MYOTONIC DYSTROPHIES AND CNS RESEARCH

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Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1

Nicolas Sergeant[1], Bernard Sablonnière[1], Suzanna Schraen-Maschke[1], Antoine Ghestem[1], Claude-Alain Maurage[1,2], Annick Wattez[1], Patrick Vermersch[3] and André Delacourte[1,*]

Hum. Mol Gen., 2001

Post-mortem brains were examined:

- ✓ 5 DM1
- ✓ 10 healthy CTR + 10 Alzheimer

Several brain regions were analysed:

1. Neurofibrillary degeneration with pathological tau protein is a common feature in DM1
2. Hyper phosphorylated tau proteins are phosphorylated at identical pathological sites found in other neurological disorders
3. Alteration of Tau expression pattern: **expression reduction of Tau isoforms containing exon 2 (mRNA and protein)**
4. Large expanded CTG repeats were present

Myotonic Dystrophy type 1 is a peculiar Tauopathy

Myotonic Dystrophies are Tauopathies

Mice transgenic for the human myotonic dystrophy region with expanded CTG repeats display muscular and brain abnormalities

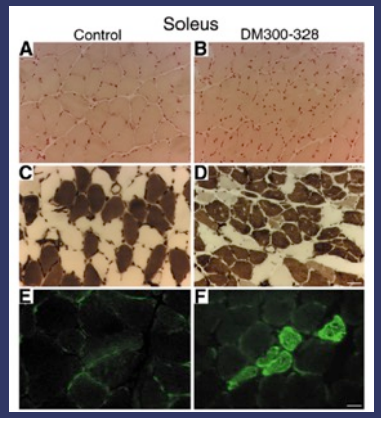
Hervé Seznec, Onnik Agbulut[1], Nicolas Sergeant[2], Cédric Savouret, Antoine Ghestem[2], Nacira Tabti[3], Jean-Claude Willer[4], Lucie Ourth, Chantal Duros, Edith Brisson, Coralie Fouquet, Gillian Butler-Browne[1], André Delacourte[2], Claudine Junien and Geneviève Gourdon*

Hum. Mol Gen., 2001

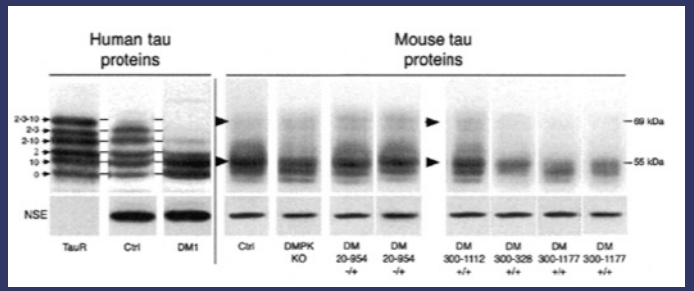
Analysis of transgenic mouse carrying the human genomic DM1 region with **350 CTG** or normal expansion of **20 CTG**

Transgenic mice DM300:

- ✓ Myotonia
- ✓ Skeletal muscle abnormalities

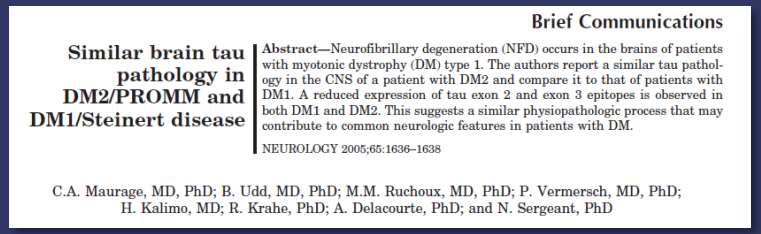


- ✓ Changes in the distribution of Tau protein isoforms



This transgenic mouse is a good model to investigate changes in brain

Myotonic Dystrophies are Tauopathies



Brief Communications

Similar brain tau pathology in DM2/PROMM and DM1/Steinert disease

Abstract—Neurofibrillary degeneration (NFD) occurs in the brains of patients with myotonic dystrophy (DM) type 1. The authors report a similar tau pathology in the CNS of a patient with DM2 and compare it to that of patients with DM1. A reduced expression of tau exon 2 and exon 3 epitopes is observed in both DM1 and DM2. This suggests a similar physiopathologic process that may contribute to common neurologic features in patients with DM.

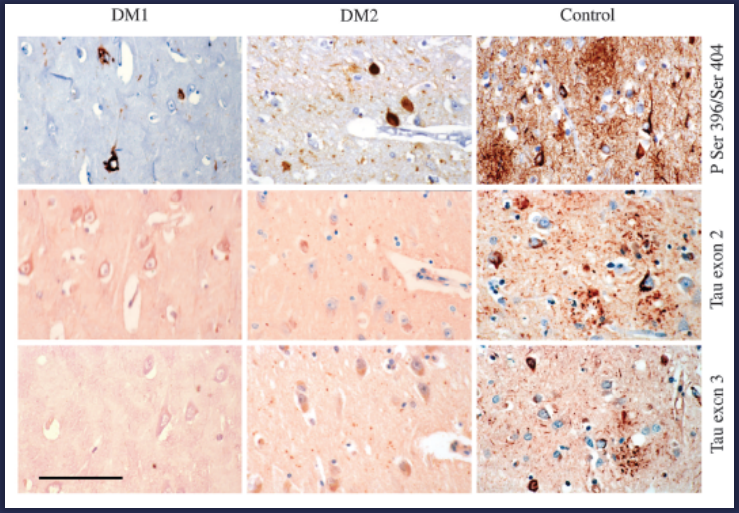
NEUROLOGY 2005;65:1636–1638

C.A. Maurage, MD, PhD; B. Udd, MD, PhD; M.M. Ruchoux, MD, PhD; P. Vermersch, MD, PhD; H. Kalimo, MD; R. Krahe, PhD; A. Delacourte, PhD; and N. Sergeant, PhD

Neurology, 2005

DM1 and DM2 patients show similar **Tau pathology** in the CNS

the occurrence of **Neuro Fibrillary Tangles (NFTs)** and **Marinesco bodies** have been reported in brain tissue from one patient with DM2



Brain tissue from patients with DM1 and DM2 lacks tau-immunoreactive NFTs containing tau-E2 and tau-E3

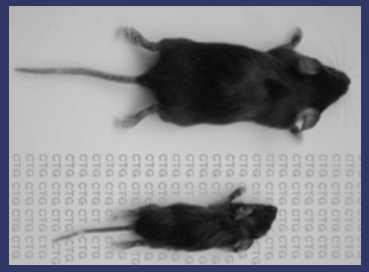
The tauopathy in a single patient with DM2 suggests an underlying physiopathologic process that is most possibly similar to that observed in DM1

Myotonic Dystrophies are Tauopathies

CTG Trinucleotide Repeat "Big Jumps": Large Expansions, Small Mice

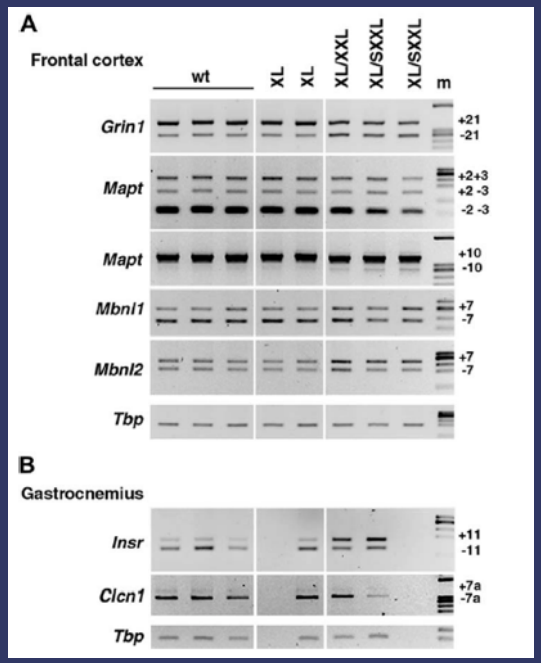
Mário Gomes-Pereira[1,2☯], Laurent Foiry[1,2☯], Annie Nicole[1,2], Aline Huguet[1,2], Claudine Junien[1,2], Arnold Munnich[1,2], Geneviève Gourdon[1,2*]

PLoS Genet., 2007



Two sublines were derived from DM300:

- ✓ **XL mice** = about 600-700 CTG
- ✓ **XXL mice** = about 900-1000 CTG



Homozygous Mice Carrying over 600CTG

Brain:

- abnormal splicing of glutamate receptor, ionotropic, N-methyl D-aspartate 1 (**Grin1/Nmdar1**), microtubule-associated protein tau (**Mapt/Tau**) and **MBNL1- MBNL2**

Skeletal muscle:

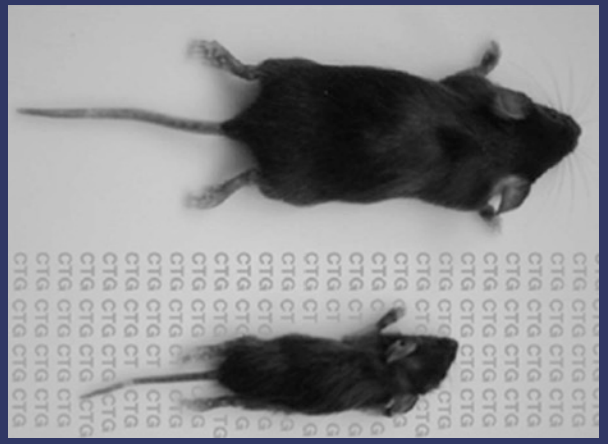
- abnormal splicing of **CLCN1** and of insulin receptor gene (**INSR**)

Myotonic Dystrophies are Tauopathies

CTG Trinucleotide Repeat "Big Jumps": Large Expansions, Small Mice

Mário Gomes-Pereira[1,2☯], Laurent Foiry[1,2☯], Annie Nicole[1,2], Aline Huguet[1,2], Claudine Junien[1,2], Arnold Munnich[1,2], Geneviève Gourdon[1,2*]

PLoS Genet., 2007



This transgenic mouse model of DM1 represents:

- ✓ a unique tool to explore the complex dynamics of simple trinucleotide repeats, the increasing phenotypic severity through generations, as well as the molecular bases of RNA toxicity in disease pathogenesis
- ✓ the first mouse model of DM1 presenting RNA splicing abnormalities in the central nervous system.

Myotonic Dystrophies are Tauopathies

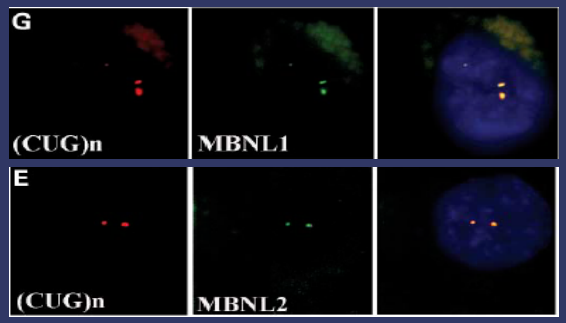
Myotonic dystrophy type 1 is associated with nuclear foci of mutant RNA, sequestration of muscleblind proteins and deregulated alternative splicing in neurons

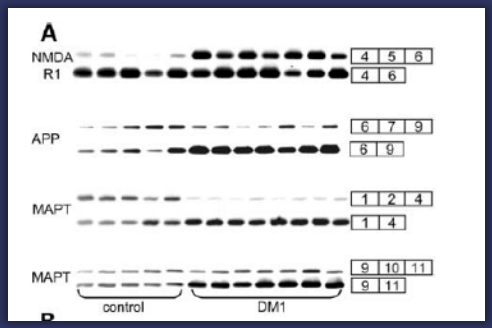
Hong Jiang[1], Ami Mankodi[1], Maurice S. Swanson[2], Richard T. Moxley[1] and Charles A. Thornton[1,*]

Hum. Mol Gen., 2004

Post-mortem brains were examined:

- ✓ **10 DM1 vs 13 CTR**: 6 with no neurologic disease, two with Alzheimer disease, four with Huntington disease and one with refractory epilepsy.





- ✓ In DM1 mutant transcripts accumulate in discrete **foci** within neuronal nuclei
- ✓ **MBNL1 and MBNL2** are recruited into the RNA foci and depleted elsewhere in the nucleoplasm
- ✓ A subset of neuronal pre-mRNAs show abnormal regulation of alternative splicing (**NMDAR1, MAPT**)

CNS impairment in DM1 may result from gain-of-function by mutant DMPK mRNA

Myotonic Dystrophies are Tauopathies

Muscleblind-Like 1 Knockout Mice Reveal Novel Splicing Defects in the Myotonic Dystrophy Brain

Koichi Suenaga[1☯], Kuang-Yung Lee[2,3☯], Masayuki Nakamori[4¤a], Yoshiki Tatsumi[1], Masanori P. Takahashi[4], Harutoshi Fujimura[5], Kenji Jinnai[6], Hiroo Yoshikawa[1], Hongqing Du[7¤b], Manuel Ares Jr.[7], Maurice S. Swanson[2], Takashi Kimura[1*]

PLoS one, 2012

RNA from brains of Mbnl1 knockout (**Mbnl1$^{\Delta E3/\Delta E3}$**) mice was analysed using splicing-sensitive microarrays and compared to the results obtained post-mortem brain from **DM1 patients.**

- ✓ Surprisingly, splicing-sensitive microarray analysis of **Mbnl1$^{\Delta E3/\Delta E3}$** brains yielded only 14 candidates for mis-spliced exons
- ✓ Only 3 of these splicing events are perturbed in both Mbnl1 knockout and DM1 brain

the extent of splicing mis-regulation in **Mbnl1$^{\Delta E3/\Delta E3}$** was significantly less than observed in DM1

Other factors, possibly other MBNL proteins, likely contribute to splicing mis-regulation in the DM1 brain.

DM brain and MBNL1

Muscleblind-like 2-Mediated Alternative Splicing in the Developing Brain and Dysregulation in Myotonic Dystrophy

Konstantinos Charizanis,[1] Kuang-Yung Lee,[1,4] Ranjan Batra,[1] Marianne Goodwin,[1] Chaolin Zhang,[5] Yuan Yuan,[5] Lily Shiue,[6] Melissa Cline,[6] Marina M. Scotti,[1] Guangbin Xia,[2] Ashok Kumar,[3] Tetsuo Ashizawa,[2] H. Brent Clark,[7] Takashi Kimura,[8] Masanori P. Takahashi,[9] Harutoshi Fujimura,[10] Kenji Jinnai,[11] Hiroo Yoshikawa,[8] Mário Gomes-Pereira,[12] Geneviève Gourdon,[12] Noriaki Sakai,[13] Seiji Nishino,[13] Thomas C. Foster,[3] Manuel Ares, Jr.,[6] Robert B. Darnell,[5] and Maurice S. Swanson[1,*]

Neuron, 2012

Mbnl1 knockout (**Mbnl1$^{\Delta E3/\Delta E3}$**) mice show modest effects on alternative splicing regulation in the brain

a Mbnl2 knockout mice (Mbnl2$^{\Delta E2/\Delta E2}$) was generated , which exhibit several phenotypes consistent with features of DM neurologic disease

- ✓ abnormal REM sleep propensity and deficits in spatial memory
- ✓ a decrease in NMDA receptor (NMDAR) synaptic transmission and impaired hippocampal synaptic plasticity
- ✓ misregulated splicing of hundreds of exons the majority of which were similarly misregulated in DM
- ✓ did not develop overt skeletal muscle pathology or motor deficits prior to 6 months of age

The major pathological changes in DM brain are attributable to toxic RNA expression, MBNL2 sequestration and dysregulation of specific alternative splicing events

DM brain and MBNL2

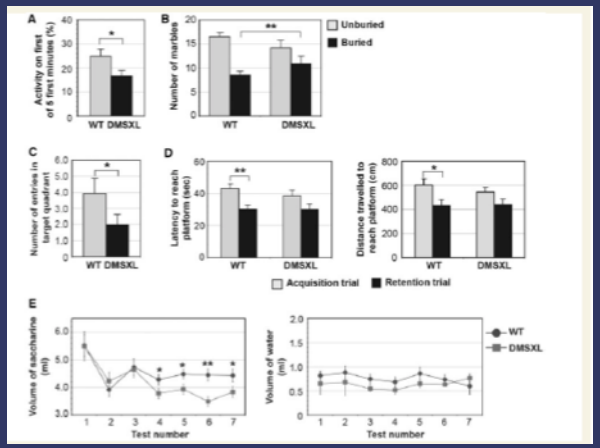
Myotonic dystrophy CTG expansion affects synaptic vesicle proteins, neurotransmission and mouse behaviour

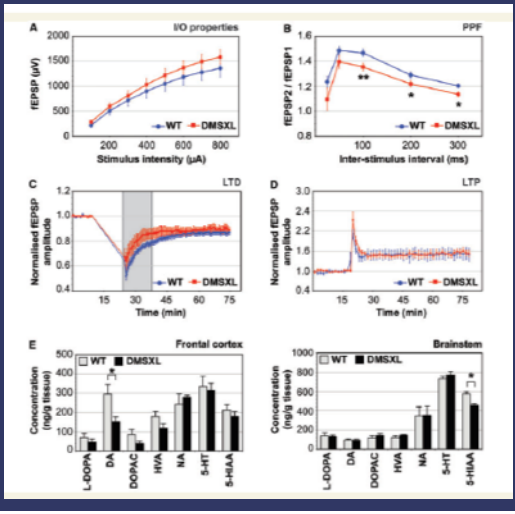
Oscar Hernández-Hernández,[1,*] Céline Guiraud-Dogan,[1,2] Géraldine Sicot,[1] Aline Huguet,[1] Sabrina Luilier,[3] Esther Steidl,[4] Stefanie Saenger,[5] Elodie Marciniak,[6] Hélène Obriot,[6] Caroline Chevarin,[7] Annie Nicole,[1] Lucile Revillod,[2] Konstantinos Charizanis,[8,9] Kuang-Yung Lee,[8,9,10] Yasuhiro Suzuki,[11] Takashi Kimura,[11] Tohru Matsuura,[12] Bulmaro Cisneros,[13] Maurice S. Swanson,[8,9] Fabrice Trovero,[3] Bruno Buisson,[4] Jean-Charles Bizot,[3] Michel Hamon,[7] Sandrine Humez,[6] Guillaume Bassez,[2,14] Friedrich Metzger,[5] Luc Buée,[6] Arnold Munnich,[1] Nicolas Sergeant,[6] Geneviève Gourdon[1] and Mário Gomes-Pereira[1]

Brain, 2012

DMSXL mice vs **DM1 patients** (n=9) were examined

The behavioural phenotyping of DMSXL mice revealed reduced exploratory activity, increased anxiety, spatial memory impairment and anhedonia, which resemble DM1 neurological manifestations





The behavioural abnormalities of DMSXL mice are associated with deficits in short-term plasticity, as well as changes in neurochemicals, suggesting altered synaptic function and neurotransmission in response to the CTG repeat expansion.

DM behavior and synaptic dysfunctions

Myotonic dystrophy CTG expansion affects synaptic vesicle proteins, neurotransmission and mouse behaviour

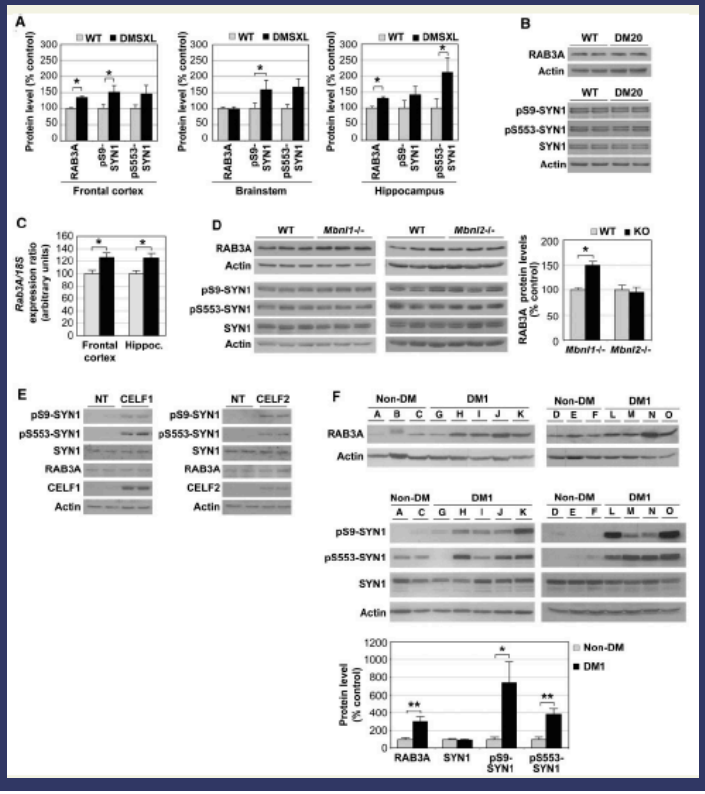
Oscar Hernández-Hernández,[1,*] Céline Guiraud-Dogan,[1,2] Géraldine Sicot,[1] Aline Huguet,[1] Sabrina Luilier,[3] Esther Steidl,[4] Stefanie Saenger,[5] Elodie Marciniak,[6] Hélène Obriot,[6] Caroline Chevarin,[7] Annie Nicole,[1] Lucile Revillod,[2] Konstantinos Charizanis,[8,9] Kuang-Yung Lee,[8,9,10] Yasuhiro Suzuki,[11] Takashi Kimura,[11] Tohru Matsuura,[12] Bulmaro Cisneros,[13] Maurice S. Swanson,[8,9] Fabrice Trovero,[3] Bruno Buisson,[4] Jean-Charles Bizot,[3] Michel Hamon,[7] Sandrine Humez,[6] Guillaume Bassez,[2,14] Friedrich Metzger,[5] Luc Buée,[6] Arnold Munnich,[1] Nicolas Sergeant,[6] Geneviève Gourdon[1] and Mário Gomes-Pereira[1]

Brain, 2012

DMSXL mice vs **DM1 patients** (n=9) were examined

In the search for disease intermediates affected by disease mutation, a global proteomics approach revealed **RAB3A upregulation** and **synapsin I hyperphosphorylation** in the CNS of transgenic mice, transfected cells and post-mortem brains of DM1 patients

a novel connection between physiological phenotypes and synaptic protein dysregulation, indicative of synaptic dysfunction in DM1 brain pathology has been demonstrated



DM behavior and synaptic dysfunctions

Myotonic dystrophy type 1-associated CTG repeats disturb the expression and subcellular distribution of microtubule-associated proteins MAP1A, MAP2, and MAP6/STOP in PC12 cells

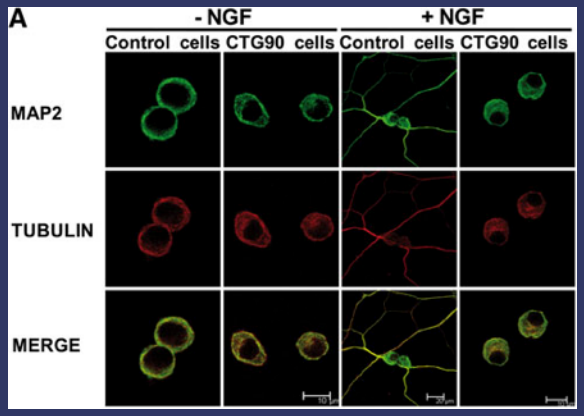
Prisiliana Velázquez-Bernardino · Francisco García-Sierra · Oscar Hernández-Hernández · Mario Bermúdez de León · Geneviève Gourdon · Mário Gomes-Pereira · Bulmaro Cisneros

Mol Biol Rep, 2012

The expression and subcellular localization of **Microtubule Associated Proteins (MAPs)** was analyzed in **PC12 neuronal cells with CTG90**

Microtubules serve as scaffolding for neurite formation and also for transporting organelles and macromolecules essential for the growth and maintenance of developing neurites

MAPs participate in microtubule stabilization and assembly as well as in regulation of interactions between microtubules and cytoskeletal elements



the expression and subcellular localization of MAP1A, MAP2, and MAP6/STOP are altered in CTG90 cells. these cells

MAPs deficiency is the main cause of the disturbed neurite outgrowth displayed by these cells

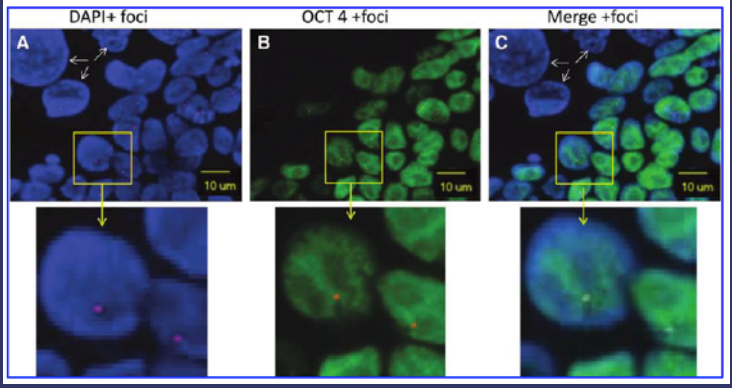
DM Microtuble Associated Proteins

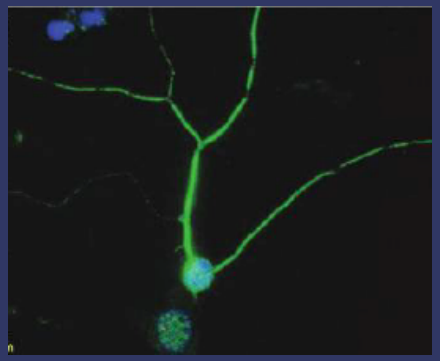
Generation of Neural Cells from DM1 Induced Pluripotent Stem Cells As Cellular Model for the Study of Central Nervous System Neuropathogenesis

Guangbin Xia,[1] Katherine E. Santostefano,[2] Marianne Goodwin,[3] Jilin Liu,[1] S.H. Subramony,[1] Maurice S. Swanson,[3] Naohiro Terada,[2] and Tetsuo Ashizawa[1]

Cellular Rep, 2013

DM1 iPSC lines were established from dermal fibroblasts by retroviral transduction of Yamanaka's four factors





- ✓ both DM1 and control iPSC expressed stem cell markers and differentiated into cells derived from three embryonic germ layers
- ✓ **iPSC lines underwent normal neural differentiation**
- ✓ intranuclear **RNA foci** were detected in DM1 iPSCs, neural stem cells (NSCs), and terminally differentiated neurons and astrocytes.

Human DM1 iPSC lines and neuronal lineages offer an unlimited cell resource for CNS mechanistic studies and a translational platform for therapeutic development

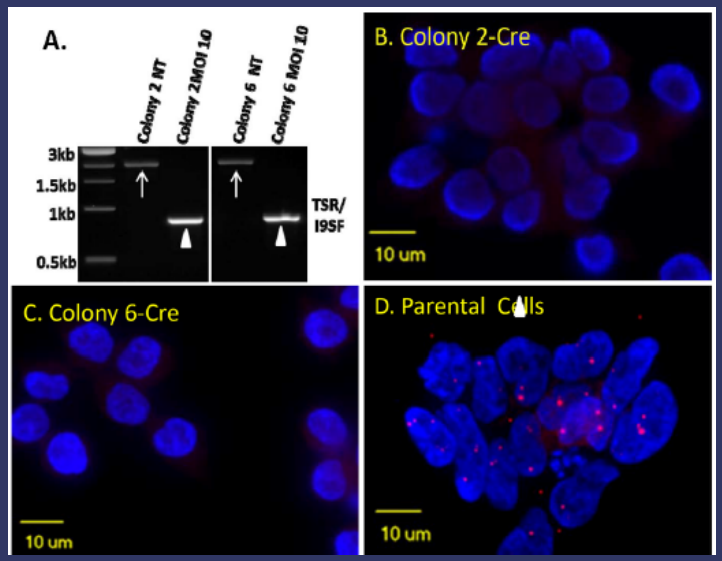
DM Induced Pluripotent Stem Cells (iPSc)

Genome Modification Leads to Phenotype Reversal in Human Myotonic Dystrophy type 1 iPS-cell Derived Neural Stem Cells

Guangbin Xia[1,2,3,4,5*], Yuanzheng Gao[1,4], Shouguang Jin[6], SH. Subramony[1,3,4], Naohiro Terada[2,7], Laura P.W. Ranum[1,3,6,8], Maurice S. Swanson[3,6,8], Tetsuo Ashizawa[1,2,3,4]

Stem Cell, 2015

in vitro genome editing to prevent production of toxic mutant transcripts and reverse phenotypes in DM1 neural stem cells derived from DM1 iPSC



An editing cassette containing SV40/bGH polyA signals was integrated upstream of the CTG repeats by TALEN-mediated homologous recombination (HR)

complete disappearance of nuclear RNA foci

MAPT and MBNL 1, 2 aberrant splicing in DM1 NSCs was reversed to normal

Genome modification may be used to generate genetically modified progenitor cells as a first step toward autologous cell transfer therapy for DM1

DM Induced Pluripotent Stem Cells (iPSc)

MBNL Sequestration by Toxic RNAs and RNA Misprocessing in the Myotonic Dystrophy Brain

Marianne Goodwin,[1,9] Apoorva Mohan,[1,9] Ranjan Batra,[1] Kuang-Yung Lee,[1,2] Konstantinos Charizanis,[1,3] Francisco José Fernández Gómez,[4] Sabiha Eddarkaoui,[4] Nicolas Sergeant,[4] Luc Buée,[4] Takashi Kimura,[5] H. Brent Clark,[6] Joline Dalton,[6] Kenji Takamura,[6] Sebastien M. Weyn-Vanhentenryck,[7] Chaolin Zhang,[7] Tammy Reid,[1] Laura P.W. Ranum,[1] John W. Day,[8] and Maurice S. Swanson[1,*]

Cell Rep, 2015

post mortem brain from DM1 vs DM2 vs CTR subjects

High-throughput sequencing-crosslinking immunoprecipitation (**HITS-CLIP**) combined with pre-mRNA processing analysis was performed

In DM1 and DM 2 brain:

- ✓ MBNL1 and MBNL2 proteins are directly sequestered by microsatellite expansion RNAs
- ✓ Toxic RNA expression results in MBNLs depletion from normal RNA targets
- ✓ MBNL loss leads to fetal patterns of splicing and polyadenylation in the brain

DM1 and DM2 human brain

- DM are tauopathy. Need for post-mortem brain study.
- Transgenic mouse model (BIG JUMP) shows abnormal splicing in CNS.
- MBNL2 knockout mouse explains several brain manifestations.
- DMSXL mice show DM behavior abnormalities and synaptic dysfunction.
- Human DM1 iPSc lines are tool for CNS mechanistic studies and translational platform for therapeutic development.

Take home message