CRISPR-Mediated Deletion of a Large Mutational Hotspot for Treatment of Duchenne Muscular Dystrophy

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BACKGROUND

Duchenne muscular dystrophy (DMD) is a severe muscle wasting disease affecting ~1 in 5,000 male live births. DMD is caused by an out-of-frame mutation in the dystrophin gene, which encodes a vital structural protein in the muscle membrane. Loss of dystrophin leads to progressive muscle degeneration, weakness and premature death in patients' 20s to early 30s. Gene therapies have aimed to provide miniaturized versions of the dystrophin gene or antisense oligonucleotides to induce exon skipping of dystrophin mRNA, which exploits a truncated, yet functional dystrophin protein, similar to Becker muscular dystrophy patients with an in-frame mutation of the dystrophin gene. We previously harnessed the capability of CRISPR/Cas9 technology to permanently excise defective exons, restoring the dystrophin reading frame and expression in a mouse model of DMD. However, this approach did not target the human dystrophin gene, nor does it address the wide heterogeneity of patient mutations. Here, we present a novel therapeutic approach, utilizing recombinant adeno-associated viruses (AAVs) to deliver CRISPR/Cas9 components targeting a large mutational hotspot (~700 kb) in the dystrophin gene that can be applied to approximately half of all DMD patients.

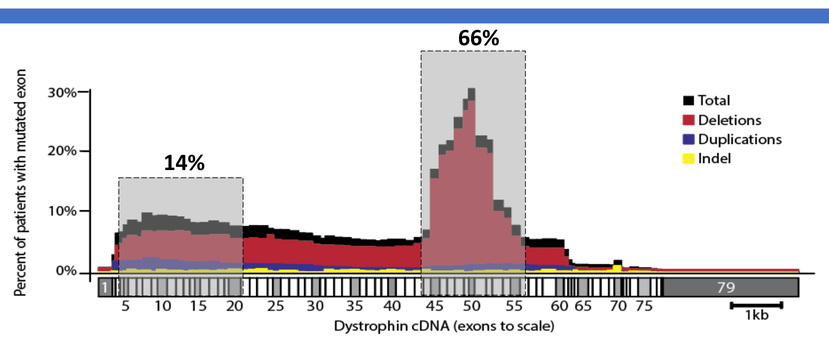
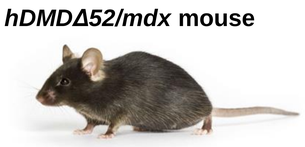
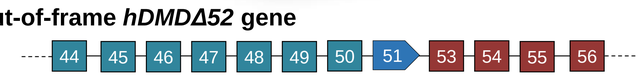


Figure 1. Patient mutations cluster in two hotspots of the dystrophin gene.

***hDMDΔ52/mdx* mouse**



Out-of-frame *hDMDΔ52* gene



Premature stop codon in murine dystrophin gene

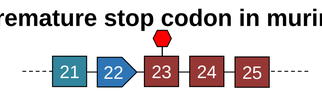
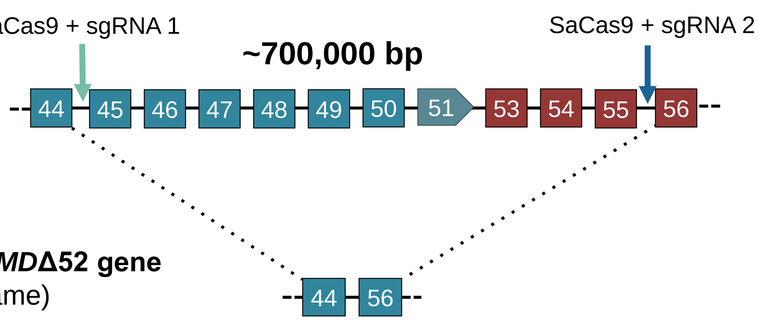


Figure 2. A humanized mouse model of DMD.

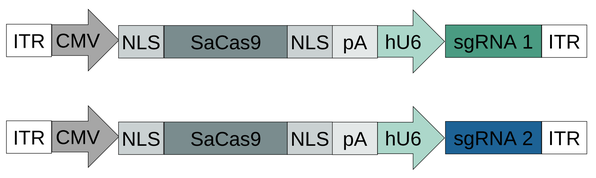
EXPERIMENTAL APPROACH

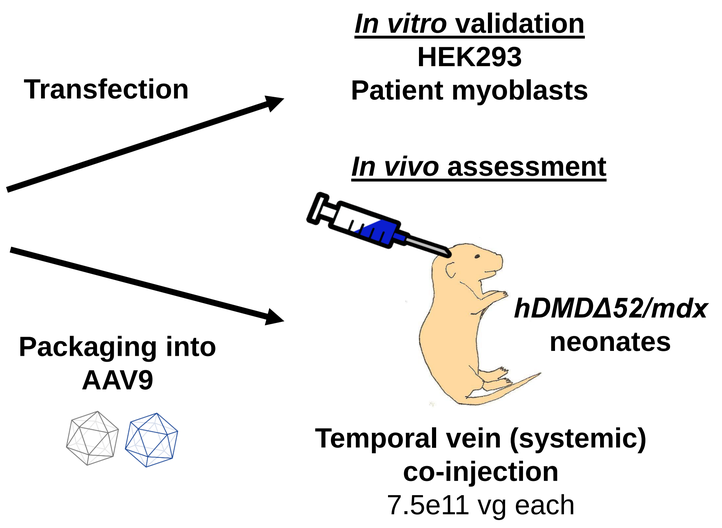
Overview of therapeutic strategy

***hDMDΔ52* mouse model**
(out-of-frame)



AAV vector constructs

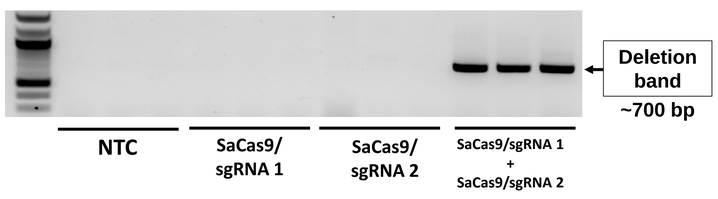




RESULTS

In vitro validation in HEK293 cells

Genomic DNA deletion PCR



Sequencing of deletion band

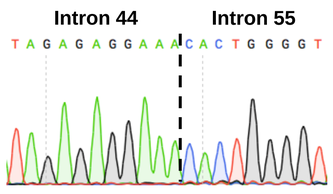
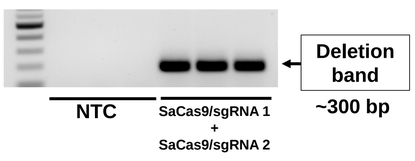


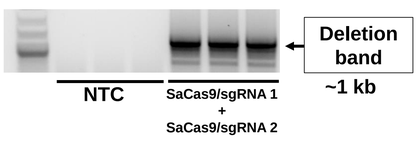
Figure 4. Deletion PCR and Sanger sequencing show genomic deletion of Exons 45-55 following transfection with vector constructs.

In vitro validation in patient myoblasts

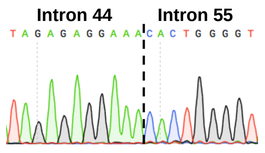
Genomic DNA deletion PCR

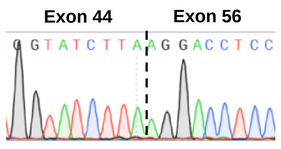


cDNA deletion PCR



Sequencing of deletion band





Western blot

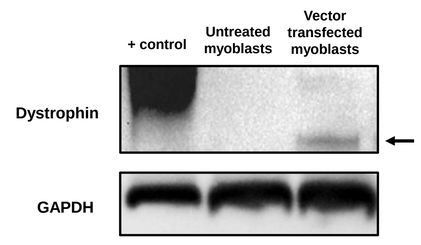
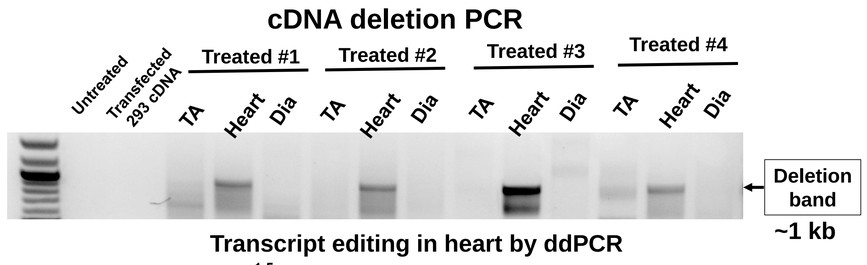


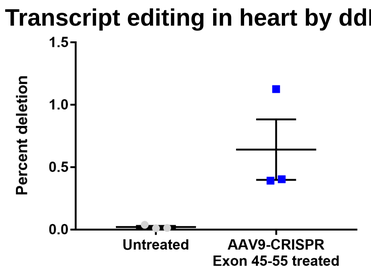
Figure 5. Deletion PCR and Sanger sequencing demonstrate genomic and transcript deletion of Exons 45-55 following transfection with vector constructs. Dystrophin expression was detected in vector-transfected cells via Western blot.

In vivo assessment in humanized _hDMDΔ52/mdx_ dystrophic mice

cDNA deletion PCR



Transcript editing in heart by ddPCR



Dystrophin expression in cardiac muscle

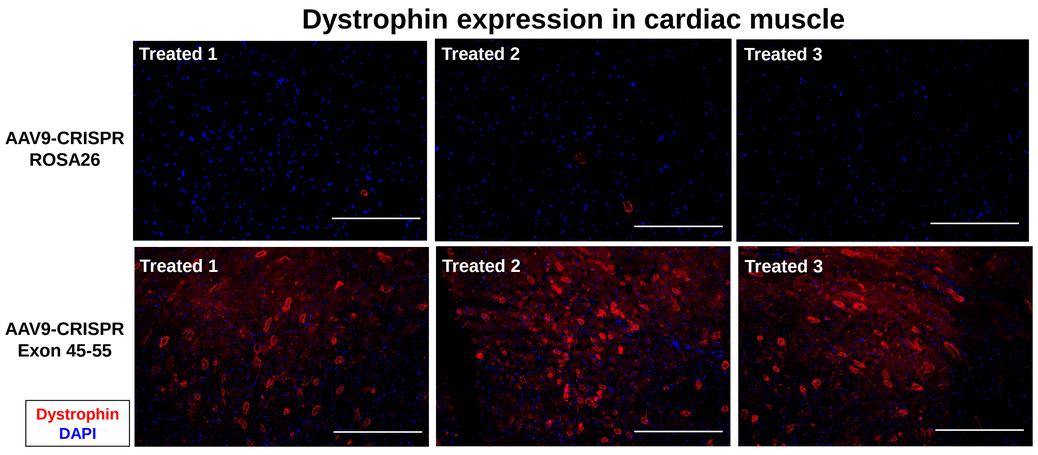


Figure 6. Deletion PCR and ddPCR analysis show deletion of Exons 45-55 in the cDNA of treated *hDMDΔ52/mdx* mice. Dystrophin expression and staining was observed in treated hearts via immunostaining. Dystrophin is shown in red and DAPI in blue. Scale bar = 100 um.

SUMMARY AND FUTURE EFFORTS

- We have generated vector constructs amenable to packaging constraints of rAAVs, which are desirable delivery vehicles for clinical application.
- We have validated deletion of exons 45 through 55 of the human dystrophin gene and transcript *in vitro* in HEK293 cells and immortalized patient myoblasts.
- We have observed and quantified excision of exons 45-55 in the dystrophin transcript of humanized *hDMDΔ52/mdx* dystrophic mice.
- Deletion of exons 45-55 effectively restores dystrophin expression in cardiac muscle of *hDMDΔ52/mdx* dystrophic mice.
- We are currently exploring ways to increase efficacy of editing through vector design optimizations and guide RNA screening.
- Overall, we have created an approach with the potential to benefit about half of DMD patients.

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References: Bladen et al, Hum Mutat, 2015. Nakamura et al, J Clin Neurosci, 2008. Nelson et al, Muscle Gene Ther, 2019. Ousterout et al, Nat Commun, 2015. Robinson-Hamm et al, unpublished.