DM1MICE
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Molecular and physiopathological consequences of CTG repeats expansion in a mouse model for myotonic dystrophy.

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Context and aims

Myotonic dystrophy type 1 (DM1) is characterised by a progressive deterioration of neuromuscular functions[1]. This multisystemic disease, which can appear in adults, children or neonates, affects various organs such as muscles, heart and the central nervous system (CNS). There is a high variability of the nature, severity and age at onset of the symptoms. The mutation causing DM1 is the abnormal expansion of a CTG repeat located in the 3'UTR of the *DMPK* gene on chromosome 19. The majority of the symptoms results from a toxic gain of function of the mutant *DMPK* RNA carrying expansions that are retained in the cell nuclei[2]. Details of the pathological mechanisms and metabolic pathways affected by the mutation are still unclear, especially in brain and for the congenital form. The aims of this project are to study the expression defects of the genes from the DM1 locus during development and to identify the metabolic pathways affected by the CTG expansion. A better understanding of the mechanisms involved in DM1 should open new routes towards therapeutic strategies.

Tools: mouse models for DM1

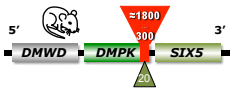


Figure 1. Transgene construct. A large fragment of human genomic DNA (~45 kb), containing the 3 genes of the *DM1* locus and a variable number of repeats (20 or >300 CTG) , was inserted in the mouse genome.

We have developped transgenic mouse models carrying the human *DMPK* gene with normal or expanded CTG repeats (DM20 or DMSXL mice). The mice carrying large CTG repeat show high mortality before 1 month, growth retardation, muscle weakness, heart abnormalities, behavioral deficits and variable splicing defects in muscle heart and brain[3,4,5].

Expression of sense and antisense *DMPK* RNA during heart development

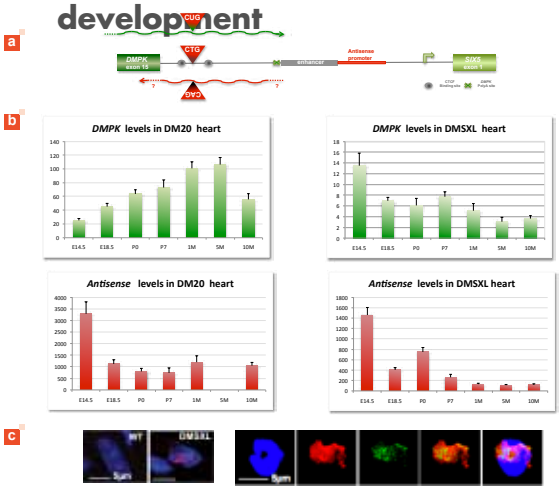
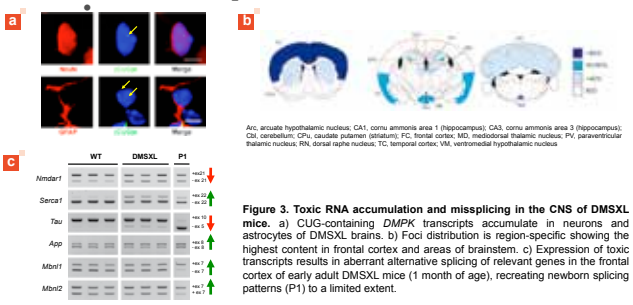


Figure 2. Transgene expression. a) Diagram of the 3' regulatory region of *DMPK*, showing sense and antisense transcripts. b) Sense and antisense *DMPK* RNA levels during heart development and ageing in mice with normal 20 repeats (DM20) or mice with large CTG repeat expansions (DMSXL). c) Antisense RNA can be translated in polyGln peptide and colocalise with caspase-8 in DMSXL cardiomyocytes and leukocytes nuclei.

* Antisense expression RNA profiles are similar between DM20 and DMSXL. However, expression of sense *DMPK* RNA is very different between DMSXL and DM20 mice[6].
* CAG containing transcripts can be translated in polypeptides. PolyGln peptides colocalise with caspase-8, an early indication of polyGln-induced apoptosis[7].

RNA toxicity in the CNS of DMSXL

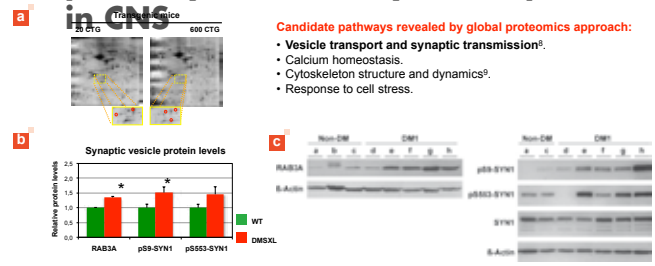


Arc, arcuate hypothalamic nucleus; CA1, cornu ammonis area 1 (hippocampus); CA3, cornu ammonis area 3 (hippocampus); Cbl, cerebellum; CPu, caudate putamen (striatum); FC, frontal cortex; MD, mediodorsal thalamic nucleus; PV, paraventricular thalamic nucleus; RN, dorsal raphe nucleus; TC, temporal cortex; VM, ventromedial hypothalamic nucleus

Figure 3. Toxic RNA accumulation and missplicing in the CNS of DMSXL mice. a) CUG-containing *DMPK* transcripts accumulate in neurons and astrocytes of DMSXL brains. b) Foci distribution is region-specific showing the highest content in frontal cortex and areas of brainstem. c) Expression of toxic transcripts results in aberrant alternative splicing of relevant genes in the frontal cortex of early adult DMSXL mice (1 month of age), recreating newborn splicing patterns (P1) to a limited extent.

* DMSXL mice recreate important aspects of DM1-associated RNA toxicity in the CNS.
* The DMSXL line provides a useful tool to investigate DM1 neuropathogenesis.

Novel disease intermediates and pathways affected by CTG repeats in CNS



Candidate pathways revealed by global proteomics approach:
- **Vesicle transport and synaptic transmission**[8].
- Calcium homeostasis.
- Cytoskeleton structure and dynamics[9].
- Response to cell stress.

Figure 4. Identification of new proteins and pathways affected by the DM1 mutation in the CNS. a) Four candidate pathways were revealed by the comparison of the brain proteomic profiles of mice carrying short CTGs or large repeat expansions. b) Higher steady-state levels of RAB3A and synapsin 1 hyperphosphorylation were confirmed in DMSXL frontal cortex by western blot. c) RAB3A and synapsin 1 abnormalities were validated in post-mortem DM1 brains (frontal cortex).

* A proteomics approach revealed relevant CNS pathways possibly affected in DM1.
* Synaptic vesicle-associated proteins are deregulated in DMSXL mice and DM1 patients.

Conclusion/Perspectives

- Possible deregulation of *DMPK* expression during heart development by large CTG repeats: in accordance with the hypothesis from Cho et al[10].
- Antisense *DMPK* is highly expressed during development, encompasses the CAG expansions and can be translated in polypeptides.
- What are the role of the antisense and the pathophysiological consequences of the polypeptides ?
- The DMSXL mice probably model the congenital form of DM1 (CDM)
- Altered synaptic transmission may contribute to DM1 neurological manifestations.
- What is the cellular and physiological impact of the molecular abnormalities detected in the CNS of DMSXL mice?

Valorisation: 11 MTA, 2 licences with Genzyme and Isis Inc.

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Publications from the group