MYOTONIC DYSTROPHIES AND CNS RESEARCH

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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

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

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

Myotonic Dystrophies are Tauopathies
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**
Two sublines were derived from DM300:

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

Homozygous Mice Carrying over 600 CTG

**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)
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

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**
RNA from brains of Mbnl1 knockout (Mbnl1ΔE3/ΔE3) 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 Mbnl1ΔE3/ΔE3 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 Mbnl1ΔE3/ΔE3 was significantly less than observed in DM1.

Other factors, possibly other MBNL proteins, likely contribute to splicing mis-regulation in the DM1 brain.
Mbnl1 knockout (Mbnl1^{ΔE3}/^{ΔE3}) mice show modest effects on alternative splicing regulation in the brain

- 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

a Mbnl2 knockout mice (Mbnl2^{ΔE2}/^{ΔE2}) was generated, which exhibit several phenotypes consistent with features of DM neurologic disease.
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

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.
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.
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.
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
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 DM2 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
• 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.