Missplicing and functional impairment in myotonic dystrophy type 1 (DM1)

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DM1 is a rare neuromuscular disorder with multi-systemic involvement[1,2]

CNS[1–5]

- Fatigue
- Excessive daytime sleepiness
- Cognitive impact
- Behaviour/personality patterns
- Emotional impact
- Sleep disturbances

Ocular[1–4]

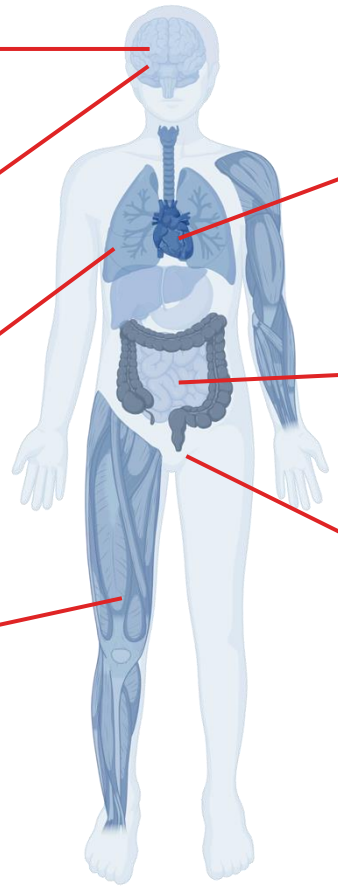
- Cataracts
- Ptosis

Skeletal muscle (respiratory)[1–4]

- Restrictive ventilatory pattern
- Shortness of breath

Skeletal muscle[1–4]

- Muscle weakness
- Myotonia
- Balance issues
- Muscle pain
- Atrophy



Cardiac[1–4]

- Conduction disturbances
- Arrythmia
- Cardiomyopathy
- Sudden death

Smooth muscle[1–4]

- Dysphagia
- Constipation
- Heartburn
- Regurgitation

Endocrine[1–4]

- Thyroid disorders
- Diabetes
- Male hypogonadism
- Vitamin D deficiency

DM1 affects virtually all organs and tissues[1–4]

Slide does not represent an exhaustive list of symptoms. Some symptoms have multi-system involvement.
CNS, central nervous system.
Figure from BioRender.

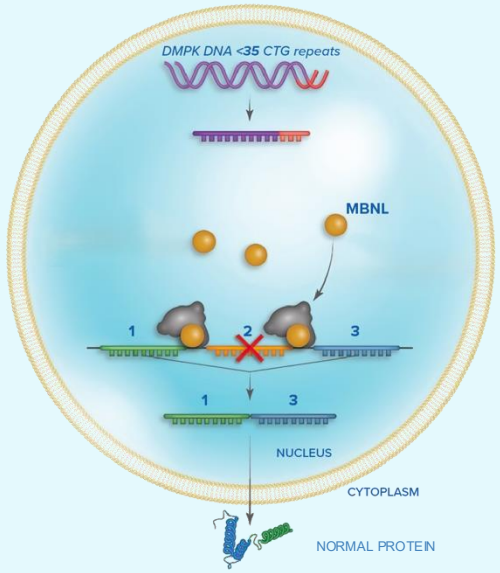
1. Thornton CA. *Neurol Clin.* 2014;32:705–719; 2. Gutierrez Gutierrez G, et al. *Neurologia (Engl Ed).* 2020;35:185–206; 3. Hagerman KA, et al. *Muscle Nerve.* 2019;59:457–464; 4. Ho G, et al. *World J Clin Pediatr.* 2015;4:66–80; 5. Laberge L, et al. *Sleep Med.* 2026;108781.

DM1 is a spliceopathy caused by an expansion of a CTG trinucleotide repeat in the 3’ UTR of the *DMPK* gene[1,2]

Unaffected individuals

***DMPK* gene contains 5–34 CTG repeats; 35–49 repeats is considered ‘premutation’[1]**

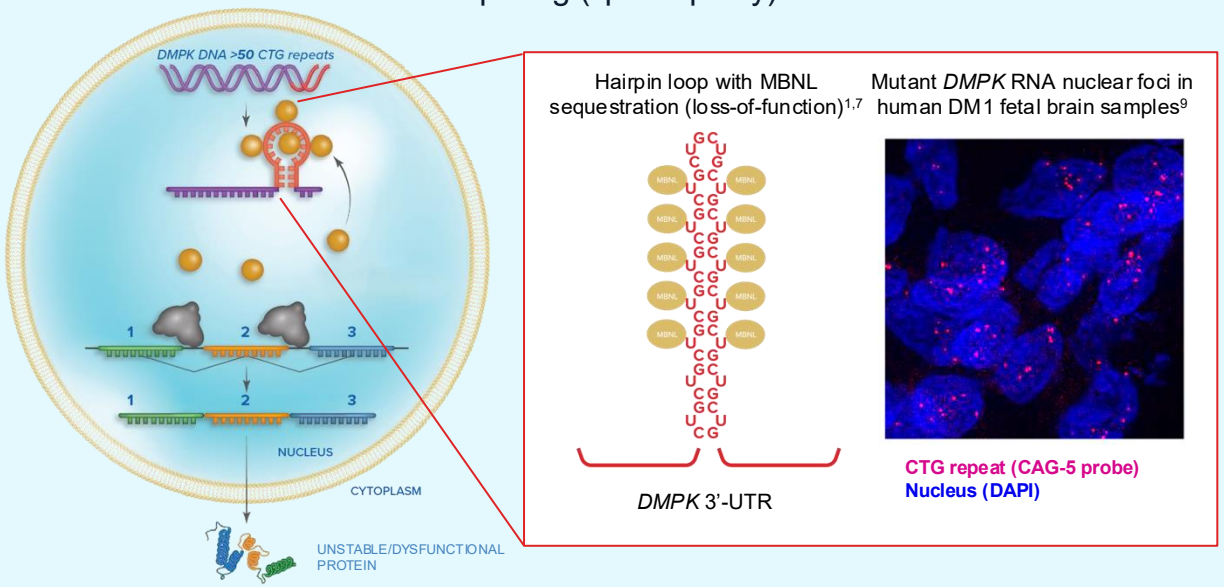
Under normal circumstances, the **MBNL family of proteins** are critical regulators of alternative splicing[2]



DM1 spliceopathy

***DMPK* gene contains ≥50 CTG repeats, and can have >1000[1,3,4]**

Mutant *DMPK* mRNA forms a stable hairpin structure that sequesters members of the MBNL family of proteins into toxic nuclear foci, leading to widespread dysregulation of RNA splicing (spliceopathy)[1,2,5–8]



mRNA transcribed from the mutated *DMPK* gene forms stable hairpin loops that sequester MBNL proteins and form nuclear foci[1,5,6,8]

CAG, cytosine-adenine-guanine; CTG, cytosine-thymine-guanine; DAPI, 4′,6-diamidino-2-phenylindole; DMPK, dystrophia myotonica protein kinase; DM1, myotonic dystrophy type 1; MBNL, muscleblind-like; mRNA, messenger ribonucleic acid; UTR, untranslated region. Image of nuclear foci from Michel L, et al. *PLoS One* 2015;10:e0137620, licensed under a CC-BY 4.0 Creative Commons license; doi:10.1371/journal.pone.0137620. Spliceopathy images used with permission of Sage Publications, from Berglund JA, et al. *J Neuromuscul Dis.* 2025:22143602251365101. Epub ahead of print; permission conveyed through Copyright Clearance Center, Inc. Images show a simplified representation of spliceopathy in individuals with DM1 and unaffected individuals; for illustrative purposes only.

1. Chau A, Kalsotra A. *Dev Dyn*. 2015;244:377–390; 2. López-Martínez A, et al. *Genes (Basel)*. 2020;11:1109; 3. Gutierrez Gutierrez G, et al. *Neurologia (Engl Ed)*. 2020;35:185–206; 4. Thornton CA. *Neurol Clin*. 2014;32:705–719; 5. Davies BM, et al. *Proc Natl Acad Sci USA. 1997*;94:7388–7393; 6. Napierala M, Krzyzosiak WJ. *J Biol Chem*. 1997;272:1079–1085; 7. Misra C, et al. *Adv Neurobiol*. 2018;20:213–238; 8. Pascual-Gilabert M, et al. *Drug Discov Today*. 2021;26:1765–1772; 9. Michel L, et al. *PLoS One* 2015;10:e0137620.

The central dogma of protein synthesis is based on several key steps[1,2]

1 Transcription

Pre-mRNA is produced, consisting of protein-coding exons interspersed with non-coding introns[2]

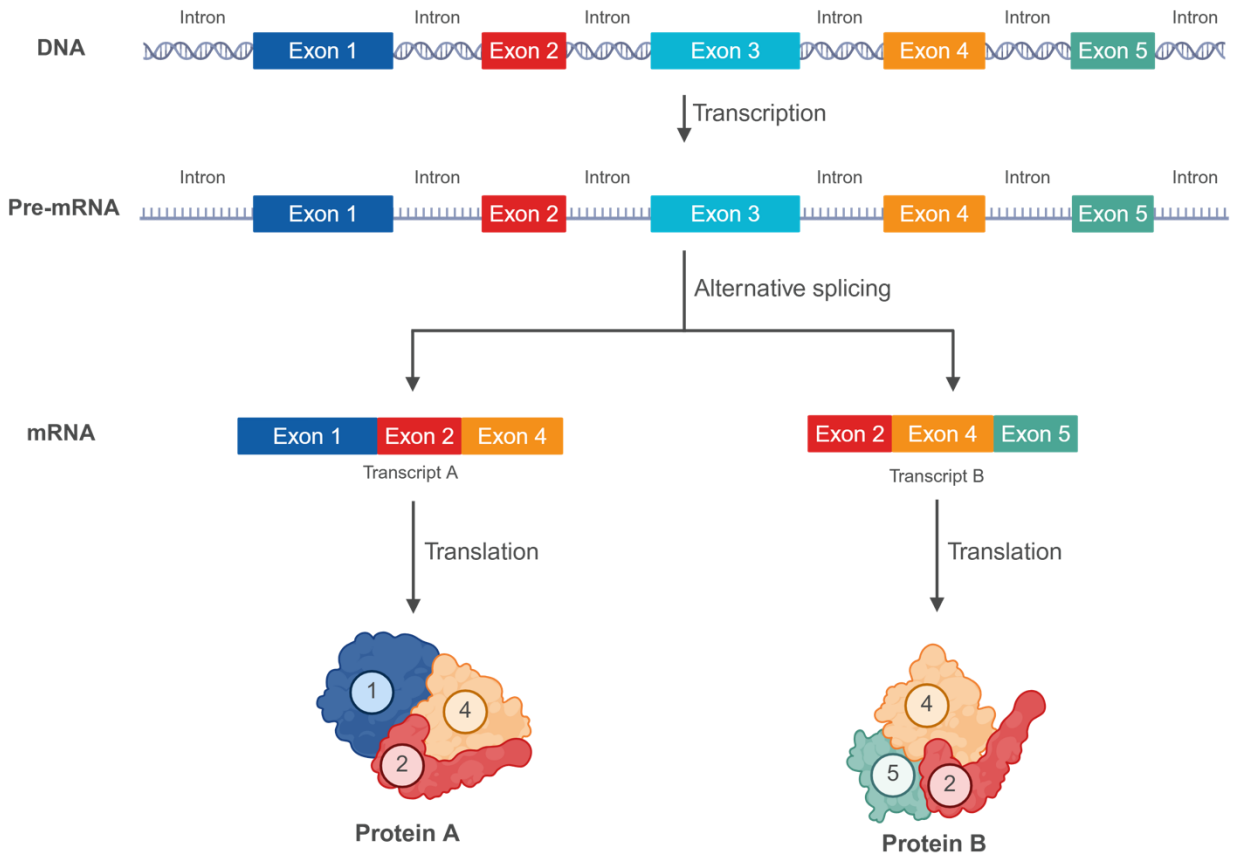
2 Splicing

Introns are removed, and exons are linked together to form mature mRNA[2]

Alternative splicing can produce different mature mRNAs from the same pre-mRNA, and therefore different proteins from the same gene.[2,3] More than 90% of human genes are alternatively spliced and most are dynamically regulated in a cell type- or developmental stage-specific manner[3]

3 Translation

Mature mRNA is exported to the cytoplasm, where the ribosome translates the mRNA reading frame into a functional protein[2]



Splicing removes introns to produce mature mRNA ready for translation; many genes are alternatively spliced to produce different mature mRNAs leading to the synthesis of diverse proteins[2,3]

DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; RNA, ribonucleic acid.
Figure created in BioRender.[1,2]
Graphic shows a simplified representation of alternative splicing; for illustrative purposes only.

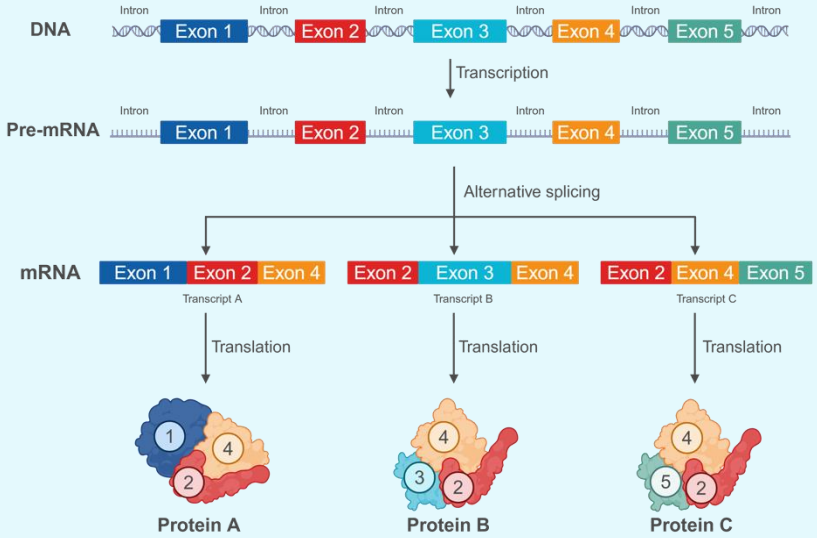
1. Central Dogma. National Human Genome Research Institute. Accessed January 7, 2026. https://www.genome.gov/genetics-glossary/Central-Dogma; 2. Alberts B, et al. *Mol. Biol. Cell*, 4th ed New York: Garland Science; 2002; 3. Choi S, et al. *Exp Mol Med.* 2023;55:755–66.

Sequestration of MBNL into nuclear foci leads to widespread dysregulation of RNA splicing (spliceopathy)[1]

Unaffected individuals

***DMPK* DNA** 5–34 CTG repeats in the 3' UTR[2]

MBNL proteins Normal function in regulating splicing[1]

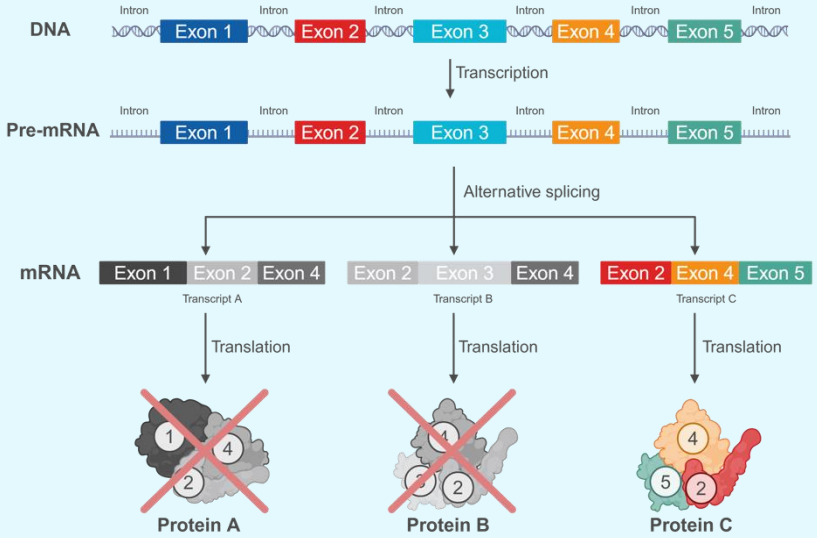


The alternative splicing of many genes is dynamically regulated in a cell type- or developmental stage-specific manner[3]

DM1 spliceopathy

***DMPK* DNA** 50 to >1000 CTG repeats in the 3' UTR[2]

MBNL proteins Sequestered in toxic *DMPK* RNA foci[1]



Spliceopathy in DM1 results in the abnormal persistence of fetal patterns of alternative splicing of hundreds of genes in adult tissues[2,4]

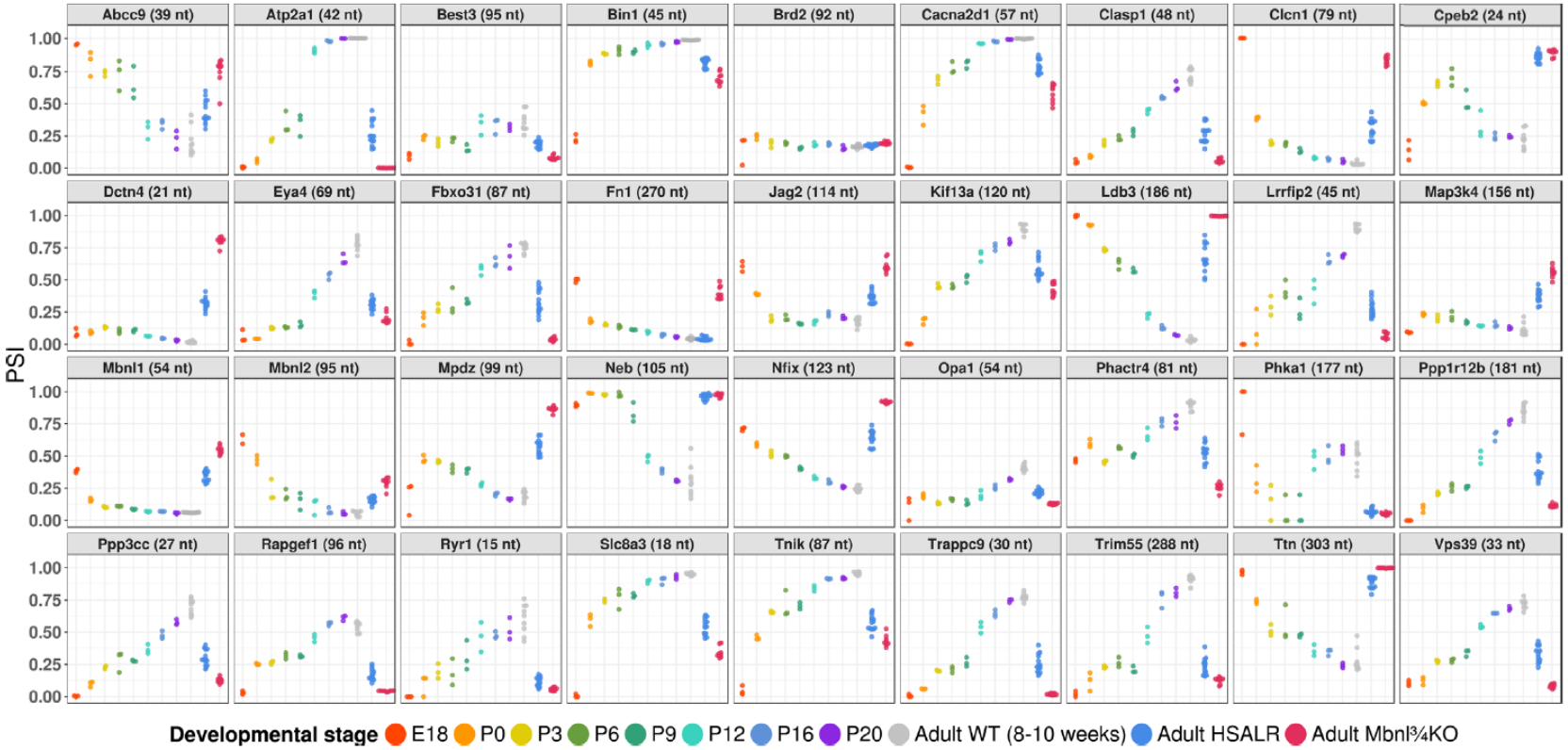
Sequestered MBNL proteins cannot perform their normal function in splicing, so the expression of many genes throughout the body is dysregulated[1,2,4]

CTG, cytosine-thymine-guanine; DM1, myotonic dystrophy type 1; DMPK, dystrophia myotonica protein kinase; DNA, deoxyribonucleic acid; MBNL, muscleblind-like; mRNA, messenger ribonucleic acid; RNA, ribonucleic acid; UTR, untranslated region.
Figure created in BioRender.[3,5]
Graphic shows a simplified representation of alternative splicing; for illustrative purposes only.

1. López-Martínez A, et al. *Genes (Basel)*. 2020;11:1109; 2. Chau A, Kalsotra A. *Dev Dyn*. 2015;244:377–390; 3. Choi S, et al. *Exp Mol Med*. 2023;55:755–766; 4. Pascual-Gilabert M, et al. *Drug Discov Today*. 2021;26:1765–1772; 5. Alberts B, et al. *Mol Biol Cell*. 4th ed New York: Garland Science; 2002.

DM1-affected alternative exons are developmentally regulated[1]

Targeted splice sequencing of hindlimb tissue (E18 and P0) or quadriceps muscle (P3 through adult) in wild-type mice vs adult quadriceps from HSALR and *Mbnl1*$^{-/-}$/*Mbnl2*$^{+/-}$ (Mbnl ¾ KO) mice[1]



The sequestration of MBNL proteins in DM1 causes spliceopathy and an abnormal persistence of fetal patterns of alternative splicing of hundreds of genes in adult tissues[2]

HSALR mice are a transgenic model of myotonic dystrophy that express a human *ACTA1* gene with ~220 CTG repeats in the 3'UTR.[1]
PSI, precent spliced in index, is the ratio between reads including or excluding exons and indicates how efficiently sequences of interest are spliced into transcripts.[3]
DM1, myotonic dystrophy type 1; EX, embryonic day X; HSALR, human skeletal actin long-repeat; KO, knockout; MBNL, muscleblind-like; PX, post-natal day X; WT, wild type.

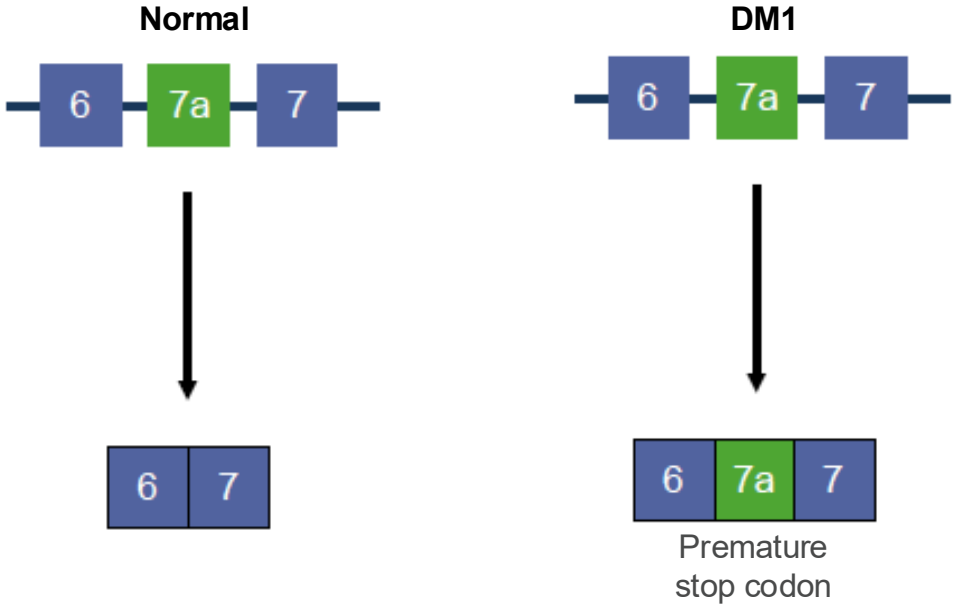
1. Tanner MK, et al. *Nucleic Acids Res.* 2021;49:2240–2254; 2. Chau A, Kalsotra A. *Dev Dyn*. 2015;244:377–390; 3. Schafer S, et al. *Curr Protoc Hum Genet* 2015;87:11.16.1-11–16.14.

Splicing dysregulation has direct functional consequences in DM1

Myotonia[1,2]

CLCN1

Muscle-specific chloride channel

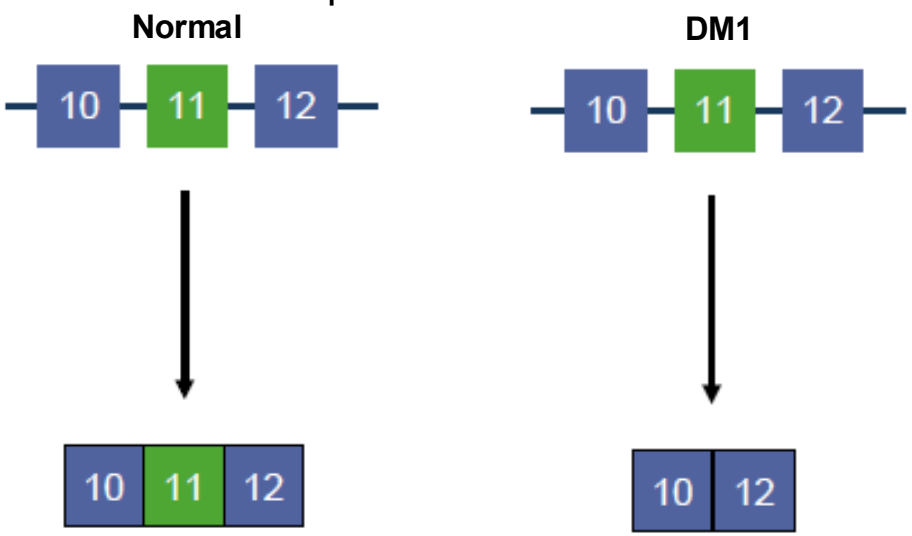


Missplicing of *CLCN1* results in the degradation of the transcript due to the presence of a premature stop codon in the spliced in exon 7a. Decrease in functional CLCN1 protein results in myotonia in DM1[1,2]

Muscle Weakness[3]

BIN1

Component of T-tubules



Missplicing of *BIN1* results in the exclusion of exon 11 and expression of an isoform that cannot bind phosphoinositides and tubulate membranes. This causes disruption of T-tubule biogenesis and muscle weakness in DM1[3]

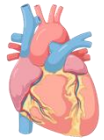
BIN1, bridging Integrator-1; CELF1, CUGBP Elav-like family member 1; CLCN1, chloride voltage-gated channel 1; DM1, myotonic dystrophy type 1; MBNL, muscleblind-like.

1. Charlet BN, et al. *Mol Cell* 2002;10:45–53; 2. Mankodi A, et al. *Mol Cell* 2002;10:35–44; 3. Fugier C, et al. *Mat Med* 2011;17:720–725.

Spliceopathy drives the multi-system clinical manifestations of DM1[1]

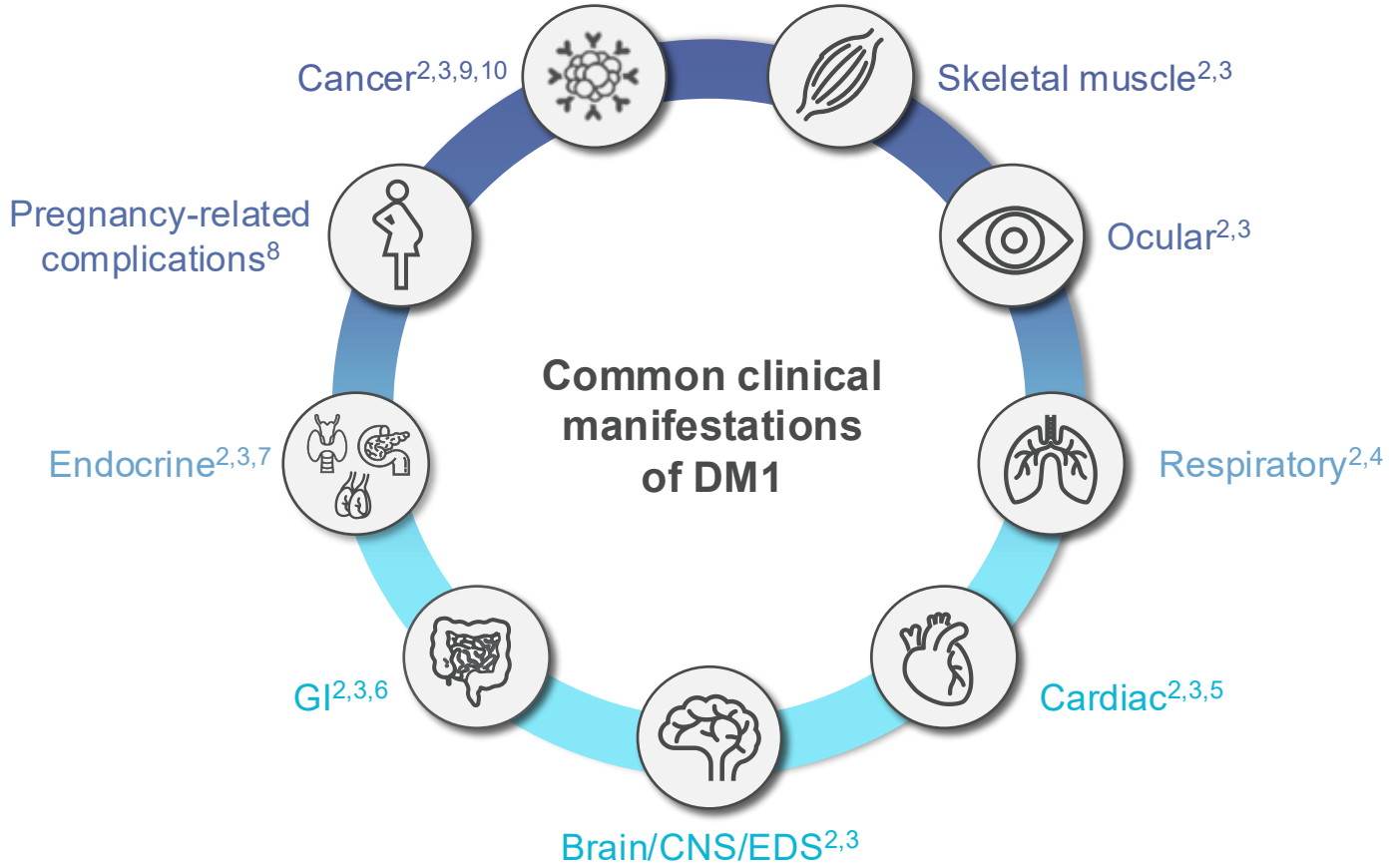
Examples of genes that are alternatively spliced in DM1:[1]

Myotonia and muscle weakness
CLCN1, DTNA, PKM2, MBNL1, BIN1, CACNA1S, RyR1, SERCA1, TNNT3, DMD, CAPN3, NEB, MTMR1, ATP5MC2, NCOR2, S0S1, NFIX



Cardiac conduction complications
SERCA2, LDB3, SCN5A, TNNT2, TTN, MYOM1, ALPK3, RBFOX2

Cognitive impairment
MAPT, NMDAR1, APP, MBNL1, MBNL2



Skeletal muscle-, cardiorespiratory-, and CNS-related effects of DM1 are the main determinants of function and survival[2,3]

CNS, central nervous system; DM1, myotonic dystrophy type 1; EDS, excessive daytime sleepiness; GI, gastrointestinal. Figuresfrom BioRender.
Table adapted from López-Martínez A, et al. *Genes (Basel)*. 2020;11:1109, licensed under a CC-BY 4.0 Creative Commons license; doi: 10.3390/genes11091109.

1. López-Martínez A, et al. *Genes (Basel)*. 2020;11:1109; 2. Bird TD. Myotonic Dystrophy Type 1. 1999 Sep 17 [Updated 2021 Mar 25]. In: Adam MP, Everman DB, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2022; 3. Thornton CA. *Neurol Clin*. 2014;32:705–719; 4. Hartog L, et al. *Front Neurol* 2021;12:658532; 5. Wahbi K, Furling D. *Trends Cardiovasc Med*. 2020;30:232–238; 6. Bellini M, et al. *World J Gastroenterol*. 2006;12:1821–1828; 7. Peric S, et al. *Acta Myol*. 2013;32:106–109; 8. Johnson NE, et al. *J Neuromuscul Dis*. 2015;2:447–452; 9. Gadalla SM, et al. *JAMA*. 2011;306:2480–2486; 10. Win AK, et al. *Mayo Clin Proc*. 2012;87:130–135.

CASI quantifies RNA missplicing in DM1[1]

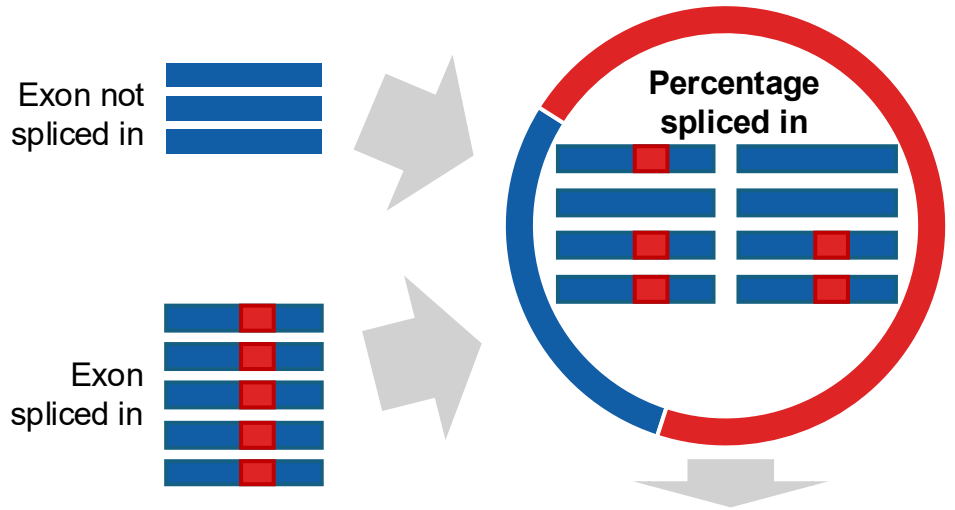
CASI quantifies RNA missplicing across a panel of 22 genes implicated in the pathophysiology of DM1[2–5]

Muscle weakness and/or altered contraction and relaxation[2,3]	Aberrant mRNA splicing[2,3]
RYR1 (calcium channel) *CACNA1S* (calcium channel) *ATP2A1* (calcium pump) *CLCN1* (chloride channel) *DMD* (muscle structure) *BIN1* (component of T-tubules) *SOS1* (cell cycle regulation) *OPA1* (mitochondrial dynamics)[4]	*MBNL1* (alternative splicing) *MBNL2* (alternative splicing)
	Insulin resistance[2,3]
	INSR (glucose metabolism)

Undefined, regulated by MBNL[2,6]

VPS39 (vesicle fusion)	*ANK2* (membrane targeting)
GOLGA4 (membrane trafficking)	*BEST3* (ion channel)
KIF13A (motor protein)	*CCPG1* (GTPase regulation)
CLASP1 (microtubule dynamics)	*CAPZB* (actin filament assembly)
GFPT1 (glucose metabolism)[3]	*NFIX* (extracellular matrix component)[3]
CAMK2B (calcium signaling)	

Calculating CASI[1,5]



Normalize to standardized reference from healthy controls and average

CASI

0 Unaffected healthy control

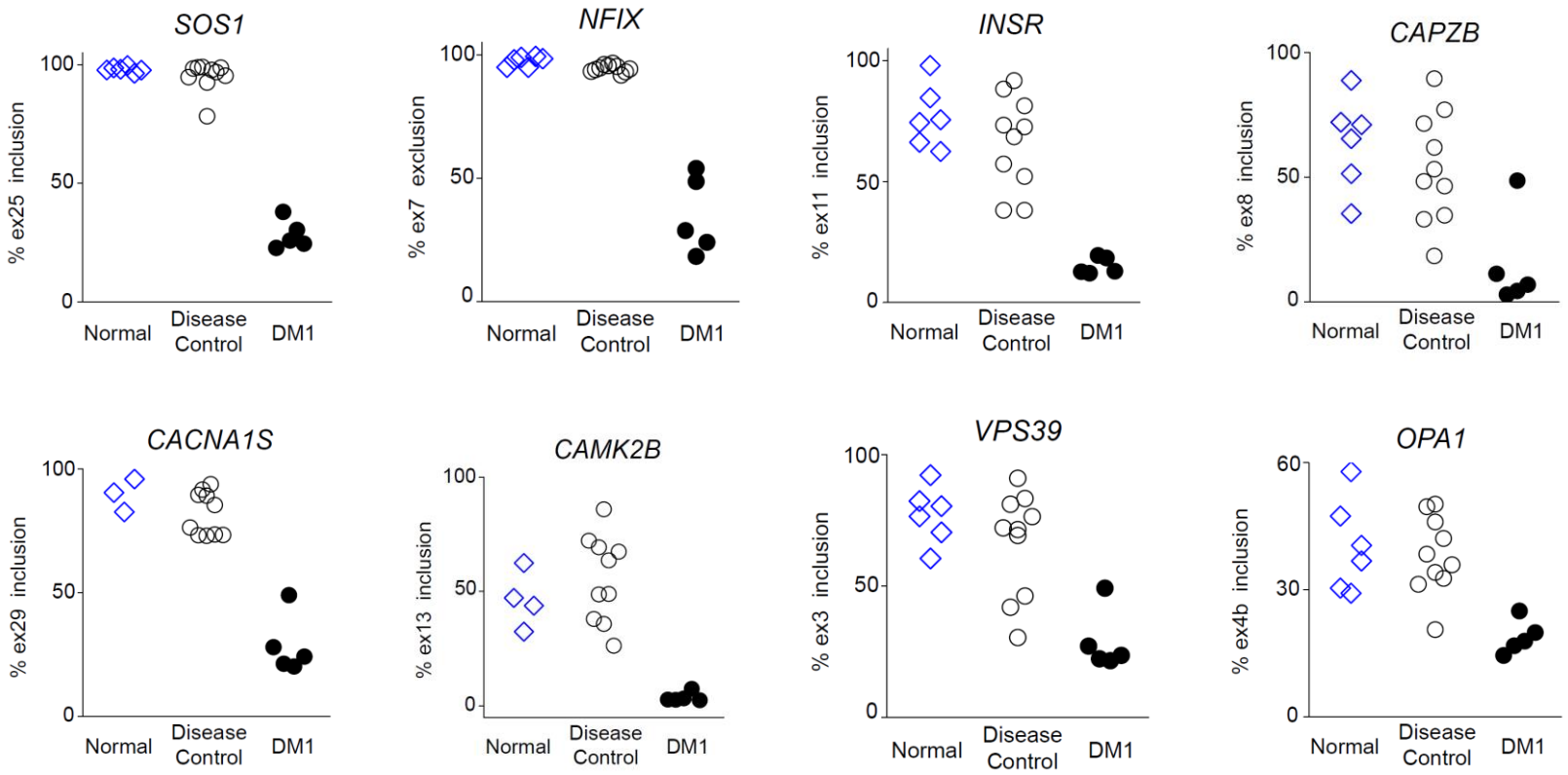
Most affected DM1 individuals **1**

CASI, composite alternative splicing index; DM1, myotonic dystrophy type 1; MBNL, muscleblind-like; mRNA, messenger ribonucleic acid.

1. Berglund JA, et al. *J Neuromuscul Dis*. Published online August 14, 2025. doi:10.1177/22143602251365101; 2. Wang W. 2017. University of Rochester School of Medicine and Dentistry PhD thesis. Accessed February 19, 2025. http://hdl.handle.net/1802/32572; 3. López-Martínez A, et al. *Genes (Basel)*. 2020;11:1109; 4. Mikhail AI, et al. *Trends Mol Med*. 2023;29:512–529; 5. Provenzano M, et al. *J Clin Invest*. 2025;135:e185426; 6. Gene functions from AmiGO 2. Accessed February 19, 2025. https://amigo.geneontology.org/amigo.

A sample panel of genes included in CASI exhibit dysregulated alternative splicing in TA muscle of individuals with DM1[1,2]

Splicing outcomes in DM1 vs healthy and disease controls*[1]



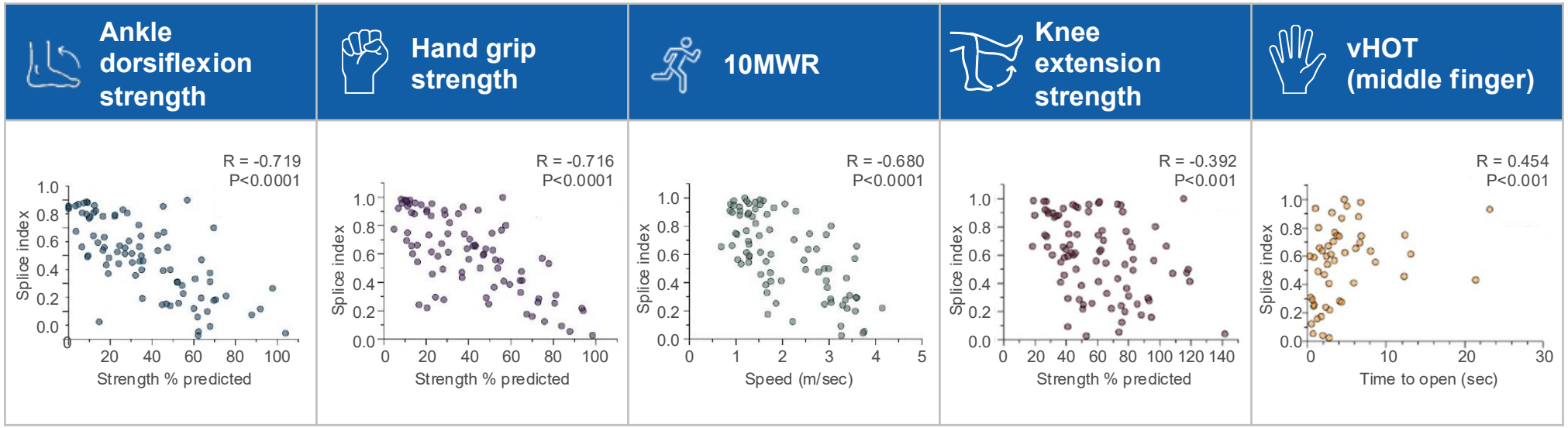
Dysregulated alternative splicing for genes included in the 22-gene panel is generally specific to DM1[1,2]

*Normal n=3–6; disease control n=9–10; DM1 n=5–6. Disease control includes patients with chronic muscle disease.
DM1, myotonic dystrophy type 1; TA, tibialis anterior.

1. Nakamori M, et al. *Ann Neurol*. 2013;74:862–872 (including supplement); 2. Provenzano M, et al. *J Clin Invest*. 2025;135:e185426.

CASI correlated with functional outcomes in individuals with DM1

- The missplicing of RNA has been shown to correlate with functional outcomes in several natural history studies of DM1 cohorts[1–4]
- In a study of 95 muscle biopsies taken from adults with DM1, a composite missplicing score was shown to correlate with common clinical outcome measures for DM1:[3]



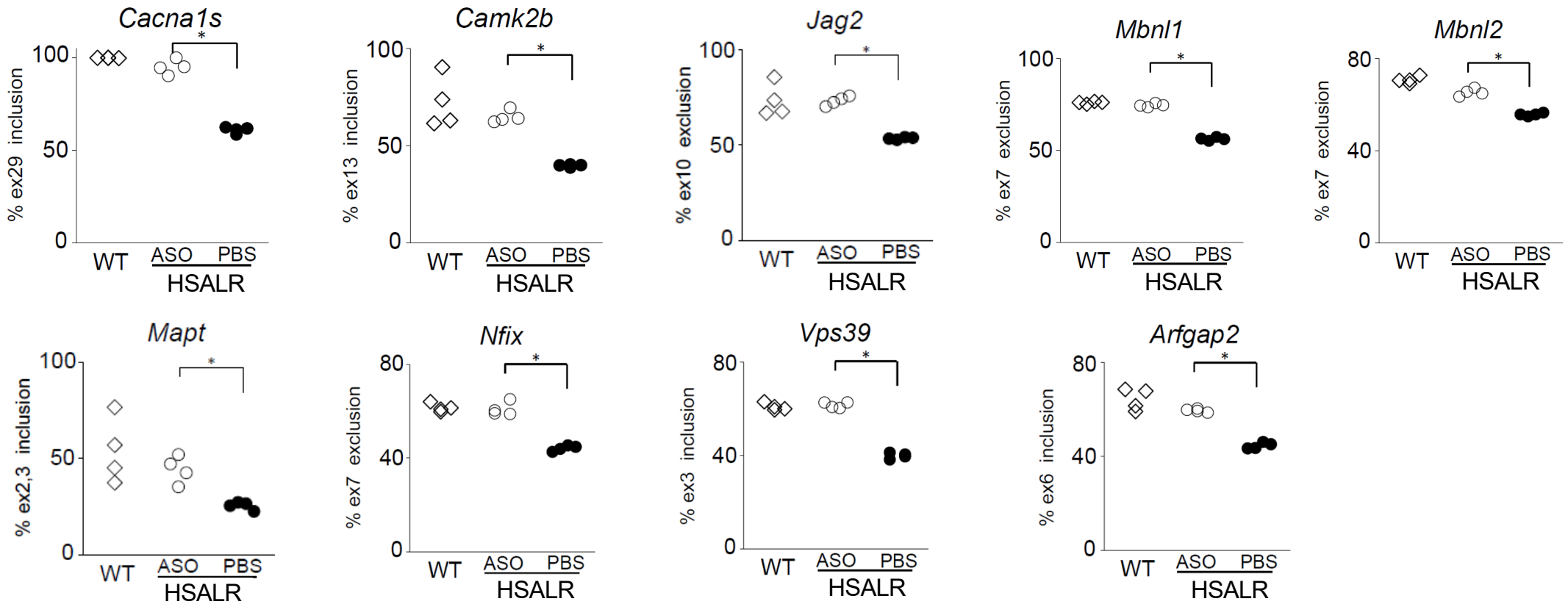
All correlations are reported as Pearson or Spearman r [95% CI] with two-tailed p-value.

10MWR, 10-meter walk/run; CASI, composite alternative splicing index; CI, confidence interval; DM1, myotonic dystrophy type 1; RNA, ribonucleic acid; SI, splice index; vHOT, visual hand opening time.

1. Nakamori M, et al. *Ann Neurol.* 2013;74:862–872 (including supplement); 2. Wang ET, et al. *Hum Mol Genet.* 2019;28:1312–1321; 3. Provenzano M, et al. *J Clin Invest.* 2025;135:e185426;
4. Hartman JM, et al. *Ann Clin Transl Neurol.* 2024;11:3175–3191.

Antisense oligonucleotide treatment corrected splicing in a panel of genes in a preclinical model of myotonic dystrophy[1]

Splicing correction in HSALR mice receiving 8 subcutaneous injections of an ASO over 4 weeks[1]



ASO treatment reversed splicing defects to levels observed in wild-type mice[1]

Splicing was evaluated in quadriceps muscle. *p<0.01.
HSALR mice are a transgenic model of myotonic dystrophy that express a human *ACTA1* gene with ~220 CTG repeats in the 3'UTR.[2]
ASO, antisense oligonucleotide; CTG, cytosine-thymine-guanine; HSALR, human skeletal actin long-repeat; PBS, phosphate-buffered saline; UTR, untranslated region; WT, wild type.

1. Nakamori M, et al. *Ann Neurol*. 2013;74:862–872; 2. Tanner MK, et al. *Nucleic Acids Res*. 2021;49:2240–2254.

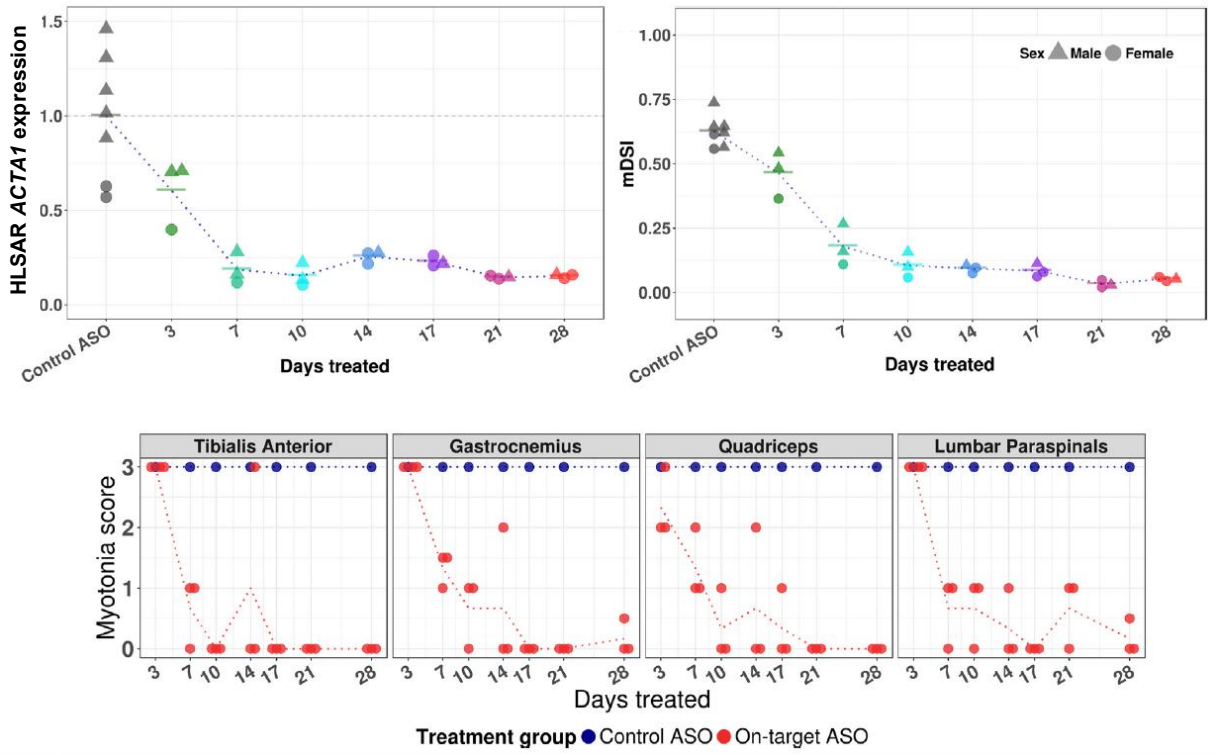
Antisense oligonucleotide treatment corrected myotonia 7 days after the first injection in a preclinical model of myotonic dystrophy[1]

Responsivity of splice index and myotonia in HSALR mice receiving 8 subcutaneous injections of an ASO over 4 weeks[1]

ACTA1 is disease causative in the HSALR mouse model, reproducing several features of DM1[2]

ACTA1 is expressed across several skeletal muscle groups[2]

HSALR mice were treated with an ASO targeting the 3' UTR of the *ACTA1* mRNA[1]



Myotonia is reversed rapidly upon correction of missplicing[1]

HSALR mice are a transgenic model of myotonic dystrophy that express a human *ACTA1* gene with ~220 CTG repeats in the 3'UTR. *ACTA1* expression and splicing were evaluated in quadriceps. Myotonia was assessed by electromyography of indicated muscles and reported as myotonic discharge score (discharges with nearly all needle insertions = 3, >50% = 2, <50% = 1, and 0% = 0). Dotted lines indicate mean transgene expression, Mouse DM1 Splicing Index, or myotonia scores in respective treatment groups.[1]

ASO, antisense oligonucleotide; CTG, cytosine-thymine-guanine; CUG, cytosine-uracil-guanine; mDSI, mouse DM1 splicing index; RNA, ribonucleic acid.

1. Tanner MK, et al. *Nucleic Acids Res*. 2021;49:2240–2254; 2. Hicks SM, et al. *Mol Ther Nucleic Acids*. 2024 Sep 13;35:102338.

Summary

- DM1 is a neuromuscular disorder with multisystemic involvement[1]
- DM1 is caused by expansions in an unstable CTG repeat region in the *DMPK* gene. The mRNA forms hairpin-loop structures that sequester MBNL splicing regulators into toxic nuclear foci, leading to widespread dysregulation of RNA splicing (spliceopathy)[2]
- Spliceopathy in DM1 causes an abnormal persistence of fetal patterns of alternative splicing of hundreds of genes in adult tissues and drives the multisystemic clinical manifestations of the disease[2]
- Splicing in DM1 is measured using a 22-gene panel which includes genes for which dysregulated alternative splicing is generally specific to DM1 and that are functionally representative of the multisystemic nature of the disease[2–4]
- Dysregulated alternative splicing in DM1 correlates with measures of muscle strength, and therapeutic correction of splicing with antisense oligonucleotides improves myotonia in a preclinical model of myotonic dystrophy[3,5]

DM1, myotonic dystrophy type 1; CUG, cytosine-uracil-guanine; DMPK, dystrophia myotonica protein kinase; MBNL, muscleblind-like; mRNA, messenger ribonucleic acid; RNA, ribonucleic acid; UTR, untranslated region.

1. Liao Q, et al. *Neuroepidemiology*. 2022;56:163–173; 2. López-Martínez A, et al. *Genes (Basel)*. 2020;11:1109; 3. Provenzano M, et al. *J Clin Invest*. 2025;135:e185426; 4. Nakamori M, et al. *Ann Neurol*. 2013;74:862–872 (including supplement); 5. Tanner MK, et al. *Nucleic Acids Res*. 2021;49:2240–2254.