy, another benefit of pre-symptomatic treatment and thus NBS may be to alleviate the high financial costs arising from living with severe SMA. Funding, screening methods, organization, and consent process are variable between SMA NBS programs. Many respondents pointed out the lack of cost/benefit data as a major obstacle to SMA NBS implementation. In the next four years, are suggested that a 24% coverage of newborns from countries where a disease-modifying drug is available and 8,5% coverage in countries with no diseases-modifying drugs[9], including the Republic of Moldova. The annual proportion of newborns to be screened in the coming years is expected to increase steadily. The experts expressed a strong need for the implementation of SMA NBS as means to improve care for patients with SMA [9].
Aim. The aim of the work is to evaluate the perspective of implementation the SMA newborn screening in Republic of Moldova.

MATERIALS AND METHODS

The NBS program for SMA involves collaboration between multiple stakeholders from the policy, diagnostic and health care systems. Methodologies that enhance interdisciplinary learning, leading to multidisciplinary, patient-centered growth, are essential in program effectiveness[10], [11][12]. Among the first steps taken in this direction was the development and approval of the national clinical protocol for SMA (Atrofia Musculară Spinală, PCN-402, approved by Health Ministry, nr. 417 on 05.05.2022) [13][14], [15]. Also, at the governmental level, negotiations are ongoing for the approval of the prices proposed by the holders of marketing authorizations for the treatments with international trade names, so that the insured can benefit from the treatments included in the list.
Thus, within the IIIth level scientific-practical Institute of Mother and Child, and Human Molecular Genetics Laboratory, together with Nicolae Testemițanu State

University of Medicine and Pharmacy, it was approved the development of an initiative for pre-symptomatic diagnosis of SMA-associated mutations, for newborns from Maternity wards of Institute whose legal representatives or parents agree to participate in the study. The initiative consists in the development of the system for testing in newborns the mutations in the genes that have been determined to be associated with spinal muscular atrophy in order to implement the pilot genetic screening for the pre-symptomatic diagnosis of the SMA disease.

Participants. The subjects that can participate are newborns from the Maternity of the Mother and Child Institute, or others which have a DBS card collection system.

Inclusion criteria:

- Age up to 3 months,
- Gender: male and female,
- Children born in Institute of Mother and Child.
- Informed consent signed by the parent or legal representative.

Exclusion criteria:

- Children whose parents or legal representatives who did not sign the Consent and Informed Agreement,
- Children whose legal representatives refuse to participate,
- Newborns with acute illness or congenital anomalies in the neonatal intensive care unit,
- Cards with bloodstains collected incorrectly,
- Samples with concentrations of less than 20 ng/ml of genomic DNA obtained from spots on filter paper.

Methods. The research methods involve molecular-genetic investigations that will be carried out within the Human Molecular Genetics Laboratory (HMGL) of the Mother and Child Institute. These involve the study of biological samples (dried blood samples on filter card) that are being collected from newborns within the Maternity of the Mother and Child Institute. Thus, the presence or absence of exon 7 in the SMN1 gene will be identified through Real-Time PCR method like a first test, together with specifying the result and quantification of the number of copies of the SMN1/SMN2 gene by MLPA (Multiplex ligation-dependent probe amplification) for a second test. 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 [16][8]. Also, MLPA method is important for the follow-up of diagnosed children because it allows the assessment of the number of copies of the SMN1/SMN2 genes, which is a decisive factor in the choice of therapy and treatment management.

Similarly, there are more commercial kits that are assay in vitro diagnostic (IVD) or research use only (RUO) designed for the detection of homozygous deletions of exon 7 in the SMN1 gene in genomic DNA isolated from human peripheral whole blood specimens or dry blood spot (DBS) cards, (such as HRM method by MRC Holland, or real time PCR by Labsystems).

Currently, consent and blood samples on filter cards are collected. And, subsequently, the DNA obtained from the collected blood spots will be subjected to analysis by the mentioned methods.

Data analysis. Such an initiative provides in itself an observational cohort analysis. In order to assess a minimum number of participants to include in this initiative, the representative research group was calculated in the EpiInfo 7.2.2.6 Program, compartment "StatCalc-Sample Size and Power". Subsequently, for the first-tier testing, through qPCR, the delta delta Ct method of quantification will be used to determine a cutoff value that can reliably distinguish the patients and unaffected newborns. In the case of screen-positive results, patients will be elected for subjection to the second-tier MLPA test. Data will be kept secure and reviewed for accuracy.

Confidentiality. The upcoming study will be conducted in accordance with the principles of the Declaration of Helsinki (Seoul, Korea, 2008) and in accordance with the Human Subjects Research Act (WMO). The personal data of the participants will be depersonalized and stored in the database of the Laboratory of Human Molecular Genetics so that it is possible to contact the participants in case they need to be informed about the results of the analyzes performed (especially in the case of a positive result).

RESULTS AND DISCUSSIONS

Spinal muscular atrophy (SMA) is a rare and devastating disease. New disease-modifying treatments have recently been approved and early treatment has been related to a better outcome. In this context, several newborn screening (NBS) programs have been implemented[17], [18].

Besides that, carrier screening for SMA being proposed as an alternative model to NBS, parental carrier status is difficult to determine in a proportion of couples because worldwide, 2% of carriers are compound heterozygotes and 6% carry two copies of exon 7 on SMN1 (including the 2 + 0 genotype). Thus, NBS programs for SMA remain essential for early diagnosis and treatment of SMA[5], [19][17]. The implementation time differs from considerations such as facilitators or barriers that influence in one way or another the implementation of such a program, Thus, it took 3 to 23 months on average to initiate the first activity in order to implement[20]. Impediments such as staffing, obtaining necessary laboratory equipment, or establishing follow-up protocols that may compromise the efficiency of the screening-diagnosis pathway include the coordination of services

that have not always been closely linked historically, such as screening/diagnostic laboratories and clinical facilities. As can be seen in the experience of countries that have implemented this type of program[5],[19][21][22][23], the involvement of interested parties is vital to overcome such barriers to the effective translation of services. Pre-establishing defined roles and pathways for laboratory and clinical stakeholders allows for the smooth transition of the newborn and their family through the process of screening, diagnosis, and clinical supervision.

Ethics. Consent for newborn screening for SMA was developed in accordance with the ethical norms of research, which includes all the necessary information for both participants and researchers. Ethics approval for consent and research was gained from the "Nicolae Testemițanu" State University of Medicine and Pharmacy Ethics Research Committee prior to commencement of the study (Notice no. 84, dated 09.09.2020) and Institute of Mother and Child, Human Molecular Genetic Laboratory (verbal process no. 2 dated: 20.12.2019).

Feasibility. To assess the feasibility of the study regarding the adoption of newborn screening for SMA, we first developed an algorithm regarding crucial stages in its development. Here, public policies, funding, the presence of a laboratory with staff, equipment and specific and sensitive methodologies, limitations, cost effectiveness, benefits, risks and later the possibility of a correct follow-up adapted to the urgency of a case of SMA were taken into consideration.

To implement and parameterize the real-time PCR method in HMGL, custom primers and probes have been designed to detect qualitative loss of exon 7 SMN1. Thus, these probes were then verified on a certain control group that includes both DNA with deletions and DNA without deletions associated with SMA. To establish or confirm the presence of deletions, the MLPA method was implemented within the HMGL. Presently, there are collected 65 consent and blood samples on filter cards.

At the same time we worked together with CLSI (Clinically Laboratory Standards Institute, USA) for elaboration of Newborn Screening for Spinal Muscular Atrophy (NBS13) Protocol. CLSI brings together the worldwide laboratory community to advance a common cause[24]. Funding. This initiative is funded by research project „Medicina Genomică și Metabolomică în serviciul profilaxiei maladiilor genetice pentru generații Sănătoase în Republica Moldova" [SCREENGEN, Cipher: 20.800009.8007.22, Contract 22-PS, 03.01.2022], also with the support of the Ministry of Health, Labor and Social Protection of the Republic of Moldova.

CONCLUSIONS

1. SMA is a devastating inherited neuro-muscular condition. Genetic testing of SMA is used in preconception, prenatal testing and newborn screening.
2. Detection and treatment of SMA is rapidly changing, with multiple interventions arising contemporaneously thus public policies and health is necessary to be available to support them.
3. The implementation of a National Neonatal Screening Program for SMA through which all newborns are tested at birth, is the only way that children who are born each year with this condition, can be diagnosed at birth, before the onset of disease symptoms and before motor neuron degeneration. Thus diagnosed, they will be able to be treated in time and will have the chance of a normal life.
4. The initiative to implement such a program within HMGL, IMandC, will help to evaluate its perspective by assessing its feasibility and profitability for the Republic of Moldova.
5. The Mother and Child Institute, being a IIIth level scientific-practical institution and part of the specialized consortia, through the contribution and support of the initiative outlined in this article, demonstrates once again, that it is fully involved in patient service and high quality healthcare.

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