Determining MyHC isoforms in WT and HD mice muscles

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Introduction

Huntington's Disease (HD) is an inherited neurodegenerative disease that causes the degeneration of nerve cells in the brains of the affected individual (1). This results in motor, cognitive, and psychiatric disorders, impairing the individual's ability to move. Most affected individuals show symptoms during middle age. However, some individuals develop symptoms before the age of 20, called juvenile HD (1). Curiously, HD patients also develop muscle atrophy, weakness, and impaired muscular function, though there is no clear understanding of why this occurs (3). This disease currently has no cure.

The purpose of this study was to better understand the impairments in muscular function associated with Huntington's Disease. Specifically, this study assessed the expression of specific protein (myosin heavy chain, MyHC) isoforms that are known to play an important role in contractile function in muscles from transgenic mice (R6/2 Huntington's disease mice) that are genetically altered to display HD symptoms and wild-type (WT) controls. The implication of this study was to clearly identify changes in protein composition occurring in HD muscles which would impact muscle function.

Hypothesis

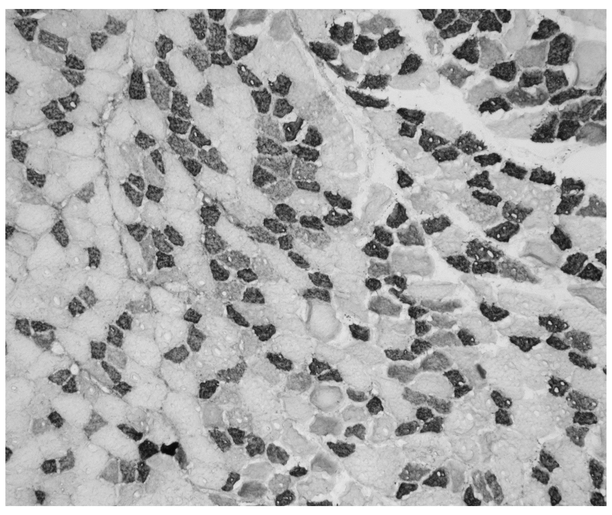
We hypothesized that HD transgenic mice would have a greater proportion of type I (slow twitch oxidative) muscle fibers compared to the WT mice muscles.

Experimental Design

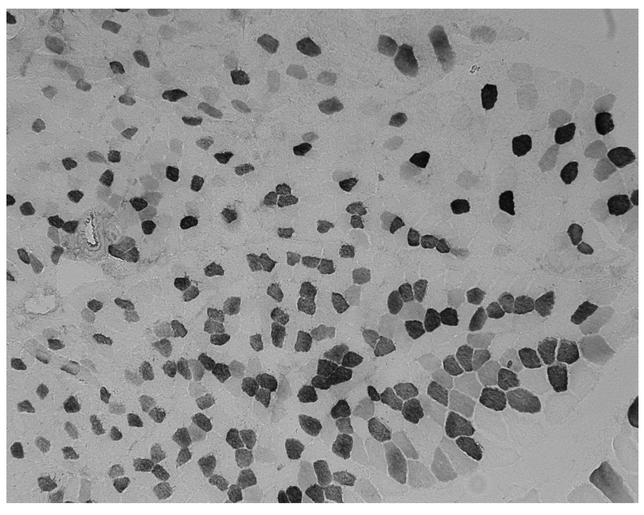
Two groups of mice were analyzed. One group consisted of R6/2 Huntington's disease mice and the other were wild-type control mice (n=5/group) for the mid-TA muscles. The second group consisted of R6/2 Huntington's disease mice analyzing the deep-TA muscles (n=3/group). The initial stages of the experimental process consisted of utilizing a cryostat to cut 10 micron thick sections of the tibialis anterior (TA) muscle and placing them on glass slides. A total experimental sample of 48 sections was obtained by organizing and placing 4 muscle sections on 12 separate slides. The individual 12 slides had 2 sections of the WT muscles and 2 sections of the HD muscle. Then processed with immunohistochemical staining of the sections for the specific MyHC isoforms (I, IIa, IIx, and IIb). The process consisted of the following steps: a) rehydrating the sections for 15 minutes; b) exposing the sections to blocking reagent; c) adding primary antibodies (directed against the specific MyHC isoforms) for 48 hours; d) washing the sections with phosphate buffered saline, e) then adding the secondary antibodies (which bind to the primary antibody); f) washing again; and g) exposing the sections to a color development reagent which stains the positively labelled fibers gray. After the stains set and washed off, we analyzed the sections under a microscope attached to a digital camera and a computer-assisted image analysis system.

The comparison between the HD and WT will be analyzed through the images captured of the stained fibers. The antibodies used in the beginning of the experiment are fiber type specific and will stain either type I, IIA, IIX, or IIB MyHC-based fibers. We compared these muscle tissue samples and quantified the relative proportions of each fiber type in a given sample to distinguish any key differences between the WT and the HD muscles .

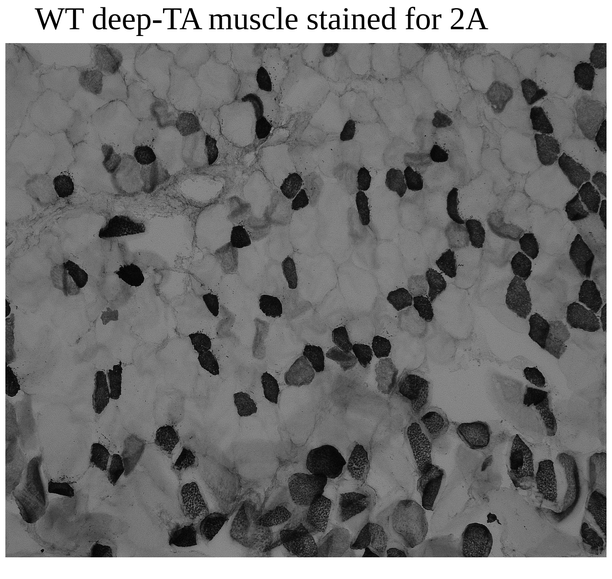
WT mid-TA muscle stained for 2A



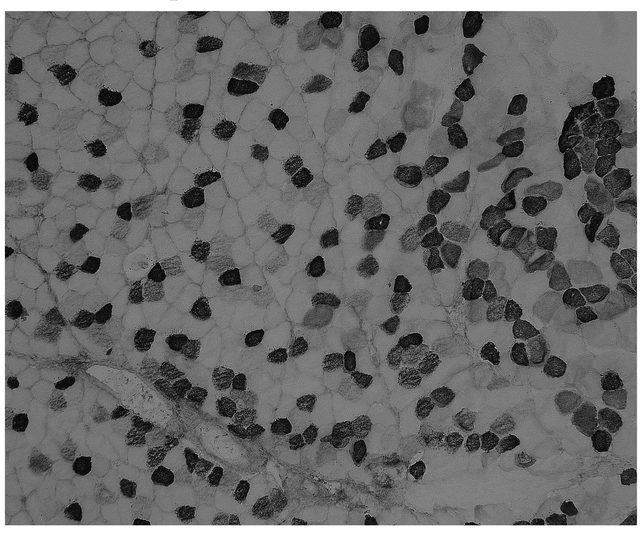
HD mid-TA muscle stained for 2A

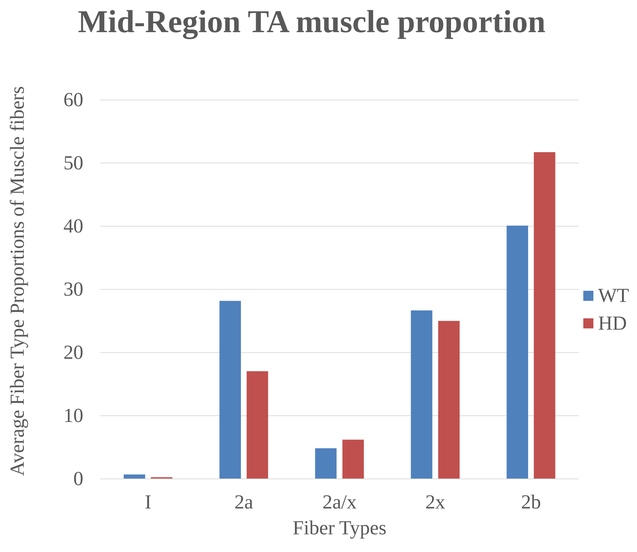


WT deep-TA muscle stained for 2A

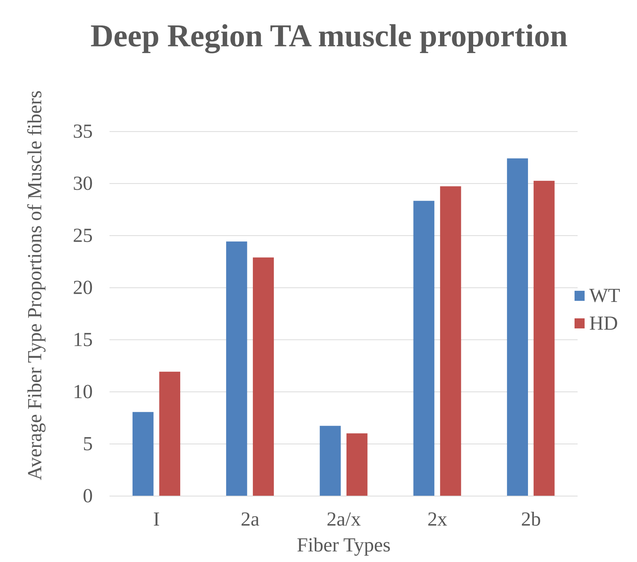


HD deep-TA muscle stained for 2A





Graph 1: Mid Region TA Muscle Proportion



Graph 2: Deep Region TA Muscle Proportion

Results

Graph 1 demonstrated that the mid-tibialis anterior muscle from the R6/2 mice had the following proportions of fibers with the R6/2 mice had a muscle proportion of MyHC-IIA (17%) compared to the WT MyHC IIA (28%). The MyHC-IIX in the TA muscle (25%) compared to the WT mice (26%). The R6/2 mice muscle fiber proportion for the MyHC-IIB (51%) compared to the WT fibers (40%). There were no significant differences in the MyHC muscle fiber proportions of the WT and R6/2 HD mice muscle fibers: MyHC-IIA ($p = 0.155$), MyHC II-X ($p=0.77$), and MyHC II-B ($p=0.18$).

Graph 2 demonstrated that the deep-tibialis anterior muscle from the R6/2 mice had the following proportions of fibers with the R6/2 mice had a muscle proportion of MyHC-IIA (22.9%) compared to the WT MyHC IIA (24.4%). The MyHC-IIX in the TA muscle (29.7%) compared to the WT mice (28.3%). The R6/2 mice muscle fiber proportion for the MyHC-IIB (30.2%) compared to the WT fibers (32.42%). There were no significant differences in the MyHC muscle fiber proportions of the WT and R6/2 HD mice muscle fibers: MyHC-IIA ($p = 0.81$), MyHC II-X ($p=0.87$), and MyHC II-B ($p=0.82$).

Discussion

Muscle fiber-type proportions can be altered based on activity level, muscle atrophy and electrical stimulation (9). Muscle atrophy is associated with a change in mRNA and MyHC protein isoform compositions, transitioning from slow to fast fiber-types in a bed-rest study (8). Our hypothesis, based on the involuntary movements associated with HD was that the heightened contractile activity experienced by the HD mice would result in a change in MyHC isoform from fast to slow. This would be consistent with the changes that occur following prolonged muscle activation (10). Our data suggests that no significant differences in MyHC isoform composition occurred in HD mice. This data suggests that the expression of MyHC isoforms in individual fibers is not altered by HD. There were several potential limitations to this study which include the following factors: There was not a large sample size, as the experiment was limited due to the access of only n=5 animals for the mid-TA and n=3 for the deep-TA. The number of fibers analyzed per muscle type was limiting. The age of HD animals may be too young to show HD associated with abnormalities. Lastly, it is possible that the TA muscle is not a good representative muscle for all muscles in the body. However, the HD muscle fibers were smaller in size suggesting that some muscle characteristics were altered due to HD. This atrophy in overall muscle fibers is one variable that should be studied in greater detail in the future. In total, this study will benefit the real world and society by providing a better understanding of the overall disease processes which ultimately may lead to the development of treatment strategies. Future studies may be aimed at determining treatment strategies or directly assessing the altered signaling mechanisms that cause muscle fiber atrophy.

Literature Cited

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