ASSESSMENT OF MYOTONIC DYSTROPHY TYPE 2 RESEARCH AND DRUG DEVELOPMENT WITH RECOMMENDATIONS FOR INVESTMENT

PROJECT BACKGROUND AND GOALS

Myotonic commissioned the development of a landscape assessment of the status of research and drug development in myotonic dystrophy type 2 (DM2) in Spring 2019. The goal of this project was to understand current opportunities and constraints in the research and drug development pipelines in order to implement projects designed to increase academic and industry interest in, and incentivize therapy development for, DM2. The below results of this assessment have been reviewed by the Myotonic Scientific Advisory Committee for veracity and scope.

Next steps will include selecting key projects to drive research and drug development for DM2, identifying and confirming funding partners, and subsequently releasing Requests for Application to engage leading investigators to carry out high-impact, time-sensitive projects.

The application of tools to study and treat DM2 has lagged significantly behind the development and application of tools for DM1, although many of the DM1 tools, and drug development efforts are likely to be applicable to DM2 as well. The DM2 challenges besides adequate funding, include the technical difficulties involved in working with the large DM2 repeat expansions, difficulty pinpointing distinct phenotype characteristics for which to monitor therapy-driven changes, the lack of biomarkers to serve as surrogate endpoints and identify early target engagement, and an understanding of the disease itself, particularly as it relates to CNS involvement. As one researcher noted, "the headwinds against DM2 have been strong."

However, research has clearly identified the predominant disease process: a gain of function in the *CNBP* gene leading to the build-up of toxic RNA which causes the sequestration of MBNL proteins. This sequestration prevents MBNL from binding with its normal RNA targets and leads to downstream consequences of alternative splicing and the diffuse, highly variable symptomatology. The below provides a review and assessment of the status of key basic and translational research efforts and opportunities to accelerate therapy development for DM2.

CURRENT RESEARCH

Current DM2 research includes both bench and lab research and clinical studies involving affected patients.

- **Bench and lab research studies:**

Lab-based studies of disease/protein/genetic modifiers and molecular interactions of DM2 are currently being conducted by several international investigators:

- *Repeat Associated Non-ATG (RAN) translation, RNA gain of function and protein gain of function in repeat expansion disorders including amyotrophic lateral sclerosis (ALS), spinocerebellar ataxia (SCA) types 5 and 8, myotonic dystrophy (DM) types 1 and 2 and Huntington's disease (HD).* Laura Ranum, PhD, at the University of Florida is building from her work in ALS to demonstrate the role of Repeat Associated Non-ATG (RAN) translation, RNA gain of function, and protein gain of function in repeat expansion disorders including (DM) types 1 and 2.
- *How do simple repeat expansions in non-coding regions result in disease*? Maurice Swanson, PhD. Maury Swanson has generated a number of knock-out, knock-in and transgenic mouse models to investigate the roles of specific RNA-binding proteins in disease pathogenesis. His lab is studying an RNA-mediated disease model in which mutant DM1 and DM2 mRNAs are trapped in the nucleus and sequester (C)CUG repeat expansion binding proteins that are essential for normal tissue development and maintenance. His lab identified the sequestered factors as the muscleblind-like (MBNL) proteins. Ongoing efforts are focused on elucidating the normal functions of these proteins as well as investigating whether RNA toxicity has a pathogenic role in other hereditary disorders.
- *Intron retention induced by microsatellite expansions as a disease biomarker:* Maurice Swanson, PhD, along with Myotonic post-doctoral fellow Dr. Łukasz Sznajder, PhD, have developed a diagnostic assay using a person's peripheral blood to assess intron retention events as disease-specific diagnostic biomarkers for microsatellite expansions such as those found in DM1 and DM2 patients. Using DM2 as a model, they showed that CNBP i1 retention occurs even with expansion sizes in the low pathogenic range, which suggests that this assay may be informative for pre-symptomatic patients.
- Myotonic Dystrophy Population-based Prevalence Study. Myotonic-funded study led by Dr. Nicholas Johnson, MD, Virginia Commonwealth University, reporting new data regarding the population-based (as opposed to diagnosed) prevalence of both DM1 and DM2. The study results, which are expected to publish in Summer/Fall 2019, will support Myotonic efforts to make the case to industry for DM2 as a desirable drug development.

- **Clinical studies:**

  There are three clinical research studies currently underway to identify biomarkers and phenotypic changes in DM2 patients. They are longitudinal and prospective without the ability to yield outcomes and information for ~ 3 years.

  - *STOPP DM2*. A natural history study with a target enrollment of 50 DM2 patients is being conducted at the University of Rochester. Dr. Charles Thornton, MD, Dr. Joanna Hamel, MD, Dr. Chad Heatwole MD, and others are looking at muscle biopsies, patient-reported outcomes of disease burden, and mechanistic changes over three years of observation. Currently 12 DM2 patients have completed the 3 years of observations. There are 28 additional DM2 patients enrolled past baseline; participant selection is designed to include DM2 patients with broad symptom burden, age, etc. to reduce bias in the study results. If the additional 10 patients can be enrolled within a year, cross-sectional data regarding baseline

readings can initially be made available. No other outcome or publication is planned until all enrolled patients complete the 3-year observational period and the data is analyzed against the full cohort of participants.

The single-site STOPP DM2 study will then move to a multi-site, multi-year study conducted via the Myotonic Dystrophy Clinical Research Network (DMCRN), likely in the next 3 years.

- *Clinical Outcome Measures in Myotonic Dystrophy Type 2 (COMEDY-2)*. Dr. Benedikt Schoser, MD, at Ludwig-Maximilian Universitat Munchen will be recruiting at least 60 DM2 patients to be evaluated through a battery of patient-reported outcomes (PROs) and clinical outcome measures in order to define suitable measurement strategies for DM2, and to propose a disease-specific severity scale. Patients will be re-evaluated after 6 months. An age and gender-matched control cohort will be assessed.

- *Identifying practical and reliable endpoints to quantitate lean tissue mass in Myotonic Dystrophy Type 1 and 2*. Dr. Araya Puwanant, M.D. at Wake Forest, is using Dual Energy X-ray Absorptiometry (DEXA) scans as a simple imaging technique, to test whether DEXA measurements in affected muscles may provide a sensitive endpoint that can be bridged to meaningful outcome measures that are sensitive and appropriate to monitor disease progression and therapeutic response in the clinical trial setting of DM2. This work was started at the University of Pittsburgh and will continue at Wake Forest.

- *Assessing Clinical Endpoints and Biomarkers in Myotonic Dystrophy Type-1 and Type-2 (ASCEND-DM).* Dr. Ami K Mankodi, M.D., at the National Institute of Neurological Disorders and Stroke (NINDS), is using results of the Myotonic Dystrophy Clinical Research Network (DMCRN) 100-patient longitudinal study data to continue work on biomarker and endpoint development in both DM1 and DM2. Her current project will recruit 50 DM2 patients at NINDS as a single site study. It will include efforts to validate custom devices she has developed for both muscle endpoint assessment and non-muscle endpoints and biomarkers, including respiratory and central nervous system (CNS) function. She will also assess selected tests, imaging, and patient-reported outcomes for their ability to quantify disease burden, detect disease progression and predict changes. Dr. Mankodi will study DM2 disease progression over 2 years. She will also examine muscle and cerebrospinal fluid (CSF) for RNA alternative splicing events that may be used as potential biomarkers of DM2 severity, and genetic modifiers of DM2 severity by genome-wide association (GWA).

RESEARCH TOOLS

- **Cell lines:**
    - DM2 cell lines commissioned by Myotonic are in progress and should be ready in Fall 2019 via a partnership with Rutgers University and NINDS. The 4 DM2 patient-derived cell lines will create induced pluripotent stem cells (iPSCs) derived from erythroblasts extracted from DM2 blood samples. The goal with the new cell lines offered by Myotonic will be to offer readily available, IP-free, at-cost

tissue-derived models to industry and academia in an effort to further encourage research and development for DM2 treatments.

- DM2 cell lines published in 2019 by Dr. Denis Furling, PhD, Institut de Myologie, and Dr. Elizabeth McNally MD, PhD, Northwestern University, were produced from muscle biopsies (myoblasts) and urine-derived cells reprogrammed into myotubes, respectively. These cell lines do not show missplicing events expected in DM2 patient-derived cells. As opposed to DM1, the DM2 field remains divided on the degree to which RNA splicing changes are related to pathogenesis.

The need for human iPSC for DM2 cannot be overstated. In the absence of human-derived DM2 cell lines, researchers have been working with and transfecting the mouse myoblast C2C12 cell line. This cell line is a subclone of myoblasts that were originally obtained by Yaffe and Saxel at the Weizmann Institute of Science in Israel in 1977. The cell line is widely available through commercial vendors such as ATCC.

- **Mouse models:**
  - Dr. Charles Thornton, MD, University of Rochester, has been working for several years to create a DM2 mouse based on the technology he used to develop the HSA^{lr} DM1 mouse. Significant technical challenges arise in making a DM2 transgenic mouse because of the expansion size and amount of toxic RNA involved. A successful DM2 mouse is likely in the next few years.
  - Dr. Maurice Swanson, PhD, University of Florida, is working on a DM2 mouse model using CRISPR-Cas9 technology to impact the germline through expression of the DM2 repeats in the mutant gene in endogenous DM2 intron 1 of CNBP.
  - Dr. Kiruphagaran Thangaraju, PhD, University of Florida, a MYOTONIC-funded Fellow in the lab of Dr. Laura Ranum, generated a patient-derived BAC library and screened ~92,000 clones. Dr. Thangaraju has identified several clones with the CNBP gene. One clone in particular contains a substantial flanking sequence and a large (~700 CCTGs) repeat expansion. Pronuclear injections were performed but genotyping did not identify any transgenic founders. This work-in-progress continues as Dr. Thangaraju continues to purify additional BAC DNA with the expansion for another round of pronuclear injections. The goal is to identify transgenic founders and build colonies
  - Dr. Ralf Krahe, PhD, University of Texas MD Anderson Cancer Center, developed an HSA transgenic DM2 mouse model. The model includes a (CCTG)121 expansion in intron 1 of the human skeletal actin gene (DM2-HSATG) with a small number of repeats. The model does not demonstrate the mis-splicing events many believe to be associated with DM2. According to Dr. Krahe, the mice do display phenotypic changes associated with DM2.
  - Dr. Lubov Timchenko PhD, Cincinnati Children's Hospital, recently published a cnbp-KO mouse created at CCH. Her focus is on downstream genetic events resulting from the role of CNBP, such as the reduction in ZNF9. Dr. Timchenko proposes that heterozygous cnbp-KO mice could be a model for DM2 because patients with DM2 have only partial reduction of ZNF9, similar to that seen in the CCH heterozygous mice.

More mouse, and possibly other animal, models are needed to demonstrate a comprehensive picture of DM2 with multiple clinical-like phenotypic changes, as well as the molecular and mis-splicing events associated with the biology and pathogenesis of DM2.

- **Other models for DM2 study**:
  - Dr. Andrew Berglund, PhD, The RNA Institute, collaborating with Dr. Karen Guillemin's lab at the University of Oregon, are developing and characterizing zebrafish models of myotonic dystrophy. The main focus of the zebrafish project is to the study the mechanisms underlying DM-related changes in gut motility and the microbiome due to GI issues suffered by DM patients.
  - Teams including Dr. Ralf Krahe, PhD, at The University of Texas and Dr. Giovanni Meola, MD, at Università degli Studi di Milano are working with Drosophila models of DM2.
- **Drug development efforts:**
  - Dr. Andrew Berglund, PhD, RNA Institute, is evaluating the feasibility of conducting a clinical trial using a repurposed antimalarial for DM2 patients. He is also developing novel small molecules around unique chemistry scaffolds to target toxic RNA in DM2.
  - Dr. Matthew Disney, PhD, Scripps Research Institute, is working on early-stage small molecule compounds with the potential to treat patients with DM2, although his lead indication at this time is for DM1. His recently-formed biotech, Expansion Therapeutics, has formally expressed interest in developing DM2 therapies.

Potential areas for study and funding in DM2 (as of 8/2019):

There are no active NIH grants working exclusively on DM2, although 2 out of the 3 aims of the NIH 5 year, $5M Wellstone grant to the University of Rochester are DM2-focused. Further, there are currently no DOD Peer-reviewed Medical Research Program grants funding DM2, although that funding stream has only been available for one year. Myotonic intends to launch a multi-year initiative to attract researchers and industry to DM2 discovery and drug development, assuming funds can be raised. Areas of potential solicitation across the spectrum of DM2 are included below.

The below were suggested by interviewees who provided content for this DM2 landscape review.

1. **Longitudinal DM2 single and multi-site studies** to drive disease understanding, and clinical trial outcome measures and design

2. **Publication and availability of animal models and cell lines**

3. **Research into modifier genes** and their potential role in DM2 disease progression

4. **Exploration of DM2 CNS symptoms** and mechanisms behind "brain fog" reported by many DM2 patients.

5. **Research into DM2 repeat size and incomplete penetrance,** collecting data from more patients with "small" DM2 expansions and known repeat tract structure to better estimate penetrance and provide for better genetic counseling for DM2 patients

6. **Research examining age and sex differences in DM2**

7. **Studies of cellular nucleic acid-binding protein** (**CNBP) as a transcription regulators**: Such studies may determine whether CNBP acts as a transcription regulator required for activating the innate immune response. Such studies may lead to the identification of specific binding motifs present in the promoter region of inflammatory cytokines that induce activity of the CNBP gene.

8. **Cancer and DM2** to explore earlier study results suggesting that cancer should be considered in the overall pathogenesis of disease manifestation in DM2 patient

9. **Overexpression of rbFOX1**: rbFOX1 has been identified as a possible molecular mechanism in DM2. More studies looking at its overexpression may lead to a greater understanding of the role it plays in releasing MBNL1 from sequestration within CCUG RNA foci and muscle atrophy.

RECENT PUBLICATIONS - 2015-2019

- Consensus-based Care Recommendations for Adults with Myotonic Dystrophy Type 2
  Schoser, B, Montagnese, F, Bassez, G et al. **Neurology Clinical Practice** April 24, 2019, DOI: https://doi.org/10.1212/CPJ.0000000000000645
- The myotonic dystrophy experience: a North American cross-sectional study.
  Hagerman, KA, Howe, SJ, Heatwole CR, Christopher Project Reference Group. **Muscle Nerve** 2019 Apr;59(4):457-464. Doi:10.1002/mus26420. Epub 2019 Feb 5.
- Sleep Complaints, Sleep and Breathing Disorders in Myotonic Dystrophy Type 2.
  Romigi A, Maestri M, Nicoletta C, Vitrani G, Caccamo M, Siciliano G, Bonanni E, Centonze D, Sanduzzi A. **Curr Neurol Neurosci Rep.** 2019 Feb 9;19(2):9. doi: 10.1007/s11910-019-0924-0. Review.
- Distinct pathological signatures in human cellular models of myotonic dystrophy subtypes.
  Kim EY, Barefield DY, Vo AH, Gacita AM, Schuster EJ, Wyatt EJ, Davis JL, Dong B, Sun C, Page P, Dellefave-Castillo L, Demonbreun A, Zhang HF, McNally EM. **JCI Insight.** 2019 Feb 7. pii: 122686. doi: 10.1172/jci.insight.122686. [Epub ahead of print]
- Body composition analysis in patients with myotonic dystrophy types 1 and 2.
  Peric S, Bozovic I, Nisic T, Banovic M, Vujnic M, Svabic T, Pesovic J, Brankovic M, Basta I, Jankovic M, Savic-Pavicevic D, Rakocevic-Stojanovic V. **Neurol Sci.** 2019 Feb 21. doi: 10.1007/s10072-019-03763-0. [Epub ahead of print]
- Repeat associated non-ATG (RAN) translation.
  Cleary JD, Pattamatta A, Ranum LPW.
  J Biol Chem. 2018 Sep 13. pii: jbc.R118.003237. doi: 10.1074/jbc.R118.003237. [Epub ahead of print]
- Heart involvement in patients with myotonic dystrophy type 2.
  Peric S, Bjelica B, Aleksic K, Kovacevic M, Cvitan E, Mandic Stojmenovic G, Rakocevic

Stojanovic V. **Acta Neurol Belg.** 2018 Dec 7. doi: 10.1007/s13760-018-1052-3. [Epub ahead of print]
- Cannabis use in myotonic dystrophy patients in Germany and USA: a pilot survey.
  Montagnese F, White M, Klein A, Stahl K, Wenninger S, Schoser B. **J Neurol.** 2018 Dec 15. doi: 10.1007/s00415-018-9159-2. [Epub ahead of print]
- Quantitative myotonia assessment using a commercially-available dynamometer in myotonic dystrophy types 1 and 2.
  Horáková M, Horák T, Parmová O, Bednařík J, Voháňka S. **Muscle Nerve.** 2018 Dec 21. doi: 10.1002/mus.26401. [Epub ahead of print]
- Comparative Sleep Disturbances in Myotonic Dystrophy Types 1 and 2.
  Romigi A, Franco V, Placidi F, Liguori C, Rastelli E, Vitrani G, Centonze D, Massa R. **Curr Neurol Neurosci Rep.** 2018 Oct 31;18(12):102. doi: 10.1007/s11910-018-0903-x.
- RNA/MBNL1-containing foci in myoblast nuclei from patients affected by myotonic dystrophy type 2: an immunocytochemical study.
  Perdoni F, Malatesta M, Cardani R, Giagnacovo M, Mancinelli E, Meola G, Pellicciari C. **Eur J Histochem.** 2009 Sep 30;53(3):e18. doi: 10.4081/ejh.2009.e18. eCollection 2009 Sep 30.
- Ribonuclear inclusions as biomarker of myotonic dystrophy type 2, even in improperly frozen or defrozen skeletal muscle biopsies.
  Cardani R, Mancinelli E, Giagnacovo M, Sansone V, Meola G. **Eur J Histochem.** 2009 Jun 29;53(2):e13. doi: 10.4081/ejh.2009.e13. eCollection 2009 Jun 29.
- Myotonic Dystrophy Type 2 - Data from the Serbian Registry.
  Bozovic I, Peric S, Pesovic J, Bjelica B, Brkusanin M, Basta I, Bozic M, Sencanic I, Marjanovic A, Brankovic M, Savic-Pavicevic D, Rakocevic-Stojanovic V. **J Neuromuscul Dis.** 2018 Sep 15. doi: 10.3233/JND-180328. [Epub ahead of print]
- Generation and Neuronal Differentiation of hiPSCs From Patients With Myotonic Dystrophy Type 2.
  Spitalieri P, Talarico RV, Murdocca M, Fontana L, Marcaurelio M, Campione E, Massa R, Meola G, Serafino A, Novelli G, Sangiuolo F, Botta A. **Front Physiol.** 2018 Jul 27;9:967. doi: 10.3389/fphys.2018.00967. eCollection 2018.
- Distribution and Structure of DM2 Repeat Tract Alleles in the German Population.
  Mahyera AS, Schneider T, Halliger-Keller B, Schrooten K, Hörner EM, Rost S, Kress W. **Front Neurol.** 2018 Jun 19;9:463. doi: 10.3389/fneur.2018.00463. eCollection 2018.
- Towards clinical outcome measures in myotonic dystrophy type 2: a systematic review.
  Rastelli E, Montagnese F, Massa R, Schoser B. **Curr Opin Neurol.** 2018 Jul 25. doi: 10.1097/WCO.0000000000000591. [Epub ahead of print]
- Towards clinical outcome measures in myotonic dystrophy type 2: a systematic review.
  Rastelli E, Montagnese F, Massa R, Schoser B. **Curr Opin Neurol.** 2018 Oct;31(5):599-609. doi: 10.1097/WCO.0000000000000591.
- Generation of neural cells from DM1 induced pluripotent stem cells as cellular model for the study of central nervous system neuropathogenesis.
  Xia G, Santostefano KE, Goodwin M, Liu J, Subramony SH, Swanson MS, Terada N, Ashizawa T. **Cell Reprogram.** 2013 Apr;15(2):166-77. doi: 10.1089/cell.2012.0086.
- Generation and Neuronal Differentiation of hiPSCs From Patients With Myotonic Dystrophy Type 2.
  Spitalieri P, Talarico RV, Murdocca M, Fontana L, Marcaurelio M, Campione E, Massa R, Meola G, Serafino A, Novelli G, Sangiuolo F, Botta A. **Front Physiol.** 2018 Jul 27;9:967. doi: 10.3389/fphys.2018.00967. eCollection 2018.

- SCN4A as modifier gene in patients with myotonic dystrophy type 2.
  Binda A, Renna LV, Bose F, Brigonzi E, Botta A, Valaperta R, Fossati B, Rivolta I, Meola G, Cardani R **Sci Rep.** 2018 Jul 23;8(1): 11058. Doi: 10.1038/s41598-018-29302-z.
- Reduction of Cellular Nucleic Acid Binding Protein encoded by a Myotonic Dystrophy type 2 gene causes muscle atrophy.
  Wei C, Stock L, Schneider-Gold C, Sommer C, Timchenko NA, Timchenko L. **Mol Cell Biol.** 2018 May 7. pii: MCB.00649-17. doi: 10.1128/MCB.00649-17. [Epub ahead of print]
- Core Clinical Phenotypes in Myotonic Dystrophies.
  Wenninger S, Montagnese F, Schoser B. **Front Neurol.** 2018 May 2;9:303. doi: 10.3389/fneur.2018.00303. eCollection 2018. Review.
- rbFOX1/MBNL1 competition for CCUG RNA repeats binding contributes to myotonic dystrophy type 1/type 2 differences.
  Sellier C, Cerro-Herreros E, Blatter M, Freyermuth F, Gaucherot A, Ruffenach F, Sarkar P, Puymirat J, Udd B, Day JW, Meola G, Bassez G, Fujimura H, Takahashi MP, Schoser B, Furling D, Artero R, Allain FHT, Llamusi B, Charlet-Berguerand N. **Nat Commun.** 2018 May 22;9(1):2009. doi: 10.1038/s41467-018-04370-x.
- Qualitative and Quantitative Aspects of Pain in Patients with Myotonic Dystrophy Type 2.
  van Vliet J, Tieleman AA, Verrips A, Timmerman H, van Dongen RTM, van Engelen BGM, Wilder-Smith OHG. **J Pain.** 2018 Mar 27. pii: S1526-5900(18)30117-2. doi: 10.1016/j.jpain.2018.03.006. [Epub ahead of print]
- Dysautonomia as Onset Symptom of Myotonic Dystrophy Type 2.
  Rossi S, Romano A, Modoni A, Perna F, Rizzo V, Santoro M, Monforte M, Pieroni M, Luigetti M, Pomponi MG, Silvestri G. **Eur Neurol.** 2018 Mar 13;79(3-4):166-170. doi: 10.1159/000487508. [Epub ahead of print]
- Evidence for a relatively high proportion of DM2 mutations in a large group of Polish patients.
  Sulek A, Krysa W, Rajkiewicz M, Lusakowska A, Kaminska A, Nojszewska M, Zdzienicka E, Kubalska J, Rakowicz M, Szirkowiec W, Kwiecinski H, Zaremba J. **Neurol Neurochir Pol.** 2018 Mar 7. pii: S0028-3843(17)30336-5. doi: 10.1016/j.pjnns.2018.02.008. [Epub ahead of print]
- Expanded [CCTG]n repetitions are not associated with abnormal methylation at the CNBP locus in myotonic dystrophy type 2 (DM2) patients.
  Santoro M, Fontana L, Maiorca F, Centofanti F, Massa R, Silvestri G, Novelli G, Botta A. **Biochim Biophys Acta.** 2017 Dec 29;1864(3):917-924. doi: 10.1016/j.bbadis.2017.12.037. [Epub ahead of print]
- Hearing impairment in patients with myotonic dystrophy type 2.
  van Vliet J, Tieleman AA, van Engelen BGM, Bassez G, Servais L, Béhin A, Stojkovic T, Meulstee J, Engel JAM, Lamas G, Eymard B, Verhagen WIM, Mamelle E. **Neurology.** 2018 Jan 17. pii: 10.1212/WNL.0000000000004963. doi: 10.1212/WNL.0000000000004963. [Epub ahead of print]
- Impeding Transcription of Expanded Microsatellite Repeats by Deactivated Cas9.
  Pinto BS, Saxena T, Oliveira R, Méndez-Gómez HR, Cleary JD, Denes LT, McConnell O, Arboleda J, Xia G, Swanson MS, Wang ET. **Mol Cell.** 2017 Oct 18. pii: S1097-2765(17)30711-6. doi: 10.1016/j.molcel.2017.09.033. [Epub ahead of print]
- Assessing the influence of age and gender on the phenotype of myotonic dystrophy type 2.
  Montagnese F, Mondello S, Wenninger S, Kress W, Schoser B. **J Neurol.** 2017 Oct 30. doi: 10.1007/s00415-017-8653-2. [Epub ahead of print]

- Genetic testing of individuals with pre-senile cataract identifies patients with myotonic dystrophytype 2.
  Rakočević-Stojanović V, Perić S, Pešović J, Senćanić I, Božić M, Šviković S, Brkušanin M, Savić-Pavićević D. **Eur J Neurol.** 2017 Nov;24(11):e79-e80. doi: 10.1111/ene.13401.
- RAN Translation Regulated by Muscleblind Proteins in Myotonic Dystrophy Type 2.
  Zu T, Cleary JD, Liu Y, Bañez-Coronel M, Bubenik JL, Ayhan F, Ashizawa T, Xia G, Clark HB, Yachnis AT, Swanson MS, Ranum LPW. **Neuron.** 2017 Sep 13;95(6):1292-1305.e5. doi: 10.1016/j.neuron.2017.08.039.
- Receptor and post-receptor abnormalities contribute to insulin resistance in myotonic dystrophy type 1 and type 2 skeletal muscle.
  Renna LV, Bosè F, Iachettini S, Fossati B, Saraceno L, Milani V, Colombo R, Meola G, Cardani R. **PLoS One.** 2017 Sep 15;12(9):e0184987. doi: 10.1371/journal.pone.0184987.
- Sarcolemmal excitability in the myotonic dystrophies.
  Boland-Freitas R, Lee J, Howells J, Liang C, Corbett A, Nicholson G, Ng K. **Muscle Nerve.** 2017 Sep 7. doi: 10.1002/mus.25962. [Epub ahead of print]
- High frequency of gastrointestinal manifestations in myotonic dystrophy type 1 and type 2.
  Hilbert JE, Barohn RJ, Clemens PR, Luebbe EA, Martens WB, McDermott MP, Parkhill AL, Tawil R, Thornton CA, Moxley RT 3rd; National Registry Scientific Advisory Committee/Investigators. **Neurology.** 2017 Aug 30. pii: 10.1212/WNL.0000000000004420. doi: 10.1212/WNL.0000000000004420. [Epub ahead of print]
- Elimination of Toxic Microsatellite Repeat Expansion RNA by RNA-Targeting Cas9.
  Batra R, Nelles DA, Pirie E, Blue SM, Marina RJ, Wang H, Chaim IA, Thomas JD, Zhang N, Nguyen V, Aigner S, Markmiller S, Xia G, Corbett KD, Swanson MS, Yeo GW. **Cell.** 2017 Aug 8. pii: S0092-8674(17)30817-6. doi: 10.1016/j.cell.2017.07.010. [Epub ahead of print]
- Magnetic resonance imaging of leg muscles in patients with myotonic dystrophies.
  Peric S, Maksimovic R, Banko B, Durdic M, Bjelica B, Bozovic I, Balcik Y, Pesovic J, Savic-Pavicevic D, Rakocevic-Stojanovic V. **J Neurol.** 2017 Jul 29. doi: 10.1007/s00415-017-8574-0. [Epub ahead of print]
- Personality traits in patients with myotonic dystrophy type 2.
  Paunic T, Peric S, Parojcic A, Savic-Pavicevic D, Vujnic M, Pesovic J, Basta I, Lavrnic D, Rakocevic-Stojanovic V. **Acta Myol.** 2017 Mar;36(1):14-18.
- Investigation of the molecular mechanisms underlying myotonic dystrophy types 1 and 2 cataracts using microRNA target gene networks.
  Shao D, Zhu X, Sun W, Huo L, Chen W, Wang H, Liu B, Pan P. **Mol Med Rep.** 2017 Jul 21. doi: 10.3892/mmr.2017.7059. [Epub ahead of print]
- Expanded CCUG repeat RNA expression in Drosophila heart and muscle trigger Myotonic Dystrophy type 1-like phenotypes and activate autophagocytosis genes.
  Cerro-Herreros E, Chakraborty M, Pérez-Alonso M, Artero R, Llamusí B. **Sci Rep.** 2017 Jun 6;7(1):2843. doi: 10.1038/s41598-017-02829-3.
- Brain positron emission tomography in patients with myotonic dystrophy type 1 and type 2.
  Peric S, Brajkovic L, Belanovic B, Ilic V, Salak-Djokic B, Basta I, Rakocevic Stojanovic V. **J Neurol Sci.** 2017 Jul 15;378:187-192. doi: 10.1016/j.jns.2017.05.013. Epub 2017 May 10.
- (CCUG)n RNA toxicity in a Drosophila model for myotonic dystrophy type 2 (DM2) activates apoptosis. Yenigun VB, Sirito M, Amcheslavky A, Czernuszewicz T,

Colonques-Bellmunt J, García-Alcover I, Wojciechowska M, Bolduc C, Chen Z, López Castel A, Krahe R, Bergmann A. **Dis Model Mech.** 2017 Jun 16. pii: dmm.026179. doi: 10.1242/dmm.026179. [Epub ahead of print]

- Biomolecular diagnosis of myotonic dystrophy type 2: a challenging approach. Meola G, Biasini F, Valaperta R, Costa E, Cardani R. **J Neurol**. 2017 May 26. doi: 10.1007/s00415-017-8504-1. [Epub ahead of print]
- Myotonic Dystrophy: Approach to Therapy. Thornton CA, Wang E, Carrell EM. **Curr Opin Genet Dev.** 2017 Apr 1;44:135-140. doi: 10.1016/j.gde.2017.03.007. [Epub ahead of print]
- CNBP acts as a key transcriptional regulator of sustained expression of interleukin-6. Lee E, Lee TA, Kim JH, Park A, Ra EA, Kang S, Choi HJ, Choi JL, Huh HD, Lee JE, Lee S, Park B. **Nucleic Acids Res.** 2017 Apr 7;45(6):3280-3296. Doi: 10.1093/nar/gkx071.
- Cardiac Autonomic Function in Type 1 and Type 2 Myotonic Dystrophy. Bienias P, Łusakowska A, Ciurzyński M, Rymarczyk Z, Irzyk K, Konwerski M, Ciąpała K, Kowalski P, Kamińska A, Pruszczyk P. **Clin Auton Res.** 2017 Mar 20. doi: 10.1007/s10286-017-0413-y. [Epub ahead of print]
- Association of peripheral neuropathy with sleep-related breathing disorders in myotonic dystrophies. Banach M, Antczak J, Rola R. **Neuropsychiatr Dis Treat.** 2017 Jan 12;13:133-140. doi: 10.2147/NDT.S123908.
- Myotonic dystrophy type 2 and modifier genes: an update on clinical and pathomolecular aspects. Meola G, Cardani R. **Neurol Sci.** 2017 Jan 11. doi: 10.1007/s10072-016-2805-5. [Epub ahead of print] Review.
- Cutaneous features of myotonic dystrophy types 1 and 2: Implication of premature aging and vitamin D homeostasis. Campione E, Botta A, Di Prete M, Rastelli E, Gibellini M, Petrucci A, Bernardini S, Novelli G, Bianchi L, Orlandi A, Massa R, Terracciano C. **Neuromuscul Disord.** 2016 Nov 16. pii: S0960-8966(16)30856-2. doi: 10.1016/j.nmd.2016.11.004. [Epub ahead of print]
- Clusters of cognitive impairment among different phenotypes of myotonic dystrophy type 1 and type 2. Peric S, Rakocevic Stojanovic V, Mandic Stojmenovic G, Ilic V, Kovacevic M, Parojcic A, Pesovic J, Mijajlovic M, Savic-Pavicevic D, Meola G. **Neurol Sci.** 2016 Nov 28. [Epub ahead of print]
- Unusual structures of CCTG repeats and their participation in repeat expansion. Guo P, Lam SL. **Biomol Concepts.** 2016 Dec 1;7(5-6):331-340. doi: 10.1515/bmc-2016-0024.
- Molecular Diagnosis of Myotonic Dystrophy. Chakraborty S, Vatta M, Bachinski LL, Krahe R, Dlouhy S, Bai S. **Curr Protoc Hum Genet.** 2016 Oct 11;91:9.29.1-9.29.19. doi: 10.1002/cphg.22.
- Peripheral neuropathy in patients with myotonic dystrophy type 2.
- Leonardis L. **Acta Neurol Scand.** 2016 Jul 12.
- Cardiac Involvement in Myotonic Dystrophy Type 2 Patients With Preserved Ejection Fraction: Detection by Cardiovascular Magnetic Resonance. Schmacht L, Traber J, Grieben U, Utz W, Dieringer MA, Kellman P, Blaszczyk E, von Knobelsdorff-Brenkenhoff F, Spuler S, Schulz-Menger J. **Circ Cardiovasc Imaging.** 2016 Jul 9.
- Focal seizures in a patient with myotonic disorder type 2 co-segregating with a chloride voltage-gated channel 1 gene mutation: a case report. Peddareddygari LR, Grewal AS, Grewal RP. **J Med Case Rep.** 2016 Jun 7.
- A Molecular Signature of Myalgia in Myotonic Dystrophy 2. Moshourab R, Palada V, Grunwald S, Grieben U, Lewin GR, Spuler S. **EBioMedicine.** 2016 May 7.

- Quality of life in patients with myotonic dystrophy type 2.
  Rakocevic Stojanovic V, Peric S, Paunic T, Pesovic J, Vujnic M, Peric M, Nikolic A, Lavrnic D, Savic Pavicevic D. **J Neurol Sci.** 2016 Jun 15.
- Drug resistant focal epilepsy in a patient with myotonic dystrophy type 2: casual or causal association?
  Giuliano L, Sofia V, Cardani R, Meola G, Zappia M. **Neurol Sci.** 2016 May 25.
- Multidimensional aspects of pain in myotonic dystrophies.
  Peric M, Peric S, Rapajic N, Dobricic V, Savic-Pavicevic D, Nesic I, Radojicic S, Novakovic I, Lavrnic D, Rakocevic-Stojanovic V. **Acta Myol.** 2015 Dec 2.
- No relevant excess prevalence of myotonic dystrophy type 2 in patients with suspected fibromyalgia syndrome.
  van Vliet J, Verrips A, Tieleman AA, Scheffer H, Cats HA, den Broeder AA, van Engelen BG. **Neuromuscul Disord**. 2016 Apr 6.
- An Age-Standardized Prevalence Estimate and a Sex and Age Distribution of Myotonic Dystrophy Types 1 and 2 in the Rome Province, Italy.
  Vanacore N, Rastelli E, Antonini G, Bianchi ML, Botta A, Bucci E, Casali C, Costanzi-Porrini S, Giacanelli M, Gibellini M, Modoni A, Novelli G, Pennisi EM, Petrucci A, Piantadosi C, Silvestri G, Terracciano C, Massa R. **Neuroepidemiology**. 2016 Feb 17.
- Comparison of temporal and stride characteristics in myotonic dystrophies type 1 and 2 during dual-task walking.
  Radovanović S, Perić S, Savić-Pavićević D, Dobričić V, Pešović J, Kostić V, Rakočević-Stojanović V. **Gait Posture**. 2016 Feb. Epub 2015 Dec 20.
- Patient-Reported Impact of Symptoms in Myotonic Dystrophy Type 2 (PRISM-2).
  Heatwole C, Johnson N, Bode R, Dekdebrun J, Dilek N, Hilbert JE, Luebbe E, Martens W, McDermott MP, Quinn C, Rothrock N, Thornton C, Vickrey BG, Victorson D, Moxley RT 3rd. **Neurology**. 2015 Nov 18.
- New Insights into the genetic instability in CCTG repeats.
  Guo P, Lam SL. **FEBS Lett**. 2015 Oct 7;589(20 Pt B):3058-63. Doi: 10.1016/j.febslet.2015.09.007. Epub 2015 Sep 16.

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