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Protocol Clinical Trial

- Title of the project
  Is low-frequency repetitive nerve stimulation a reliable test to evaluate the neuromuscular junction in myotonic dystrophy type 1?
- Date
  18/11/2021.
- Objective of the study
  The study aims to evaluate the neuromuscular junction in DM1 using low-frequency RNS on several nerve-muscle pairs.
- Investigator(s)
  Dr. De Ville Jella (jella.deville@uzbrussel.be): investigator
  Prof. Dr. Veronique Bissay (Veronique.Bissay@uzbrussel.be): investigator, promoter.
  Prof. Dr. Sebastiaan Engelborghs (Sebastiaan.engelborghs@uzbrussel.be): head of the department
- Sponsor
  UZ Brussels
- Departments/laboratories involved in the study
  Department of Neurology, UZ Brussels

1 Introduction

1.1 Clinical features

Myotonic dystrophy type 1 (DM 1) or Steinert 's disease is the most prevalent adult onset dominant inherited muscular disease. Based on the age of onset, 5 subtypes are recognized: congenital, infantile, juvenile, adult-onset, and late-onset DM1. [1] The adult-onset DM1 is the most prevalent form. It is characterized by progressive muscle weakness primarily affecting the distal, axial, facial, pharyngeal, and respiratory muscles; myotonia; and systemic involvement including cataracts, cardiac arrhythmia, endocrine disorders, cognitive dysfunction, and excessive daytime sleepiness. Cardiac and respiratory complications are often the cause of shortened life expectancy.

1.2 Pathogenic mechanisms

1.2.1 Genetics

DM1 is an autosomal inherited disease that results from CTG trinucleotide repeat expansion in the myotonic dystrophy protein kinase (*DMPK*) gene on chromosome 19. The CTG repeat length ranges from 5 to 37 in healthy individuals and 50 to thousands in DM1. The CTG repeat size seems to be a prognostic marker for disease severity and onset age. [2] [3]

1.2.2 Molecular background

The pathogenic mechanism of DM1 due to the CTG repeats is complex. Different mechanisms have been proposed thus far, including *DMPK* haploinsufficiency [4], silencing neighboring genes like *SIX5* and RNA toxicity. The latter is now widely accepted as the major pathogenic mechanism of DM1. [5]

In DM1 the CUG expanded *DMPK* RNA transcripts aggregate into RNA inclusions, also called RNA foci, which are sequestered in the cell nucleus. These aggregations are responsible for splicing alterations, leading to missplicing or deregulation of the alternative splicing and consequently loss of function of several genes. Recent findings suggest that the longer the CTG repeats, the more genes might be affected. [6] Misregulation of alternative splicing probably explains most of the DM1 multisystem manifestations. [7]

1.2.3 Molecular mechanisms leading to multi-organ involvement and DM1 related symptoms

According to Lopez-Martinez et all. [8], over 30 deregulating events of alternative splicing are identified in DM1**,** explaining the DM1 multiorgan dysfunction and related symptoms [9, 1]. Brain, cardiac, and muscle tissue are preferentially affected due to altered transcripts of different genes expressed in these organs. [8] Despite multi-organ impairment, the most prominent clinical signs are those linked to muscular dystrophy and myotonia, after which the disease is named. This is probably due to the fact that skeletal muscle has one of the highest variable exon expression. [10]

Aggregation of the mutant DMPK RNA may inflict missplicing of the insulin receptor (IR) RNA [11]**,** leading in a decreased metabolic response to insulin in DM1 patients. Another example of the multiorgan affection in DM1 is the cardiac conduction disturbances induced by altered transcripts of different genes expressed in cardiac tissue, including sodium voltage-gated channel alpha subunit 5 (*SCN5A)* and truncating titin (*TTN)* gene, among others. [12] [13]

The most important splicing regulators that are identified in this muscle thus far are the musclebind-like (*MBNL*) and *CELF1* (CUG-BP and ETR-3-like factors) factors, acting as antagonist regulators. [14] [8] Sequestration of *MBNL* by the CUG expanded RNA foci, resulting in a loss of function, induces CUGBP up-regulation which promotes alternative fetal splicing. This inversion from adult to foetal splicing patterns is one of the main characteristics of missplicing of different genes expressed in DM1 muscles. [15] [16]

Muscle weakness and wasting in DM1 may result from misregulation of the alternative splicing of the bridging integrator-1 (*BIN1*) pre-mRNA respectively due to *MBNL1* loss-of-function [17] [18], which in turn creates disorganized T-tubules in the muscles. Missplicing of dystrobrevin-α (*DTNA*), a component of the dystrophin-associated protein complex responsible for the sarcolemma stability, may also contribute to muscle weakness [8]. Transcriptional alterations in the ryanodine receptor 1 (*RYR1*) and *SERCA1*, involved in the calcium homeostasis, result in reduced contraction strength of the skeletal muscle. [8]

The aberrant splicing of the skeletal muscle-specific Chloride Channel 1 (*CLCN1*) leads to downregulation of *CLCN1* mRNA which causes hyperexcitability of skeletal muscles, resulting in myotonia [19] [20] [7].

1.3 Myotonic dystrophy and the neuromuscular junction

Some scientific reports suggest that splicing alterations might not only be responsible for myopathic features but might also affect the neuromuscular junction, contributing to muscle weakness and fatigue. [21] [22]

1.3.1 Genetic evidence of the neuromuscular junction involvement

Toxic CUG expanded RNA foci are highly expressed in the subsynaptic nuclei of muscle fibers, which might result in aberrant splicing of genes involved in the function and stability of the neuromuscular junction function. [21]

1.3.2 Histological evidence of neuromuscular junction involvement

Several histological abnormalities were described at the level of the neuromuscular junction, including abnormal large endplate size, decrease of post-synaptic acetylcholine receptor density and decrease of presynaptic vesicles. However, it is unclear whether these findings are secondary to progressive muscle atrophy or primarily linked to the missplicing of genes related to the function or maintenance of the neuromuscular junction. [23] [21]

1.3.3 Clinical evidence of neuromuscular junction involvement

Chronic fatigue and exercise-related fatigability are often pronounced symptoms in DM1 patients. The underlying pathophysiological mechanism is insufficiently understood and very complex, including central and peripheral mechanisms. The possible role of neuromuscular junction is poorly documented. [24]

1.4 Electrophysiological evaluation of the neuromuscular junction

Repetitive nerve stimulation is used to evaluate the neuromuscular junction. Repeatedly activating a motor nerve allows studying the extent to which the neuromuscular junctions manage to transmit nerve impulses to the muscle. The amount of available Acetylcholine released by the presynaptic nerve endings decreases at low stimulation frequencies (2-5 Hz) and lead to reductions in motor endplate potentials. Typically, the amplitude of the plate potential is far greater than the minimum depolarization necessary for a muscle action potential to occur. However, this safety factor is lower when neuromuscular transmission is impaired, and the endplate potential will not reach the threshold for triggering a muscle action potential in a number of muscle fibers. Therefore, these fibers will no longer participate in the overall motor response (compound muscle action potential, (CMAP)), resulting in an amplitude decrement during the shock train. A reproducible decrement of CMAP of at least 10% after the fourth or fifth stimulation is universally accepted as abnormal and correlates with muscle weakness and fatigue. To obtain a reliable evaluation of the NMJ,

at least 4 nerve-muscle pairs are tested as standard in distal and proximal muscles of both upper and lower extremities.

The RNS can also be performed at high frequency (10-50 Hz) and is considered as a mimic of short maximal muscle exercise. High-frequency RNS is valuable for disease affecting the presynaptic membrane, but together with the short exercise test (SET) of Fournier, it is also a provocation test to distinguish the different subtypes of myotonia and demonstrate muscle fiber inexcitability. [25]

In addition to repetitive stimulation, single fiber (SF)-EMG can also be applied. The latter is a more sensitive test to detect neuromuscular junction dysfunction but less specific than repetitive nerve stimulation, as abnormal results can also be seen in conditions other than those affecting the neuromuscular junction, such as myopathies and neuropathies.

1.5 Electrofysiological studies of the neuromuscular junction in myotonic syndromes, and particularly in myotonic dystrophy type 1

If a decrement is observed in myotonic syndromes, it is usually gradual and persistent for many seconds and occurs with a delay of several seconds after the onset of repetitive stimulation. The higher the stimulation rate, the faster and larger the decrement [26] [27] [28]. These findings were observed mainly in recessive myotonia congenital (rMC) and to a lesser extent DM1. At low stimulation frequencies (2-5 Hz), prolonged stimulation is required to obtain a decrement. This decrement translates an inexcitability of some myotonic muscle fiber membranes in a refractory condition and not an neuromuscular junction dysfunction. [19] A characteristic rapid onset decrement after low-frequency RNS as observed in myasthenia gravis was never described in DM1 and other myotonic disorders, except by Bombelli et al. in 2016. [29] In this study, 4 of the 12 studies DM1 patients showed a significant and rapidly occurring decrement at 3Hz repetitive stimulation, suggesting an neuromuscular junction disorder. However, only the ADM muscle was tested, a distal muscle where myotonic discharges are usually strongly present.

2 Study rationale and design

The study design is a prospective cohort study. It aims to evaluate the neuromuscular junction in DM1 using low-frequency RNS on several nerve-muscle pairs of the one side including proximal and distal muscles of upper and lower extremities. First, it will be investigated whether a decrement with 3 Hz stimulation, as described by Bombelli [29], is reproducible in our patient population. If this is the case, it will be examined whether it is the consequence of a dysfunction of the neuromuscular junction or rather linked to a hypo-excitability of some muscle fibers due to myotonia. For this purpose, additional tests including SET (to observe any decrement resulting from an inexcitability in myotonic muscle fibers) and needle EMG (for mapping myotonic discharges in the muscles tested with RNS) will be performed. SF-EMG will not be provided in this study as an abnormal result does not necessarily indicate a dysfunction of the neuromuscular junction but could just as well be due to the muscular dystrophy in the context of DM1. Finally, it will be investigated if there is a correlation between the decrement

with 3 Hz stimulation and clinical signs as fixed muscle weakness (via Medical Research Counsil (MRC) scale, DM-activ scale [30]) and fatigue (via MG-ADL scale).

3 Investigational plan (tables: see annex)

3.1 Study population

We aim to include 10-30 subjects with genetically confirmed DM1. The patients will be recruited from UZ Brussels and surrounding hospitals. There will be no exclusion criteria other than minor age, auto-immune diseases, and known medical conditions involving the neuromuscular junction (myasthenia gravis, Lambert-Eaton myasthenic syndrome, congenital myasthenia syndromes). The study will be submitted to the local ethics committee of the UZ Brussels. In accordance with the Declaration of Helsinki, written informed consent will be gained from all patients.

3.2 Clinical evaluation

Al patients will be evaluated by dr. De Ville Jella, supervised by prof. dr. Bissay Veronique and with help of Eva Parys (nurse), during one visit within a period of 12 months at the UZ Brussels. Genetic data (CTG repeats) will be reported from all the participants. Further, the muscle impairment and wasting will be assessed by a disease-specific rating scale (MIRS) based on manual muscle testing to specify the disease stage. (**TABLE 1**) [31]

Muscle strength will be evaluated for all DM1 subjects via rating on the 5-point Medical Research Counsil (MRC) scale. (**TABLE 2)**
Further, clinical evaluation of the neuromuscular junction will be performed by testing the muscle fatigability, as followed [32]:
- The Barre test: the patient will be asked to maintain his arms at a 90° angle with completely extended elbows and hands in supination. The time until the arms begin to drop will be timed. The test lasts a maximum of 4 minutes.
- The Mingazzini test: the patient will be asked to hold the legs at a 90° angle at the hips and knees. The time until the legs begin to drop will be timed. The test lasts a maximum of 100 seconds.
- Hand-held dynamometer (HDD, Biometrics Microfed 2): The isometric strength will be tested by using a dynamometer on the grip strength. The instrument will be held in the hand of the individual being tested. All testing will be performed while correcting or eliminating gravity. The subject will be asked to perform an increasing force against the dynamometer over a period of several seconds, alternating left and right (three times).
Normative reference values are available for interpreting measurements of muscle strength obtained using HHD. [33]

Patients with DM1 will be asked to complete a questionnaire to rate a 25-item activity scale (DM1-Activ) (**TABLE 3**) and the Myasthenia Gravis Activity of Daily Life scale (MG-ADL) (**TABLE 4**) to rate their level of functional burden. For the DM1-Activ a score of 40 alludes no impairment and a score of 0 indicates the highest functional burden of physical activity. This scale has proven to be practical, reliable and valid [30]**.** For the MG-ADL the total score

ranges from 0 to 24, a score of 0 denotes no and 24 the highest functional burden. It should be noted that this scale is not adjusted for DM1. The rationale is to gain information about muscle fatigue and consequently the neuromuscular junction. [34]

3.3 Electrophysiological investigations

The electrophysiological studies will be performed under a skin temperature maintained above 32°C to prevent a decrease of the CMAP. Neuromuscular transmission will be tested by using short-lasting low frequency RNS (10 stimuli at 3 Hz). To prevent hypoexcitability of the myotonic muscle fibers the stimulation will not be preceded by exercise. [25] The test will be applied on the abductor digiti minimi (ADM), anterior tibial, orbiculis oculi, trapezius, anconeus and EDB muscles of one side, in this particular order, by supramaximal stimulation of the corresponding nerve. When the patient can't tolerate the exam, he/her will still be included in the study when the test can be performed on the first two muscles. The muscles were selected based on their different probabilities of myotonic characteristics. [36] After a rest period of 10 minutes, a SET will be executed, according to the protocol of E. Fournier and colleagues [25]. The subject will be asked to contract the ADM muscle as hard as possible in isometric conditions for 10-seconds. CMAP's will be recorded 2 seconds after the end of the exercise and then every 10 seconds for 50 seconds. [25]

Myotonic discharges will be mapped by needle EMG in the muscles tested with RNS. The electrical myotonia of each examined muscle will be scored according to the Streiss and Sun scale [36]. (**TABLE 5**)

3.4 Study analysis

3.4.1 Analysis of the samples

Dr. Jella De Ville: database screening, data analysis and interpretation.
Prof. Dr. Veronique Bissay: database screening, data analysis and interpretation.

3.4.2 Statistical analysis

We opt for descriptive statistic analysis because of the small study population. The analyses will be performed by Prof. Dr. Veronique Bissay and Dr. Jella De Ville.

3.4.3 Quality control and quality assurance

- Dr. De Ville Jella: study concept and medical writing; acquisition, analysis and interpretation of data.
- Prof. Dr. Veronique Bissay: study concept; drafting and revising of the manuscript for content, analysis and interpretation of data; study supervision and coordination.
- Prof. Dr. Sebastiaan Engelborghs: revising of the manuscript for content, analysis and interpretation of data; study supervision and coordination

4 Primary and secondary endpoints

4.1 Primary endpoints:

Reproducibility of Bombelli's findings, especially rapid decrement at low stimulation frequency.

4.2 Secondary endpoints if decrement confirmed:

- Correlation of decrement with grade of EMG-myotonia in order differentiate muscle fiber hypoexcitability in the context of myotonia, from neuromuscular junction block resulting in decrement of the CMAP.
- Is there any clinical expression (muscle fatigability) of a possible neuromuscular junction dysfunction

5 Possible side effects

Electromyography studies are generally well tolerated and pose little risk to patients. Nerve conduction studies often cause procedural discomfort. Needle EMG commonly causes self-limiting bruises and mostly asymptomatic hematoma. The most serious, very rare, complications are clinically significant infections, compartment syndromes and pneumothorax. Performing a needle EMG on a limb with significant lymphoedema causes an increased risk of infection. [38]

6 Publication policy

Final results will we published.

7 Bronnen

[1] Ho G, „Myotonic dystrophy type 1: clinical manifestations in children and adolescents.,” *Epub ,* 2019.

[2] De Antonio M, „Unravelling the myotonic dystrophy type 1 clinical spectrum: A systematic registry-based study with implications for disease,” *Rev Neurol (Paris),* 2016.

[3] Masuda A, „ CUGBP1 and MBNL1 preferentially bind to 3’ UTRs and facilitate mRNA decay,” *Sci rep ,* 2012.

[4] Taneja KL, „ Foci of trinucleotide Repeat Transcripts in Nuclei of Myotonic-Dystrophy Cells,” *J. Cell Biol,* 1995.

[5] . Mariaelena Pistoni, „Alternative splicing and muscular dystrophy,” *PMC,* Jul 2010.

[6] Yin Q, „Dosage effect of multiple genes accounts for multisystem disorder of myotonic dystrophy type 1.,” *Cell Res. ,* 2020.

[7] BN, „Loss of the muscle-specific chloride channel in type 1 myoyonic dystrophy due to misregulated alternative splicing.,” *Mol cell,* 2002.

[8] A. López-Martínez, „An Overview of Alternative Splicing Defects,” 2020.

[9] X. Fu en M. Ares, „Context-dependent control of alternative splicing by RNA-binding proteins,” *National Rev genetics,* 2014.

[10] Bachinski LL, „Most expression and splicing changes in myotonic dystrophy type 1 and type 2 skeletal muscle are shared with other muscular dystrophies.,” *Neuromuscul Disord,* pp. 2014 Mar;24(3):227-40, 2014.

[11] W. Dansithong, S. Paul, L. Comai en S. E. Ready, „MBNL1 Is the Primary Determinant of Focus Formation and Aberrant Insulin Receptor Splicing in DM1,” *MOLECULAR BASIS OF CELL AND DEVELOPMENTAL BIOLOGY,* 2005.

[12] S. B. Chaitali Misra, „Aberrant Expression of a Non-muscle RBFOX2 Isoform Triggers Cardiac Conduction Defects in Myotonic Dystrophy,” 2020.

[13] Yadava RS, „RNA toxicity in myotonic muscular dystrophy induces NKX2-5 expression.,” *Nat Genet. ,* 2008.

[14] Brook JD, „Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member.,” *Cell,* 1992.

[15] Cox DC, „ Increased nuclear but not cytoplasmic activities of CELF1 protein leads to muscle wasting.,” *Hum Mol Genet. ,* 2020.

[16] Y. 4. Barash, J. Vaquero-Garcia, J. González-Vallinas, H. Xiong, W. Gao, L. Lee en B. Frey, „A webtool for the prediction and analysis of alternative splicing.,” *Genome Biol. ,* 2013.

[17] Fugier C, „Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy,” *Nat. Med,* 2011.

[18] L. e. al., „Amphiphysin 2 (BIN1) and T tubule biogenesis in muscle.,” *Science ,* 2002.

[19] Lueck JD, „Lueck JD, Lungu C, Mankodi A, et al. Chloride channelopathy in myotonic dystrophy resulting from loss of posttranscriptional regulation for CLCN1.,” *Am J Physiol Cell Physiol.,* p. 292:C1291–1297, 2007.

[20] Boland-Freitas R, „Sarcolemmal excitability in the myotonic dystrophies.,” *Muscle Nerve. ,* pp. 57(4):595-602., 2018.

[21] Wheeler TM, „Ribonuclear foci at the neuromuscular junction in myotonic dystrophy type 1.,” *Neuromuscul Disord. ,* pp. 17(3):242-7, 2007.

[22] . Thomas JD, „Disrupted prenatal RNA processing and myogenesis in congenital myotonic dystrophy.," *Genes Dev. ,* 2017 .

[23] G Panaite PA, „Myotonic dystrophy transgenic mice exhibit pathologic abnormalities in diaphragm neuromuscular junctions and phrenic nerves.," *J Neuropathol Exp Neurol. ,* pp. 7(8):763-72. , 2008.

[24] -Y. Boe¨rio D, „Central and peripheral components of exercise-related fatigability in myotonic dysthrophy type 1," *Acta neurology Scand,* 2012.

[25] Fournier E, „Electromyography guides toward subgroups of mutations in muscle channelopathies.," *Ann Neurol. ,* pp. 56(5):650-61, 2004.

[26] Aminoff MJ, „The declining electrical response of muscle to repetitive nerve stimulation in myotonia.," *Neurology. ,* pp. 27(9):812-6, 1977.

[27] Michel P, „Comparative efficacy of repetitive nerve stimulation, exercise, and cold in differentiating myotonic disorders. Muscle Nerve.," *Muscle nerve.,* pp. 36(5):643-50, 2007.

[28] B. C., „Muscle weakness after rest in myotonic disorders.," *Journal of neurology, neurosurgery and psychiatry.,* pp. 1336-1342., 1974..

[29] Bombelli F, „Neuromuscular transmission abnormalities in myotonic dystrophy type 1: A neurophysiological study.," *Clin Neurol Neurosurg. ,* pp. 150:84-88., 2016.

[30] Hermans MC, „Rasch-built myotonic dystrophy type 1 activity and participation scale (DM1-Activ).," *Neuromuscul Disord. ,* pp. 20(5):310-8., 2010.

[31] Mathieu J, „Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy.," *Neurology. ,* pp. 13;56(3):336-40. , 2001.

[32] Landon-Cardinal, „Responsiveness to Change of 5-point MRC Scale, Endurance and Functional Evaluation for Assessing Myositis in Daily Clinical Practice," 2019.

[33] R. W. Bohannon, „Considerations and Practical Options for Measuring muscle strength: a narrative review," *BioMed Research International,* 2019.

[34] de Meel RHP, „Sensitivity of MG-ADL for generalized weakness in myasthenia gravis.," *Eur J Neurol.,* 2019.

[35] de Meel RHP, „ Sensitivity of MG-ADL for generalized weakness in myasthenia gravis.," *Eur J Neurol. ,* 2019.

[36] S. S. Streib EW, „ Distribution of electrical myotonia in myotonic muscular dystrophy," *Ann Neurol.,* 1983 Jul;14(1):80-2.

[37] Muscle weakness after rest in myotonic disorders; an electrophysiological study.," *Neurol Neurosurg Psychiatry. ,* p. 37(12), 1974.

[38] Modoni A, „Low-rate repetitive nerve stimulation protocol in an Italian cohort of patients affected by recessive myotonia congenita.," *J Clin Neurophysiol. ,* pp. 28(1):39-44., 2011.

[39] The histology of the neuromuscular junction in dystrophia myotonica.," *Brain. ,* pp. 84:75-84, 1961.

[40] a. Dupré N, „Clinical, electrophysiologic, and genetic study of non-dystrophic myotonia French-Candanians.," *Neuromusul Disord. ,* p. 19:330 –334, 2009.

[41] Nicolau S, „Trouble at the junction: When myopathy and myasthenia overlap.," *Muscle Nerve,* 2019.

[42] Whiting EJ, „Characterization of myotonic dystrophy kinase (DMK) protein in human and rodent muscle and central nervous tissue.," *Hum Mol Genet ,* 1995.

[43] Pham YC, „Localization of myotonic dystrophy protein kinase in human and rabbit tissues using a new panel of monoclonal antibodies.," *Hum Mol Genet,* 1998.

[44] Tan, „In vivo assessment of muscle membrane properties in myotonic dystrophy," *Muscle Nerve 54 ,* p. 249–257, 2016.

[45] C. R. Meola G, „Myotonic dystrophies: An update on clinical aspects, genetic, pathology, and molecular pathomechanisms.," *Biochim Biophys Acta.,* 2015.

[46] Dansithong W, „Cytoplasmic CUG RNA foci are insufficient to electit key DM1 features.," *PLoS One ,* 2008.

[47] Reddy S, „Mice lacking the myotonic dystrophy protein kinase develop a late onset progressic myopathy.," *Nar Genet.,* 1996.

[48] C. T. Ranum LP, „RNA-mediated neuromuscular disorders.," *Annual revision neuroscience.,* 2006.

[49] Malatesta, „RNA transcription and maturation in skeletal muscle cells are similarly impaired in myotonic dystrophy and sarcopenia.," *Aging Neuroscience,* 2014.

[50] A. J. Mateos-Aierdi, „Muscle wasting in myotonic dystrophys: a model of premature aging.," *Aging neuroscience,* 2015.

[51] M. Christian Coërs, M. Nicole Telerman-Toppet en J.-M. Gerard, „Terminal Innervation Ratio in Neuromuscular Disease," *1973,* Arch neurology.

[52] F. T. M. Fardeau, „Tome, Light and electron microscopic study of motor endplates in the

adult and neo-natal formas of dystrophia myotonica," *J. Taxi,* 1980.

[53] Whiting EJ, „Characterization of myotonic dystrophy kinase (DMK) protein," *Human molecular genetics,* 1995.

[54] M. Van der Ven PF, „Myotonic dystrophy kinase is a component of neuromuscular junction," *Human molecular genetics,* 1993.

[55] JChakkalakal JV, „Localizing synaptic mRNAs at the neuromuscular junction: it takes more than transcription," *Bioessays,* 2003.

[56] Allen DE, „The intramuscular nerve endings in dystrophia myotonica-- a biopsy study by vital staining and electron microscopy," *J. anat,* 1969.

[57] F. D. A. Drachman DB, „Are muscle fibers denervated in myotonic dystrophy?," *Eur J neuroscience,* 2004.

[58] R. J. Amato Anthony, Neuromuscular disorders, McGraw-Hill Education, 2016.

[59] Yuan Y, „Muscleblind-like 1 interacts with RNA hairpins in splicing target and pathogenic RNAs.," *Nucleic Acids Res.,* 2007.