EFFECT OF AAV-GAA GENE THERAPY ON DIAPHRAGM NEUROMUSCULAR JUNCTION

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INTRODUCTION

- Pompe Disease is a genetic disorder in which GAA is not able to be produced in enough quantities or is not able to be produced at all in a cell. Without GAA, glycogen cannot break down, filling the cell lysosomes. Organelles within the cell are pushed to the side to fit the glycogen filled lysosomes, causing the cell to not be able to function properly.
- Neuromuscular Junction, known as NMJ, is the connection between the synapse of a motor neuron and a muscle cell.
- AAV gene therapy is a form of gene therapy in which a gene is inserted into a virus vector and given to a patient to add an extra gene in the DNA. Instead of just two sets of DNA the patient will have three.

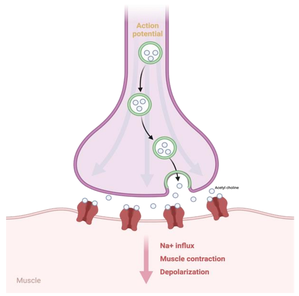

Figure 1. Neuromuscular junction. in the presynaptic terminal of motor neurons, synaptic vesicles carry and release neurotransmitters, such as acetyl choline to bind to acetyl choline receptors within the muscle. This leads to muscle contraction.

HYPOTHESIS: AAV-GAA gene therapy will be helpful in preserving NMJ's in the diaphragm of mice models.

METHODS

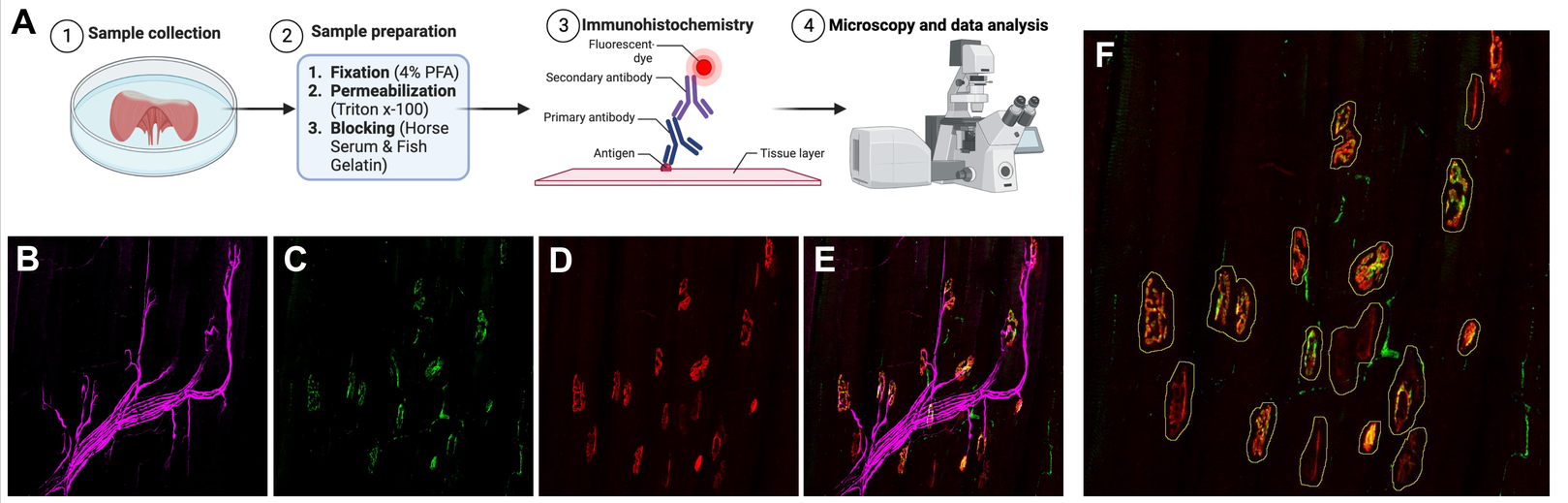

Figure 2. (A) Diaphragms from four different mouse groups (WT, AAV-A, AAV-B, KO) were removed, fixed, permeabilized, and blocked. Antibodies labeled neurofilament heavy peptide (NF-H) **(B,E)** and synaptotagmin (znp-1) **(C,E)** and α-bungarotoxin identified acetyl choline receptors **(D)**. 3-5 regions were imaged from each diaphragm and the NMJs were identified **(F)** and analyzed using FIJI Coloc 2 algorithm to obtain the Pearson's R Value, Mander's tM1, Mander's tM2, area, and the number of NMJ's.

RESULTS

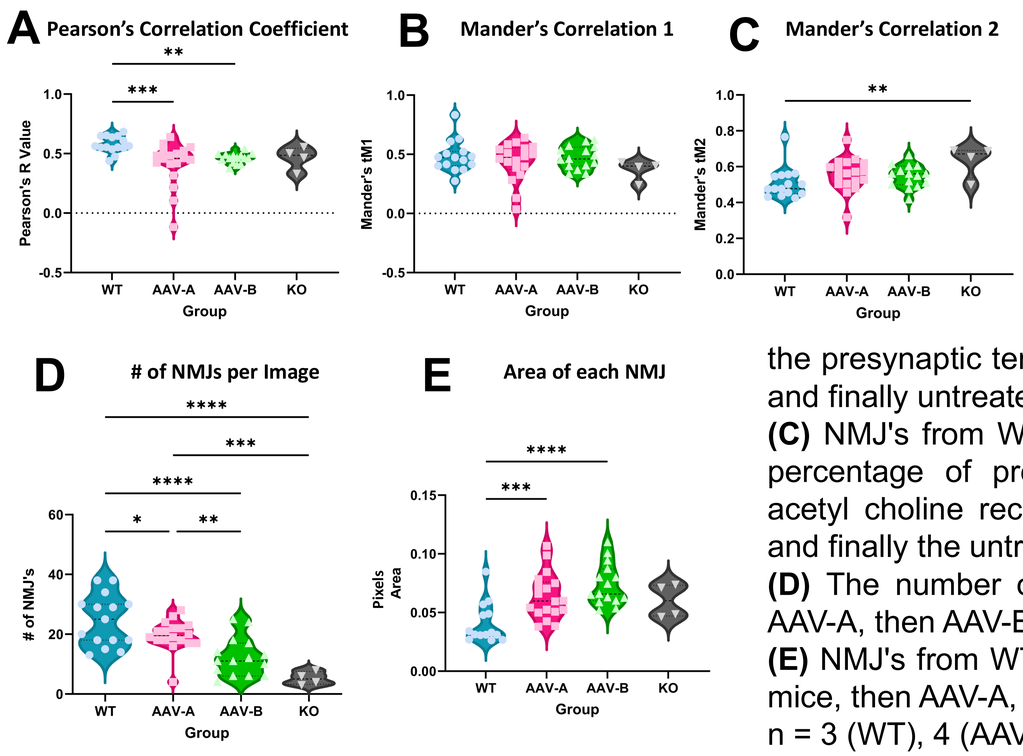

Figure 3. (A) NMJs from WT mice have the largest Pearson's Correlation Coefficient (PCC), which is the correlation in intensity of the presynaptic terminal (green) and the acetyl choline receptors (red), followed by untreated KO, AAV-B, and AAV-A.
(B) NMJ'S from WT mice have the largest Manders tM1, which is the percentage of acetyl choline receptors (red) that overlap with the presynaptic terminal (green), followed by AAV-B, then AAV-A, and finally untreated KO mice.
(C) NMJ's from WT have the lowest Mander's tM2, which is the percentage of presynaptic terminal (green) that overlap with acetyl choline receptors (red), followed by AAV-A, then AAV-B, and finally the untreated KO mice.
(D) The number of NMJ's is greatest in WT mice, followed by AAV-A, then AAV-B, and finally the untreated KO mice.
(E) NMJ's from WT mice have the smallest area, followed by KO mice, then AAV-A, and finally AAV-B mice.
n = 3 (WT), 4 (AAV-A), 3 (AAV-C), 1 (KO)

CONCLUSIONS

- Neither AAV's did not improve PCC values.
- AAV treated mice had Manders tM1 and tM2 values in between that of WT and KO mice.
- AAV treated mice maintained a moderate amount of NMJ's and had smaller area compared to that of KO mice, making them more like WT mice.
- AAV treated mice were healthier than KO mice meaning the developed AAV's were effective, however AAV-A is slightly more effective than AAV-B.

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