KEYSTONE SYMPOSIA

MASH and Fibrosis: From Molecular Phenotypes to Precision Therapeutics
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Digital Pathology Image Analysis Accurately detects the Anti-Fibrotic effects of Gene Therapy in Mouse Models of Duchenne Muscular Dystrophy

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PHARMANEST DIGITAL PATHOLOGY

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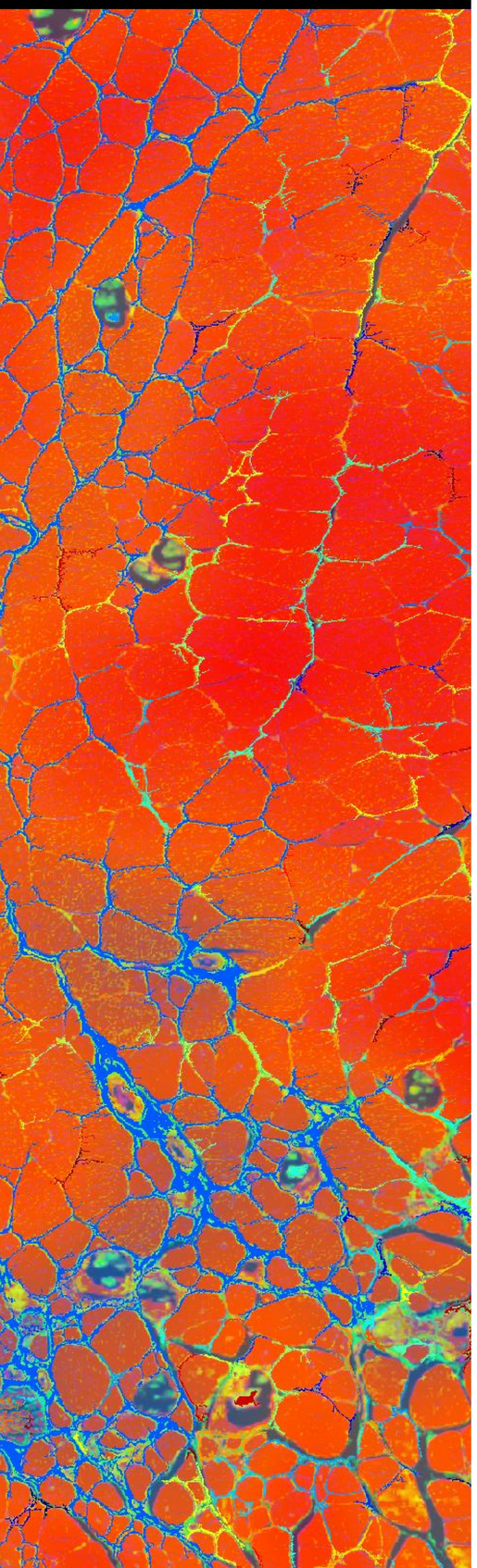


1 Introduction

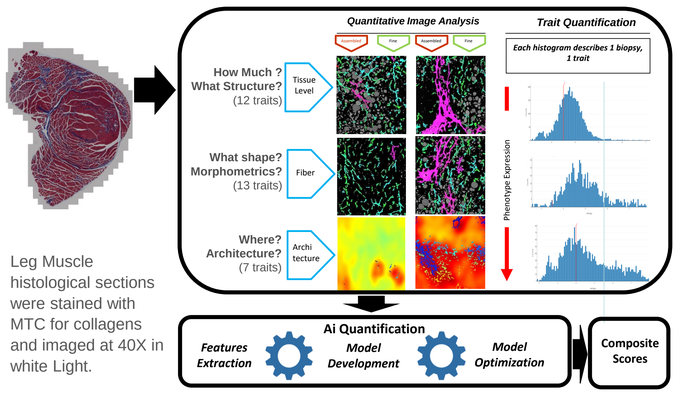
Duchenne Muscular Dystrophy (DMD) is a degenerative genetic muscular disease, causing fibrosis and necrosis of the muscle tissue. If left untreated, the disease causes impacted individuals with inability to walk by age 12 and death in teen years. Due to advances in care, life expectancy has drastically increased, but still lacks a permanent cure.

2 Aim

A recent avenue of research has been in gene therapies, including gene inhibition in animal studies. For this study we used automated single-fiber quantitative image analysis (FibroNest™, Princeton, USA) to quantify the changes of the fibrosis and tissue necrosis phenotypes and evaluate the effects of the treatment that may lead to a better therapeutic method.

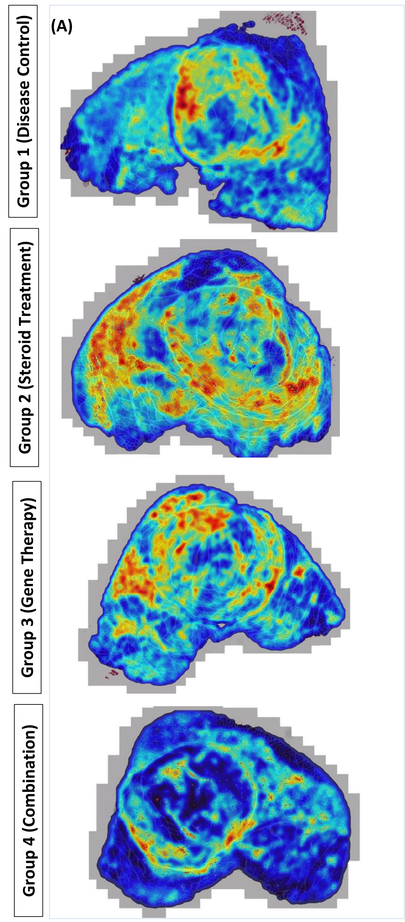
3 Method

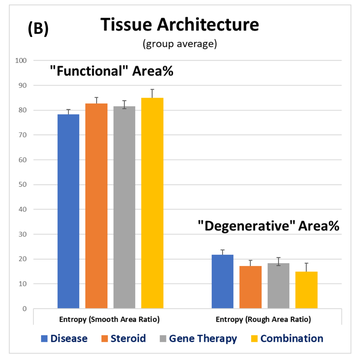
- Leg muscle samples were taken from 4 different groups of mice with DMD.
- DMD mice were treated with either **PBS** (**Control**, n=9), a **steroid** (**reference drug**, n=6), (n=17), or a **Combination** of steroid and gene therapy (n=11).
- For gene therapy, a novel gene hypothesized to be involved in DMD and fibrosis was inhibited.
- The sections were stained with Masson Trichrome for collagens and imaged at 40X with Zeiss Microscope Imaging System.
- FibroNest™, a cloud-based image analysis platform, was used to quantify the fibrosis phenotype.
- -This include 32 traits for collagen Deposition, Morphometry (fiber shape and size), and fibrosis Architecture (measures the organization and buildup of complex fibers).
- Principal quantitative fibrosis traits (up to 315 qFTs) are automatically detected and combined into a Phenotypic Fibrosis Composite Score (Ph-FCS).



Leg Muscle histological sections were stained with MTC for collagens and imaged at 40X in white Light.

4 Representative Images and Results





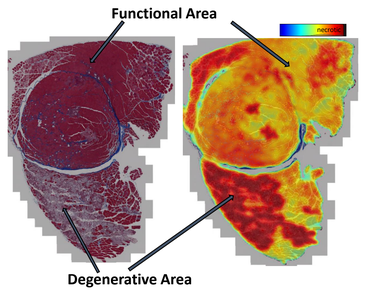


Fig B. Necrotic tissue measurements. There is a demonstrable difference in the amount of tissue necrosis where the Control (disease group) had necrotic tissue covering approximately 47% of the total tissue area, and the Combination Group shows improvement with necrotic Tissue covering 34% of the total area.

Fig A. Representative images from each of the four groups with the architectural complexity of the collagen deposition displayed as an overlay

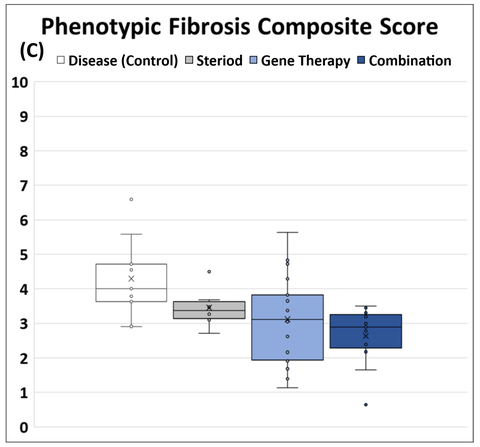


Fig C. Phenotypic Fibrosis Composite Score. Box and whisker plot of the three treatment groups show significant improvement when compared with Disease Control. The Ph-FCS for Control was 5.3, 4.5 (p=0.1) for Steroid, 4.1 (p=0.03) for Gene Therapy, and 3.7 (p=0.004) for the Combination.

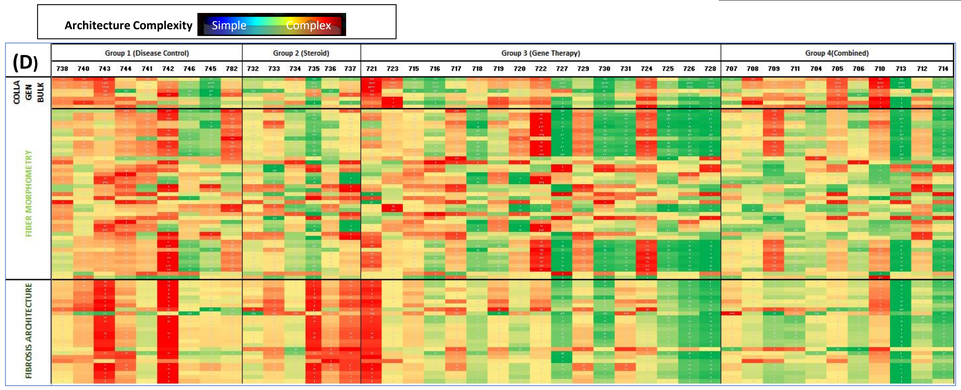


Fig D. Phenotypic Heat chart showing the progression of qFTs through the different treatments. While all groups (Steroid, Gene Therapy, and Combination) showed improvement as compared to Disease Control (untreated) in almost all aspects that we measure (Collagen Deposition, Morphometry and Architecture). It is clear to see the combination group consistently had the highest improvement.

5 Conclusions

-Gene inhibitory therapy has a clear demonstrable impact on degenerative muscle disease.
-The behavior of the collagen, but not the amount, shows the progression of necrosis and fibrosis.
-This indicates fascinating areas of research, along with a hopeful light for the potential treatments for inherited muscular dystrophies.

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