NEUROSCIENCE
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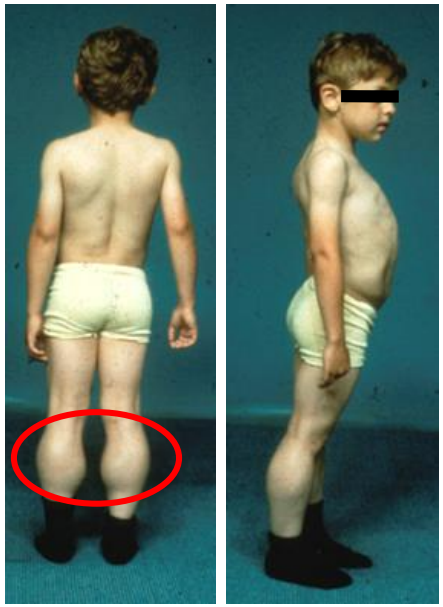
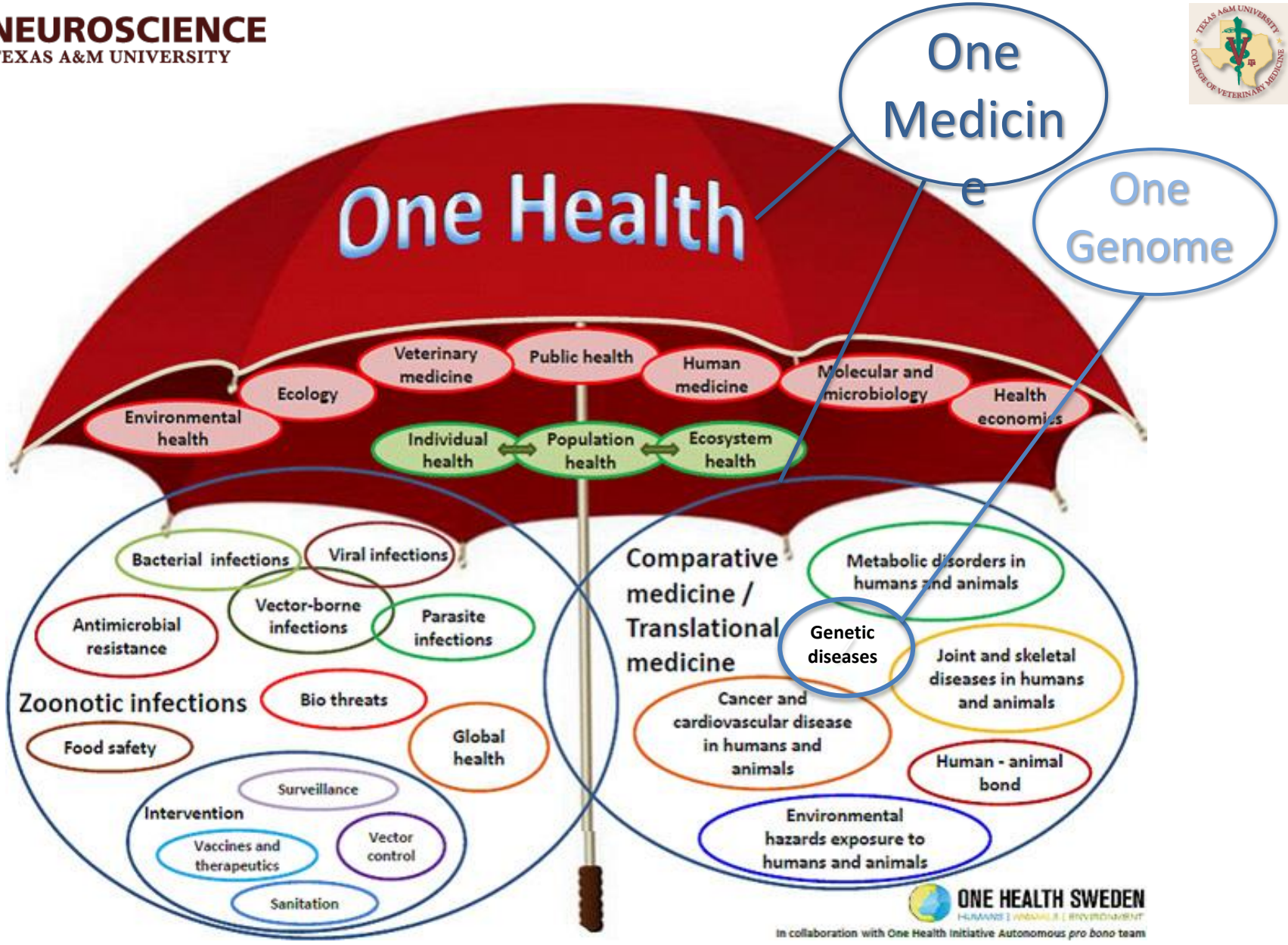
One Man's View of One Health

Translational Lessons Learned from a Canine Model of Duchenne Muscular Dystrophy




Joe N. Kornegay, DVM, PhD, DipACVIM (Neurology)
Texas A&M University

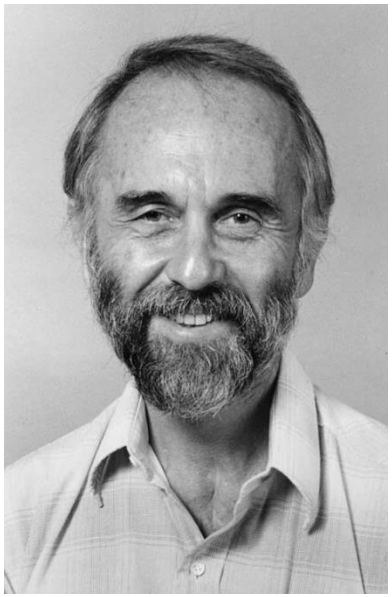
AAVMC 2014 Recognition Lecture
Alexandria, VA
March 15 , 2014



http://onehealthinitiative.com/about.php

Veterinary Medicine and Human Health
Calvin Schwabe

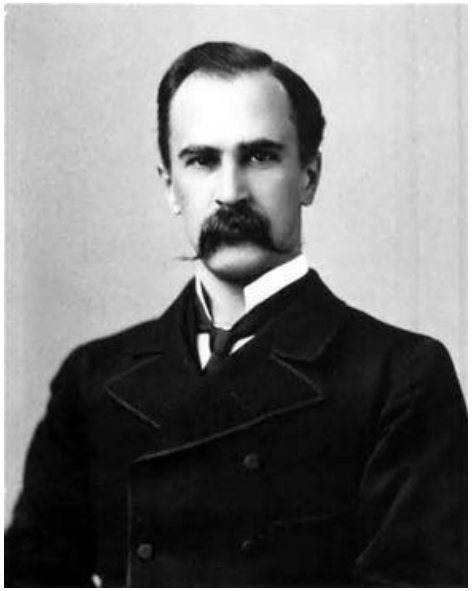
- *1st Edition* (1964) had sections on population medicine, epidemiology, and food and hygiene.
- *2nd Edition* (1969) included, under Epidemiology, a subsection on "Comparative Approaches to Diseases of Unknown Etiology" in which cardiovascular disease and cancer were discussed.
- *3rd Edition* (1984) included an initial section on the "Challenges of *One Medicine*" and a multifaceted discussion of animal models.



"My second fixed idea is the uselessness of men above sixty years of age, and the incalculable benefit it would be in commercial, political, and in professional life, if as a matter of course, men stopped work at this age."

William Osler – *The Father of Modern Medicine*

(Vol. I, Ch. 24 : *The Fixed Period – The Life of Sir William Osler*; 1925)

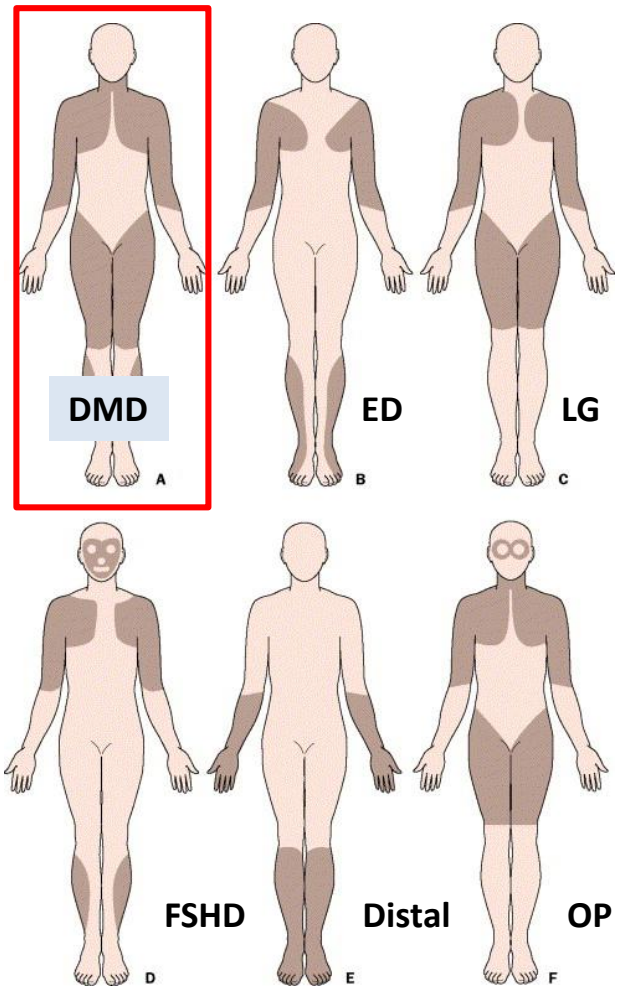


Biomedical Models
National Research Council (1985, 1998)

- "A surrogate for a human being, or a human biologic system, that can be used to understand normal and abnormal function from gene to phenotype and to provide a basis for preventive or therapeutic intervention in human diseases."
- "Can be many types – from animal models of human diseases to animal, in vitro, or modeling systems for studying any aspect of human biology or disease."

Muscular Dystrophy

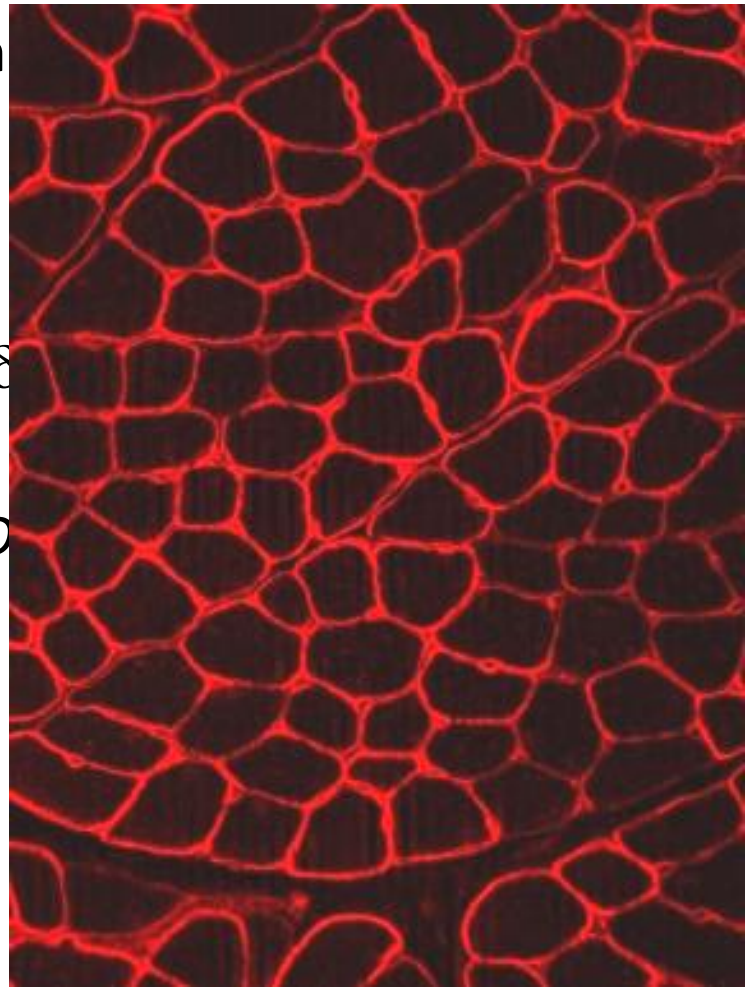
- Group of inherited, progressive myopathies in which major signs relate primarily to skeletal muscle.
- Originally classified based on clinical features (pattern of inheritance, age at onset, and muscles involved).
- Classification system has been revised based on molecular testing.
- Duchenne muscular dystrophy (DMD) – proximal distribution.



Emery AEH: ***Lancet*** 359: 687–95, 2002.

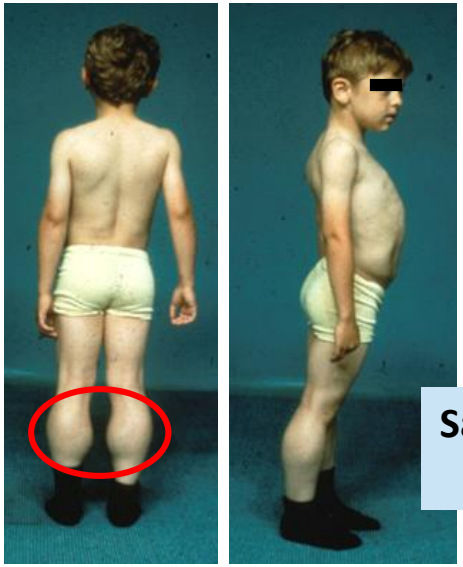
Dystrophin-Glycoprotein Complex

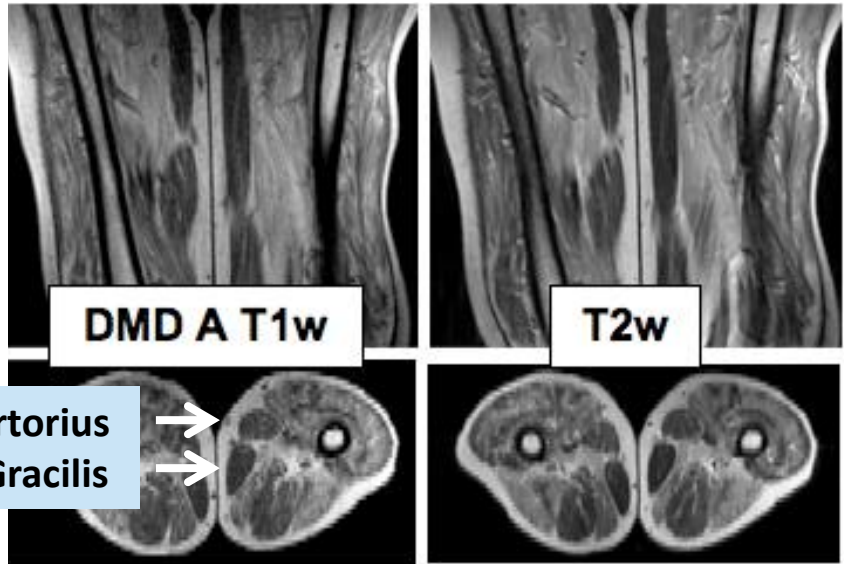
- Muscular dystrophies tied to proteins in a complex that spans the muscle cell membrane.
- Major components include dystrophin, α and β dystroglycans, and α, β, γ, and δ sarcoglycans.
- Dystrophin protein is coded by the *DMD* gene and connects cytoskeleton to the extracellular matrix via a transmembrane complex.

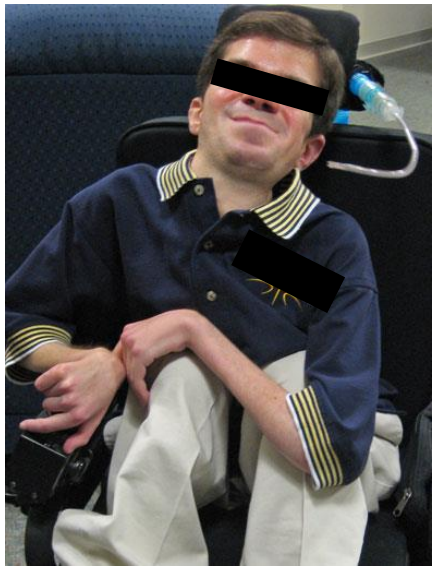


Duchenne Muscular Dystrophy

- X-linked; males affected, females are carriers.
- ~ 1 in 5,000 live male births.
- Boys in wheelchairs by teens; most die by early 20s.
- Cardiomyopathy; respiratory.
- Spinal lordosis.
- *Pseudohypertrophy* of the calf muscles.
- Selective muscle sparing.
- Agonist-antagonist imbalance contributes to contractures.







Mammalian Animal Models of DMD

- Mdx mouse – a nonsense point mutation causes premature termination of translation within exon 23. Mild phenotype.
- Feline hypertrophic muscular dystrophy – deletion of the dystrophin muscle and cerebellar Purkinje cell promoters in one case.
- GRMD – splice site mutation in intron 6 causes exon 7 to be skipped in transcription and a stop codon; multiple other dog breeds.

GRMD Colony Development

- 1981 – GRMD littermates (*Rusty* and *Dusty*) seen at UGA (Kornegay JN et al: *Muscle Nerve* 11:1056-1064, 1988).
- 1982 – *Rusty* and *Dusty* transferred to NC State.
- 1985 – GRMD dog (*Rusty*) first seen at UGA provided to Barry Cooper at Cornell to develop colony.
- 1987 – GRMD dog and carriers provided to NCSU for second colony.
- 1994 – NCSU colony to Missouri.
- 2007 – Missouri colony to UNC-CH.
- 2012 – UNC-CH colony to Texas A&M.
- Colonies in Australia (closed), France, Japan, Brazil, the Netherlands, Missouri, and at the Fred Hutchinson Cancer Center (Seattle).

The Role of Animal Models in Treatment Development for DMD

- *In vitro* studies (cell culture, etc).
- *In vivo* systems not involving specific X-linked animal models.
- Mdx mouse; mdx/utrophin DKO mouse.
- GRMD dog and/or primate.
- DMD patients.

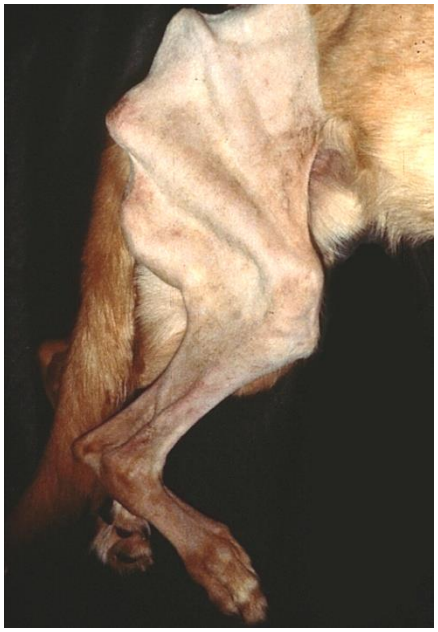
A balancing act – should the dog be driving the bus (tricycle)?

Predictive value of animal models. Will the mdx or GRMD model better predict outcome in DMD?

GRMD – General Features

- Progressive disease
- Postural instability/contractures
- Muscle atrophy and hypertrophy
- Kyphosis/lordosis
- Respiratory
- Cardiomyopathy

Phenotypic Variation: Species, Individual, and Muscle (confounds preclinical trials but offers insight on disease pathogenesis).



Primary vs. Secondary Effects of Dystrophin Deficiency and Identification of Modifier Genes as *Druggable* Targets

Therapeutic Approaches

- Gene Therapy
  - Viral Vectors
  - Plasmid DNA
  - Antisense oligos
- Cell Therapy
  - Myoblasts
  - Stem cells
- Pharmacologic
  - Prednisone (Std of Care for DMD)
  - ↑ utrophin (surrogates)
  - NF-κB inhibitors
  - Calpain inhibitors
  - Membrane sealants
  - Myostatin (GDF-8) inhibition

Muscular dystrophy comes in at least 20 forms — with D common — and progressive par affecting about

There are no e an Italian team experiments on Duchenne musc caused by a la muscle fibres.

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months, but stem-cell around with other dogs

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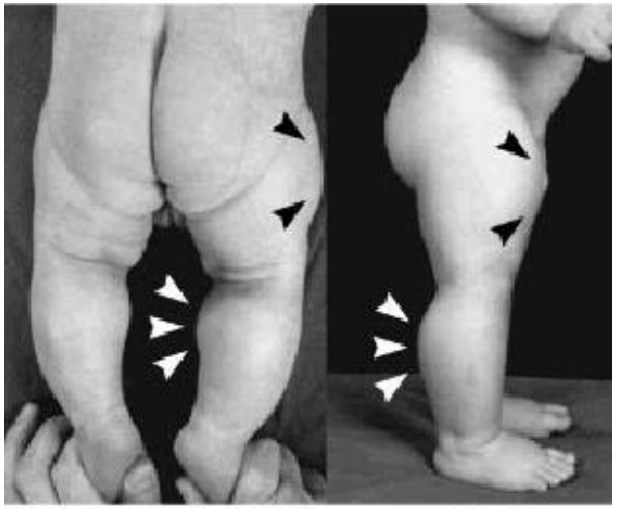
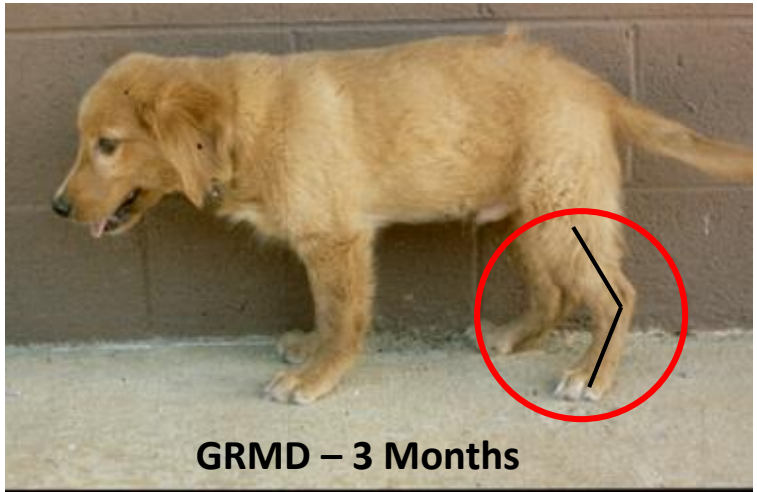
Myostatin Inhibition



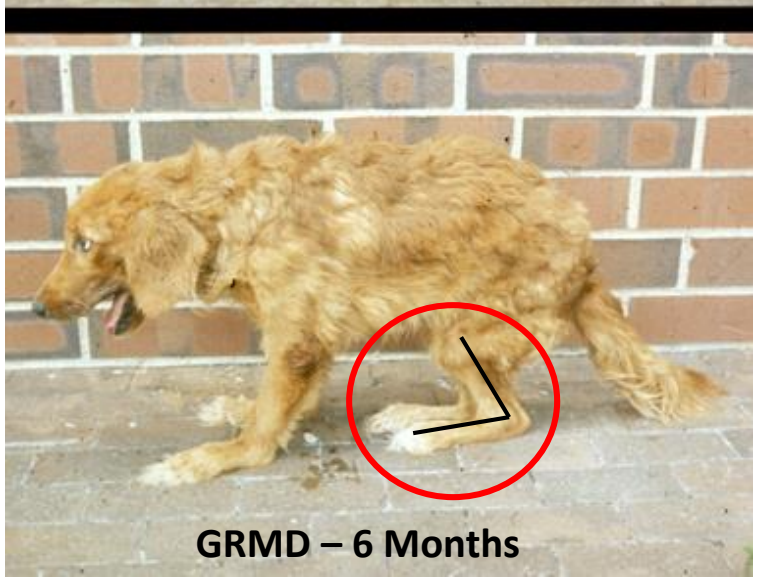
Schulke M, et al: ***NEJM*** 350:2682-8, 2004

- Myostatin (Growth/differentiation factor 8; GDF-8). Negative regulator of muscle growth; mutations lead to muscle hypertrophy (*double muscled cattle; human; sheep; whippets*).
- Knocking out myostatin improves function in mdx mice BUT…
- Adult dystrophy myostatin-inhibition (MYO-29) trial ambiguous
- Murine models of LGMD and CMD either did not improve or had differential effects in young vs. old mice and/or muscles.

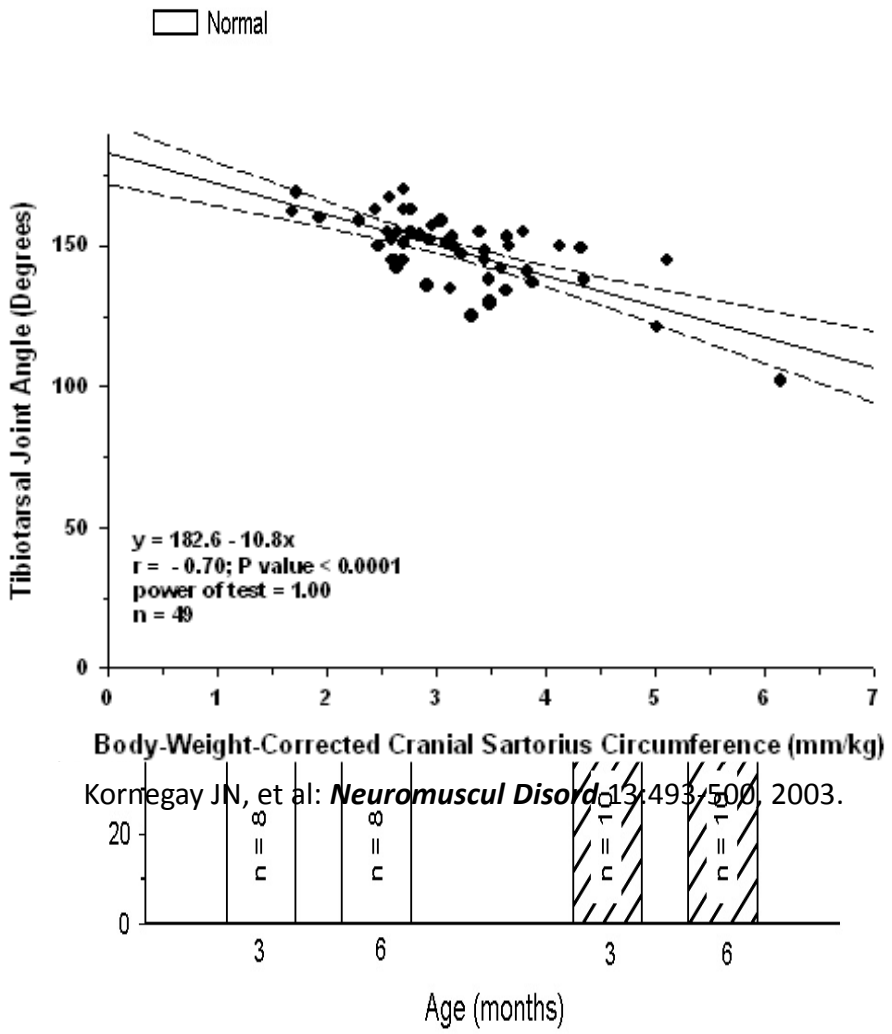
Hypertrophy of the GRMD Cranial Sartorius (and Other Flexor Muscles) May Have Deleterious Effects.



GRMD – 3 Months



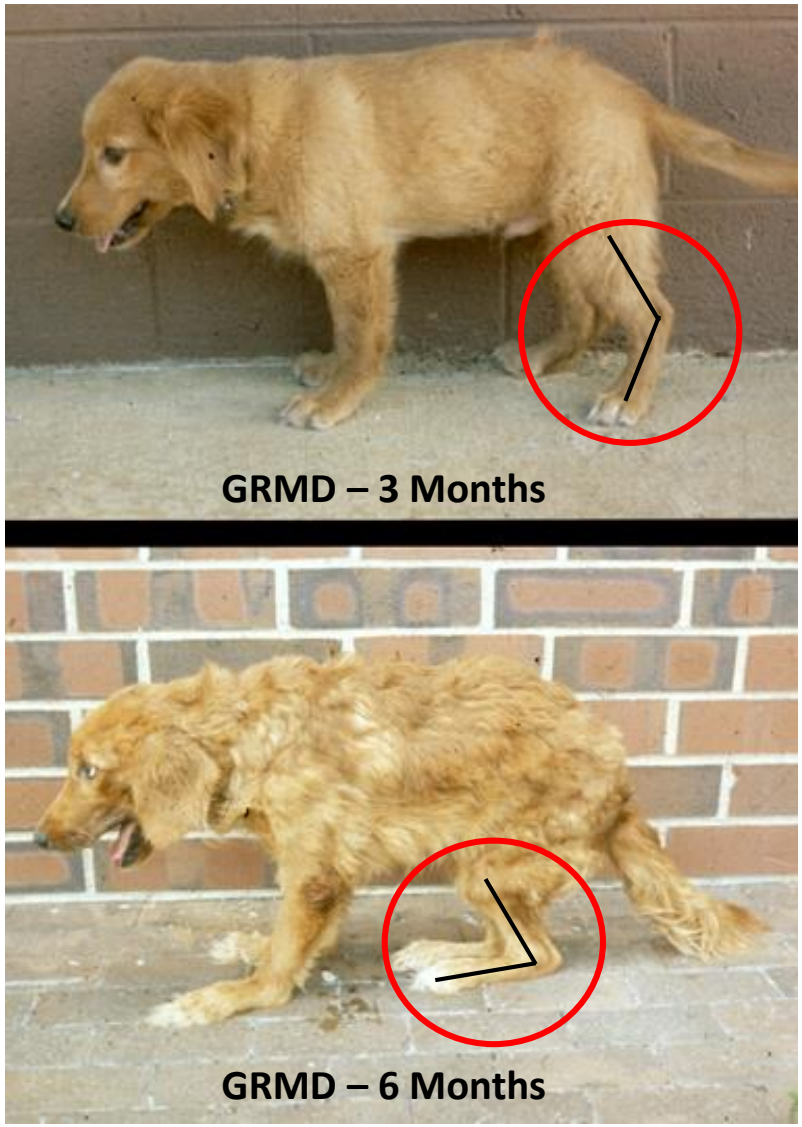
GRMD – 6 Months

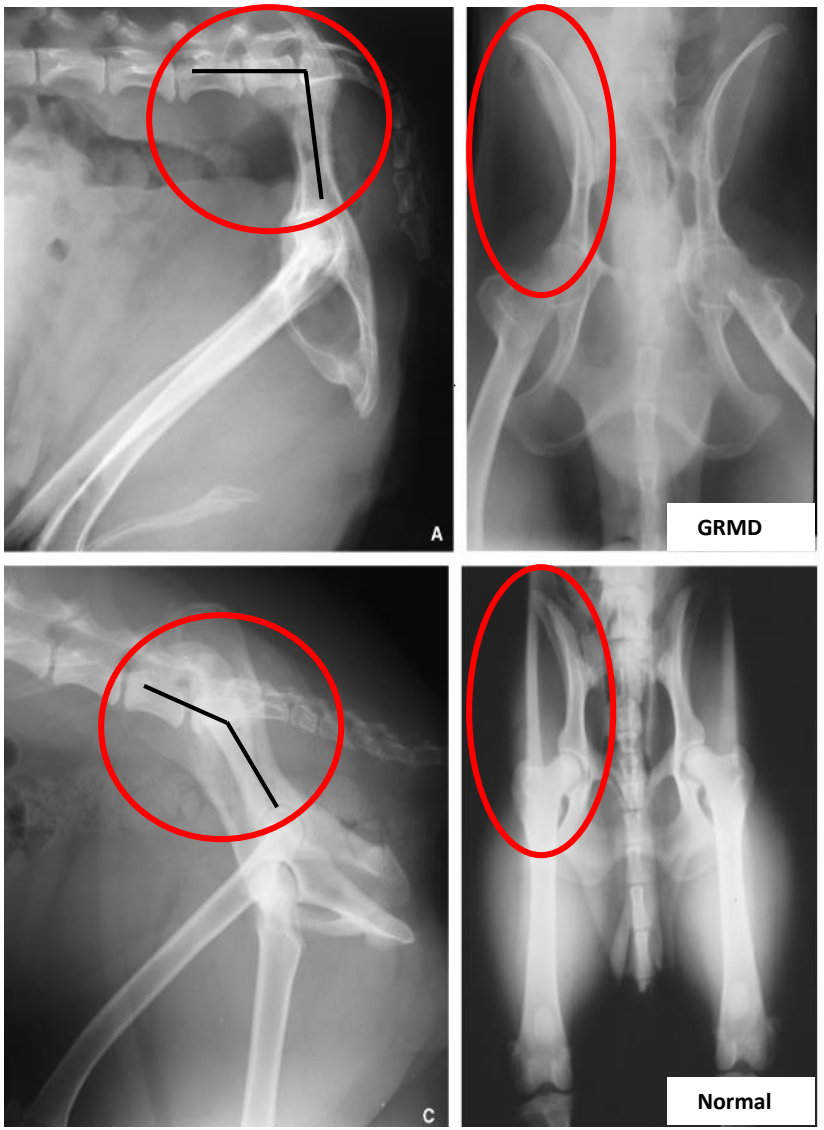


Kornegay JN, et al: ***Neuromuscul Disord*** 13:493-500, 2003.

Kornegay JN, et al: ***Lab Anim Sci*** 44:331-333, 1994.

GRMD – Postural Changes

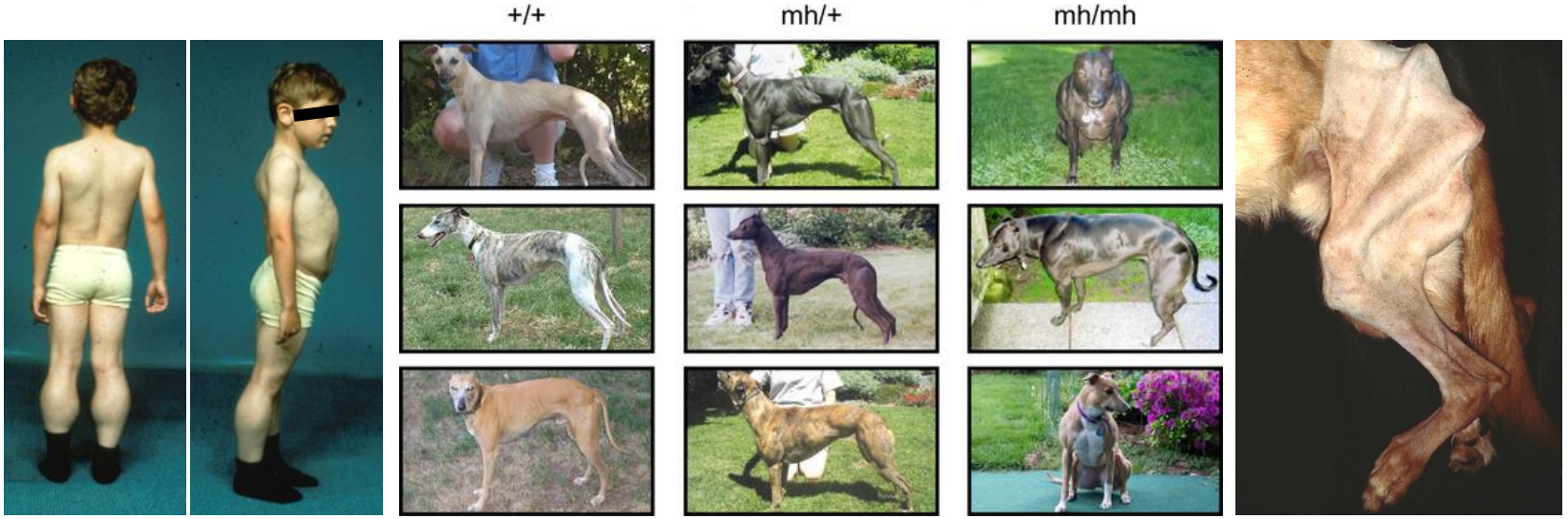




Kornegay JN, et al: ***Lab Anim Sci*** 44:331-333, 1994.

Brumitt JW, et al: ***Vet Radiol Ultrasound*** 47:574-580, 2006.

Myostatin Inhibition



Mosher DS, et al: ***PLoS Genet*** 3:779-786, 2007.

- GRMD data suggest muscle hypertrophy can be harmful, BUT….
- Whippet dogs that are heterozygous for myostatin are better athletes; homozygous-null dogs have gross muscle hypertrophy, i.e. so-called *bully whippets*.
- Transgenic/knockout technology in dogs is not widely utilized.
- Potential to cross breed GRMD and heterozygous myostatin whippet dogs (*GRippets*).

Myostatin-Heterozygous (*Mstn*$^{+/-}$) GRMD Dogs; *GRippets*

- Collaboration with Kathryn Wagner and Se-Jin Lee of Johns Hopkins.
- First litter – GRMD carrier bred to sire (*Mstn*$^{+/-}$) of "Bully Whippet" (*Mstn*$^{-/-}$).
- Second litter – GRMD male bred to *Speedy* (double mutation).

Table 1. GRMD-Myostatin Status

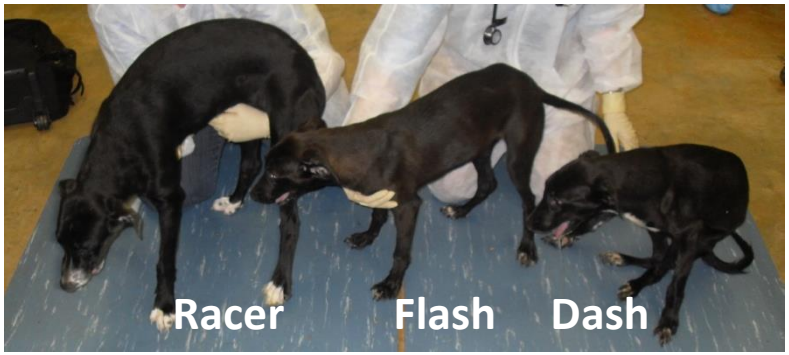
Dog Name	Gender	GRMD Status	Myostatin Status
First Litter ("Racing")			
Racer	Male	Normal	Normal
Flash	Male	Affected	Normal
Dash	Male	Affected	Heterozygote
Speedy	Female	Carrier	Heterozygote
Lightning	Female	Carrier	Normal
Zippy	Female	Normal	Heterozygote
Second Litter ("Bewitched")			
Endora	Female	Carrier	Normal
Esmerelda	Female	Carrier	Heterozygote
Samantha	Female	Affected	Normal
Hagatha	Female	Affected	Normal
Tabitha	Female	Affected	Heterozygote
Derrwood	Male	Affected	Heterozygote
Abner	Male	Affected	Heterozygote

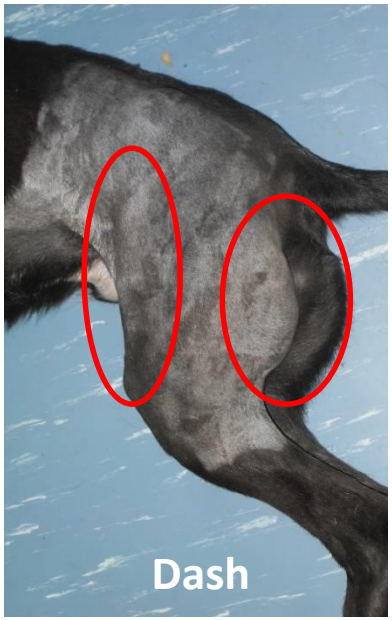
Non-dystrophic Controls (n = 3)
GRMD Myostatin normal (n = 3)
GRMD Myostatin heterozygous (n = 4)

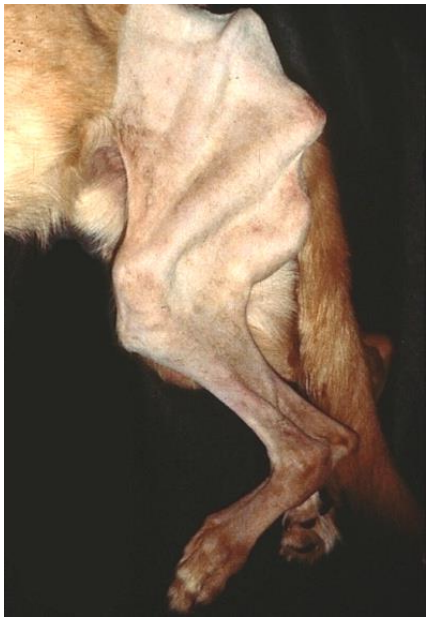
Kornegay JN, et al, submitted.

Myostatin-Heterozygote (*Mstn*$^{+/-}$) GRMD (Litter 1 – "Racing")

- Similar phenotype for *Flash* and *Dash* until ~ 4.5 mos.
- *Dash* developed contractures.
- Hypertrophy of the cranial sartorius, semitendinosus, and semimembranosus muscles.
- *Dash* had features of a severe GRMD phenotype.
- Contractures – unbalanced agonist/antagonist muscles.
- Bigger is not necessarily better.







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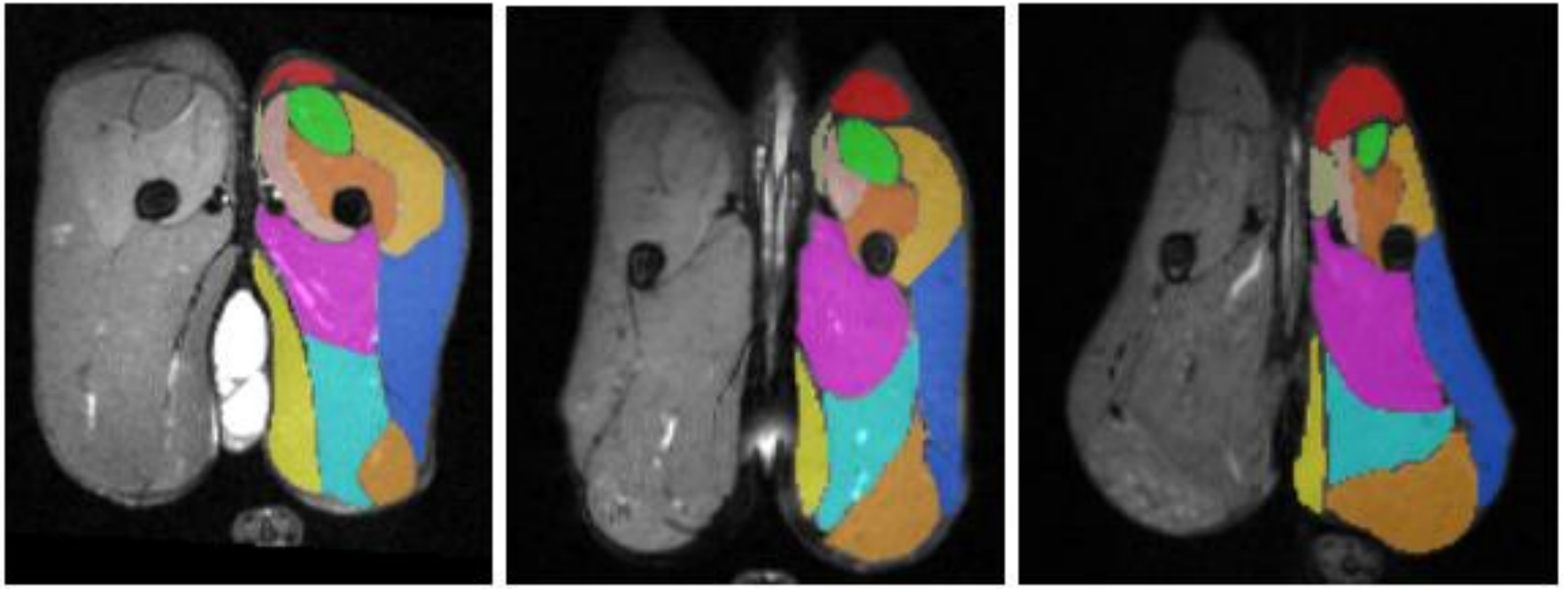
Segmentation



- Overall body-weight-corrected (mm^3/kg) muscle mass comparable among the three groups of dogs.
- Marked variation among muscles; trend whereby relative lack of myostatin exaggerates pre-existing trends for muscle atrophy/hypertrophy.

Kornegay JN, et al: ***Phys Med Rehabil Clin N Am*** 23:149-72, 2012.

The Realities of Drug Development

- The success rate for Phase II human clinical trials fell from 28% in 2006-2007 to 18% for 2008-2009 (Arrowsmith 2011).
- Over half (51%) of 108 reported Phase II failures occurred due to insufficient efficacy, even though most drugs were assessed in animal models (Plenge et al. 2013).
- Only 28 of 76 (37%) of *highly-cited* studies that investigated a preventive or therapeutic intervention in an *in vivo* animal model over the 1980-2000 period were replicated in human randomized trials (Hackam and Redelmeier 2006).

Lost in Translation

(Ergorul and Levin 2013)

- The *Butterfly Effect* (chaotic behavior whereby small differences in the animal model lead to substantial differences in clinical results);
- The *Princess and the Pea* problem based in variability of effect size when progressing from biochemical findings through tissue culture and animal and human studies (the pea does not indent the mattress to the same degree as the princess);
- The *Two Cultures* problem evident in preclinical and clinical research (need for more rigorous experimental design in preclinical studies).

Biomedical Models
National Research Council (1985, 1998)

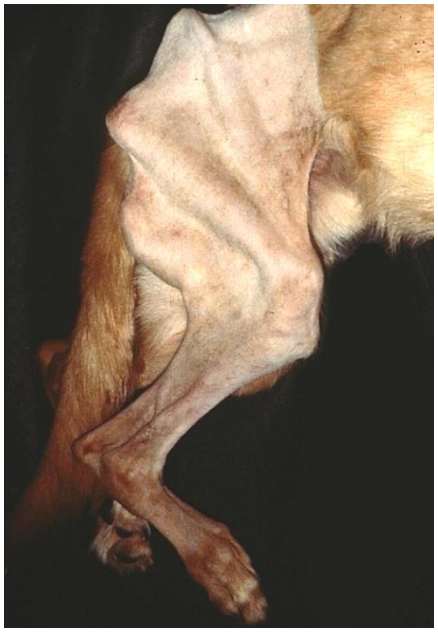
- Biological (animal) models can be based in *analogy* or *homology*.
  - Analogy implies a point-by-point relationship.
  - Homology implies a shared evolutionary history and DNA makeup.

To be functionally useful, homologous models must be good models by analogy.

- Models may be *one-to-one* (disease in humans and a species that share the same clinical features) or *many-to-many* (findings from more than one species or organ system model disease features).

GRMD – General Features

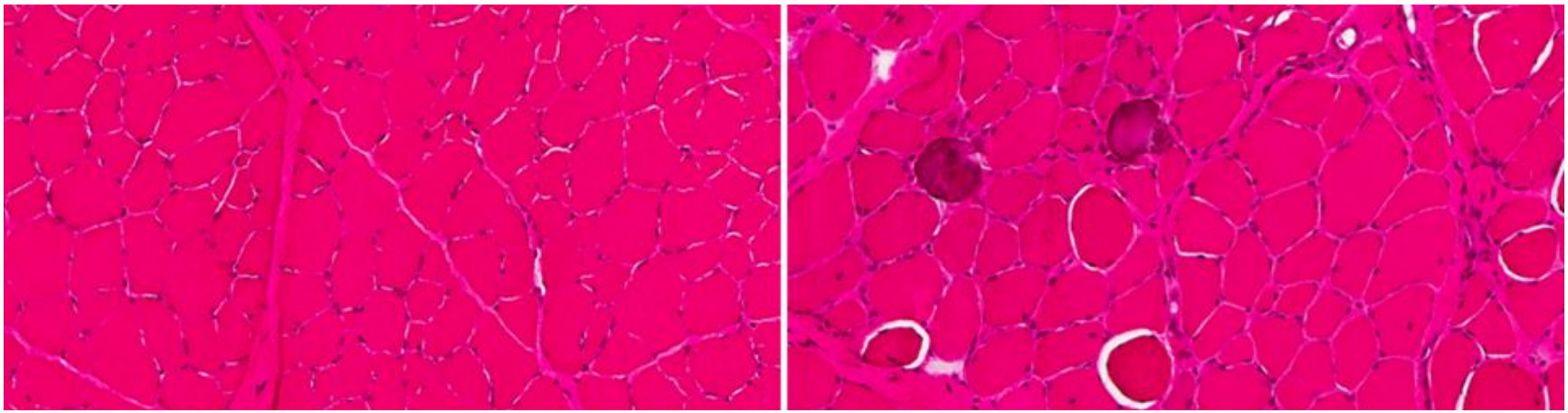
- Progressive disease
- Postural instability/contractures
- Muscle atrophy and hypertrophy
- Kyphosis/lordosis
- Respiratory
- Cardiomyopathy



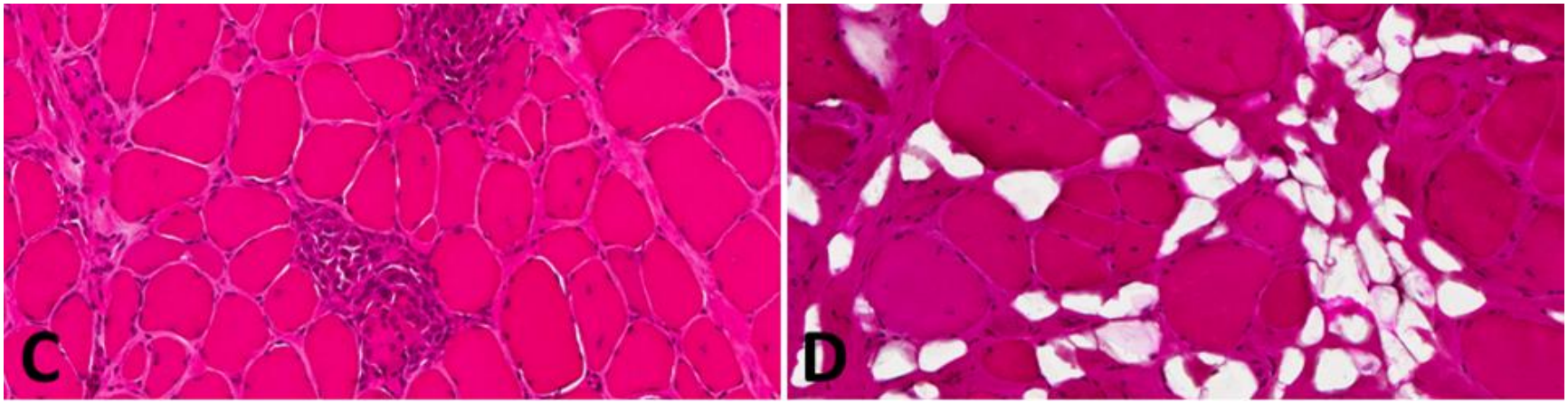
Phenotypic Variation: Species, Individual, and Muscle (confounds preclinical trials but offers insight on disease pathogenesis).

Primary vs. Secondary Effects of Dystrophin Deficiency and Identification of Modifier Genes as *Druggable* Targets

Traditional Drug Targeting – Histopathologic Lesions and Presumed Pathogenetic Mechanisms



A New Model – Targeting Genetic Mechanisms such as Modifier Genes through mRNA Array and GWAS Studies



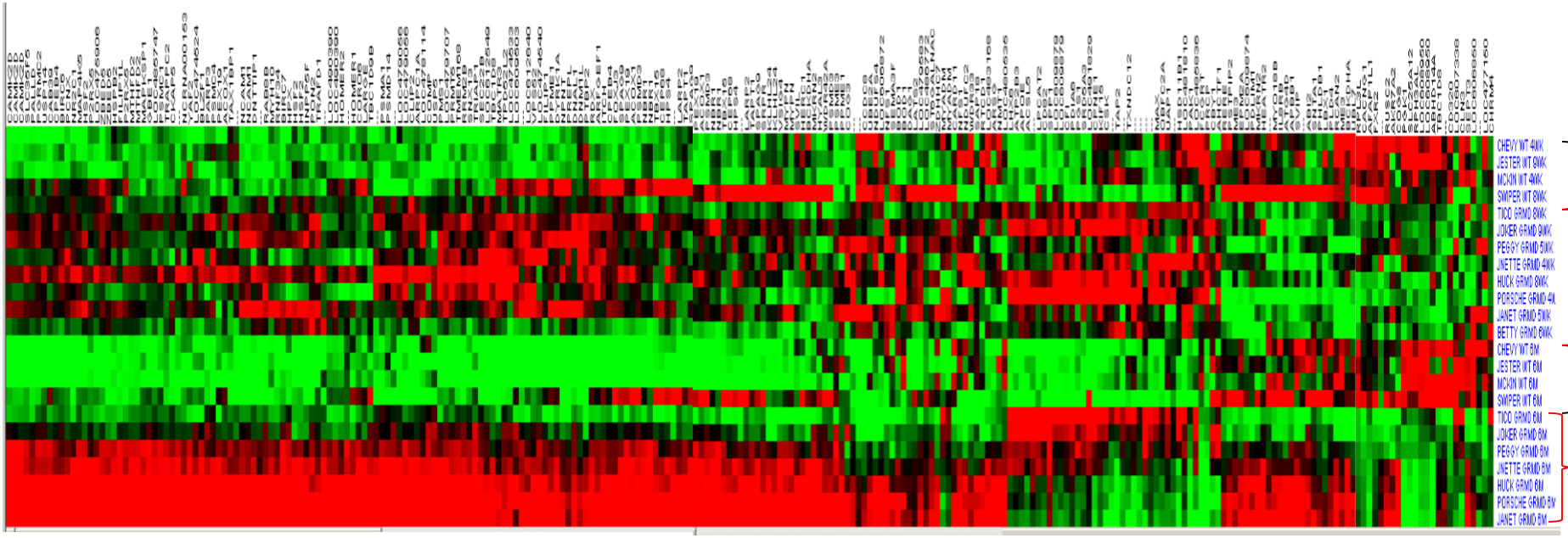
Kornegay et al: ***ILAR J***, in press.

Phenotypic Data from GRMD Dogs Assessed with mRNA Microarrays

- Collaboration with Scott Schatzberg and Peter Nghiem at UGA and Eric Hoffman at Children's National Medical Center in Washington, DC.
- 8 GRMD and 4 normal dogs with muscle biopsies at 8 wks and 6 months.
- Biopsies from the vastus lateralis, cranial sartorius, and long digital extensor muscles.

GRMD Dogs (6 mos)	Left TTJ Angle (degrees)	Tetanic Extensor Force (N/kg)	Tetanic Flexor Force (N/kg)	Body Weight (kg)	CS Circumference (mm/kg)	Overall Rank (1= least severe, 8= most severe phenotype)
Tico	159	3.138	0.296	15.2	2.303	1
Joker	155	2.135	0.331	16.3	2.653	2
Peggy	155	2.109	0.489	13.1	2.774	3
Jeannette	151	1.669	0.489	13.2	3.102	4
Huckleberry	141	1.144	0.508	13.2	3.298	5
Porsche	130	1.041	0.575	14.6	3.493	6
Janet	142	1.583	0.667	12.6	3.598	7
Betty	102	2.29	0.72	9.3	6.156	8

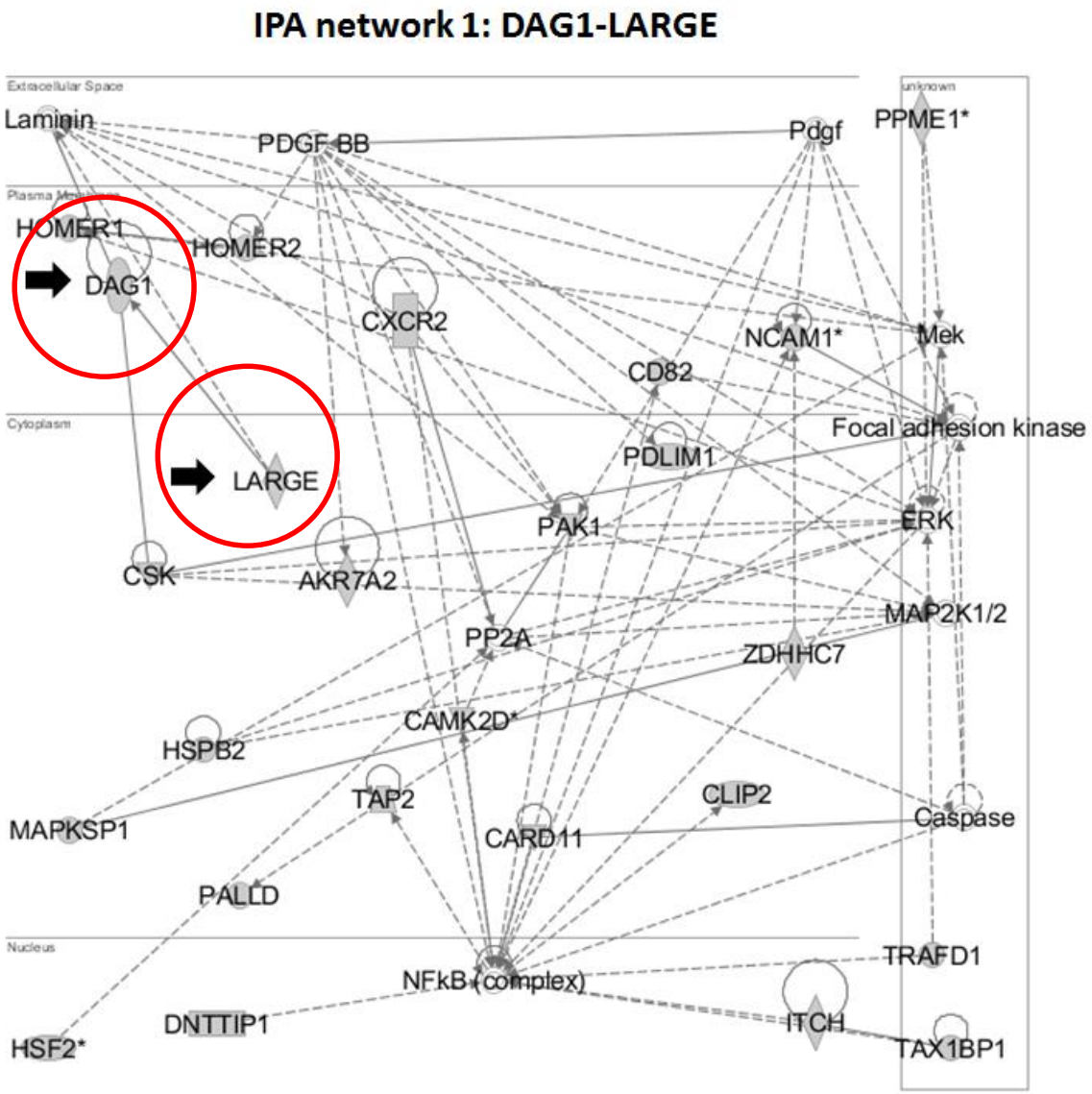
Supervised hierarchical clustering of **250 genes** from **cranial sartorius (CS)** array correlated to CS size ($p = 0.001$; $r = > 0.94$) in CS profiles of normal and GRMD at 4-9 Wk and 6 Mo **Red = up-regulated; Green = down-regulated**



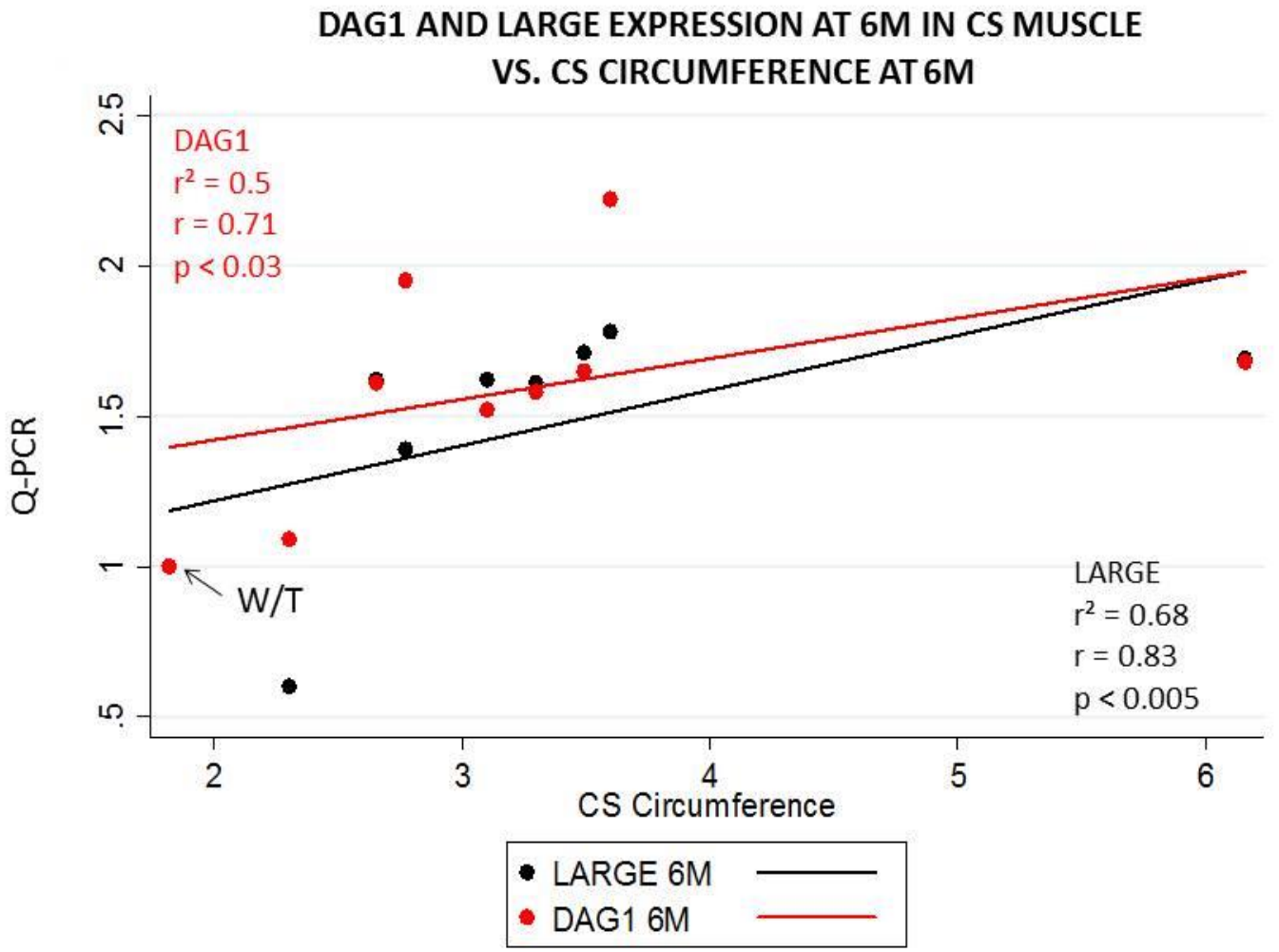
1 = Normal CS 4-9 Wk (n = 4)
2 = GRMD CS 4-9 Wk (n = 8)
3 = Normal CS 6 Mo (n = 4)
4 = GRMD CS 6 Mo (n = 8)

Nghiem PP, et al: ***Am J Pathol*** 183:1411-24, 2013.

Ingenuity Pathway Analysis (IPA) of 250 genes associated with CS hypertrophy generated the top-ranked network, DAG1 & LARGE.



Nghiem PP, et al: ***Am J Pathol*** 183:1411-24, 2013.



Nghiem PP, et al: ***Am J Pathol*** 183:1411-24, 2013.

Additional Studies

Table 2. Total Spectral Counts for Spectrin, Myotrophin, and Laminin-α2 from Proteomic Profiling Results

Genotype	Age	SPTAN1	SPTBN1	MTPN	LAMA2
Normal	4-9 wks	43	7	7	54
	6 mos	36	1	5	3
GRMD	4-9 wks	65	19	12	12
	6 mos	65	3	15	0

Sum of spectral counts for each group is shown. There are n = 3 profiles for normal and GRMD at 4 to 9 weeks and at 6 months (12 total profiles). LAMA2, laminin-α2; MTPN, myotrophin; SPTAN1, α-spectrin; SPTBN1, β-spectrin.

- Proteomic profiling (mass spectrometry) was done to identify additional dystrophin surrogates and/or hypertrophic factors.
- Membrane proteins α- and β-spectrin, as well as the muscle growth factor, myotrophin, were selectively upregulated in the CS (Confirmed by IHC and/or Western blots).

Nghiem PP, et al: ***Am J Pathol*** 183:1411-24, 2013.

What about Myostatin?

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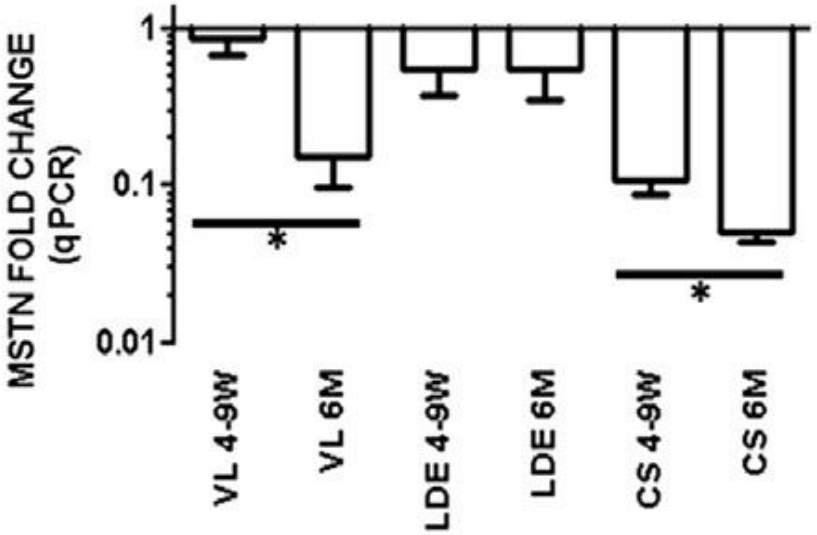

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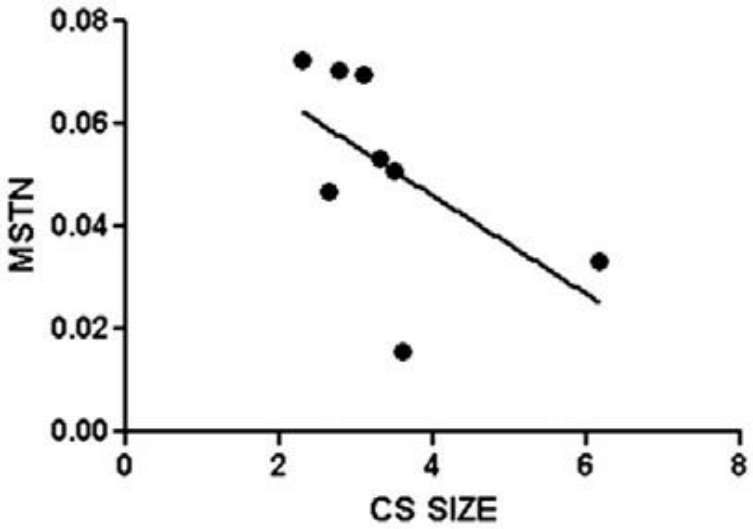


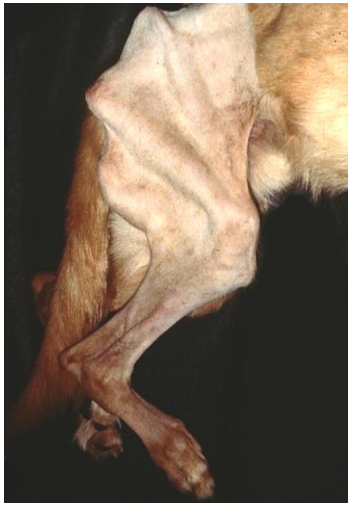
C: MSTN qPCR expression revealed decreased expression in GRMD muscle, with the greatest decrease in the hypertrophied CS at 6 months (6M; n = 8 arrayed dogs). **D**: MSTN mRNA was inversely correlated with CS size at 6 months in the CS of GRMD dogs of the discovery data set ($r = 0.73$; $r^2 = 0.53$; $P < 0.05$; n = 8 arrayed dogs). *P < 0.05, **P < 0.01, and ***P < 0.001.

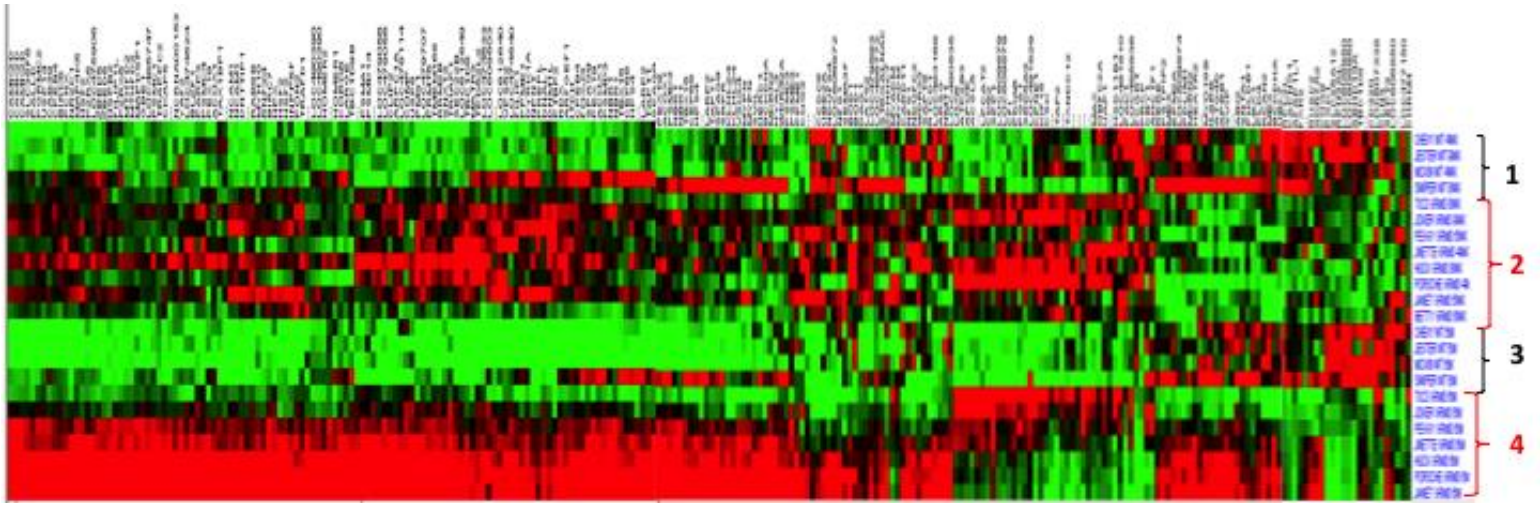
- Myostatin was downregulated on qPCR in all GRMD muscles but especially in the CS.
- Myostain mRNA levels were inversely correlated with CS muscle circumference at 6 mos.

Nghiem PP, et al: ***Am J Pathol*** 183:1411-24, 2013.

Overall Conclusion: Cranial sartorius hypertrophy in the GRMD model is driven and supported by a complex set of genes whose manipulation could have (*favorable or deleterious*) clinical significance.

Summary





- Animal models are a powerful example of the *one medicine* concept.
- Preclinical studies often do not translate to humans.
- *Homologous* genetic models are not necessarily *analogous*.
- Genetic studies, including mRNA analysis, offer an additional tool to identify potential drug targets, especially if coupled with phenotypic data.

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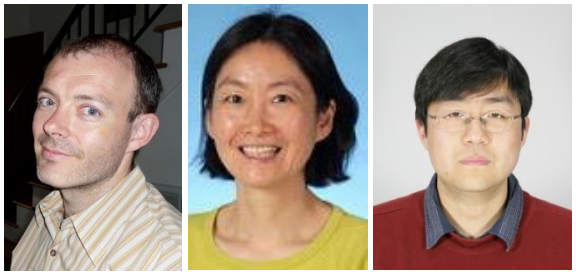

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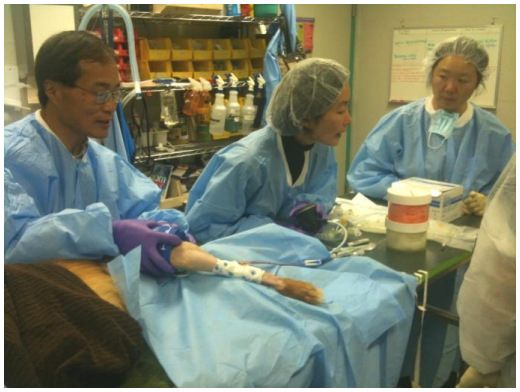
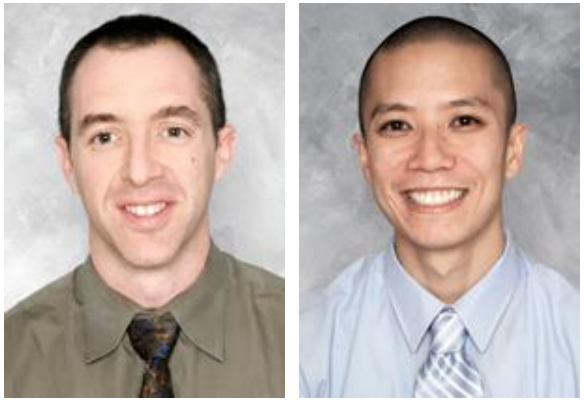


GRMD AAV-Minidys: Xiao Xiao and Juan Li



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Parent Project Muscular Dystrophy
Leading the Duchenne muscular dystrophy community

Funding

- Muscular Dystrophy Association
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- Parent Project Muscular Dystrophy
- National Institutes of Health (NIAMS, NINDS, NHLBI, NCMRR)

MDA
Muscular Dystrophy Association

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QUESTIONS?

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