A roadmap for bringing FKRP gene therapies into clinical use

Jeffrey S. Chamberlain, Ph.D.
McCaw Chair in Muscular Dystrophy
Wellstone Muscular Dystrophy Research Center - Seattle
Depts. of Neurology, Medicine and Biochemistry
University of Washington
Seattle, WA USA

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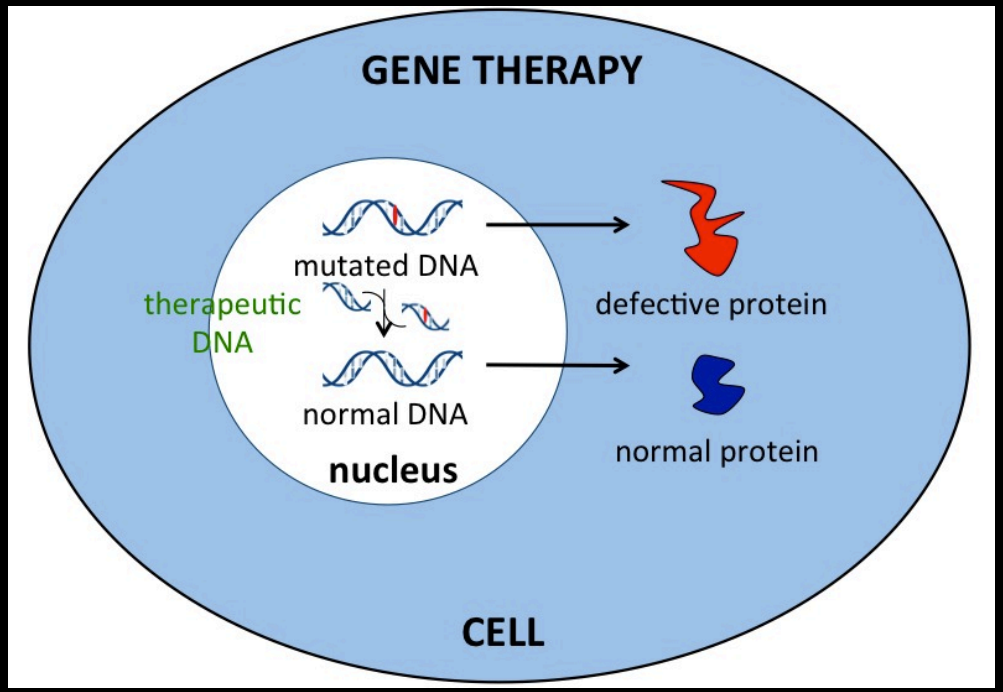
Gene therapy for muscular dystrophy

- **Gene therapy approaches are showing promise for several neuromuscular disorders**
  - **Spinal muscular atrophy and Duchenne muscular dystrophy**
- **These encouraging results suggest that similar approaches might be successful for many other dystrophies**
- **Mutations in >20 different genes lead to 'dystroglycanopathies'**
  - **Each of these leads to a failure to properly process a critical protein needed for maintaining healthy muscle cell walls (dystroglycan -Campbell lab)**
- **Gene therapy could restore normal protein production**
  - **Thus fixing the problem with altered dystroglycan function**

Why gene therapy?

- **Fix the actual *cause* of genetic disorders**
- **Potential for a permanent treatment**
- **Can be applied to muscles, and/or to muscle stem cells**
- **Many of the challenges in getting it to work are shared among different dystrophies**



Gene Therapy for Dystroglycanopathies

- **Among the most common dystroglycanopathies are those resulting from mutations in the FKRP gene**
  - **LGMD2i, MDC1C, WWS, MEB disorders**
  - **All result from defective production of the FKRP protein**
- **Can gene therapy be used to restore production of normal FKRP?**
- **Similar approaches may work for many or all dystroglycanopathies, and for many other types of muscular dystrophy**

- **What is needed to achieve these goals?**
  - **Examples from DMD and FKRP**

Gene Therapy –different types

- **Gene replacement therapy: deliver a new version of a gene to the target tissue (gene addition)**
- **Gene editing: directly modify a gene to fix or bypass a mutation**
  - **CRISPR/Cas9**
- **Gene knockdown: Shutdown the function of a mutant gene**
  - **Dominant disorders only- probably not applicable to dystroglycanopathies**
- **RNA modification, e.g. ‘exon skipping’**
  - **Sarepta’s drug for DMD- *not applicable to FKRP***

Gene Therapy – clinical results

- **Potential for gene therapy supported by multiple recent successes in clinical trials:**
  - **Hundreds of AAV gene therapy trials to date; Strong safety profile**
  - **Two gene therapies recently approved by the FDA: a form of blindness; Car-T cells for leukemia**
  - **Several more close to approval (e.g. hemophilia)**
  - **15 infants with spinal muscular atrophy successfully treated with gene therapy (Mendell/Kaspar/Avexis)**
  - **Encouraging early data with Duchenne muscular dystrophy (DMD)**

Gene Therapy for FKRP disorders

- ***Goal:*** **Develop methods to *replace* or *repair* FKRP gene**

- ***Gene replacement:*** **AAV/FKRP**

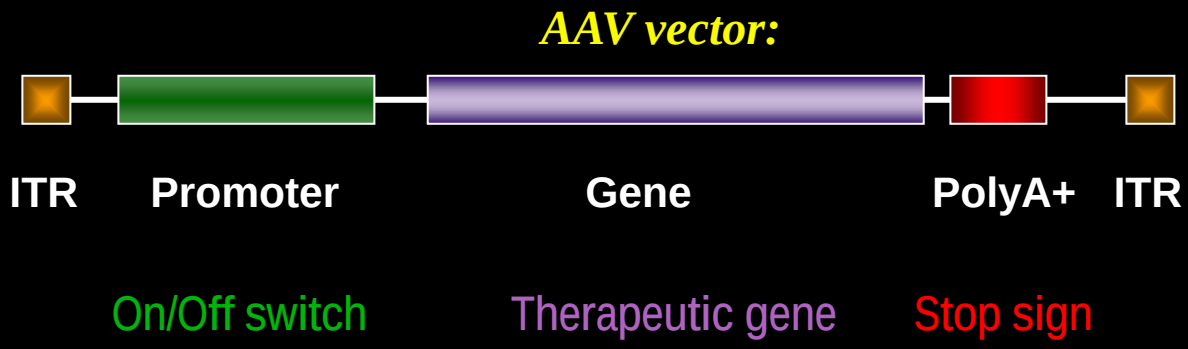
- ***Gene editing: CRISPR/Cas9***
  - **Must be adapted for each mutation**
  - **Best way to deliver remains uncertain (maybe AAV??)**

- **Gene replacement approaches – in human trials for several disorders**
- **Gene editing with CRISPR/Cas9 - future potential?**
  - **Not ready for prime time**

Challenges for gene therapy of MDs

- **How can you safely deliver a new gene to muscles (& brain?) bodywide?**
  - **Development of delivery VECTORS by manipulating viruses**
  - **Remove viral genes, replace with gene of interest (*e.g.* FKRP)**

- **Vectors derived from adeno-associated virus are promising**
  - **Some types enable systemic gene delivery *via* the bloodstream**
  - **AAV vectors have a small carrying capacity; gene size is important**



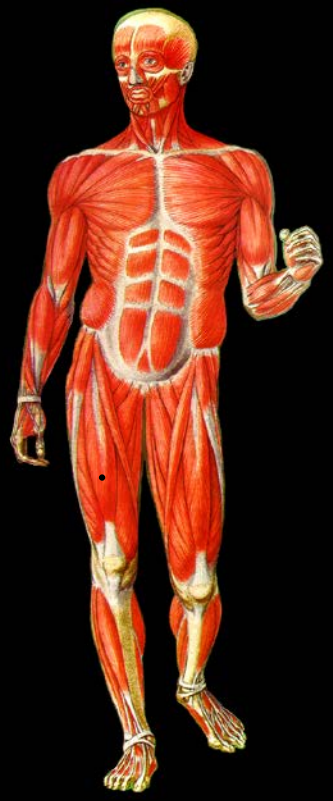
Adeno-associated viral (AAV) vectors

***PROS*:**

- **Numerous ‘serotypes’, many target muscle (AAV6, 8 & 9; rh74)**
- **Relatively easy to produce; scalable to bioreactor production**
- **Can be used for bodywide gene delivery, especially to muscles**
- **Some types cross the blood-brain barrier**

***CONS*:**

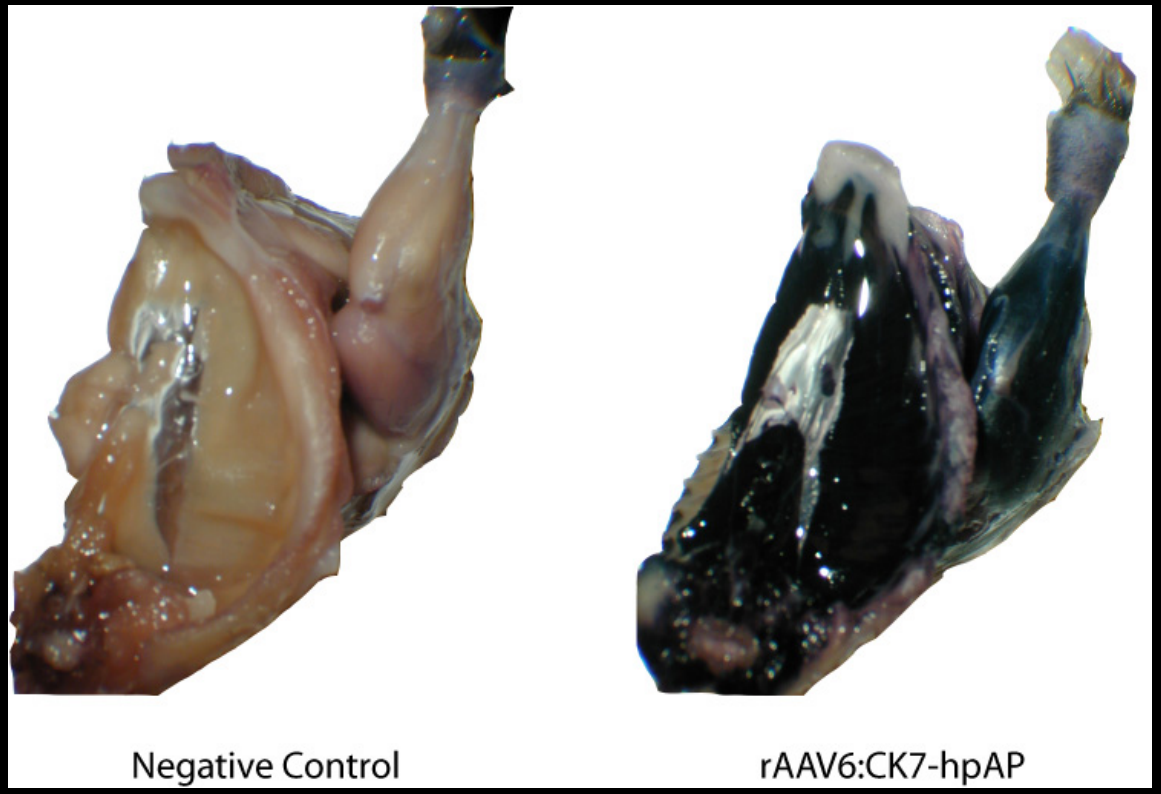
- **Small carrying capacity (problems with large genes)**
- **Generally poor results in stem cells**
- **Up to one-third of older patients may be immune to AAV**
- **Difficult to administer more than once**



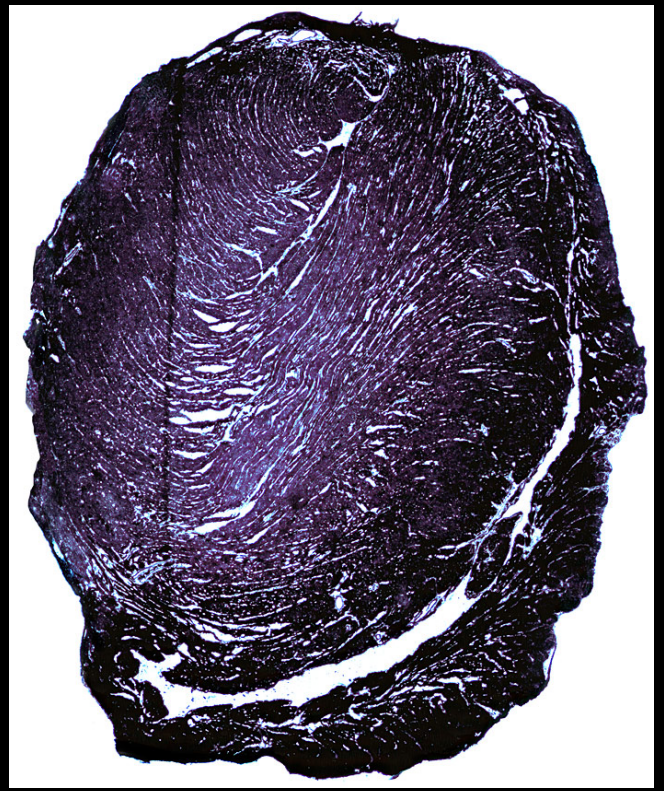
AAV vectors can deliver genes bodywide to muscles "systemic delivery"

- **One IV injection of AAV/AP into adult mice; effect lasts more than 2 years**

AP stain - 2 mos after injection



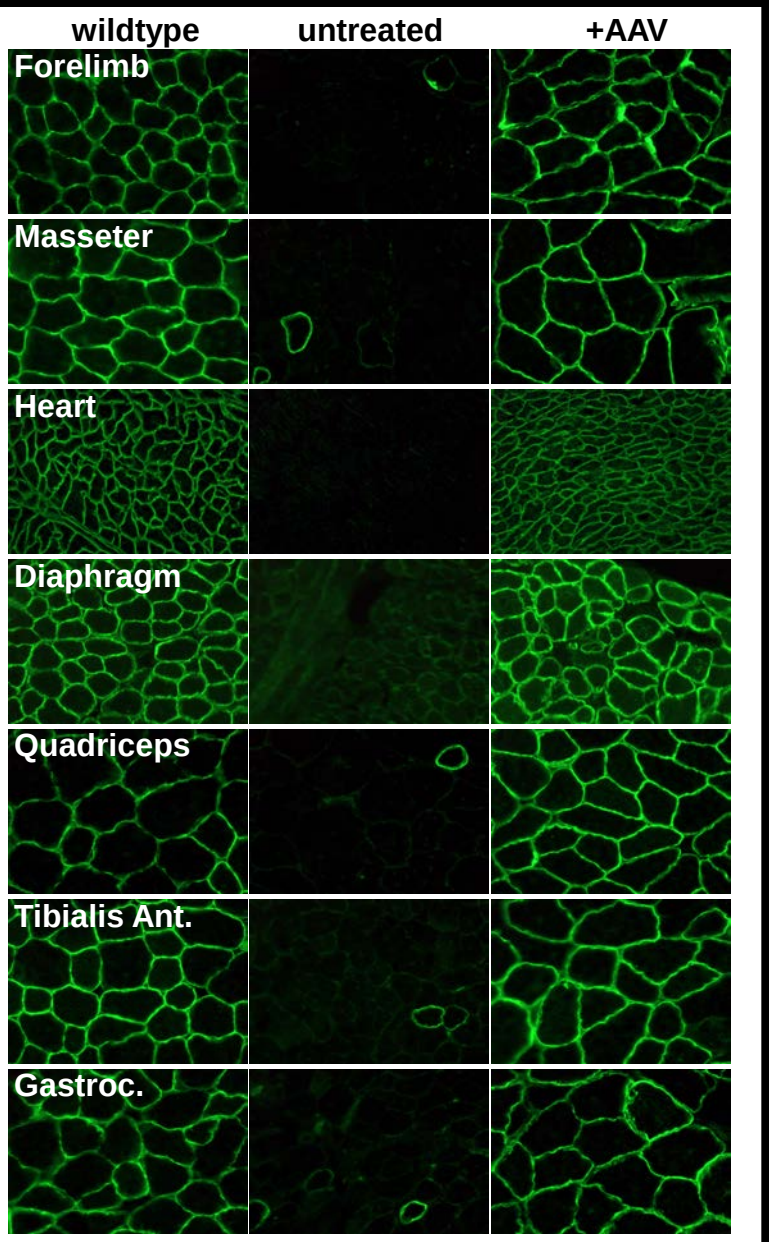
AP Stain, heart, 2 years later

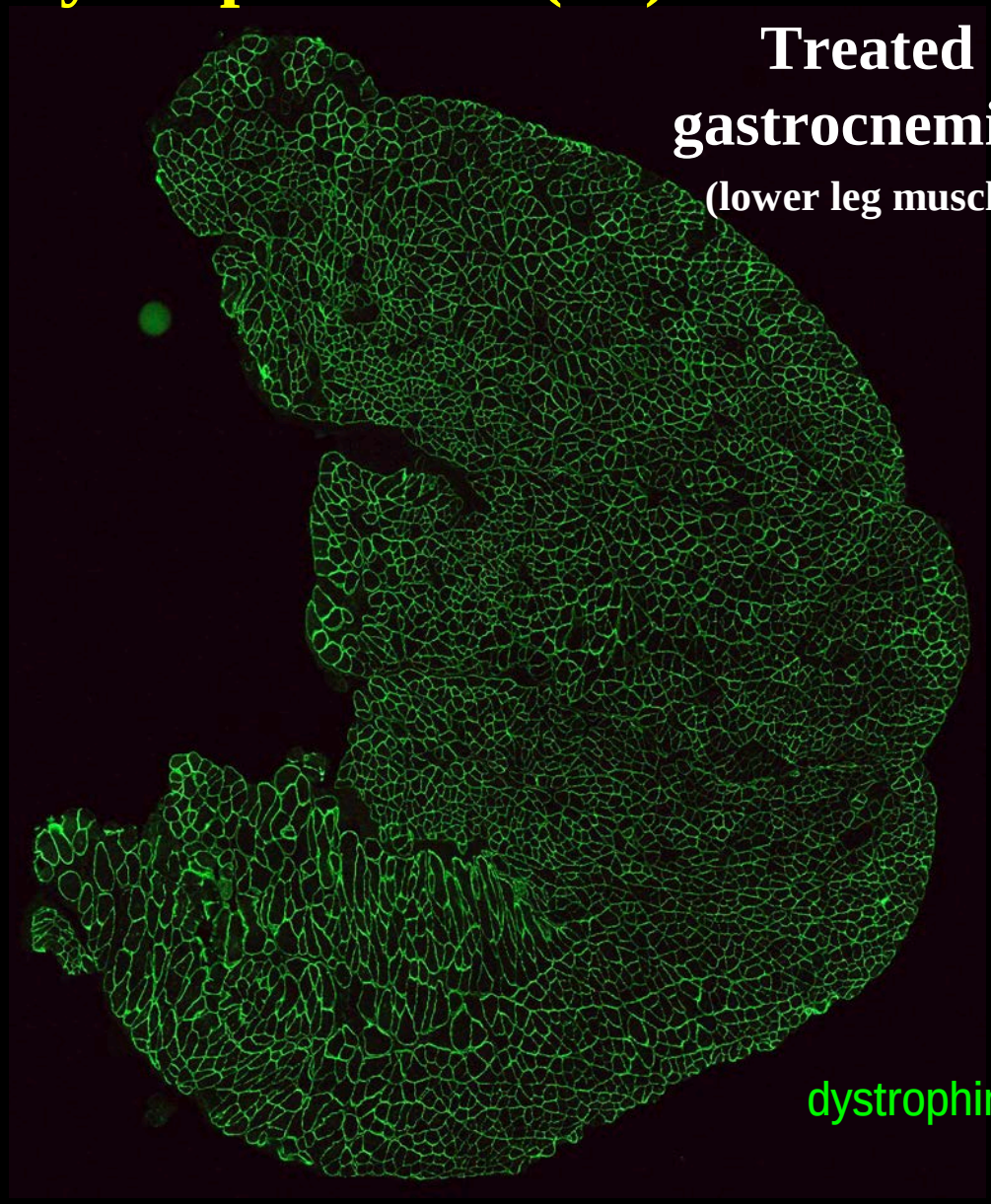


First identification of a way to deliver genes bodywide

Paul Gregorevic, J Chamberlain, et al. Nature Medicine, 2004

Expression of micro-Dystrophin one year after AAV infusion into dystrophic mice (IV)





Gregorevic, Chamberlain et al: Nature Medicine, 2006

AAV-mediated gene therapy for DMD

- **AAV-µDystrophin stops muscle loss, protects from exercise-injury and improves strength**
- **Efficient bodywide delivery has been achieved in mice and large animals**
- **No reduction in efficacy after at least 2.5 years**
- **High dose AAV delivery was safely achieved in humans (e.g. SMA- Nationwide Children's)**
- **Clinical trials of AAV/µDys were started this year by 3 groups (DMD)**

Clinical trials planned / in progress

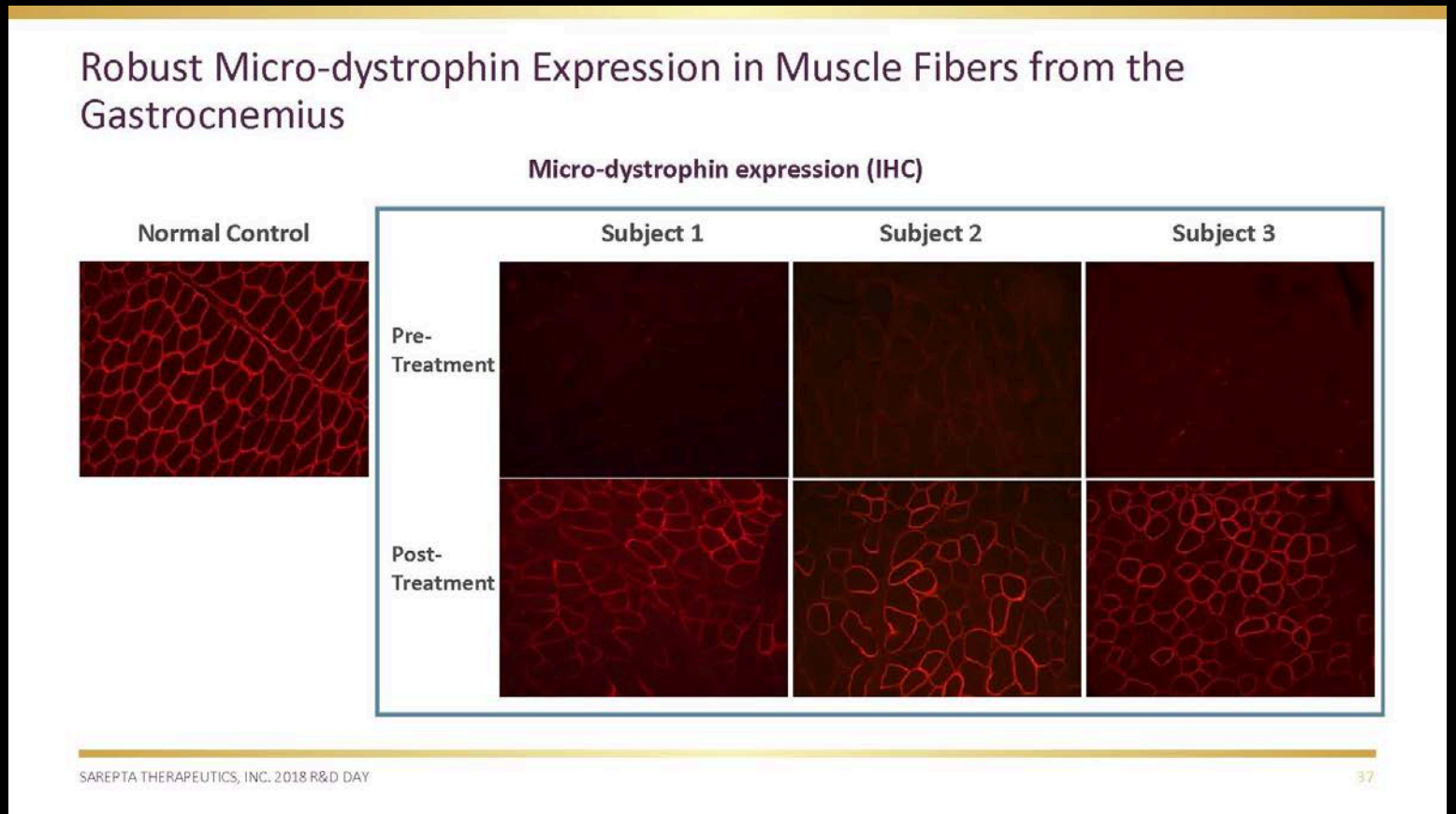
- **Solid Biosciences - Byrne et al (Chamberlain uDys); AAV9**
- **Nationwide Children's – J Mendell et al (Sarepta) (Chamberlain uDys-1st gen); AAV-rh74**
- **Pfizer– E. Smith et al (X. Xiao uDys); AAV9**
- **Genethon planning a trial in 1-2 years - Dickson et al (Chamberlain µDys 1st gen; AAV8)**

All are using bodywide delivery of an AAV vector to patients

- **Early focus is on safety, and production of dystrophin**
- **Unclear on how long it will take to observe a *functional* benefit**

Preliminary results from DMD gene therapy trials

- **Sarepta: data from 3 patients announced late June 2018**
  - **1 biopsy from each: widespread micro-dystrophin observed in the gastrocnemius muscle**
  - **~35-55% of normal dystrophin levels (2 patients)**
  - **Serum CK levels reduced ~85% (3 patients)**



Jerry Mendell et al unpublished

Preliminary results from DMD gene therapy trials

- **Solid Biosciences and Pfizer have not yet announced results**
  - **Parents report *anecdotal* improvement, but not a reliable result**
  - **Reporting data on Facebook/Twitter?**
- **Data reported so far matches data from large animal studies**

Future:

- **At least 12 more patients to be studied over the next year**
- **Future results will include strength measurements**
- **Testing in older patients**
- ***Continued positive results will simplify gene therapy for other muscular dystrophies***

Gene therapy for FKRP mutations

- **Consortium formed to plan trials in March 2018**
- **Initial meeting - U Mass**
  - **Qi Lu, Isabelle Richard, Katherine Matthews, Kathryn Wagner, Francesco Muntoni, Jeff Chamberlain, Volker Straub, Brenda Wong, Terry Flotte, Jean-Pierre Laurent, Kelly Brazzo, Herb Stevenson**
- **Possibility of two trials (France, USA)**
- **Details of vector/gene/dosing being explored**
- **Immediate goal to begin screening patients for antibodies (immunity) to AAV vectors**

Vector development and testing for gene therapy with AAV-FKRP in mouse models

Several groups have designed AAV vectors to make FKRP

Current studies are asking:

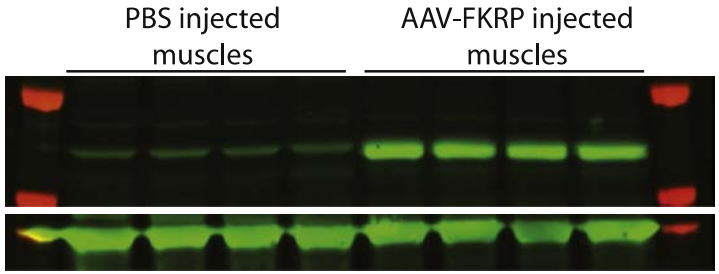
- **Which type of AAV is best**
- **Best promoter to use (on/off switch)?**
- **Design of FKRP gene?**
- **How much FKRP is needed**
- **Does the stage of disease (& age) affect therapy?**

AAV vector:

ITR **Promoter** **FKRP gene** **stop** **ITR**

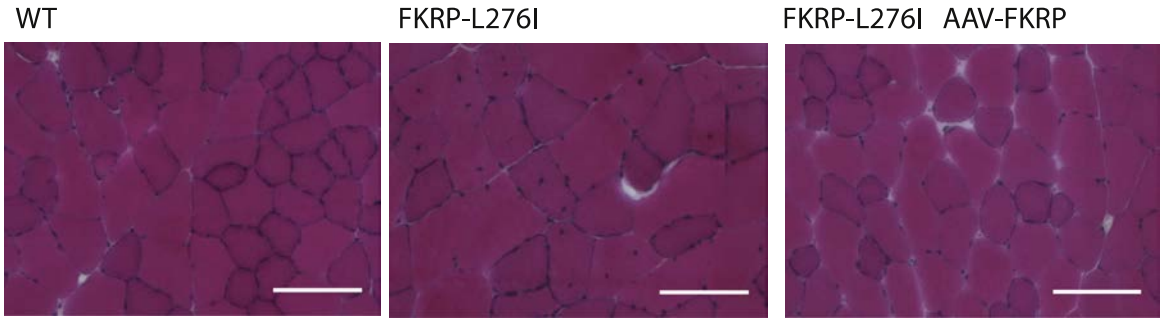
AAV-FKRP gene delivery improves muscle structure in a mouse model for LGMD2i

A



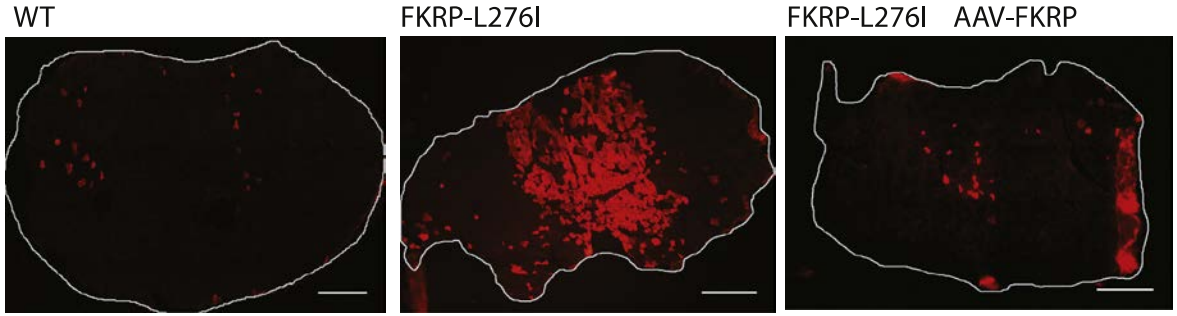
Western blot- FKRP production

B



Muscle structure

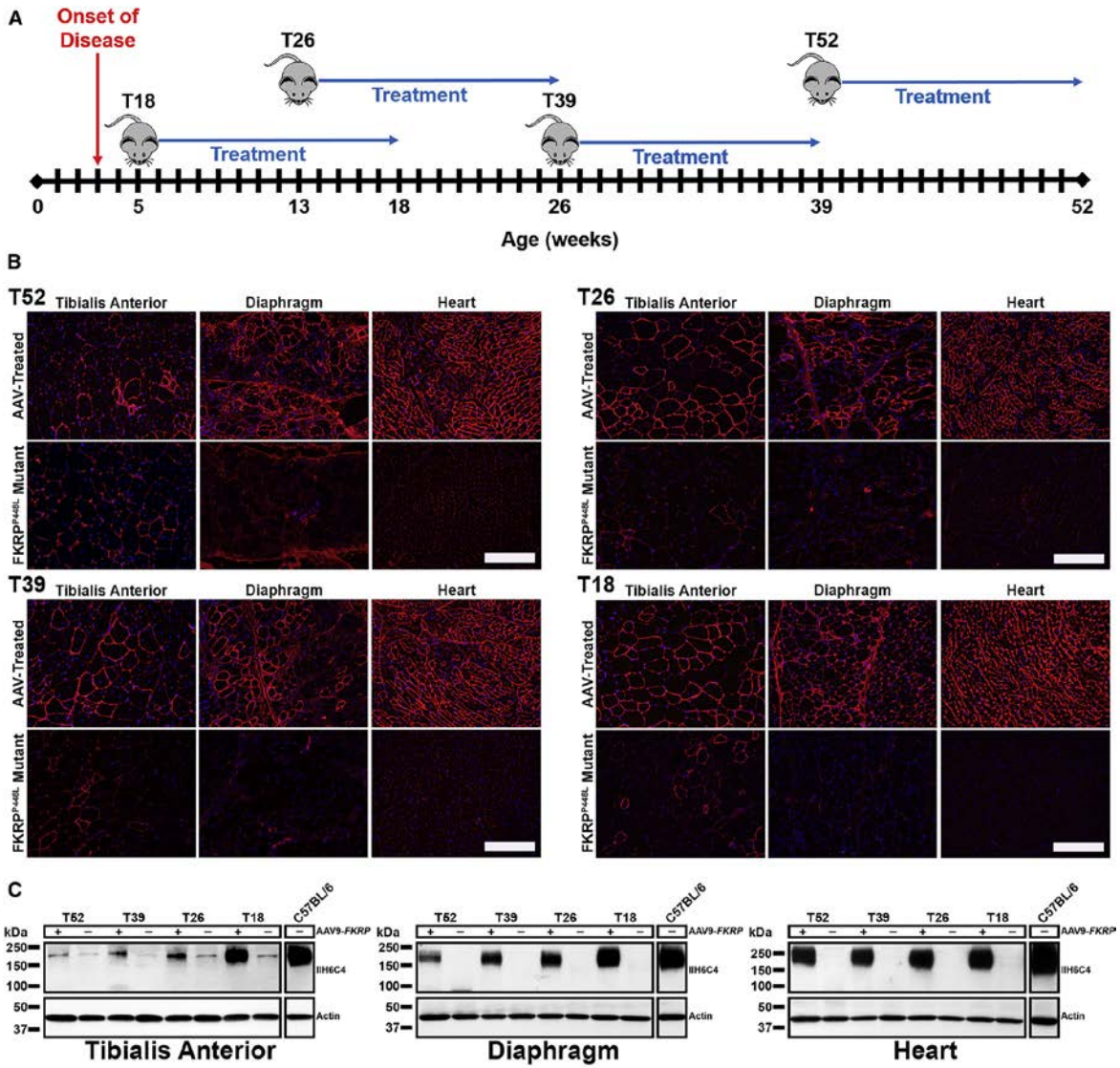
C



Muscle damage

- **Data from Isabelle Richard's lab; Genethon (France)**

AAV-FKRP gene delivery improves muscle function at a variety of mouse ages (MDC1C model)



Red = glycosylated alpha-dystroglycan (shows restored function of FKRP)

- **Qi Lu lab, Mol Ther Meth Clin Dev 2017**

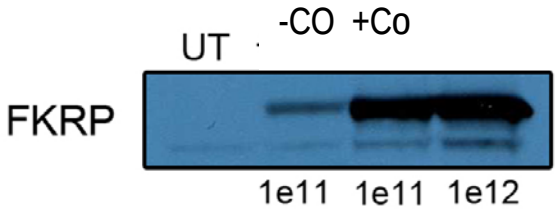
Production of FKRP using AAV-FKRP

-promoter: muscle-restricted promoter/enhancer (on/off switch)

-Same basic vector backbone as in Solid Biosciences DMD trial

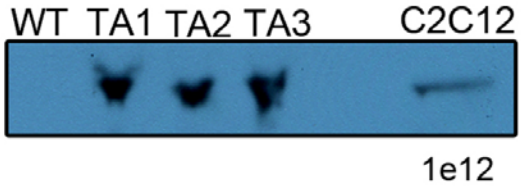
Western blots for FKRP protein:

C2C12s transduction - untagged rAAV6-CK8-FKRP



Muscle cells in a petri dish

Intramuscular injection (1e11vg) - untagged rAAV6-CK8-FKRP

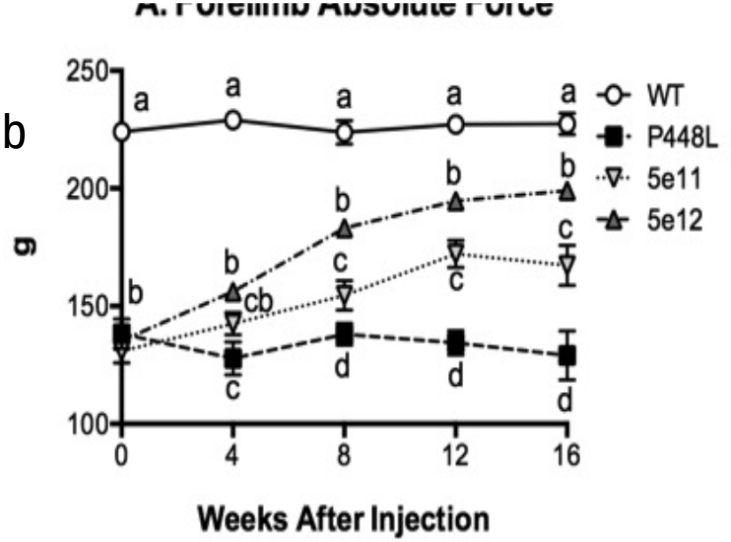


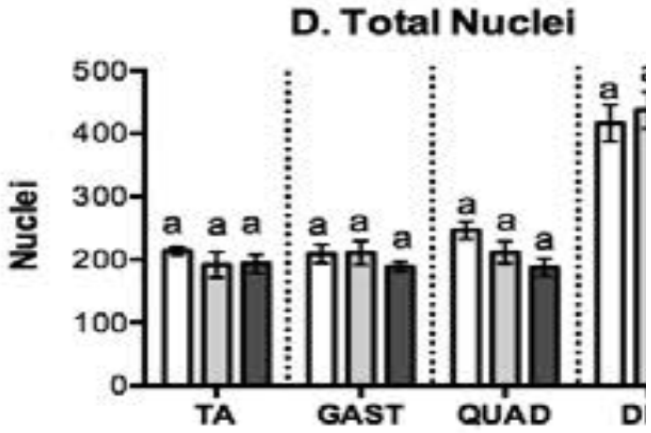
Whole mouse

Jane Seto & J Chamberlain, unpublished

AAV-FKRP gene delivery improves muscle function (*MDC1C* mouse model)

Forelimb force





AAV vector:

ITR Promoter FKRP gene stop ITR

Dan Rodgers & J Chamberlain, unpublished

Gene therapy with AAV-FKRP shows significant promise in mouse models

-what is needed to begin clinical trials?

- **Re-visit issue of how much FKRP is needed and if too much is bad (e.g. Tucker & Lu, 2018)**
- **Choose ‘best’ version of AAV-FKRP vector**
  - **on/off switch; version of FKRP gene; type of AAV**
- **Safety studies in large animal models**
  - **Rat? Monkey?; choose dose**
  - **Seeking guidance from the FDA**
- **Identify contractor to produce clinical grade AAV**
- **Recruit patients**
- **Begin phase I/II clinical trials**

Patients and FKRP gene therapies

- **What age/stage of disease to enroll?**
- **LGMD2i or MDC1C?**
- **Maintain and update patient databases**
- **Do we have adequate assays/biomarkers to measure outcome? How long will it take to see an effect?**

- **Screen for AAV neutralizing antibodies**
- **Develop informed consent protocols/forms**
- **IRB and FDA approval**
- **Enroll patients**

Future prospects for FKRP Gene

- Basic science, proof of principle is in place
- SMA & DMD trials provide hope for efficacy and safety
- Several issues still need to be resolved
  - Dose, final vector design, etc
  - Biomarkers and outcome measures needed
- Major efforts now shifting to manufacturing, regulatory approval, trial design

- Gene therapy for many other dystrophies can also move towards the clinic
- *However, development of these therapies is still early and highly experimental!*
  - *Let's not abandon alternatives*

Thank you for coming to the Iowa Wellstone Center 2018 Dystroglycanopathy Patient and Family Conference!

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The Muscular Dystrophy Association, and
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