Alternative Splicing of the Bin1 Gene of Wild Type and Huntington's Disease Affected Mice

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Abstract

Huntington's Disease (HD) is an autosomal dominant neurodegenerative disease that causes the degeneration of nerve cells in those with a mutation in the *HTT* gene. HD is characterized by an extended trinucleotide repeat that contains 40 or more repeats of CAG segments. As a result of this mutation, the individual experiences chorea, dementia, and impaired motor and cognitive functions. HD patients also develop uncontrollable involuntary movements, as well as muscle atrophy and weakness. The proteins expressed from a gene called Bin1 in muscle cells is thought to be involved in the formation of T tubules which play a role in muscle contraction and relaxation. A study on myotonic dystrophy, an RNA-mediated disease, showed expression of mutant RNAs containing CUG repeats. The study revealed that alternative splicing of BIN1 pre-mRNA is altered in skeletal muscles samples of humans. The purpose of my study is to further investigate the mechanisms of the muscular dysfunctions associated with Huntington's Disease by examining replication of the Bin1 gene in HD affected mice. Multiple muscles were examined using PCR testing of samples of wild type and HD affected mice. Three significant differences in replication were found in the gastrocnemius, tibialis anterior, and denervated gastrocnemius.

Introduction

Huntington's Disease (HD) is an inherited genetic mutation in the *HTT* gene. This disease is associated with many symptoms including neurological, behavioral, and muscular disorders (1). HD is caused by a loss of neurons in the basal ganglia especially in the caudate nucleus and putamen. Cognitive and emotional dysfunction can be seen in HD patients along with a loss of overall movement coordination and chorea. Muscle atrophy occurs in affected individuals resulting in a loss in lean body mass (2). Ultimately, the leading cause of death among HD patients is aspiration pneumonia with suicide following as the second leading cause of death (3). Aspiration pneumonia arises from problems related to reflexive movements such as swallowing which causes choking and materials from the mouth to enter the lungs.

Introduction (continued)

HD is characterized by a CAG trinucleotide repeat expansion in the *HTT* gene (4). Unaffected humans naturally have up to about 27 repeats, but once the expansion reaches 40+ repeats, symptoms will be expressed. Cellular degeneration can be due to the trinucleotide repeat expansion from gene aberrant splicing in which case the CAG repeat expansion would be classified as toxic RNA. Since the CAG encodes for glutamine, the mutant HTT protein product contains an expanded poly-glutamine tract. Degeneration may also be due to the increased poly-glutamine tract in which case the protein with the expanded poly-glutamine tract would be classified as a toxic protein.

An animal model for HD, the R6/2 transgenic mouse line (5), was used for this study. This line contains the first exon of the *hHTT* gene with an expanded trinucleotide repeat region of about 120 CAG repeats. The R6/2 mouse muscles are atrophic, and demonstrate denervation-like symptoms which are in part due to altered gene splicing. Our preliminary data demonstrate a reduction in t-tubule volume which is also consistent with denervation. The protein, Bridging Integrator 1 (Bin1) plays an important role in t-tubule formation and shows altered splicing during development (6). Therefore, this study analyzed the splicing patterns of Bin1 mRNA in HD and denervated muscles.

Hypothesis

1. It is hypothesized that muscles from R6/2 mice will show an elevation in aberrantly spliced Bin1.
2. It is hypothesized that denervated muscle will also show an elevation in aberrantly spliced Bin1.

Materials and Methods

Animals

Muscles from R6/2 and denervated mice were obtained from Wright State University in collaboration with Dr. Andrew Voss and Dr. Mark M. Rich. The muscles were denervated for a period of 14 days. Muscle masses were obtained at the time they were removed from the animals at Wright State University. The muscles were then rapidly frozen in liquid nitrogen and stored at -80°C. The muscles were shipped to Cal Poly Pomona packaged in dry ice and subsequently stored at -80°C until analyzed. All procedures used for animal care and maintenance were approved by the Wright State University Animal Care and Use Committee.

Muscle Preparation

Gastrocnemius (Gast), tibialis anterior (TA), and soleus (Sol) muscles were removed from euthanized R6/2 mice, frozen in liquid nitrogen, and stored at −80°C. Gast from denervated mice were obtained in a similar fashion. Total RNA was isolated from muscle samples using the Trizol technique. One µg of RNA was reverse transcribed to synthesize cDNA. For analysis of alternatively spliced Bin1 gene products, PCR was performed for 26 cycles, denaturing at 94°C for 45 sec, annealing at 54°C for 45 sec, and extension at 72°C for 45 sec using *Taq* DNA polymerase protocol.

Forward Primer: 5'-ATGTCCTGGTCAGCCTAGAGAAG-3'
Reverse Primer: 5'-ATTCACAGTTGCGGAGAAGGTC-3'

PCR products were separated on 1.5% agarose gels, and the amplicons representing normal and aberrantly spliced mRNA products were quantified using a FluorChem SP (Alpha Innotech).

Statistics

The relative percentage of splice variants including exon 17 were quantified and data expressed as means ± the SEM. Student's t-tests were used to determine significant differences between treatment groups with the alpha-level set at $p \leq 0.05$

Results

Muscle Masses

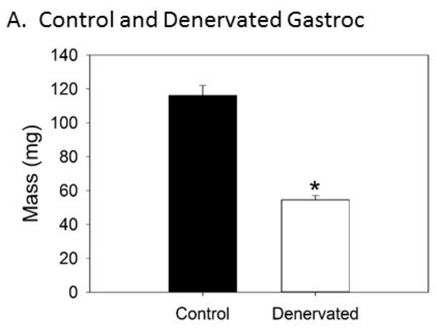
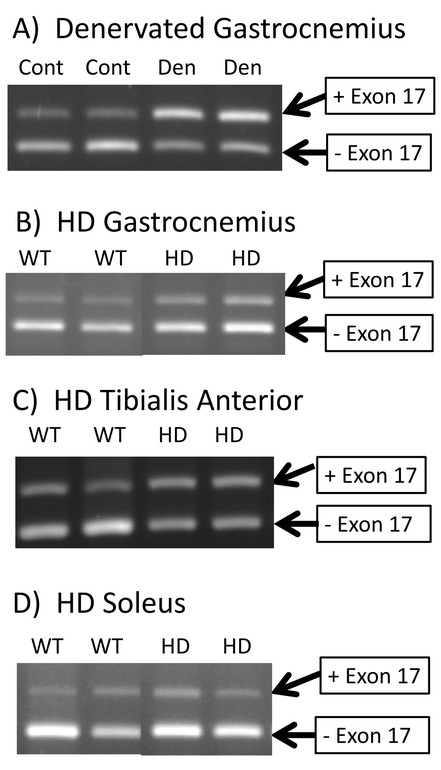


Figure 1. Muscle masses from control and denervated mice (A), and wild-type (WT) and R6/2 mice (B). Gastrocnemius (Gastroc), Tibialis anterior (TA), Soleus (Sol). *, $p \leq 0.05$.

Representative gel images



Splicing Quantification

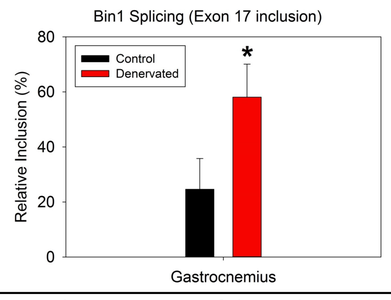


Figure 2. Relative percentage of aberrantly spliced Bin1 mRNA with exon 17 inclusion. There was a significant increase in exon 17 inclusion in denervated gastrocnemius ($p \leq 0.05$).

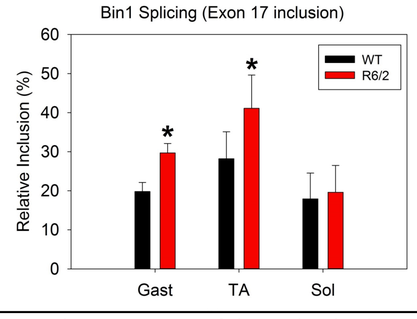


Figure 3. Relative percentage of aberrantly spliced Bin1 mRNA with exon 17 inclusion. There was a significant increase in exon 17 inclusion in HD gastrocnemius (Gast) and tibialis anterior (TA), but not soleus (Sol) ($p \leq 0.05$).

Discussion and Conclusion

Clear atrophy can be seen in Huntington's Disease affected mouse muscles and denervated mouse muscles which was expected. The denervated gastrocnemius showed aberrant splicing in Bin1 mRNA. Similarly, there was an increase in aberrant splicing of Bin1 in the gastrocnemius and tibialis anterior of R6/2 mice. Curiously, there was no difference in Bin1 splicing in the soleus muscle. Since gastrocnemius and tibialis anterior are both considered fast muscles; where as, soleus is considered a slow muscles, it appears that fast muscles are impacted more than slow muscles. While both denervation and fast muscles of R6/2 mice show aberrant splicing of Bin1, the relative amount of aberrant splicing in denervation is significantly greater than what occurs in the R6/2 mouse model. Ultimately, there is an increase in aberrant splicing in the gastrocnemius and tibialis anterior in R6/2 mice, as well as in the denervated gastrocnemius. This suggests that Huntington's Disease does show some symptoms that are denervation-like.

References

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