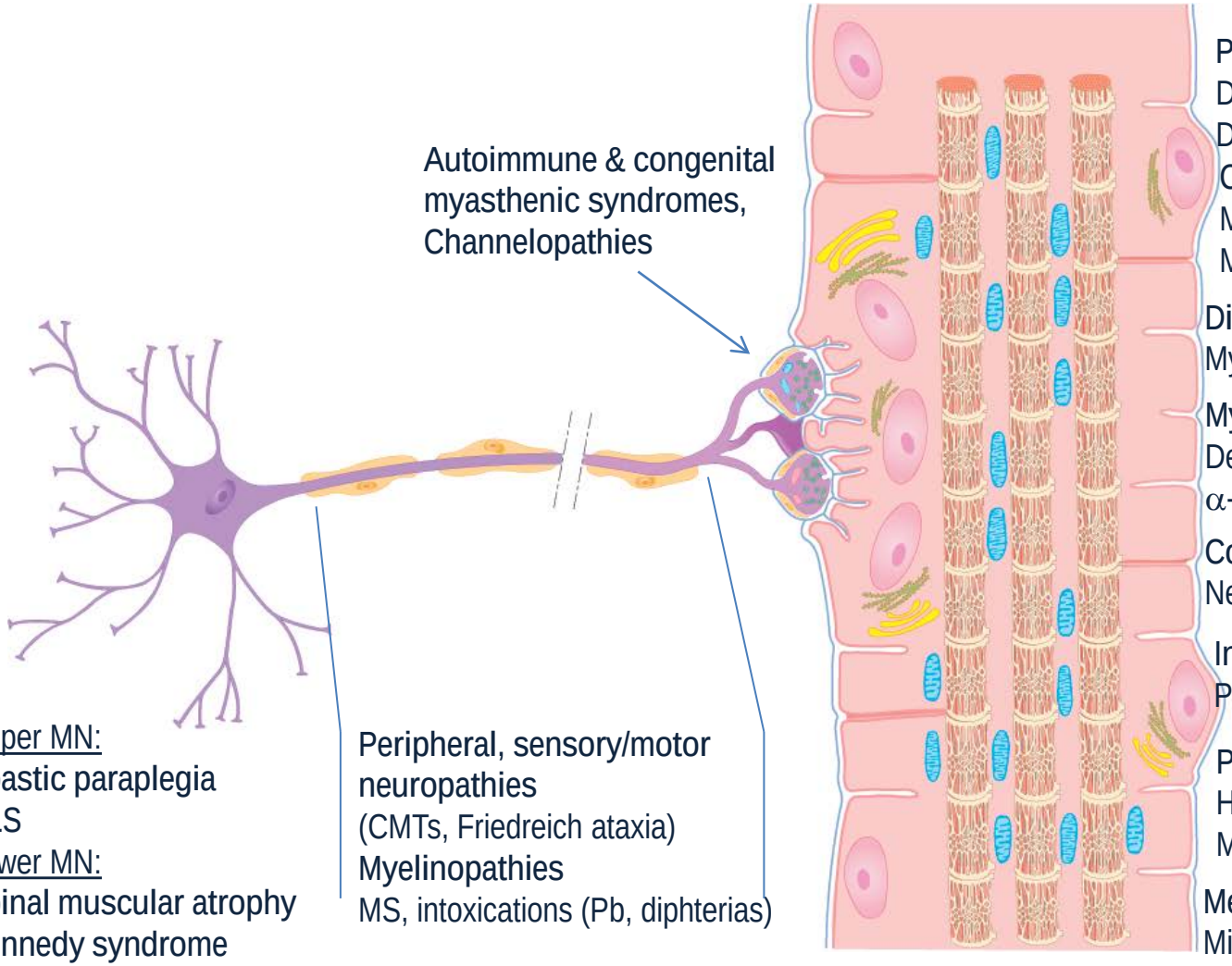
AFMTELETHON CURE THROUGH INNOVATION 7&8 DECEMBER 2012

From yeasts to large mammalian models of neuromuscular diseases. *No_body is perfect*

Serge Braun, PharmD, PhD

AFMTÉLÉTHON 7&8 DECEMBER 2012
CURE THROUGH INNOVATION

> 300 diseases



Autoimmune & congenital myasthenic syndromes, Channelopathies

Progressive muscular dystrophies
Duchenne, LGMDs, FSHD, DM1/2, DMOP

Congenital muscular dystrophies
Merosine, Fukuyama, Ullrich, Bethlem, MEB diseases

Distal myopathies
Myoshi, Nonaka, Laing

Myofibrillar myopathies
Desminopathy, zaspopathy, α-B cristallinopathy

Congenital myopathies
Nemaline, central core, centronuclear

Inflammatory myopathies
Polymyositis, IBM, Dermatomyositis

Periodic paralysis / channelopathies
Hyper/hypo-kaliemic periodic paralysis
Myotonias

Metabolic myopathies
Mitochondrial, Lipid/Glycogen storage dis

Toxin/drug/endocrine/carcinoma-myopathies
Steroid, alcohol, colchicine, cachexia

Upper MN:
Spastic paraplegia
ALS

Lower MN:
Spinal muscular atrophy
Kennedy syndrome
CMT2
(ALS)
Congenital Amyotrophies
(lower, upper legs)

Peripheral, sensory/motor neuropathies
(CMTs, Friedreich ataxia)
Myelinopathies
MS, intoxications (Pb, diphterias)

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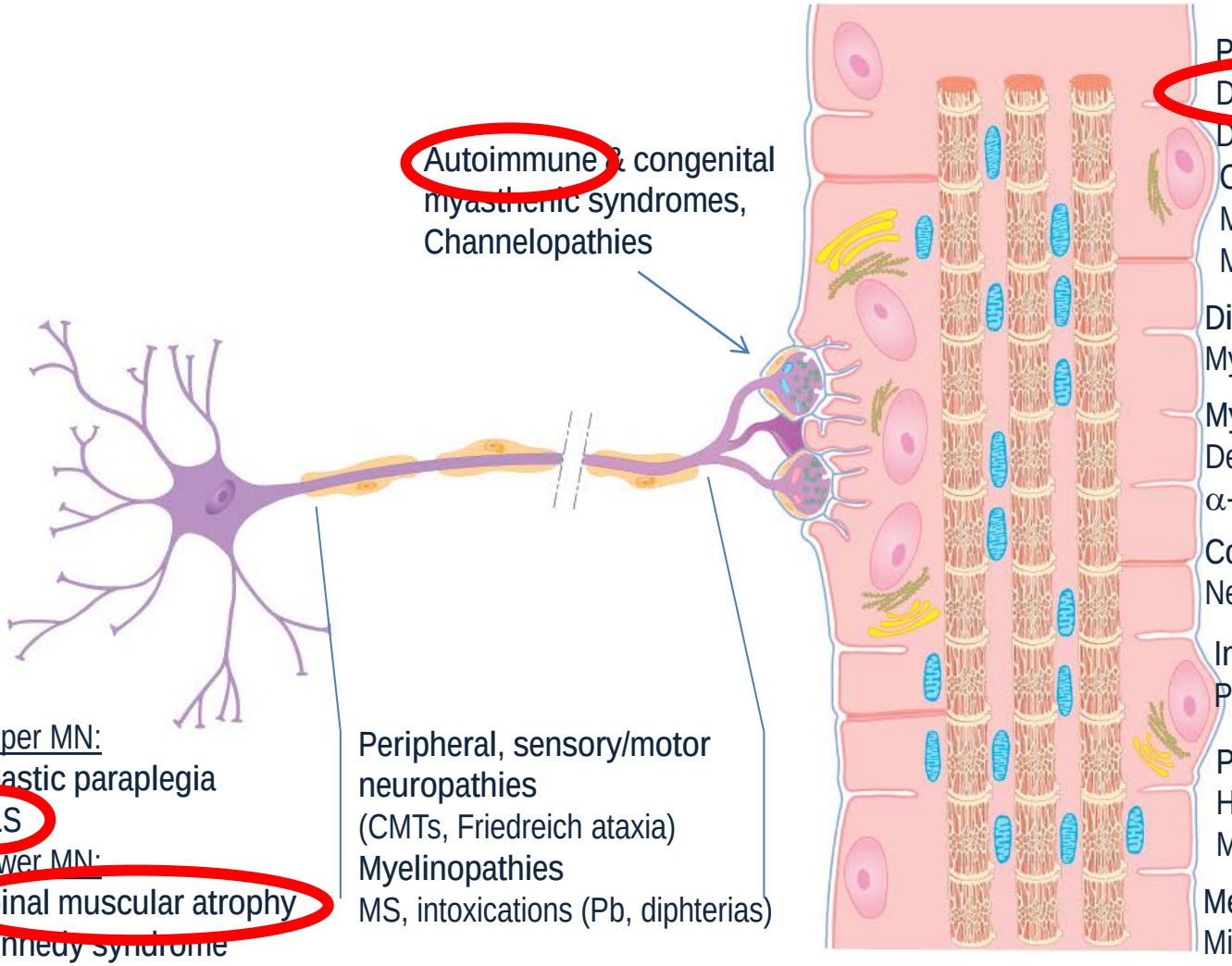

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« A model is something simple made by scientists to help them in understanding something complicated » (Segev, 1993)

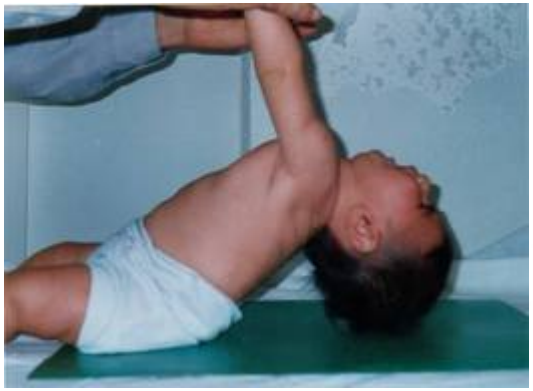





	Yeast	Worm	Fly	Zebrafish	Mouse	Human
Genes	5-6000 (12Mb)	21 000 (100Mb)	17 000 (123Mb)	15 700 (1.2Gb)	~ 23 000 (2.8Gb)	~ 21 000 (3.3Gb)
Life cycle	2 hours	3 days	12 days	3 months	4 months	*Too long*
Genes homology with human	20%	50%	60%	80%	85%	100%
Conserved pathways	50%	70%	90%	95%	99%	100%
Nervous system	**No**	Yes	Yes	Yes	Yes	Yes
Survival motor neuron pathway	**No**	Yes	Yes	Yes	Yes	Yes
Potential for high-throughput screening	Yes	Yes	Yes	Yes	**No**	**No**

Spinal muscular atrophy

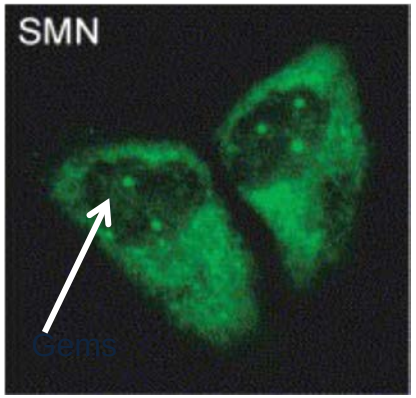
SMA Type	Age of Onset (months)	Motor Milestones	Age of death (years)
I	<6 months	Never sit	<2 years
II	<18 months	Sit, but never stand, non ambulant SMA	>2 years
III	> 18 months	Stand, ambulant SMA	Juvenile, Adult



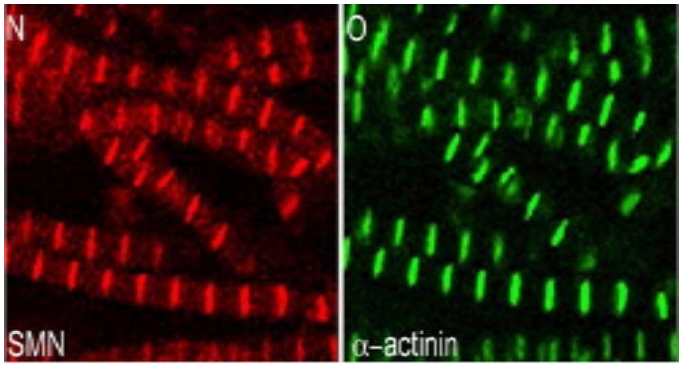
- Incidence: ~1:6000
- Carrier prevalence in the general population 1/40.

SMN protein localization

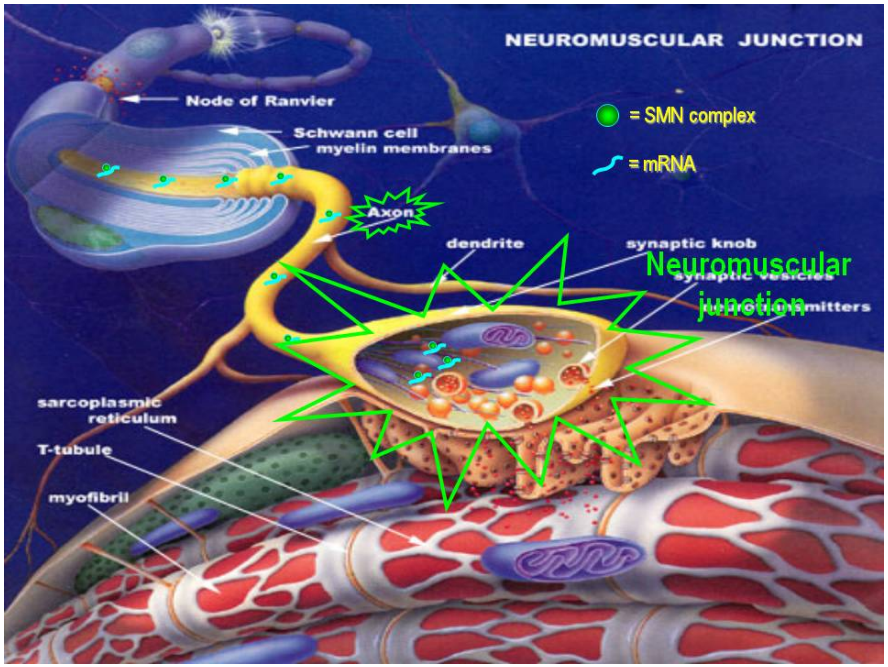
- Widely expressed
- Diffusely in cytoplasm and within small punctate nuclear structures (gems)
- Present and moves rapidly and bi-directionally in the axon. Also enriched in the growth cone
- NMJ endplate and to the Z-line of myofibrils.



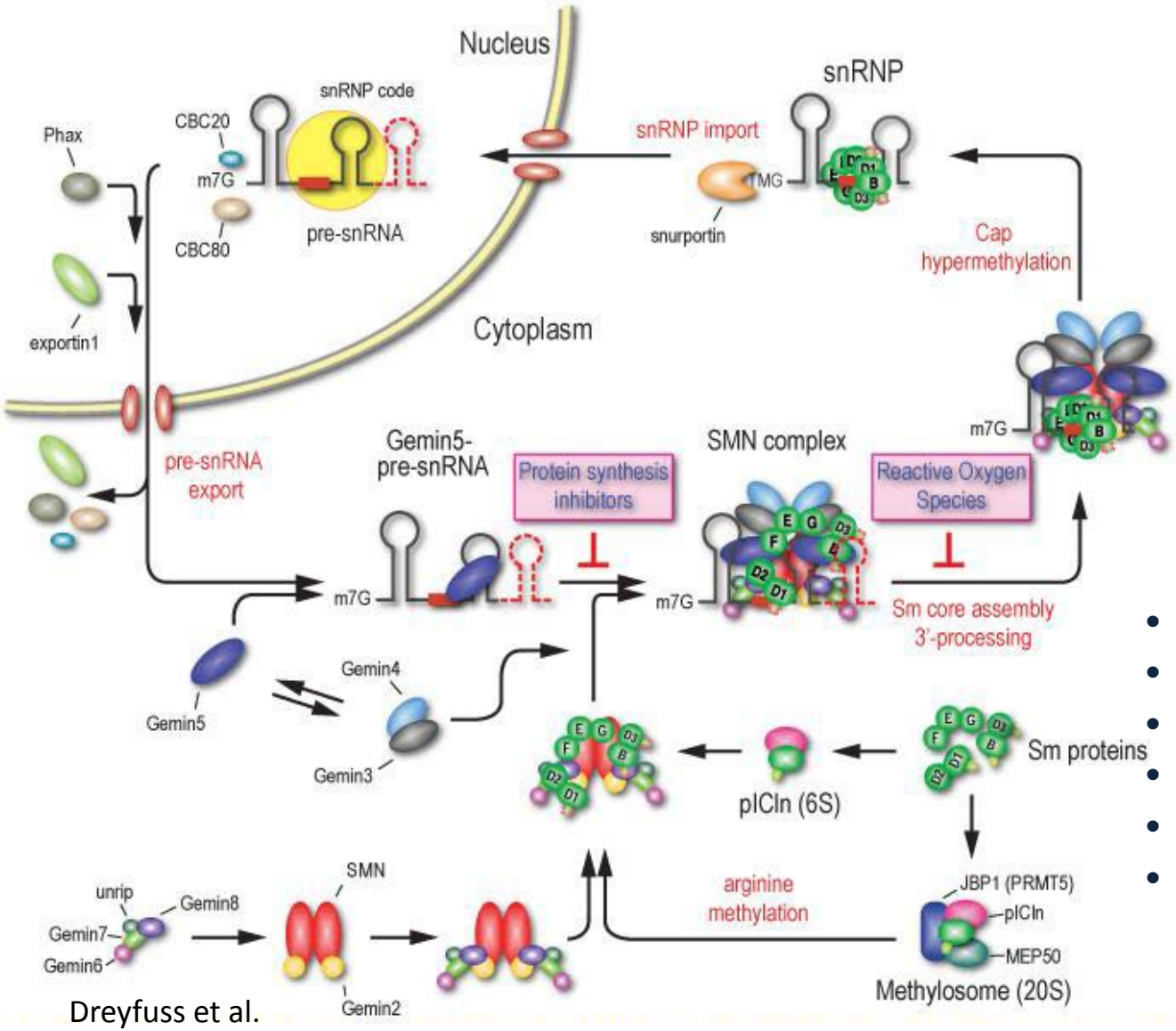
Gubitz et al. 2004



Rajendra et al. 2007



SMN complex and spliceosomal snRNPs assembly

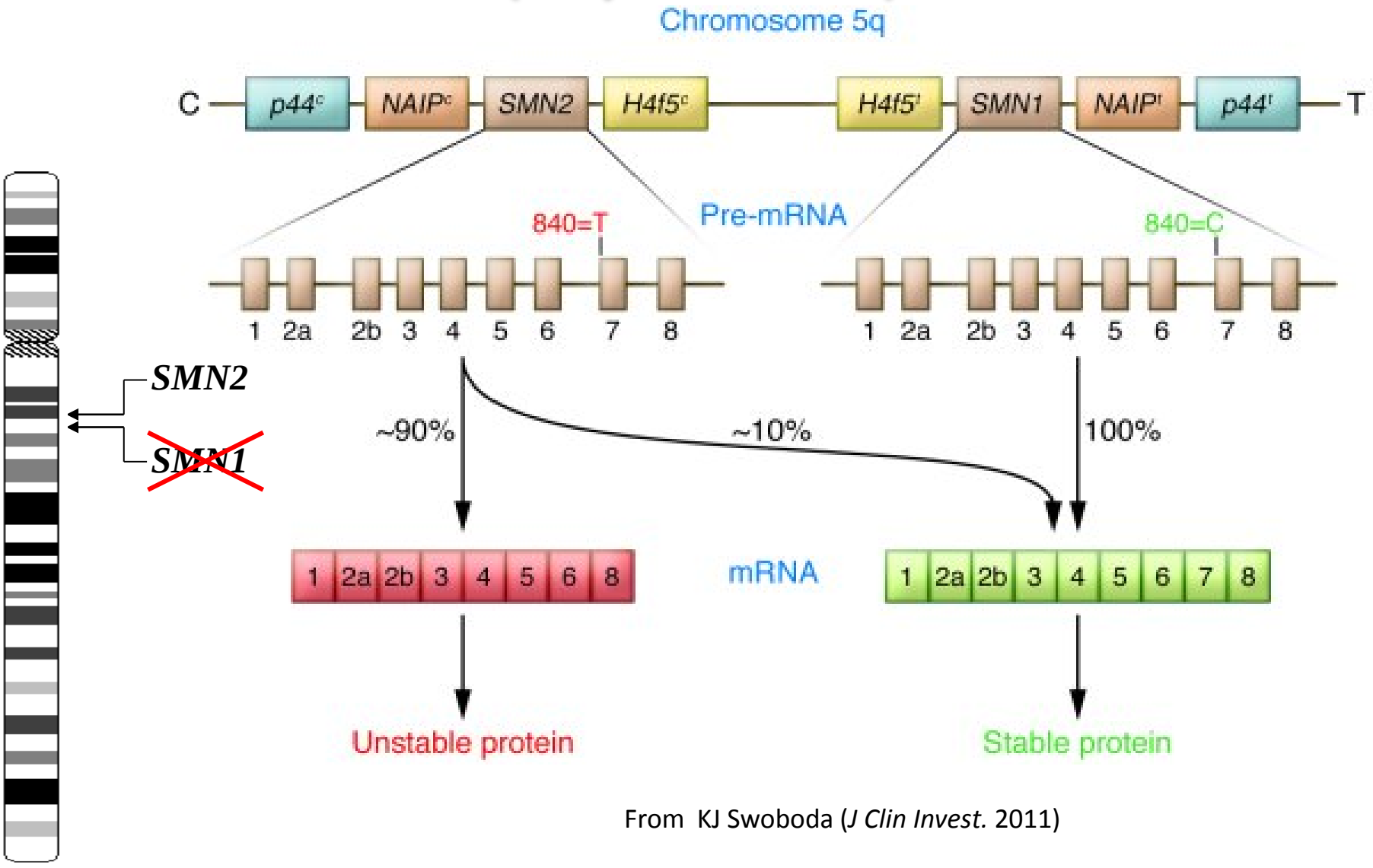


Dreyfuss et al.

SMN Functions :

- Assembly of snRNPs
- Transcription
- Neurone outgrowth
- Cytoskeleton dynamics
- Axonal transport of mRNA
- NMJ formation and maintenance

A tale of two SMNs (only in humans)



From KJ Swoboda (*J Clin Invest.* 2011)

	Ortholog gene	Role in snRNP assembly		Defective strains	Impact on viability	Neuromuscular phenotype	Deleterious impact of SMN over-expression
		TUDOR RNA binding domain	Ortholog of gemin2				
Schizosaccharomyces pombe 	Yab8 (ySMN)	-	YIP1p	mutant	retarded growth	0	+
Saccharomyces cerevisiae	no ortholog		Brr1p				
C. elegans 	C41g7.1	+	SMI-1	egl-32	decrease progeny	Neuronal defects, uncoordinated locomotion, poor muscle tone. Partial rescue with neuronal SMN	++
 **D. melanogaster**	Pos. 73A9			SMN^{E33}	late larval lethality	Pre- and postsynaptic mismatching, desorganized thoracic muscles, no flying/jumping (due to disrupted SMN-actin interaction). **Rescue if SMN in both muscles and nerve**	no
Danio rerio (~same with xenopus laevis) 	smn	snRNP assembly		Morpholino smn knockdown Gemin 3 null mutations	20%lethality	Spinal motoraxon defects only (Smn high early due to maternal RNAs/protein). Defective synaptic maintenance. **Rescue with only nerve expression + an sn-RNP-independent function of SMN on axon outgrowth**	
				smnY262stop smnL265stop smnG264D missense	survival until second week larval	Same defects **+ rescue with hSMN driven by the motoneuron-specific** zebrafish hb9 promoter	
						Plastin 3 rescues axon defects (also seen in SMA unaffected siblings)	
				smnY262stop h*SMN2*	same	Disruption of an intronic splicing silencer --> modest increase in survival, and delay in the presynaptic defect	

Mouse models

Genotype	Severity	
Smn−/−	++++	**Death of embryo** occurs prior to uterine implantation.
Smn+/−	+	**Early acute loss of lumbar spinal cord motor neurons** (~30% within 5 weeks), with subsequent slowly progressive reduction over an extended time scale.
Smn−/−; *SMN2+/+*; *SMN1(A111G)+/−*	+	Transgene containing the *SMN1* allele seen in Type I and II patients; survival with no obvious phenotype.
Smn−/−; *SMN2+/+*; *SMN1(VDQNQKE)+/−*	+++	Transgene containing *SMN1* exons 1–6 with an additional motif; has little effect on lifespan extension.
Smn+/−; *Gemin2+/−*	+	**Mice with heterozygous deletion of *Smn* and *Gemin2*** display an **accelerated loss of motor neurons** compared with *Smn+/−* mice.
Smn−/−; *SMN2(89Ahmb)+/−*; *SMN1(A2G)+/−*	+	Mean survival of mice with a single *A2G* transgene and one copy of ***SMN2*** is 227 days, whereas mice homozygous for *A2G* are relatively indistinguishable from controls.
Smn2B/−	++	*Smn* transgene harboring three nucleotide substitutions within the exonic splicing enhancer of exon 7. Mean lifespan: 28 days.
SmnF7/F7; *Alfp-Cre+*	++++	*SmnF7/F7* mice with **Cre-*loxP*-mediated deletion of *Smn* exon 7 in hepatocytes.** Causes **late embryonic lethality** at E18. Heterozygous deletion has no obvious effect.

Short presymptomatic period followed by a fulminant decline

Partially related to the snRNP assembly mechanism

Embryonic lethality reflects neuronal and non-neuronal cell deficit

But strain-background differences
→ may significantly confound interpretation
→ suggest gene modifiers

SMA is a disease of low levels of SMN protein

Optimized animal model of SMA needs both a deletion/dysfunction of the ***SMN1*** gene and the presence of the ***SMN2*** gene

Only humans have the *SMN2* gene

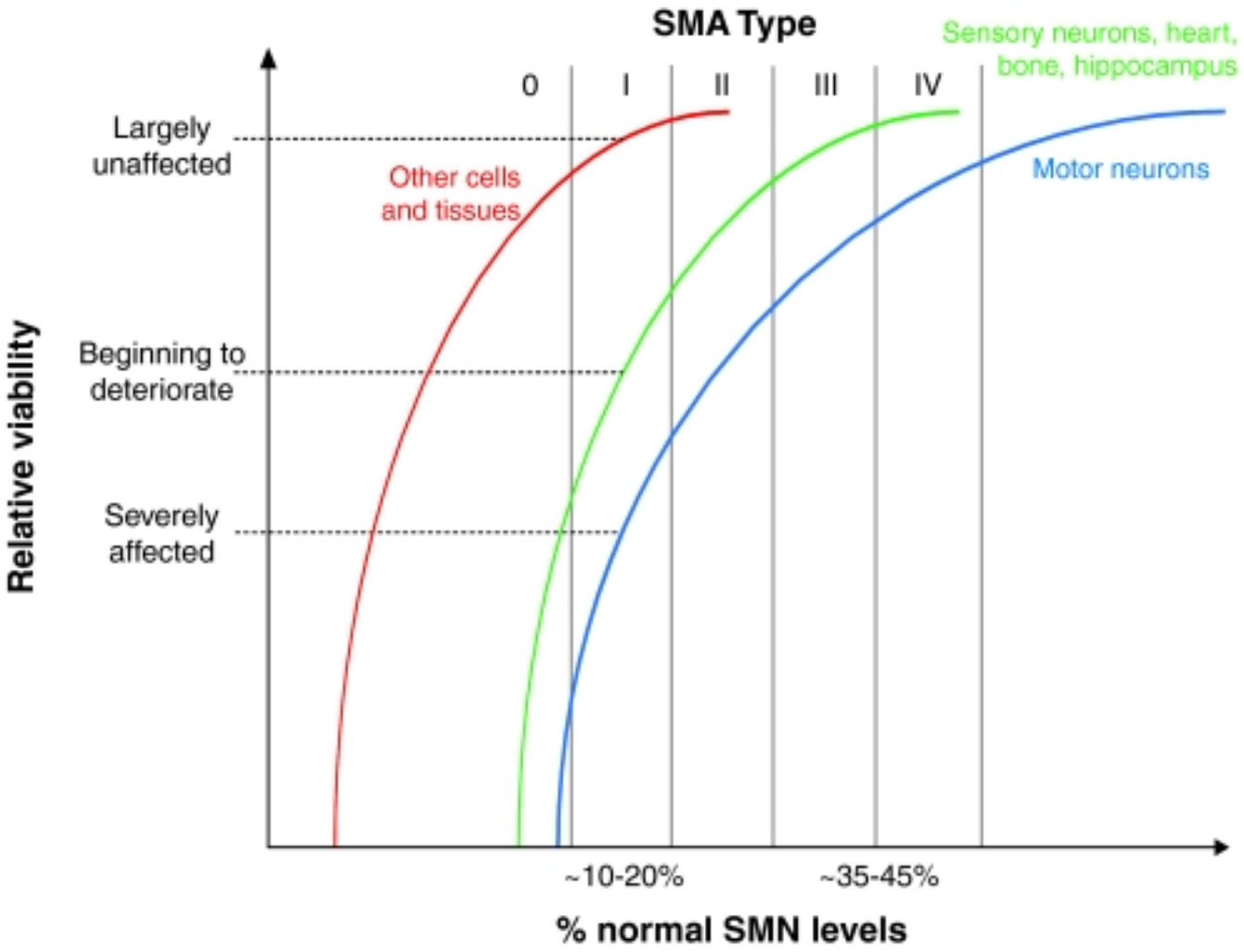
The best way to generate an animal model of SMA is to add the ***hSMN2* as a transgene to an animal with a deleted/mutated *SMN1***

SMN2 copy number correlates with disease in humans and mice

Genotype	Severity	
Smn −/−; *SMN2(2Hung)* +/+	+ to +++	Transgene including **human *SMN2***, *SERF1* and part of *NAIP*; **rescues embryonic lethality of *Smn* −/−.**
		Transgene **copy number correlates with disease severity**, which ranges from death within 1 week to normal survival
Smn −/−; *SMN2(89Ahmb)* +/+	+ to +++	Transgene containing only the *SMN2* locus, rescues *Smn* −/− embryonic lethality.
		42/56 mice with one or two transgene copies were stillborn or died before 6 hours, with the remainder dying between 4–6 days.
		Mice with eight copies of the transgene reach adulthood
Smn −/−; *SMN2(89Ahmb)* +/+; *SMNΔ7* +/+	+++	Transgene containing **human *SMNΔ7*, the predominant isoform produced by *SMN2*; improves the phenotype** *Smn* −/−; *SMN2* +/+.
		Mean lifespan: 13.3±0.3 days.
Smn −/−; *SMN2(N11)* +/−; *SMN2(N46)* +/−	+++	Mice with three copies of *SMN2* generated by crossing strains with two (N11) and four (N46) copies.
		Mean lifespan: 15.2±0.4 days.
Smn1 *tm1Cdid/tm1Cdid*; *Cre* *Esr1* and *Smn1* *tm2Cdid/tm2Cdid*; *Cre* *Esr1*	++++	Inducible *Smn* alleles that mimic *SMN2* splicing are homozygous embryonic lethal (E12.5–E15.5) and normal when heterozygous.
		In the presence of Cre recombinase, *loxP*-flanked *neomycin* (*Neo*) gene resistance cassettes situated in *Smn* intron 7 are excised, producing full-length Smn.
		When crossed with a tamoxifen-inducible *Cre* allele (*Cre* *Esr1*), **early embryonic induction of full-length *Smn* by tamoxifen can rescue embryonic lethality.**

SMN2 is able to complement the embryonic lethality and reduces severity in a dose-dependent manner

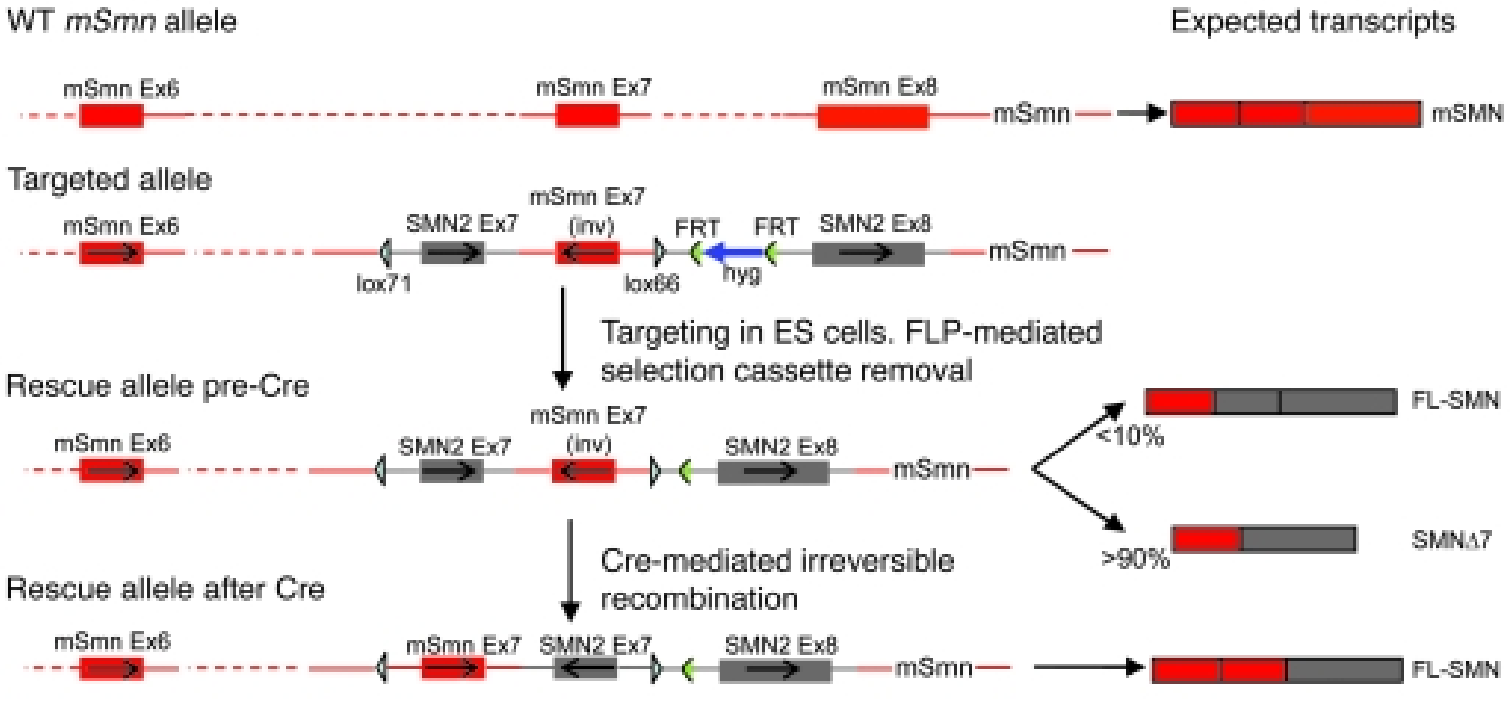
The threshold hypothesis of SMN to partially explain selective motoneuron death



(From Sleigh et al., Dis. Model Mech. 2011)

A time window for the treatment of SMA ?

Hybrid Inducible smn « rescue » allele (Lutz et al. JCI 2011)



Restoration of SMN postsymptomatically → NMD phenotype rescued

But a limited therapeutic window (1/2)

(Lutz et al. JCI 2011)

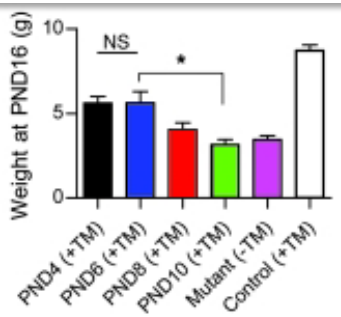
Embryonically activated :

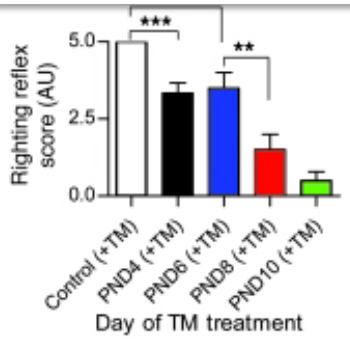
- **E2a-Cre allele → no embryonic lethality**
- **Sox2-Cre allele (expressed in epiblast at E6) → healthy**

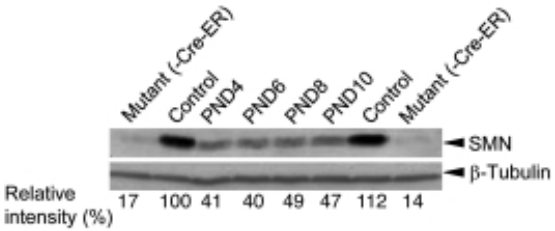
Postsymptomatic restoration : P4, P6 → NMD phenotype rescued but slighty lower body weight

P8 →lower benefit

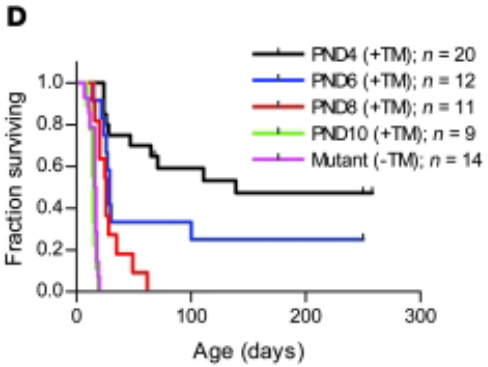
P10 →no rescue

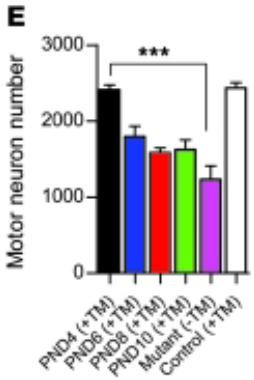


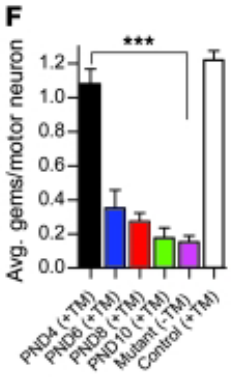




40% WT SMN in SC of all treated mice

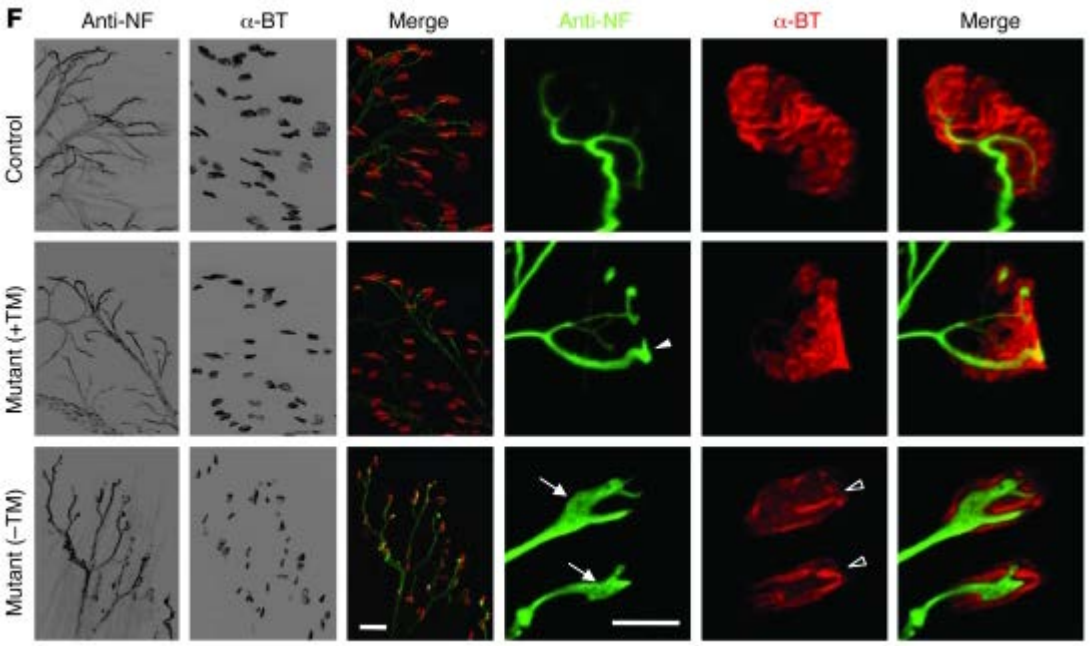




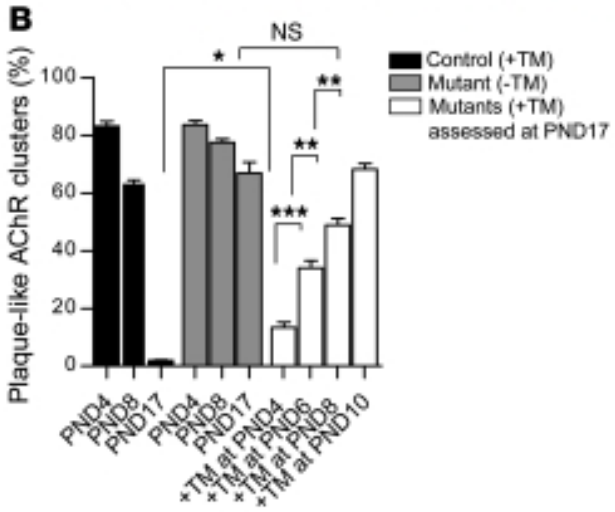


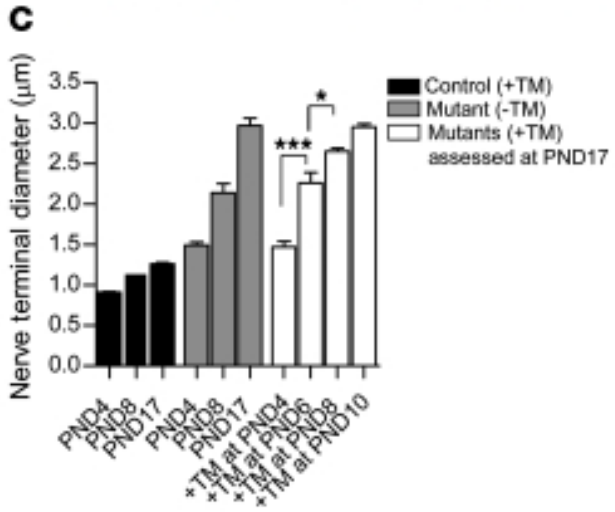
But a limited therapeutic window (2/2)

(Lutz et al. JCI 2011)



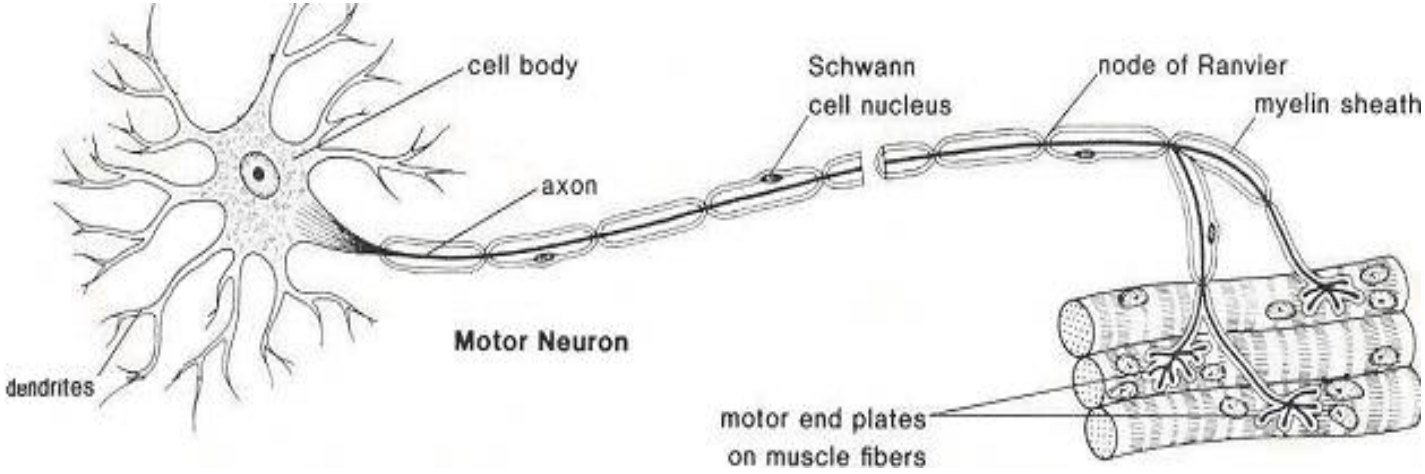
The earlier the protein is restored the lower the defect of the neuromuscular synapse

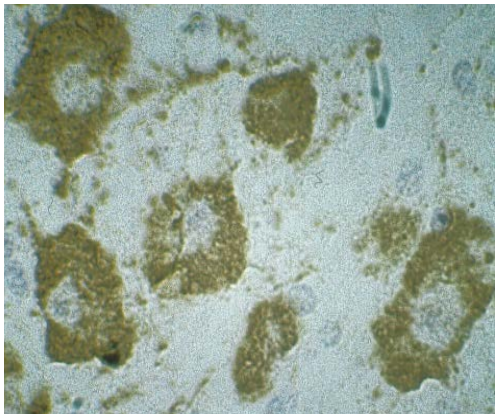




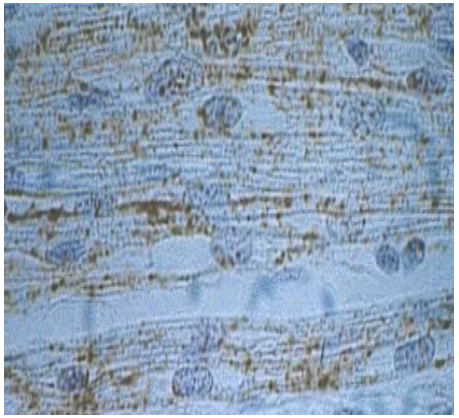
Tissue-specific ?

SMN is expressed ubiquitously

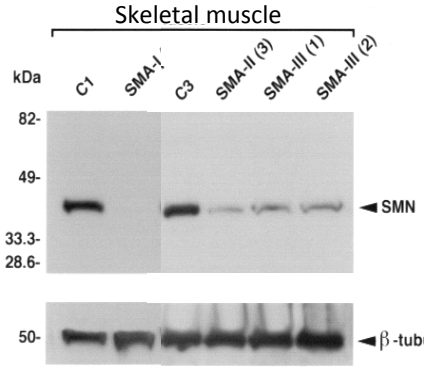




SC

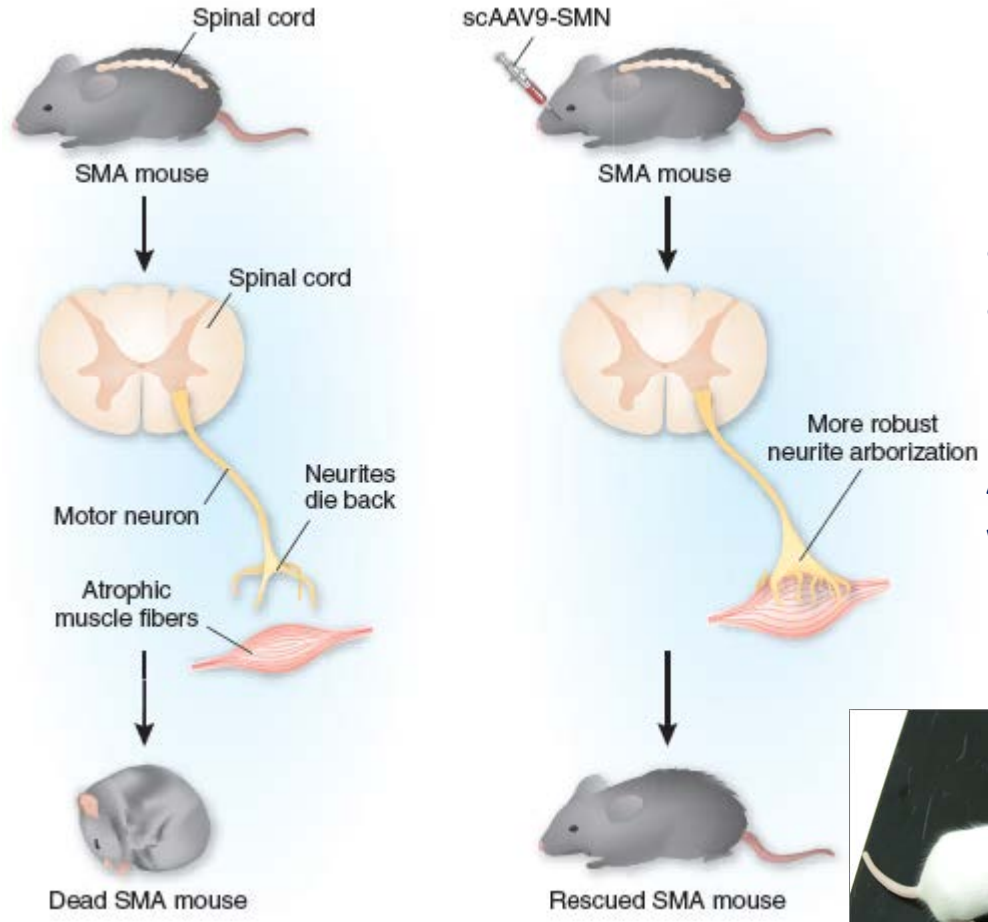


Skeletal muscle



Genotype	Severity	
Smn F7/Δ7 ; NSE-Cre +	++	*Smn F7/Δ7* mice with **Cre-*loxP*-mediated deletion of *Smn* exon 7 in neuronal tissue.**
		Mean lifespan: **25 days**.
Smn F7/Δ7 ; HSA-Cre +	++	*Smn F7/Δ7* mice with **Cre-*loxP*-mediated deletion of *Smn* exon 7 in myoblasts and post-mitotic fused myotubes of skeletal muscle**.
		Mean lifespan: **33 days**.
Smn F7/F7 ; HSA-Cre +	+	*Smn F7/F7* mice with Cre-*loxP*-mediated deletion of *Smn* exon 7 in post-mitotic fused myotubes of skeletal muscle.
		Without heterozygous deletion of *Smn* exon 7 in all somatic cells, animals live for a median of 8 months.
Smn F7/F7 ; NSE-Cre +	++	*Smn F7/F7* mice with Cre-*loxP*-mediated deletion of *Smn* exon 7 in neuronal tissue.
		Mean lifespan: 31±2 days.
Smn F7 /–; SMN2(89Ahmb) +/+; Olig2-Cre +	+	*Smn F7 /–*; ***SMN2* +/+ mice (i.e. *Smn* +/–; *SMN2* +/+) with Cre-*loxP*-mediated deletion of *Smn* exon 7 in spinal cord** motor neuron progenitor cells.
		~**70% of mutants survived to 12 months**, yet were clearly distinguishable from controls.

- Motoneuron cell body loss is due to a **dying back axonopathy**
- **Muscle** (satellite cells) **also plays a primary role** (also seen in the drosophila)
- Why primary impact on lower motoneurons ? (Dose ? Specific splicing defects ?)



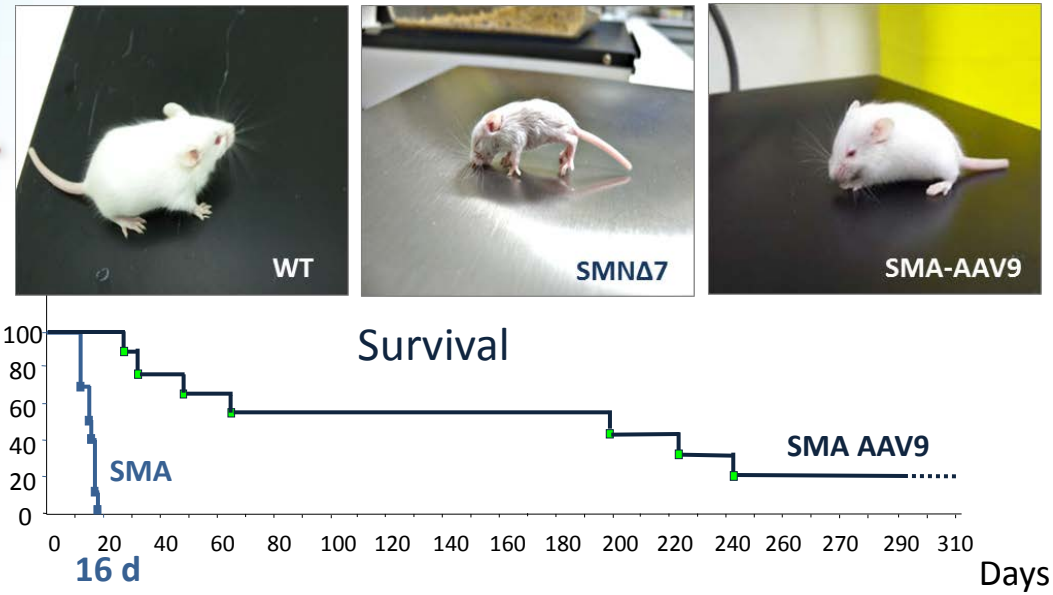
(From MacKenzie A., Nat. Biotech. 2010)

Gene therapy of SMN

- Abolition of SMN is incompatible with life
- Multiple copies of *SMN2* may compensate for loss of *SMN1*

A common requirement: a proper time window

- P0: rescue
- P5: slight increased survival
- P10: no increased survival



Barkats et al.

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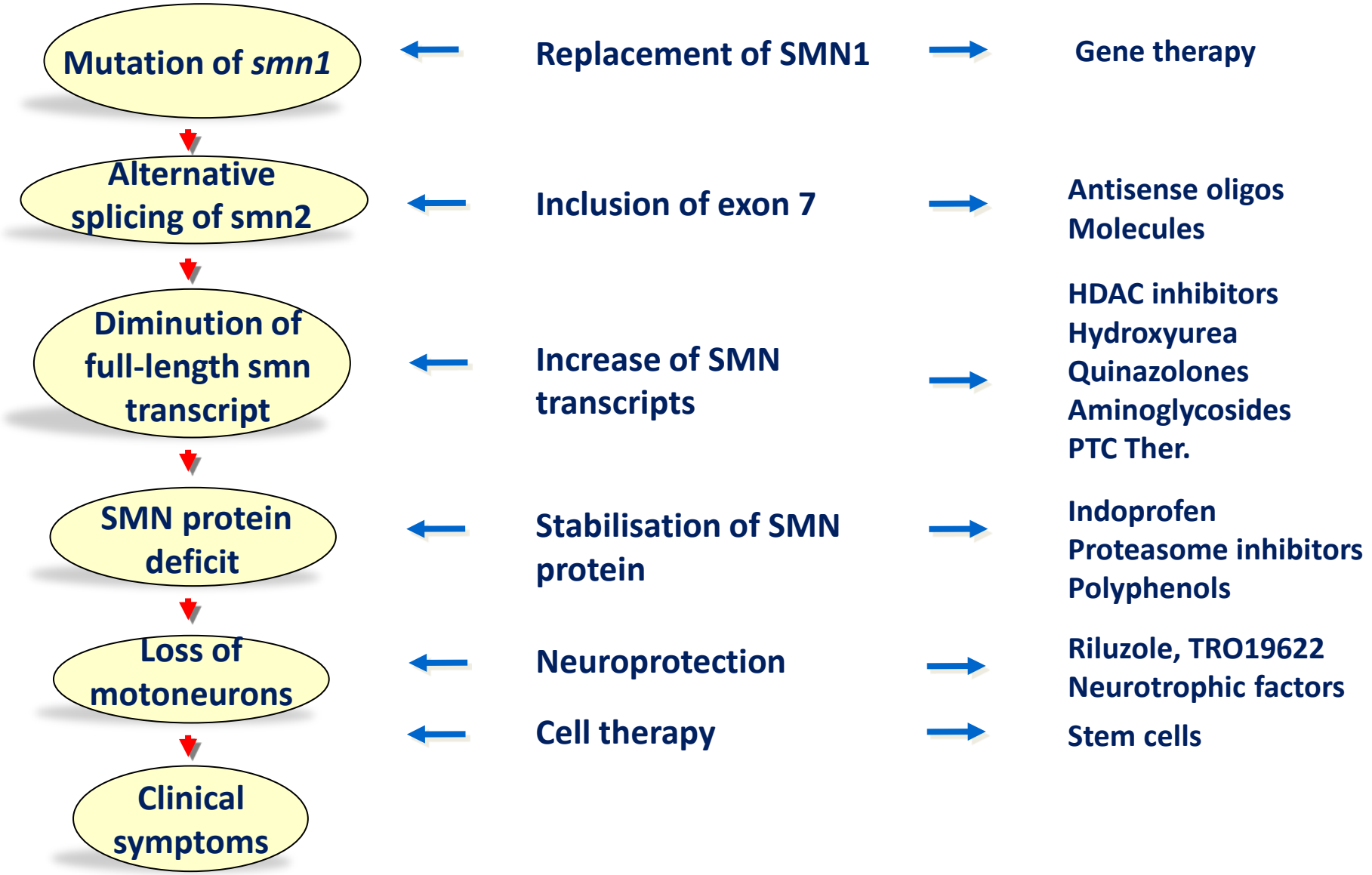
Therapeutic strategies for SMA



What the animal models told us about SMA

SMN serves more as a **MN maintenance** factor rather than being a critical component of the neurodevelopmental process

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The disease may be **treated postsymptomatically**

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Therapy to be delivered **chronically or acutely ?**
(to be addressed by temporally depleting SMN)

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Therapy to be delivered **chronically or acutely ?**
(to be addressed by temporally depleting SMN)

A reduction in SMN levels by 50% worsens motor performance and survival of the SODG93A mouse model of ALS:

→ **SMN is required throughout life**

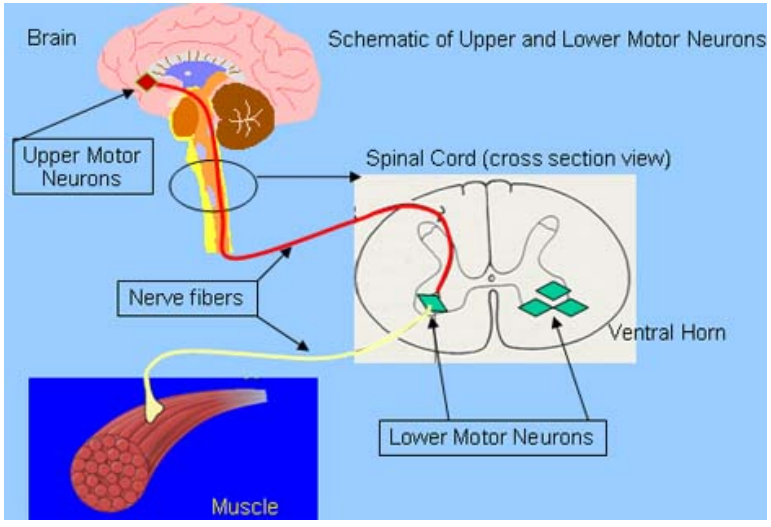
SMN a therapeutic target of ALS ?

Amyotrophic Lateral Sclerosis

Late onset neurodegenerative disorder
Incidence 1-3 / 100.000 / y

Degeneration of **cortical and spinal cord motoneurons** → progressive muscular weakness and death within 3-5 years

- **5 to 10% familial**
- Predominantly sporadic due to a complex gene-environment interactions not yet completely clarified



Related MN models ?

Name	Mutated gene	Gene product	Inheritance	Human disease	Pathway
Wobbler	*VPS54*	Subunit of the GARP complex	recessive		endosome-derived transport vehicles to the trans-Golgi network
Nmd	*IGHMBP2*	Immunoglobulin *μ*-binding protein 2	recessive	SMARD1	RNA processing
Pmn	*TBCE*	tubulin-specific chaperone E	recessive	motor neuropathy HRD/SSS	tubuline-specific chaperone
Loa	*DYNC1H1*	dynactin	dominant	sensory neuropathy	Axonal transport
Cra	*DYNC1H1*	dynactin	dominant	sensory neuropathy	

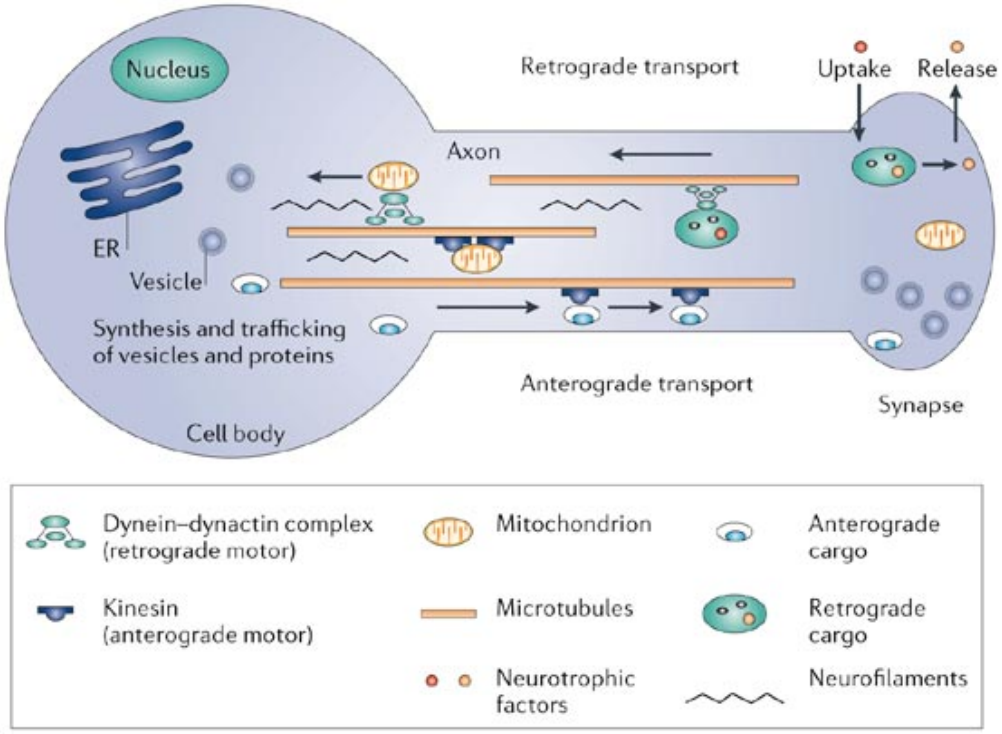
SMARD: spinal muscular atrophy with respiratory distress,
HRD: hypoparathyroidism-retardation dysmorphism syndrome,
SSS: Sanjad-Sakati syndrome,

ALS subtype	Gene symbol	Protein	Protein function	Human phenotype	Animal model	Animal phenotype	Targets
ALS1	*SOD1*	Cu/Zn superoxide dismutase	Detoxification enzyme	Varies among mutations from typical ALS type to atypical ALS	numerous mouse and rat mutants (more rapid progression) including overexpressing hSOD1 G93A	MN death by gain of function. Selective expression models --> **interplay between different cell types**	**Protein misfolding Oxydative stress not the initiating factor.** APP/caspase-6 . Microglia, macrophages, astrocytes, Schwann cells, muscle.
ALS4	*SETX*	Senataxin	Helicase, Repair mechanisms, FGF8 path.	recessive mutations cause ataxia and dominant mutations cause juvenile ALS	yeast orthologue: Sen1p		Transcritpion and RNA metabolism
ALS6 (recessive)	*FUS/TLS*	fused in sarcoma/ translocated in liposarcoma	RNA metabolism and transcription		FUS/TLS shRNA mice (no overexpressing models available)	**No motor phenotype**	Transcritpion and RNA metabolism
ALS10 and FTLD-U	*TARDBP*	Transactivation response DNA-binding protein 43kD (TDP-43)	RNA splicing (hnRNPs)		**Neuronal overexpression** mouse **mutants**, induced rats	**Motor phenotype but not all ALS features**	Transcritpion and RNA metabolism
ALS12	*OPTN*	Optineurin	membrane trafficking, protein secretion, cell division	Mainly Japanese families		**Overexpression --> glaucoma mouse**	Protein trafficking, NF-κB pathway, colocalized with FUS
ALS2 (recessive)	*ALS2*	Alsin	Guanine nucleotide exchange factor (GEF) signaling	Juvenile onset, progressive muscle weakness and paralysis	Als2 -/- mice.	Late-onset degeneration of Purkinje. FVB, but B6 have shorter lifespan. **No MN phenotype**	Endosomal
ALS5 (recessive)	*SPG*	Spatacsin		autosomal recessive juvenile amyotrophic lateral sclerosis and long-term survival	**Morpholino KO Zebrafish**	Early neural development	
ALS8 and SMA	*VAPB*	Synaptobrevin-associated membrane protein B (VAPB)	Vesicular trafficking; acts during ER-Golgi transport and secretion.	Adult onset, slowly progressive upper and lower motor neuron disease. Phenotype varies from SMA type to ALS type	PrP-VAPBP56S	**No MN phenotype**. TDP-43 accumulation in lower MN	Vesicular trafficking
ALS	*CHMP2B*	Charged multivesicular body protein 2B (CHMP2B)	Vesicular trafficking; acts as a component of the ESCRTIII (endosomal secretory complex required for transport) complex	Lower dominant motor neuron disease	KO mice	**Similar to patients**	Vesicular trafficking
CMT4J / ALS 11	*FIG4*	PI(3,5)P2 5-phosphatase FIG4		spongiform degeneration of the central nervous system (CNS) and substantial loss of peripheral neurons from sensory and sympathetic ganglia	**Spontaneous Fig4 (pale tremor) mouse**	extensive spongiform degeneration of the CNS and substantial **loss of peripheral neurons** from sensory and sympathetic ganglia	Vesicular trafficking
ALS 9	*ANG*	hypoxia-inducible factor angiogenin	Angiogenesis and MN survival	ALS + Parkinson	**transgenic VEGF**	**late onset loss of MN**	Angiogenesis

A complex array of interconnected pathological processes :

- Dying back pattern
- Glutamate excitotoxicity
- Dysregulation of - neurotrophic factors and axon guidance proteins
  - axonal transport defects
- Mitochondrial dysfunction
- Deficient protein quality control
- RNA processing

Genetic modifiers ?

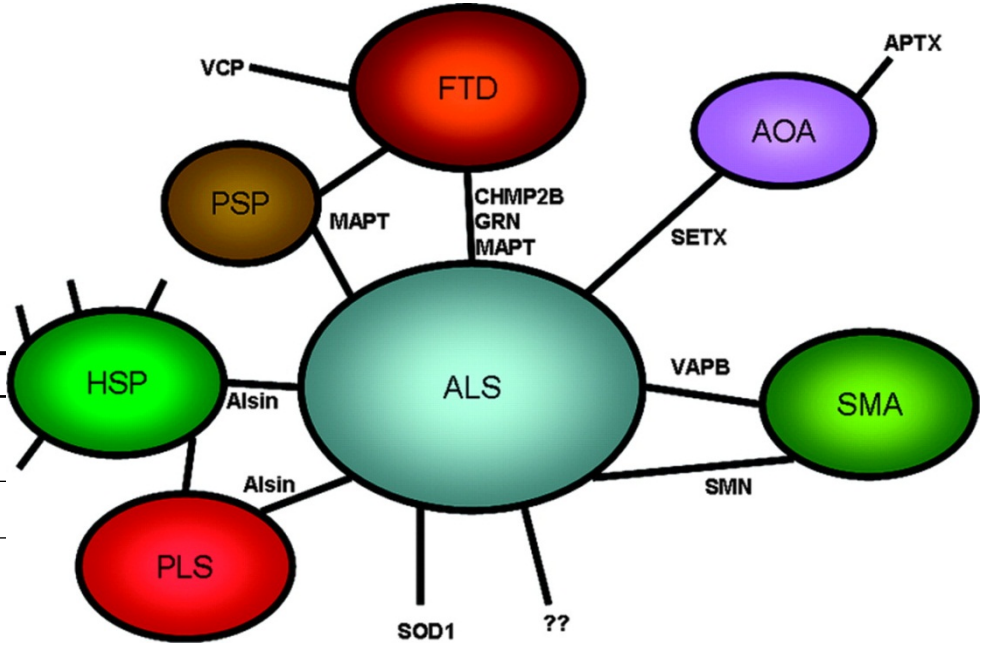


« Mechanistic » mutants

Target	Neuropatholgy
Protein misfolding	
Synuclein mutant	IF and SOD1 aggregates, parikaryal inclusions and speroid-like inclusions in motor neurons
Intermediate neurofilament abnormalities	
Human NF-H or NF-L overexpressor	Perikaryal accumulation of NF and axonal atrophy
Mutant NF-L (CMT2E)	Massive degeneration of spinal motor neurons
NF-L knockout	Developmental loss of 20% motor neurons
Peripherin overexpressor	Loss of spinal motor neurons
Microtubule abnormalities	
p50 dynactin subunit (dynamitin) overexpressor (p150(Glued mice)	Loss of motor axons
KIF5A knockout	NF accumulations
Dynein mutations	Loss of motor neurons
Short tau overexpressor	Loss of motor axons
Angiogenesis	
VEGF δ-HRE	Late-onset loss of motor neurons

From Laguna et al.,
BioValleyMonographs Vol 2 (2008)

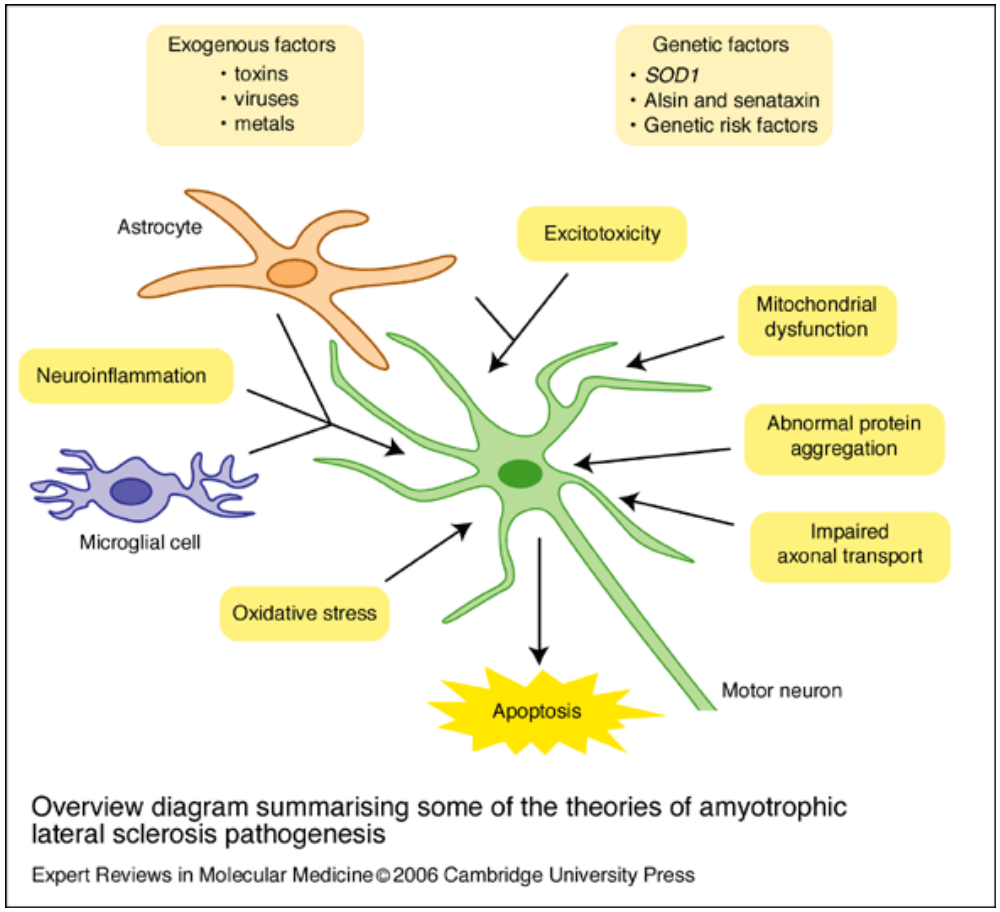
Mouse models point to a complex interplay between MN diseases



Human	Gene	Protein	Function	Phenotype		
SBMA	*AR*	Androgen receptor	DNA-binding transcription factor	Slowly progressive lower motor neuron disease		
FALS	*DAO -/-*	D-amino acid oxidase	D-serine regulation	loss of lower motoneurons		
SPG3	*ATL1*	Atlastin	Vesicular trafficking; Axonal transport	Early-onset pure, slow progression hereditary spastic paraplegia (HSP		
SPG4	*SPAST*	Spastin	Microtubule dynamics; axonal transport	Mainly pure HSP with variable onset		
SPG10	*Kif5A*	Kinesin (K1F5A)	Anterograde axonal transport	Early onset progressive weakness and leg spasticity		
Lower motor neuron disease	*DCTN1*	Dynactin-1	Retrograde axonal transport	Slowly progressive lower motor neuron disease	Dominant negative mutant (p150glued)	MN loss, astrogliosis
					Thy-1 overexpressing mutant	MN phenotype, muscle weakness, death
IBMPFD + FALS	*VCP*	valosin-containing protein	ATPase	Inclusion Body Myopathy associated with Paget's disease of bone and frontotemporal dementia	VCP(R155H/+) mouse + yeast, drosophila	MN phenotype

sALS genetic factors

ALS, sporadic	6q12	*VEGF*
	22q12.1-q13.1	*NFHC*
	6q21.3	*HFE*
	7q21.3	*PON1*
	5q13	*SMN*
	7q36	*DPP6*
	12p11.23	*ITPR2*
	1p32.1	*FLJ10986*
	17q21	*PGRN*
ALS, familiar and sporadic	14q11.2	*ANG*
ALS, familiar and sporadic	1p36	*TARDBP*



Overview diagram summarising some of the theories of amyotrophic lateral sclerosis pathogenesis

Environmental factors ?

Pesticides
Heavy metals (i.e. Mb, Hg)
Excessive physical activity
Head injuries
Cigarette smoking
Electromagnetic fields

AFMTELETHON 7&8 DECEMBER 2012
CURE THROUGH INNOVATION

Therapeutics proposed from transgenic models

> 150 clinical trials

…1 registrated drug

Table 13. Neuroprotective agents with potential effect on ALS.

- Antiaggregation
  - Scriptaid
  - Trehalose
- Anti-apoptosis
  - Activated protein C
  - Calpain inhibitors
  - Caspase inhibitors
    - Minocycline
  - Clusterin
  - DNA binding drugs
  - Lithium
  - Rasagiline
  - TCH 346
- Antiepileptic drugs
  - Levetiracetam
  - Valproic acid
- Anti-excitotoxicity
  - AMPA receptor antagonist
    - Memantine
    - NBQX
    - RPR 119990
  - EAAT2 promoter activity
    - Ceftriaxone
  - Glutamate carboxypeptidase II inhibitor: 2-MPPA
  - Glutamate inhibitor
    - Riluzole
    - Talampanel
  - Metabotropic glutamate receptor modulators
    - Cannabinoids
    - Dexanabinol (HU-211)
    - Glutathione
  - NMDA NR2B subunit receptor antagonists
    - Ifenprodil
    - Magnesium
    - NAALADase inhibitors
- Anti-inflamation
  - Interleukin-1 antagonists
  - COX inhibitor
    - Celecoxib
    - Rofecoxib
    - Sulindac
  - Protein kinase C inhibitor
    - Tamoxifen
- Antioxidants
  - AEOL 10150
  - Co-enzime Q10
  - Lipoic acid
  - N-acetyl-L-cysteine
  - Synthetic SOD catalase
- Cell based therapy
  - Umbilical cord blood cells
  - BM transplant
- Chemotherapy
  - Cyclosporine A
  - Vincristine
- Cupper chelator
  - Trientine
  - d-penicillamine
- Gene therapy
  - RNAi-based therapy
- Hormones
  - Selective estrogen receptor modulators
  - Receptor-independent neuroprotective effects of estrogens
- Immunomodulator
  - Copaxone/glatiramate
  - Thalidomide
- Ion Channel modulators
  - Calcium channel blockers
    - Nimodipine.
  - Na^+ channel blockers.
  - Neuroprotective potassium channel inhibitors
- Mitochondria-targeted
  - Arimoclomol
  - Glutathione
  - Heat shock proteins
  - Hyperbaric oxygen therapy
  - Omega-3 fatty acids
  - Vitamine E
- Neurotrophic factors
  - Brain-derived neurotrophic factor (BDNF)
  - Ciliary neurotrophic factor (CNTF)
  - Fibroblast growth factors (FGF)
  - Glial cell line-derived neurotrophic factor (GCDNF)
  - Insulin-like growth factor (IGF)
  - Nerve growth factor (NGF)
  - Neurotrophins (NTF)
  - Vascular endothelial growth factor (VEGF)
- Transcranial magnetic stimulation
  - Combination therapy
    - Drugs
      - Rofecoxib and creatine
      - Rasagiline and riluzole
      - Minocycline, riluzole and nimodipine
    - Growth factor and virus
      - AAV-CTI
      - AAV-GDNF
      - AAV-IGF
      - AVR-GDNF
      - LV-VEGF
      - VEGF

AAV: adeno-associated-virus; AVR: Adenovirus;
LV: Lentivirus; BM: bone marrow.

Validity of the animal models ?

- Artifacts due to i.e. **synthesis rate of mutant human SOD1** in mice (40-fold endogenous mouse SOD1)
- Need **later onsets in mice** and **lower copy numbers**
- **Mice do not truly reflect human ALS** (i.e. rare upper MN defects; anyway UMN have different functional consequences; + too aggressive in mice)
- Mutant SOD1 mice **do not model sporadic ALS**

- Differences in **pharmacokinetics**
- **Effects observed in the mouse are small** and can be missed in a clinically and genetically heterogeneous human ALS
- **Underpowered studies** (before onset in mice)→ guidelines (ALS, 2010)

Scott et al. (ALS 2008): 70 drugs screened in 18000 mice accross 221 blinded studies in 3 distinct facilities (basic, clean, SPF) :

- **Gender effects confirmed** (but required > 200 animals)
- **No active compound** (rather measurements of noise in the distribution of survival means; 134+/-10 days). Including Riluzole (requires many thousands of patients)
- **Student's *t*-test/ANOVA not appropriate** (to survival studies in general + cannot address litter clustering inherent to SOD1 mice)
- **Effects on other non-ALS related illness ?** (i.e. many are antibiotics or anti-inflammatory)

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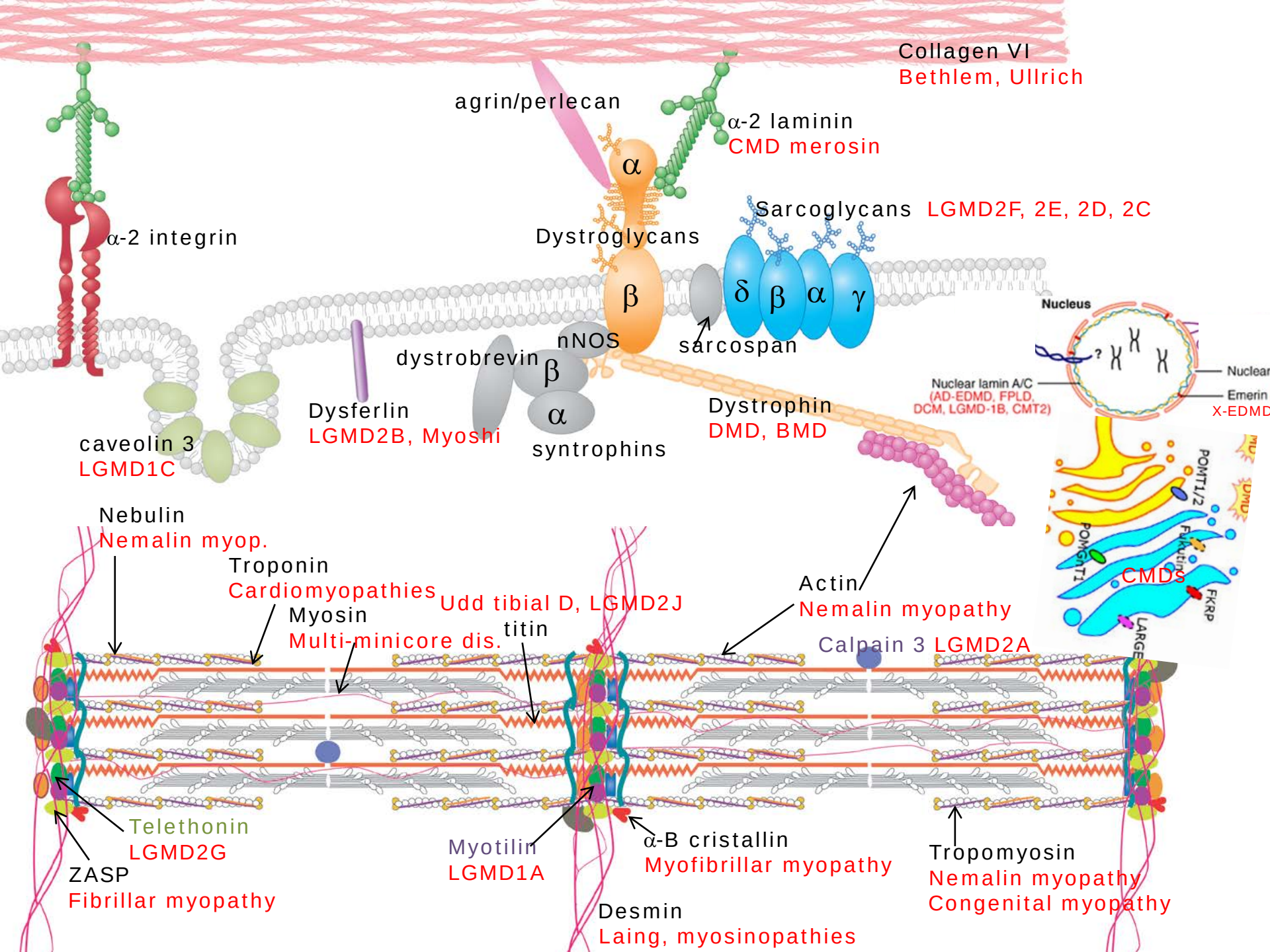
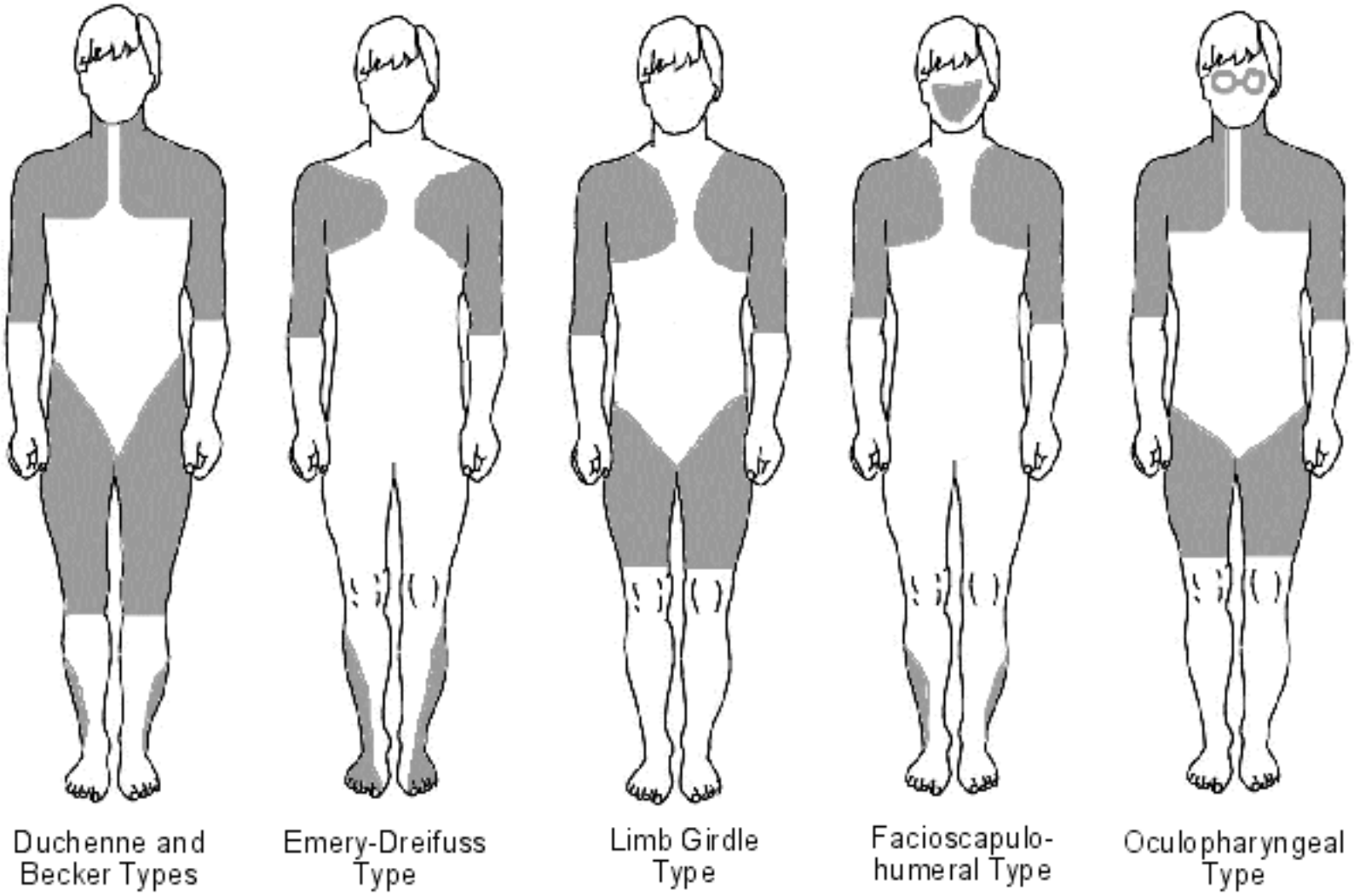
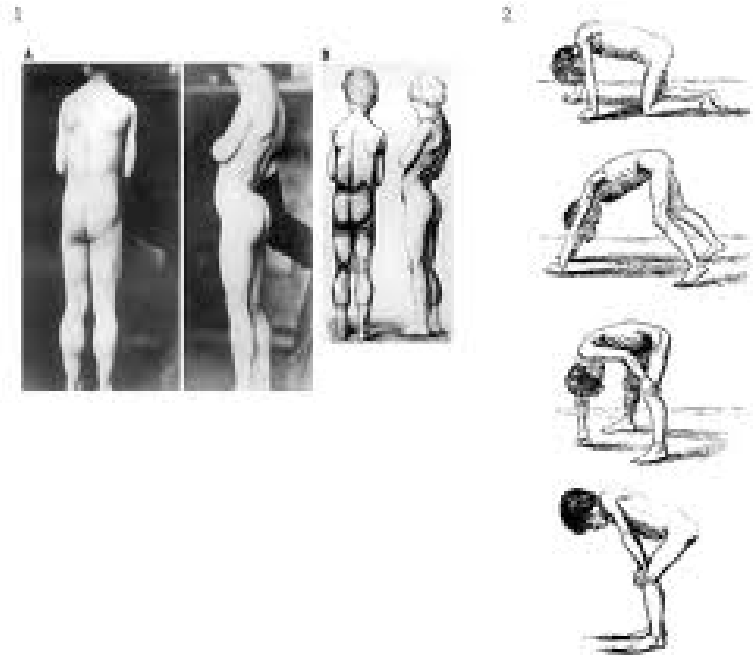
Recommandations :

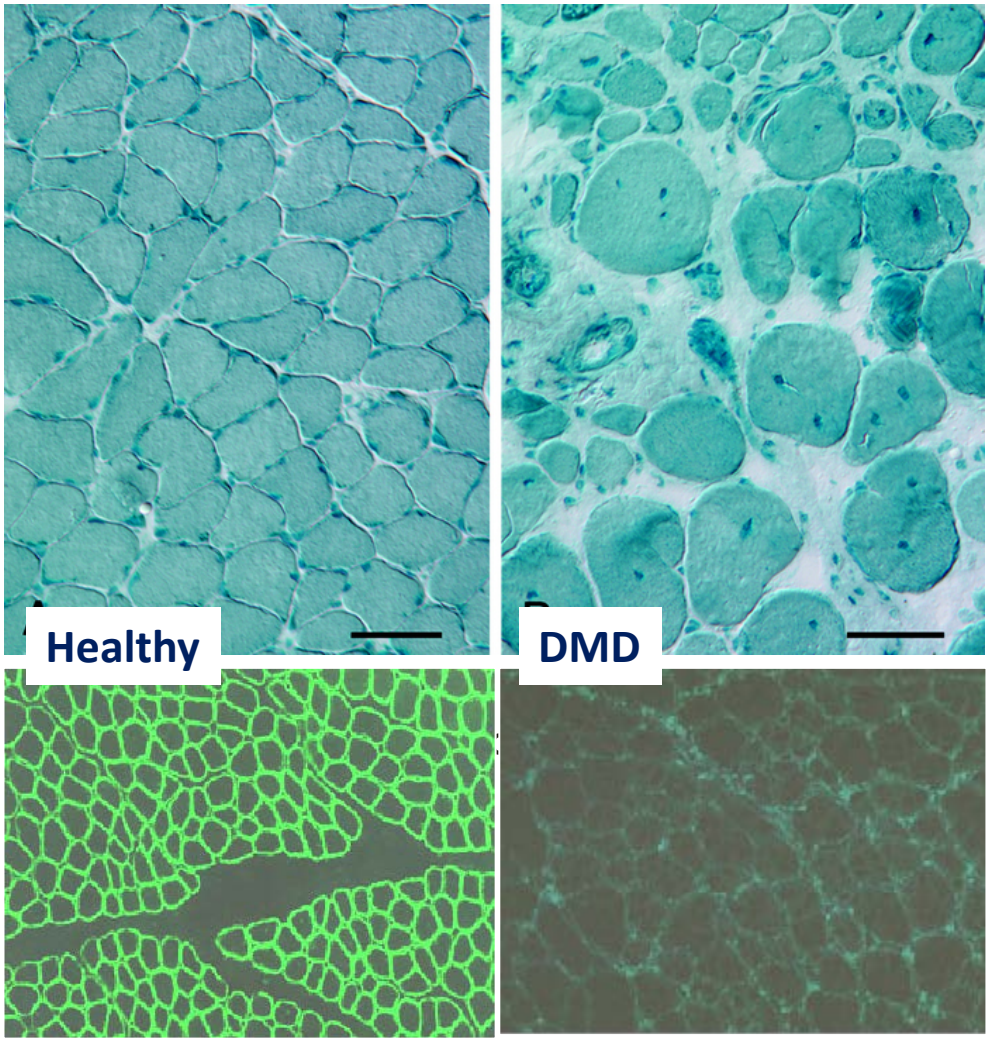
- ✓ Each cohort should have **at least 24 litter-matched gender-balanced mice**
- ✓ Study should be **double-blinded**
- ✓ Need a **single uniform endpoint** criterion
- ✓ **Non-ALS deaths** must be tracked and excluded from final analysis
- ✓ **Exclude long-lived animals** due to a low copy number of transgene copies
- ✓ Statistical analysis: **Cox proportional hazards model**
- ✓ **Age at study start**


Collagen VI
Bethlem, Ullrich
agrin/perlecan
α-2 laminin
CMD merosin
α-2 integrin
Dystroglycans
Sarcoglycans LGMD2F, 2E, 2D, 2C
nNOS
sarcospan
dystrobrevin
Dysferlin
LGMD2B, Myoshi
caveolin 3
LGMD1C
syntrophins
Dystrophin
DMD, BMD
Nucleus
Nuclear lamin A/C
(AD-EDMD, FPLD,
DCM, LGMD-1B, CMT2)
Nuclear
Emerin
X-EDMD
POMT1/2
POMGnT1
Fukutin
FKRP
CMDs
Nebulin
Nemalin myop.
Troponin
Cardiomyopathies
Myosin
Multi-minicore dis.
Udd tibial D, LGMD2J
titin
Actin
Nemalin myopathy
Calpain 3 LGMD2A
Telethonin
LGMD2G
ZASP
Fibrillar myopathy
Myotilin
LGMD1A
α-B cristallin
Myofibrillar myopathy
Desmin
Laing, myosinopathies
Tropomyosin
Nemalin myopathy
Congenital myopathy



Main areas of muscle weakness in different types of dystrophy



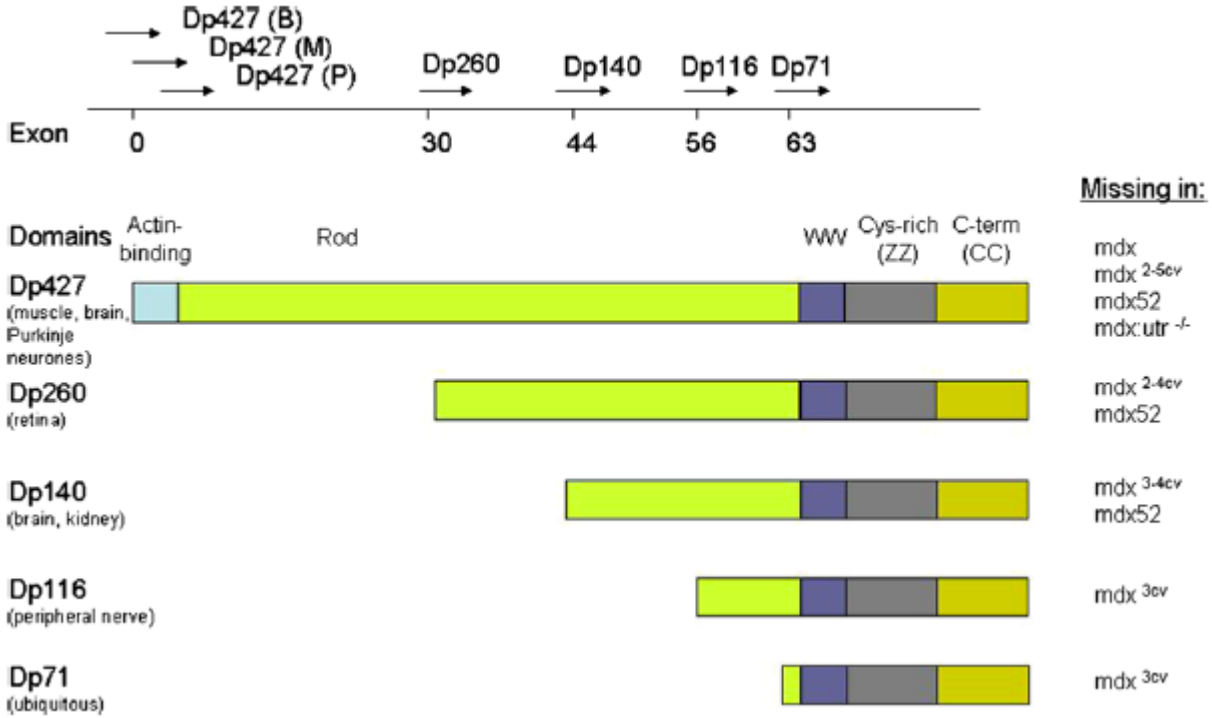


Duchenne muscular dystrophy:

- 1 /3,500 male births
- Progressive muscle waisting. Death from DMD usually occurs by age of 30
- Mental retardation (30%), smooth muscle disorders, cardiomyopathy
- Diagnosis: 16 months - 8 years

J Neuropathol Exp Neurol. 2009;68:762-73

Becker muscular dystrophy: later onset, mostly milder



C56BL/10ScSn *mdx*: naturally occuring dystrophin-deficient mutant
2700 publications

- **Slightly shorter life span**
- **Muscle degeneration in waves** (not a continuum like in DMD pts)
- **~not symptomatic** (but aggravated by forced exercice)
- **Very mild fibrosis**
- **Mild and late cardiomyopathy**
- Variations (strain, experimental conditions,…)

- Very robust **calcium homeostasis**
- **Utrophin** overexpression
- **More regeneration**
- Age-dependent **revertant fibers**
- mdx$^{2cv, 3cv, 4cv, 5cv}$ mdx52

Table 1
Qualitative and quantitative measurements in *mdx* mouse model

Parameter	*mdx*
General	
Life span	reduced by 17% in females and 19% in males [7] 23m [65]
Clinical signs at onset	CK levels increase, necrosis, regeneration
First signs of pathology	2-3w
First signs of cardiomyopathy	6m
Breeding	normal
Kyphosis	9m [22]
Body weight	normal, increases after 8w [14] drops by 25% after 6m [65]
Muscle Physiology	
Fore-limb strength (g)	12w: 35g [10,14,68] 4w: 95g; 8-12w: 156g [17] 10w: 30g [10] 4w: 103g; 8-12w: 138g [17] ①
Fore-limb strength per body weight (g strength/g BW)	1.5 at 8w [14] 1.6 at 3w, 10w [69] 1.1 at 10w [10] 75 at 4m (whole body) [16] 5.34 at 4w, 6.5 at 8w [70] 4.59 at 4w, 5.07 at 8w [70]①
Twitch force (mN)	Edl, 16w: 130 [72] Edl, 8w: 12 [20] Sol, 8w: 12 [20] Stm, 8w: 50 [46]
Normalized twitch force (mN/mm^2 CSA)	Edl, 10w: 50 [19] Sol, 10w: 40 [19] Dia, 10w: 20 [19] Dia, 7w: 5.8 [22] Stm, 8w: 6.6 [46] Edl, 8w: 82 [20] Edl, 90d: 50.2 [16]
Tetanic force (mN)	Edl, 16w: 370 [72] Edl, 8w: 52 [20] Sol, 8w: 57 [20] Edl, 10w: 170 [20] Edl, 6w: 200 [21]
Normalized tetanic force (mN/mm^2 CSA)	Sol, 10w: 200 [19] Dia, 10w: 130 [19] Dia, 7w: 32 [22] Edl, 3-32w: decreased [74] Edl, 90d: 147 [16]
Muscle Physiology	
Force drop after eccentric contraction②	Edl, 16w: 72% [21] Edl, 90d: 69% [16] Edl, 10w: -41% [19]
Force drop after fatigue protocol	Sol, 10w: 75% [19]
Normalized peak force (mN/mm^2 CSA)	Edl: 272 [19]
Biochemistry	
CK	1200 U [42] 5000 U [16]①8000 U/l [15]①10000/ 12000 U/l [17,40]
Hydroxyproline (fibrosis marker)	Dia, 24w: 19 µg/mg [75] Dia, 7w: 2.5 µg/mg [22] Int, 7w: 0.9 µg/mg [22]
Intracellular [Ca^{2+}]	normal in Fdb, Sol at 4-9w (in [76]) normal in isolated Fdb fibers [31] 60 µM (higher) in Edl at 8-12w [18] 80 nM (2x higher) in Edl at 8-12 w [18] ①
Ca^{2+} sparks after hypotonia	Fdb: irreversible at 4w [77]
Parvalbumin	fast muscles: mRNA increases [32] TA, 24w: 9 µg/mg [33] Gas, 24w: 7 µg/mg [33] Sol, 24w: 50 ng/mg [33] Edl, 32w normal levels [74]
Respiratory Physiology	
Respiratory rate	altered at 16m [23]
Response to hypercapnia/hypoxia	reduced at 7m [78]

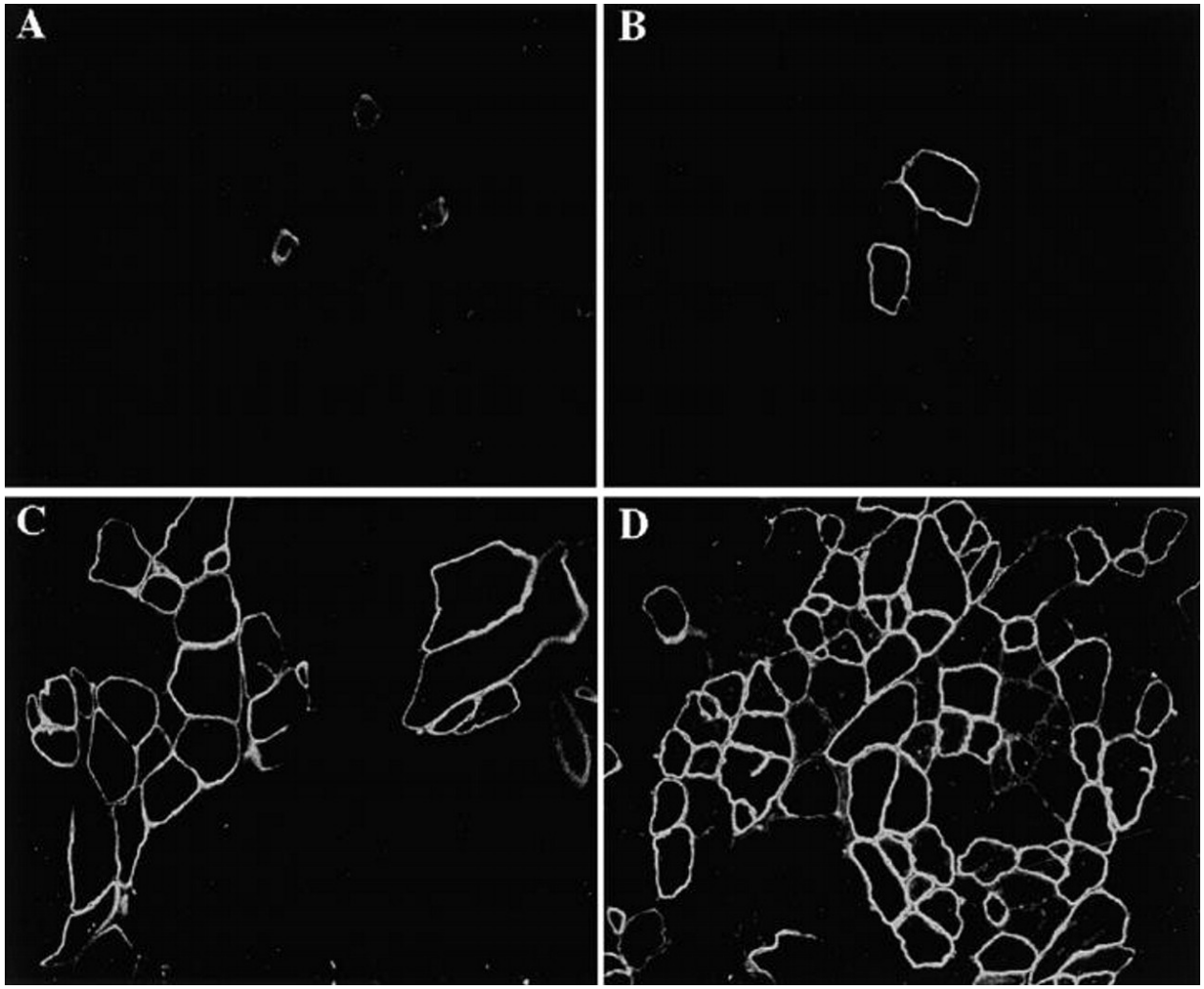
Table 1 (*continued*)

Parameter	*mdx*
Histology	
% of centronucleated fibers	TA, 7w: 50% [79] Dia, 7w: 25% [79] Sol, 7w: 56% [79] hind-limb, 4-14w: average 35% [8] Edl, 6w: 58%; TA, 90d : 75% [16] Edl, 10-12w: 64% [80] TA, 10-12w: 74% [80] Dia, 10-12w: 60% [80] Fdb, 12w: 25% [81] Gas, 8-10w: 50% [40] ①
Histology	
Fiber size	TA, 3w: 302 [79] ③ Dia, 3w: 254 [79] ③ TA, 7w: 403 [79] ③ Dia, 7w: 433 [79] ③ Sol, 7w: 267 [79] ③
Fibrosis	Dia at 16m [13] Glut at 24w [14] TA, 8-13w: 6% [15] TA, 8-13w: 9% [15] ①
Necrosis	TA, 3w: 20% [9] Edl, Bic, 10w: 5% [10] TA, Sol, Edl: peaks at 30-60d [84], decreases after 60d [11] Gas, 8-10w: 20% [17] ①
Fiber type composition	Edl: 50% I, 50% IIA [20] Edl: 80% IIB, 20% IIX [19] Sol: 65% IIA, 35% I [19] Dia: 90% IIX, 5% IIB [19] TA 90d: 57% IIB [16]
Cardiac Physiology	
Dilated cardiomyopathy (LVEDD/LVESD in mm by echocardiography)	3.6/2 at 42w (increased) [24] 3.7/2.6 at 24m (no change) [29] 3.9/2.9 at 9-10m [27] 3.83 at 10m [30]
Heart rate	decreased, 612 bpm at 42w [24] 460 bpm at 9-10m [27] 454 bpm at 10m [30]
Force output (mN/mm^2)	25 at 8w [85]
Heart fibrosis (% of area)	7% at 7m [24] 8% at 12m [29] 12% at 24m [29] 1.68% at 11w [41] 6.23% at 11w [41] ① 0.7-3.4% at 10m [30]
Hydroxyproline content	4.5 µg/mg at 15m [28] 4 µg/mg at 12-14m [26]
ECG	abnormal at 6 and 12m [25]
Left ventricular developed pressure (mm Hg)	80 at 15m [28]
Rate of pressure development (mm Hg/s)	2500 at 15m [28]
Rate of relaxation (mmHg/s)	−2000 at 15m [28]

Abbreviations used are: Bic: biceps, Dia: diaphragm, Edl: *extensor digitorum longus*, Fdb: *flexor digitorum brevis*, Gas: *gastrocnemius*, Glut: *gluteus*, Int: intercostals, Pl: *plantaris*, Sol: *soleus*, Stm: sternomastoid, TA: *tibialis anterior*. d: days; w: weeks; m: months; y: years; BW: body weight; CSA: cross sectional area; CK: creatine kinase; LVEDD; Left ventricular end diastolic diameter; LVESD: left venticular end systolic diameter; bpm: beats per minute.
① exercised *mdx*.
② after 5th elongation [19].
③ measured as the minimal Feret's diameter, units in variance coefficient (vc) [79].

From Willmann et al. Neuromusc. Dis. 2009

Age-related expansion of RF in *mdx* mouse muscles



Massive Idiosyncratic exon skipping corrects the nonsense mutation in dystrophic mouse muscle and produces functional revertant fibers by clonal expansion

Lu Q et al. J Cell Biol 2000;148:985-996

Canine dystrophinopathies have also been reported in many pure bred and mixed breed dogs :

Golden Retriever, Rottweiler, German Shorthaired Pointer, Japanese Spitz dogs,…

- Progressive, with the **gradual loss of muscle mass**
- **Contractures** that often lead to skeletal deformities
- **Enlargement of the base of the tongue**
- **Pharyngeal and esophageal dysfunction** (→ drooling, dysphagia, and regurgitation)
- Skeletal muscles: - **EMG** : spontaneous high frequency discharges and complex repetitive activity
  - **Degeneration/regeneration**

GRMD:

Rapidly progressing fatal disease
Fibrosis, Cardiomyopathy
Selective muscle involvement (tongue, masticatory, trunk muscles most affected) like in human DMD

CXMD: **slower progression**, survival increased, **milder** cardiomyopathy

Cats: clinically different :

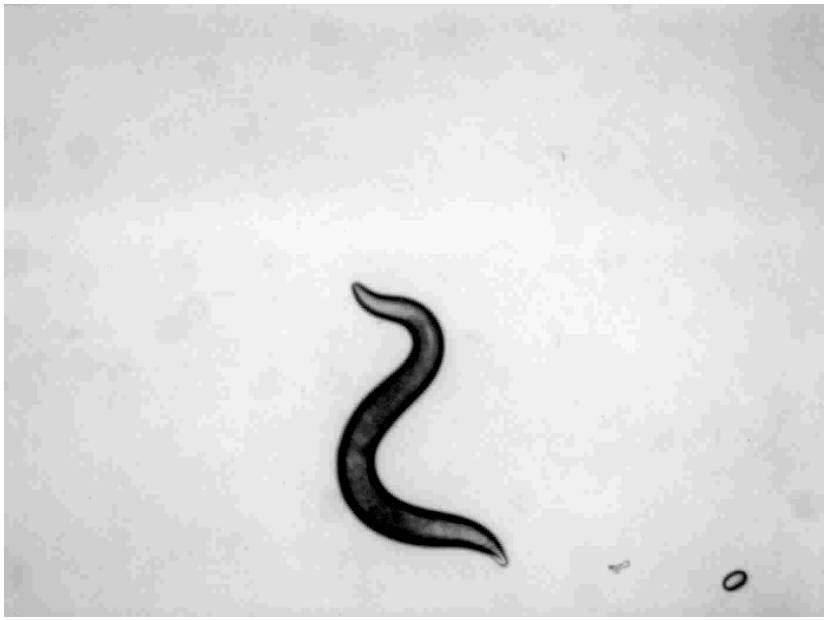
- no fibrosis, some cardiomyopathy (hypertrophy)
- restricted shoulder, neck muscle hypertrophy
- dramatic tongue enlargement

Antisense oligo-mediated exon skipping using morpholinos in the CXMD dog

Screening plateforms
i.e. *C. elegans*

Dystrophin-deficient worm (dys-1)

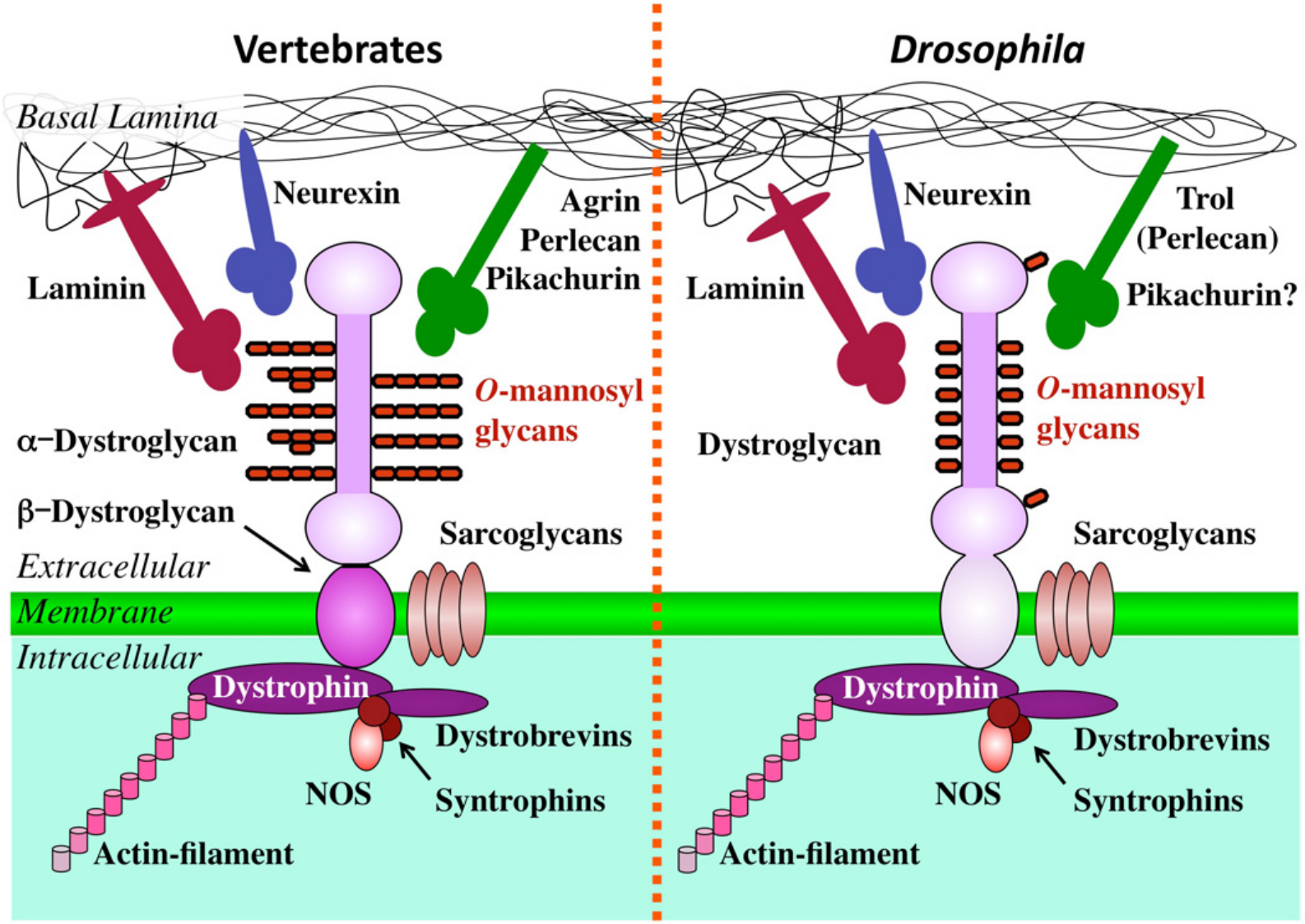
Segalat et al. (2005)



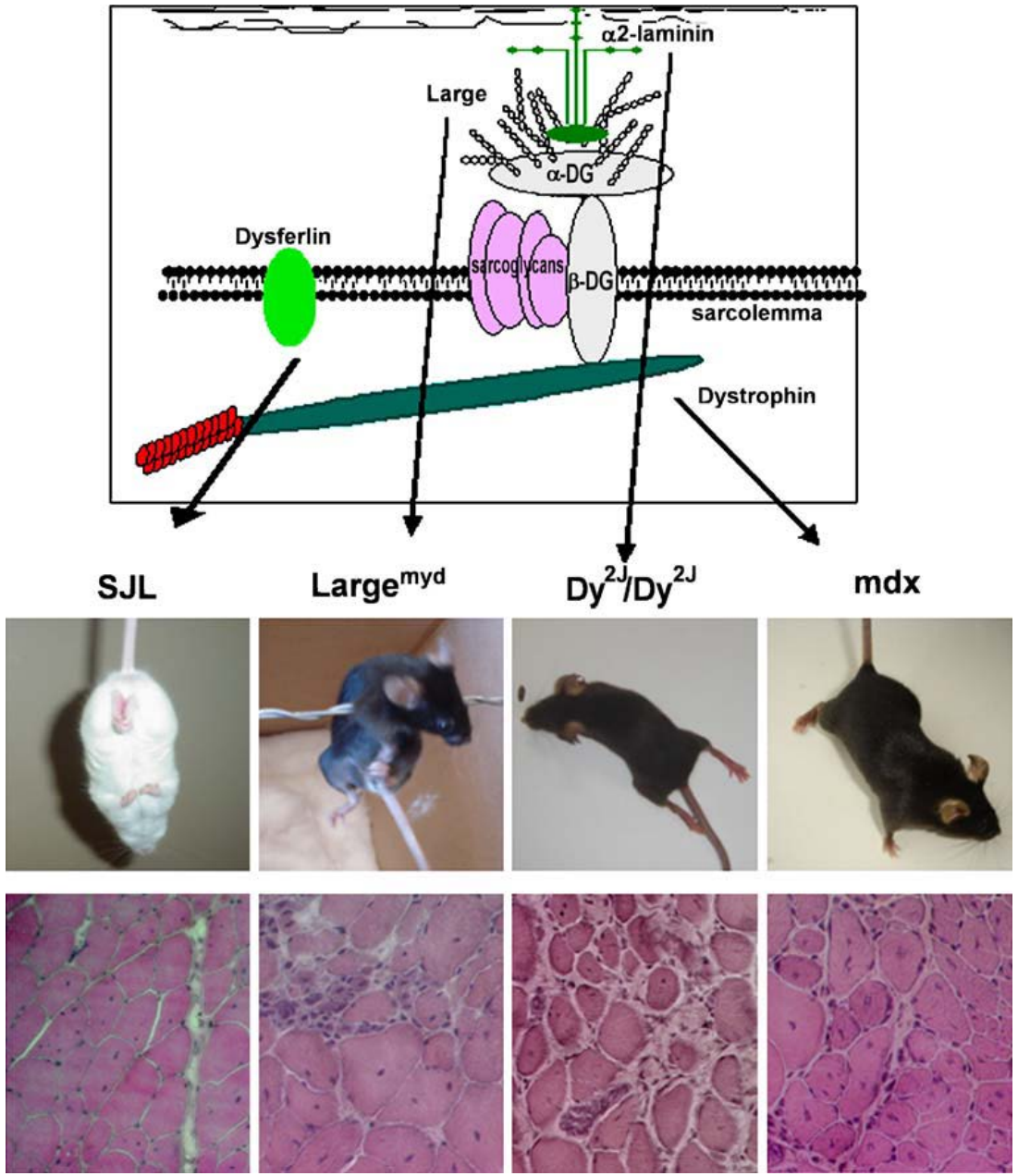
Treated worm

Identification of lead molecules

Evolutionary conservation of the dystrophin-glycoprotein complex

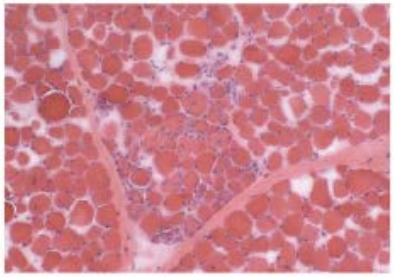


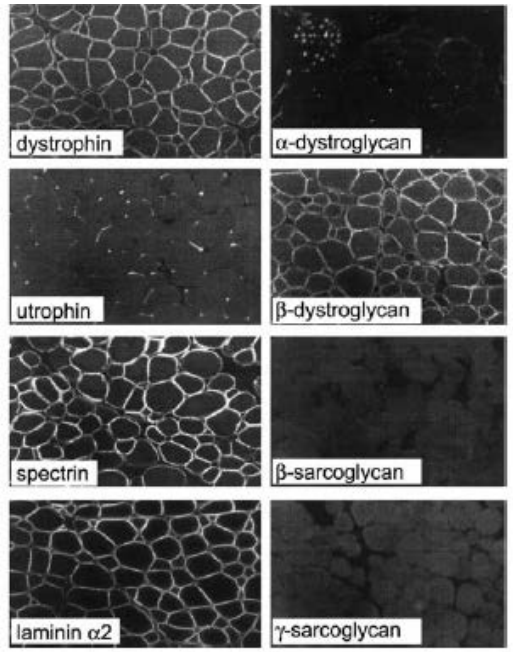
From Nakamura et al., Semin Cell Dev Biol. 2010



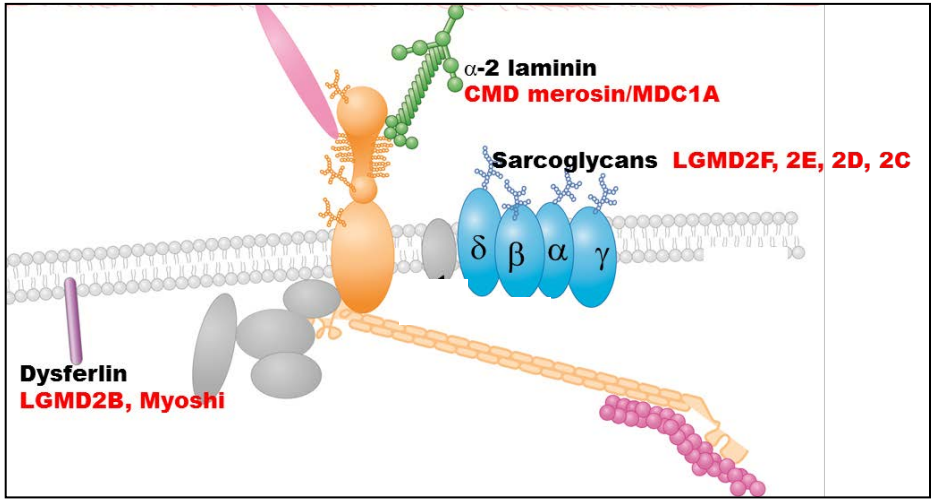
From Wainzof et al. 2008

Genotype (protein absent)	Lifespan	Muscle dystrophy	Cardiomyopathy	Fibrosis	Human disease	C. elegans	Drosophila	Zebrafish	
Mdx (dystrophin), mdx2,3,4,5 cv	>1 year	Mild/Moderate	Very mild	Poor, late	DMD	**Dys-1 (hyperactive)**	**Reduced lifespan + muscle and heart defects.** DLP1,2,3. neuromuscular transmission defects	***sapje*. Mutation in dnAChR suppresses the muscle defect.** Other morpholino of RNAi mutants	Revertant fibers
MyoD/mdx (MyoD, dystrophin)	1 year	Severe	severe	Poor, late		**Dys-1;hlh-1 (muscle defects)**			
Utrn-/-mdx (utrophin, dystrophin)	4-20 weeks	Severe	severe	moderate				DRP2. synaptic transmisisondefects	
Sgca-/- (α-sarcoglycan)	>1 year	Moderate	None	severe	LGMD2D	RNAi			
Sgcb-/- (β-sarcoglycan)	>1 year	Severe	severe	severe	LGMD2E	RNAi	**+ Notch, TGFb, EGFR genetic modifiers**		
Sgcg-/- (γ-sarcoglycan)	20 weeks	Severe	severe	severe	LGMD2C	RNAi			
Sgcd-/- (δ-sarcoglycan)	>1 year	Severe	severe	severe	LGMD2F	RNAi	**RNAi. Reduced lifespan +muscle and heart defects**	**Severe musle and heart defects**	**Bio14,6 / J2-NK / CHF147 / TO-2 hamsters**
DG-/- (dystroglycan)	**Embryonic lethal**	NA	NA			**DGN-1 not expressed in muscle**	**3isoforms, RNAi: Reduced lifespan +muscle and heart defects**	**similar to muscle-eye-brain disease and Walker-Warburg syndrome**	
POMT1, POMT2, POMGnT1, FKTN, FKRP, LARGE, (defective O-linked glycosylation of αDG)	Reduced	Moderate	None		CMD1D, MEB dis., LGMD2K/I, WWS		**muscle defects and degeneration, also causing neurological phenotypes**	isoprenoid synthase domain-containing (ISPD) --> muscle defects similar to WWS	
Dy/dy (α2-laminin)	6 months	Severe		moderate	CMD1A	laminin alpha ?	**LAMA2 porrly correlated with fly LAMA**	**candyfloss. Early muscle degeneration**	
Dy^{2J}/dy^{2J} (α2-laminin)	Reduced	Moderate/severe		moderate	CMD1A				
Calpain-3		Moderate		severe	LGMD2A				
SJL (dysferlin)	>1 year	Mild		severe	LGMD2B	fer-1		Muscle disorganization	
PABPN1 (polyalanine expansion in poly(A) binding protein nuclear 1)		Severe	Severe		OPMD	muscle cell degeneration and abnormal motility	progressive, age dependent muscle degeneration with rimmed vacuoles and nuclear inclusions		





A 5-month-old female Chihuahua dog with muscular dystrophy associated with a sarcoglycan deficiency



Mouse	Cat	Dog	Human
dy	Siamese Maine Coon Mixed breed	Mixed breed	MDC1A FCMD
myd	---	---	MEB WWS
α-SG ko β-SG ko γ-SG ko δ-SG ko	---	Chihuahua Boston Terrier Cocker spaniel	LGMD2D LGMD2E LGMD2C LGMD2F
mdx	Several breeds	Many breeds	DMD BMD

From Shelton and Engvall (2005)

Guillain-Barré Syndrome animal model: a first proof of molecular mimicry in human autoimmune disorder ?

The most frequent cause of acute neuromuscular paralysis
Limb weakness and areflexia
20% immobile after 6 months
3-10% death

Acute inflammatory demyelinating polyneuropathy
Acute motor axonal neuropathy

Gastrointestinal or upper respiratory symptoms 1-3 w prior onset of the neurological symptoms
Trigger infectious agent ?

(Cell Mol. Life Sci. 2000)

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Trigger infectious agent ?

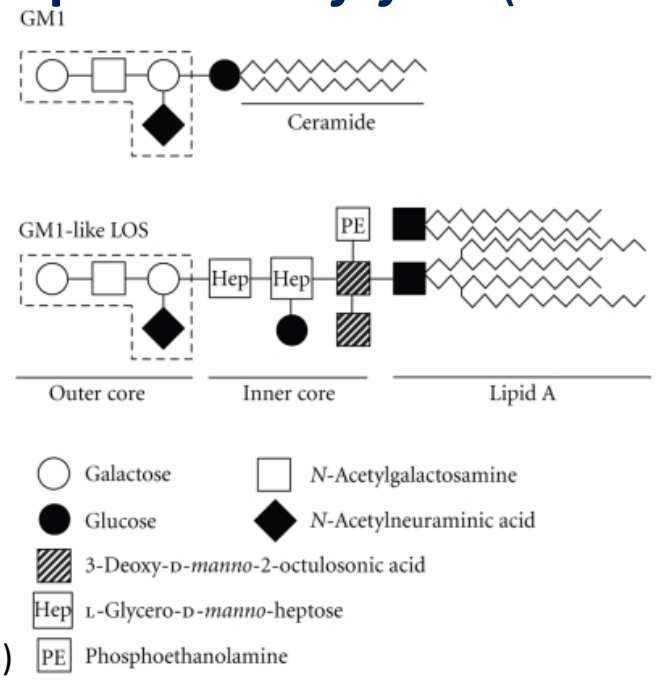
Criteria for molecular mimicry:

- **Epidemiological association between infectious agent and the immune-related disease**
- **T cells or Ab against the patients's target antigens**
- **Microbial mimics of the target antigen**
- **Reproduction of the disease in an animal model**

(Cell Mol. Life Sci. 2000)

In GBS:

- *Campylobacter jejuni*: leading cause of acute gastroenteritis
- The most common antecedent micororganisms in GBS:
  - 26% GBS patients
  - 2% household controls
  - 1% age-matched hospital controls
- **AutoAbs** in GBS (plasma exchange an effective treatment)
- **IgG deposits** on the axolemma of the SC anterior roots
- **IgG against GM1** in AMAN patients **subsequent to *C. jejuni*** (and titers decrease with clinical course)
- No autoAb in *C. Jejuni* patients with no neurological sequelae
- Terminal structure of ***C. jejuni*** Lipo-OligoSaccharide **similar to GM1** :



Shahrizaila & Yuki
J. Biomed. Biotechnol. (2011)

AMAN model : sensitization of rabbits with bovine brain GM1

→ High titers of anti-GM1 Ab followed by acute flacid limb weakness
→ Wallerian-like degeneration
→ Macrophage infiltration and IgG deposits on the anterior root axons

Model to verify molecular mimicry :

1. Active immunization against components of antecedent infectious agents:
C. Jejuni LOS bearing a GM1-like structure in Rabbits
→ IgG anti-GM1 Ab and flacid limb weakness
→ Macrophage infiltrates in the periaxonal spaces surrounded by an intact myelin sheat
→ Axonal degeneration

2. Passive model: ex vivo nerve-muscle preps. from GM1-overexpressing mice exposed to mouse IgG anti-GD1 mAb + complement
→ Ab+C deposits on the presynaptic axons + ultrastructural damages and EMG blockade (same with human sera) (disappearance of Na+ channel clusters)
Nafamostat mesilate , a potential therapeutic agent

Animal models can lead to the identification of an homologous gene in humans

Identification of a Mutation in Porcine Ryanodine Receptor Associated with Malignant Hyperthermia

JUNICHI FUJII,* KINYA OTSU, FRANCESCO ZORZATO,†
STELLA DE LEON, VIJAY K. KHANNA, JANICE E. WEILER,
PETER J. O'BRIEN, DAVID H. MACLENNAN‡

Malignant hyperthermia (MH) causes neurological, liver, and kidney damage and death in humans and major economic losses in the swine industry. A single point mutation in the porcine gene for the skeletal muscle ryanodine receptor (*ryr1*) was found to be correlated with MH in five major breeds of lean, heavily muscled swine. Haplotyping suggests that the mutation in all five breeds has a common origin. Assuming that this is the causal mutation for MH, the development of a noninvasive diagnostic test will provide the basis for elimination of the MH gene or its controlled inclusion in swine breeding programs

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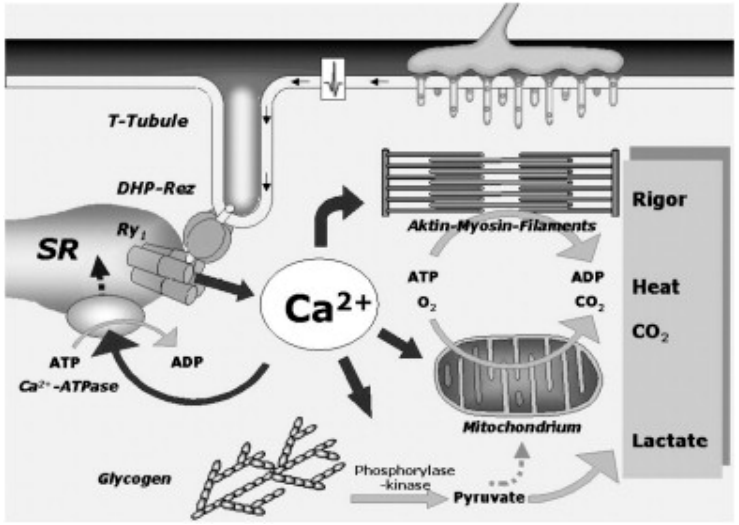


1/12000-50000 anesthetic events (massive rhamdomyolysis, acidosis, hyperthermia, often fatal)

Major economic losses in the swine industry

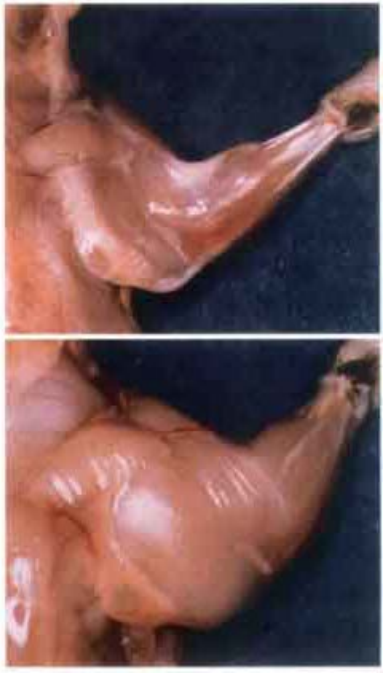
Dantrolene as a therapy

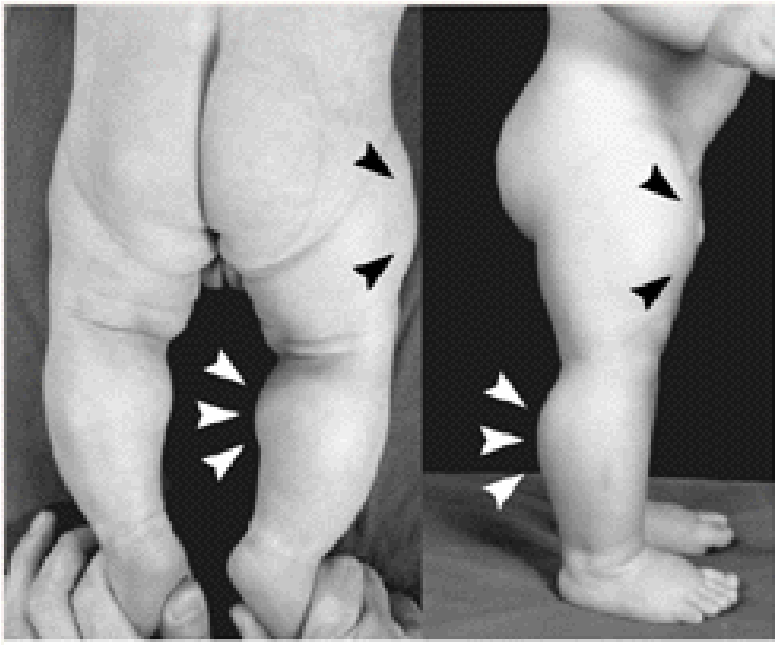
Myostatin, *the Schwarzenegger gene*

Grobet et al, 1997
Kambadur et al., 1997

Heterozygot Whippet dog
(premature STOP codon at aa 313)
Mosher et al., 2007

Myostatin mutations





Neonate 7 Months

McPherron et al., 1997; Zsabo et al., 1998; Zhu et al., 2002

Schuelke et al., 2004

Bovines

nt821(del11) Hyperplasia
nt419(del7-ins10)
C313Y
Q204X
E226X
F84L
(Grobet *et al.*, 1997; Kambadur *et al.*, 1997)

Mouse

Knockout Hypertrophy + hyperplasia
(McPherron *et al.*, 1997)

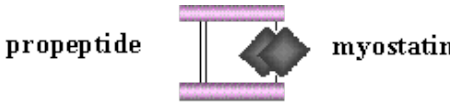
nt821(del11) Hyperplasia
(Nishi *et al.*, 2002)

Proteolytic clivage site
RSRR → GLDG
(Zhu *et al.*, 2000) Hypertrophy

nt775(del12)
(*Cmpt*)
(Szabo *et al.*, 1998) Hypertrophy

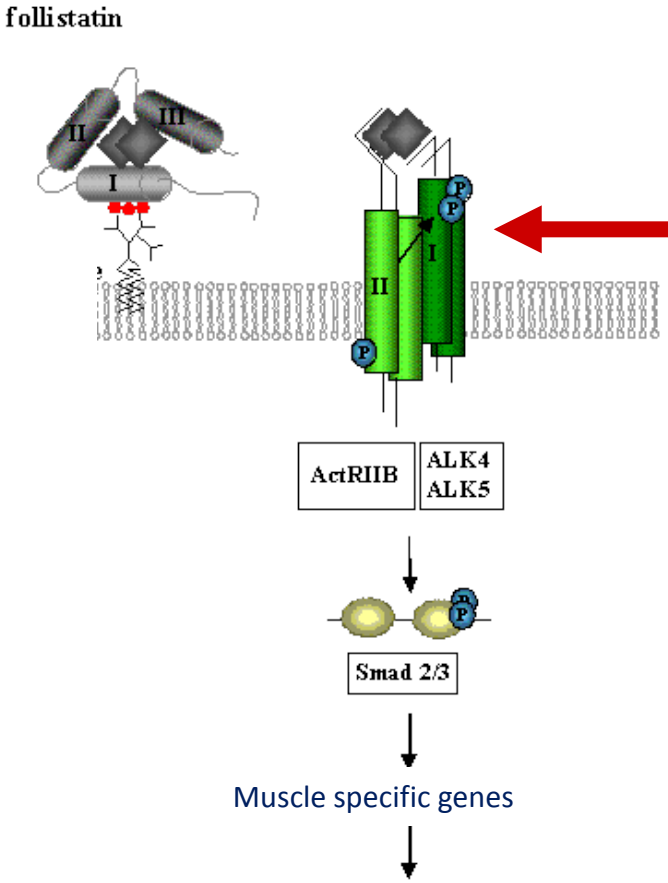
Human

G → A
Nucléotide 5 'intron 1
(Schuelke *et al.*, 2004)



Monoclonal antibodies :

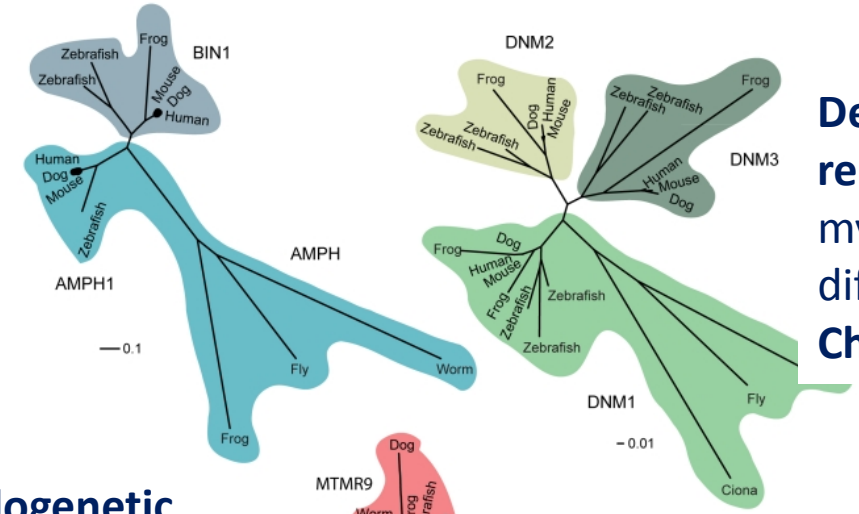
anti-myostatin (Wyeth, Eli Lilly
(DMB, FSH, LGMD)



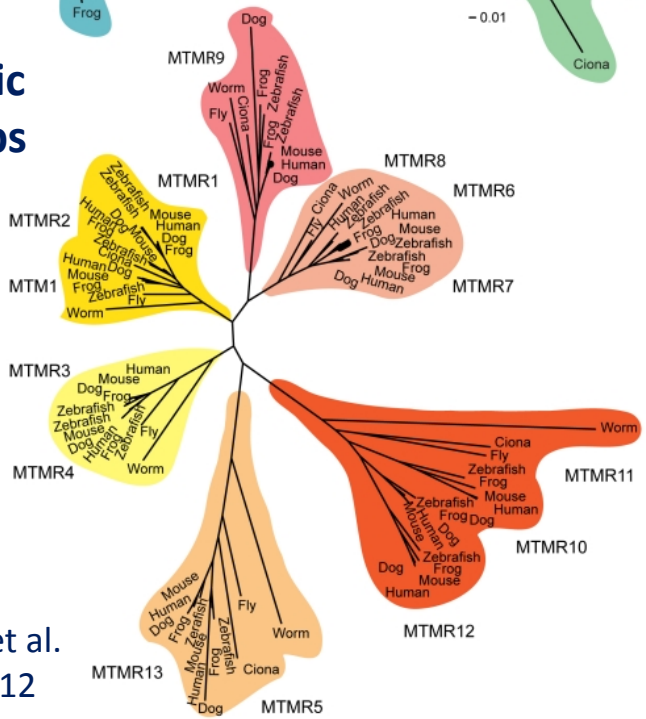
anti-ActRII
(Acceleron/Shire, Amgen, Novartis)

From genetic muscle diseases
to muscle wasting disorders
(cancer/AIDS cachexia, ageing)

Animal models instrumental to decipher cellular and physiological functions in the PNS



Phylogenetic relationships



From Cowling et al. PLoS Genet. 2012

Defects in proteins involved in membrane remodeling (i.e. amphiphysin 2 (BIN1), dynamin 2, myotubularin and MT-related proteins) → different forms of **centronuclear myopathy**, **Charcot-Marie-Tooth neuropathies**

Different proteins leading to similar diseases → common mechanisms

- Cell/cell adhesion
- T-tubule biogenesis
- Synaptic vesicule formation, endocytosis, and recycling
- Myelin sheat formation

In spite of the existence of differences in some phenotypes, and **provided careful standardisation**, animal models bring important clues to the understanding of the pathogenesis of NMD and are very valuable for testing strategies for therapeutic approaches.