Investigating Repeat Expansion in Huntington's (HD) Model Mice: Implications for Treating HD & Other Repeat Expansion Disorders

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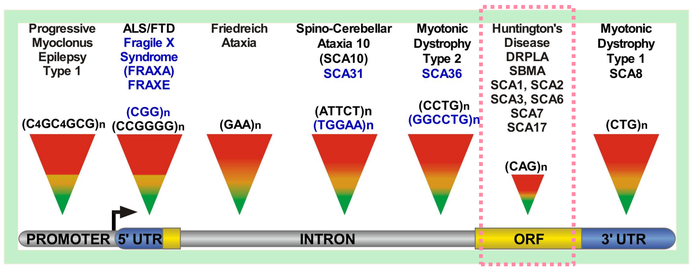
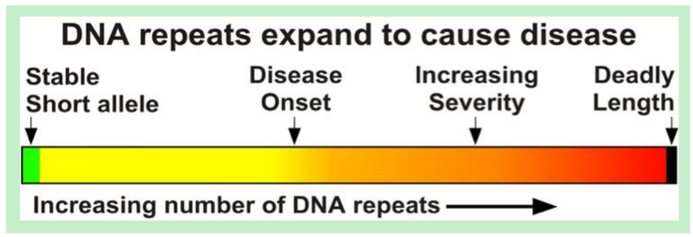
Introduction

DNA Repeat Expansion
- Genetic disorders with abnormal expansion of repeated DNA sequence

Huntington's Disease (HD)
- Dominantly inherited, incurable, neurodegenerative disorder[1]
- CAG repeat expansion in HTT gene



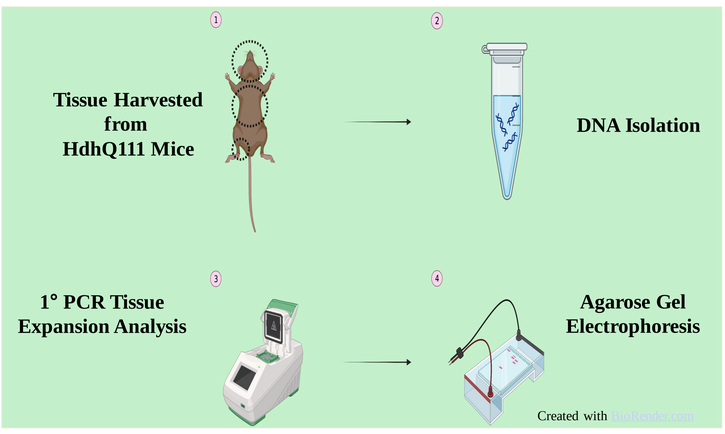


Purpose
- Understand the underlying mechanism of repeat expansions & identify in which tissues these expansions occur

Hypothesis

In HD patients, the striatum (located within the cerebral cortex) is one of the most affected tissues[2]. Therefore, the cerebral cortex tissue is expected to show extensive expansion

Methods



Results

Tissue Specific Expansion Analysis of HD Mice through Agarose Gel Electrophoresis

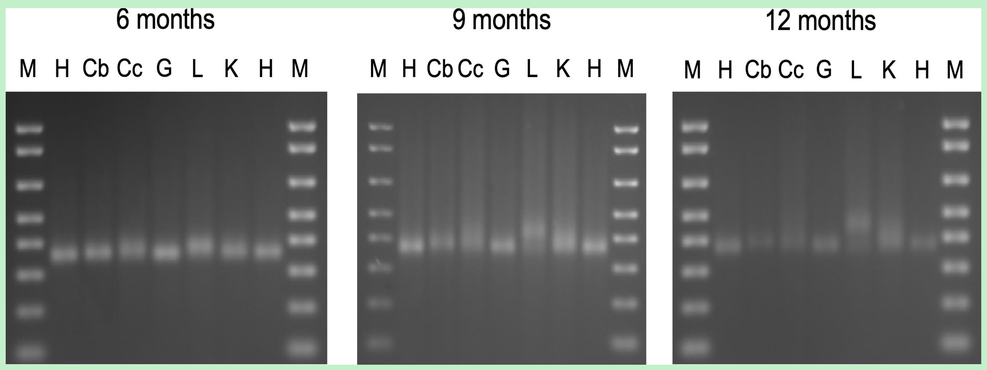


Figure 4: Images of HdhQ111 Mice at 6 months, 9 months, and 12 months. PCR products contain the expanded repeats from the indicated tissues: Cerebellum (Cb), Cerebral Cortex (Cc), Gastrocnemius (G), Liver (L), and Kidney (K). The sizes of the DNA markers, from top to bottom, are: 1 Kb, 850 bp, 650bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp

Tissue Specific Expansion Analysis of HD Mice through High Performance Capillary Electrophoresis

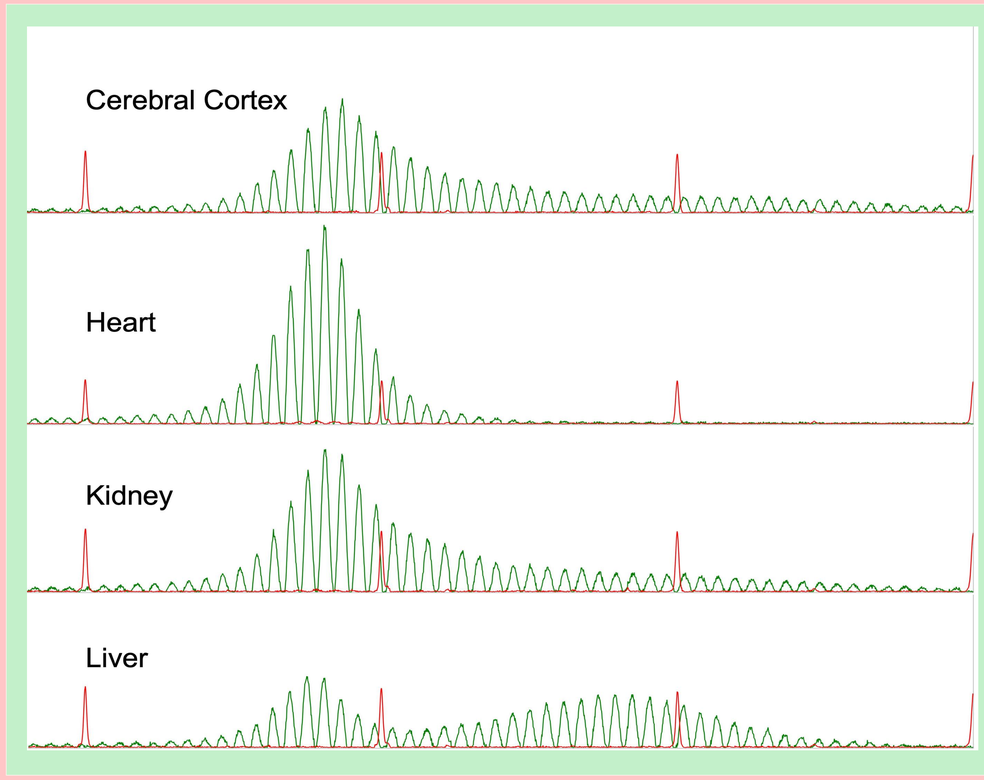


Figure 5: High Performance Capillary Electrophoresis Tissue Images of 9-month HdhQ111 Mouse. The four tissues highlighted are the Cerebral Cortex (Cc), Heart (H), Kidney (K), and Liver (L). Green peaks indicate areas of CAG repeats. Each red marker represents base pairs. From left to right, markers represent 300 bp, 350 bp, 400 bp, and 450bp. Images of the Cerebral Cortex and Kidney tissue look similar, indicating similar CAG expansion.

Discussion

Agarose Gel Electrophoresis Analysis
- Smearing indicates expansion present
  - Prevalent in Cc, L, and K tissue
- Higher degree of CAG expansion observed in older mice, especially within the Cc and K tissue

High Performance Capillary Electrophoresis
- a higher resolution technique to visualize CAG repeat expansions
- As base pairs increase, you can identify CAG repeats in the Cc and K tissue, just as you do in the Gel Electrophoresis imaging

Conclusions & Future Directions

Conclusions
- As expected, repeat expansion is extremely prevalent in the cortex, consistent with HD patients suffering from progressive cognitive decline
- Compared to HD patients, there is a wide variation of tissue specific expansion
  - Striatum/cerebral cortex tissue has greater extent of CAG expansion – demonstrating tissue specific factors contribute to expansion in HD patients

Future Directions
- Therapy targeting CAG repeat expansions within the striatum, which lies within the cerebral cortex
- Results can be applied to other repeat expansion disorders (e.g. Friedreich Ataxia)

References

1.Mouro Pinto, R., Arning, L., Giordano, J. V., et al. (2020). Patterns of CAG repeat instability in the central nervous system and periphery in Huntington's disease and in spinocerebellar ataxia type 1. Human molecular genetics, 29(15), 2551–2567.

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3. Roy, J. C.L., Vitalo, A., Andrew, M. A., et al. (2021, March 22). Somatic CAG expansion in Huntington's disease is dependent on the MLH3 endonuclease domain, which can be excluded via splice redirection. *Nucleic Acids Research*, *49*(7), 3907-3918. doi:10.1093/nar/gkab152

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