Using Transgenic Mouse Models to Track DNA Repeat Expansion of Friedreich's Ataxia Patients

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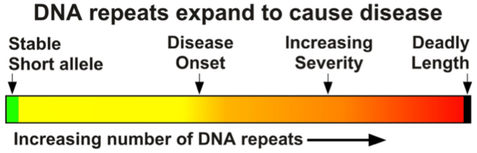
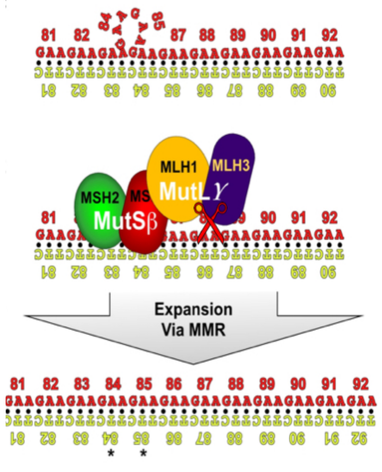
DNA repeats expand to cause disease



DNA Mismatch Repair (MMR)



A) After transcription the two strands can re-associate out-of-register forming a loop

B) This small loop is a target for this particular MMR complex which makes a cut on the short strand

C). Two trinucleotides (*) have been added to the shorter strand and the DNA sequence has expanded

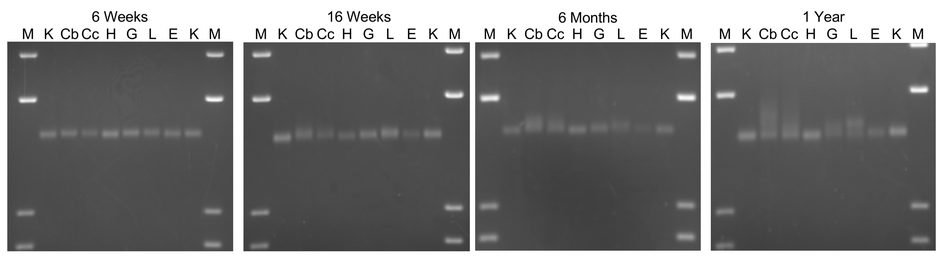
Although MMR maintains crucial genome integrity elsewhere, the process facilitates the instability in DNA expansion. Research has identified the role of MMR molecule MLH3 in the expansion of GAA•TTC repeats in the FXN gene. Importantly, one isoform of MLH3 does not cause repeat expansion. Because of this unique property, MLH3 is the target of our therapeutic answers to FRDA.

Mouse Models

Identifying Expansion

Determining the exact location and speed of repeat expansion is a crucial step prior to any therapeutic solution. In this study, transgenic mouse models were used to track the length of repeats in the human frataxin (hFXN) gene over time. Tissues from mice of different ages were analyzed to determine the extent of somatic expansion and to visualize the discrepancies between different tissues.

Tissue-specific and age-dependent expansion in Transgenic FRDA mouse model



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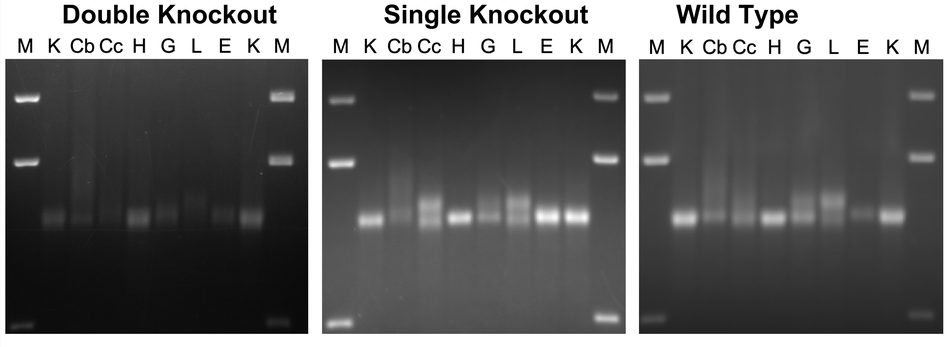
Transgene sizing of 18-month-old mice



As seen in these mouse models compared to the younger mice, the phenomenon of expansion is one that compounds over time. The older mice continue the trend of accelerating expansion.
Noticeably the two brain regions (cerebellum and cortex) show the most dramatic expansion. However, the heart barely expands at all in these mice which is inconsistent with human DNA expansion. This reminds us that although mice are useful, they can never be a perfect model for humans.

Method

DNA Extraction & PCR

Three male 18-month old mice were dissected and each tissue was placed in a boil-proof tube with Proteinase K to digest proteins and remove contamination from the sample. Additionally stainless-steel beads were used to homogenize tissues prior to extraction.

Once the DNA of each tissue was isolated, the FXN gene could be selectively cut with primers and amplified via PCR. Finally a gel electrophoresis was run to image the length of expanded DNA fragments.

Each of the three 18-month old mice represent a different genotype of the mouse FXN gene: one with both copies, one missing one copy, and one lacking both copies. This last type, the "double knockout" had to rely completely on the hFXN transgene to produce frataxin since it had no FXN gene of its own

Conclusions / Next Steps

Hope for a Cure?

As hypothesized, there is no unexpected change in expansion in older mice. Although the rate of expansion increases to dangerous levels, this pattern is expected based on trends in younger mice. Because of this predictable progression of the disease and the compounding nature of expansion, an early delay or even a halt to expansion can be life changing for FRDA patients.

Further Questions?

Are any discrepancies in expansions due to the different mouse frataxin genotyping?

Since the double knockout mouse genotype must transcribe from the hFXN gene for all its frataxin production, would expansion be accelerated in these cells?

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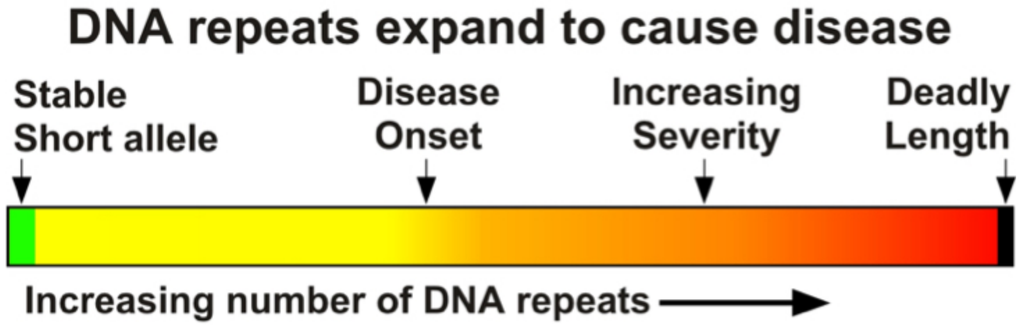

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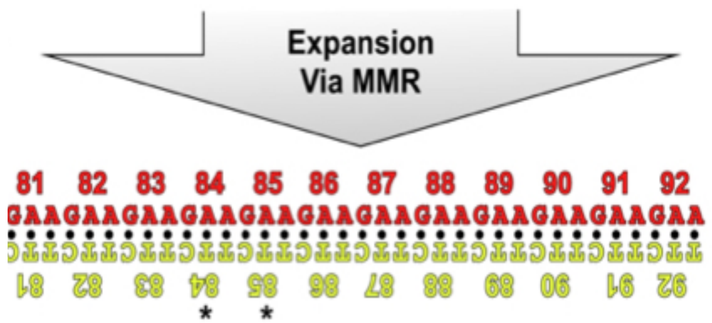

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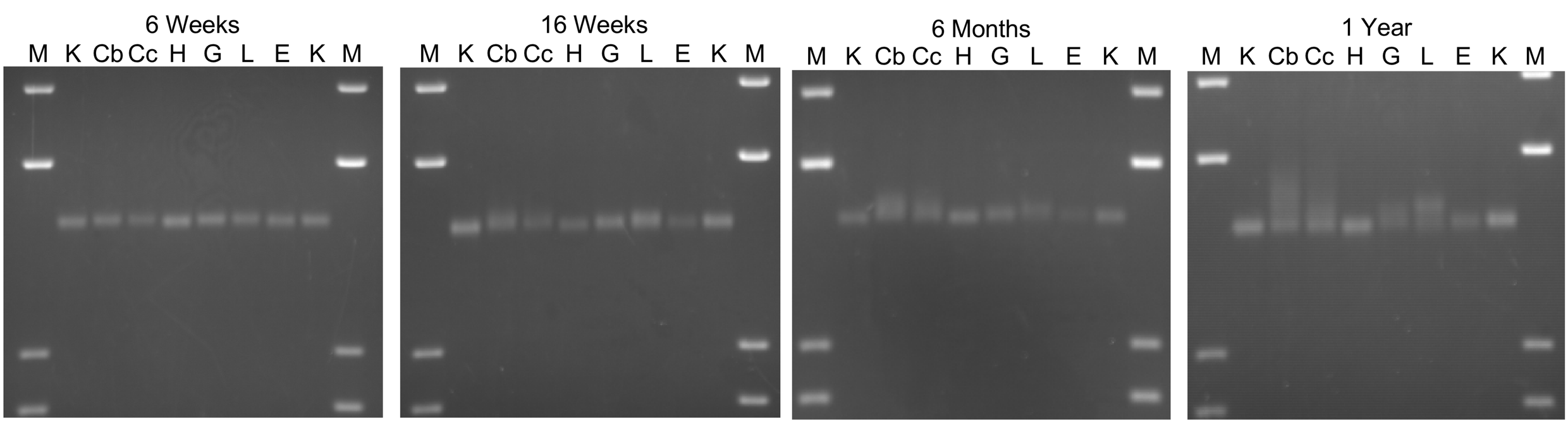

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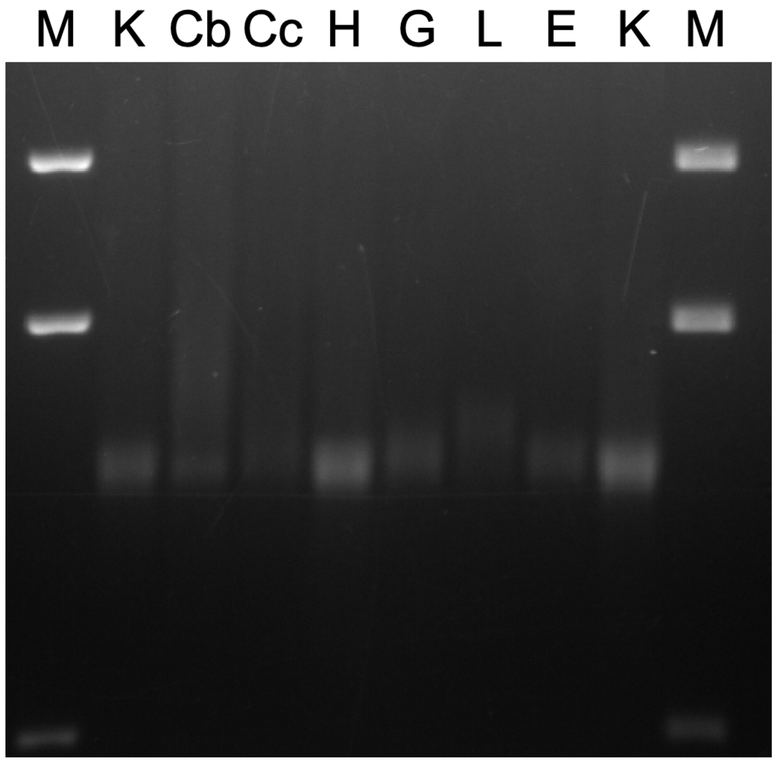

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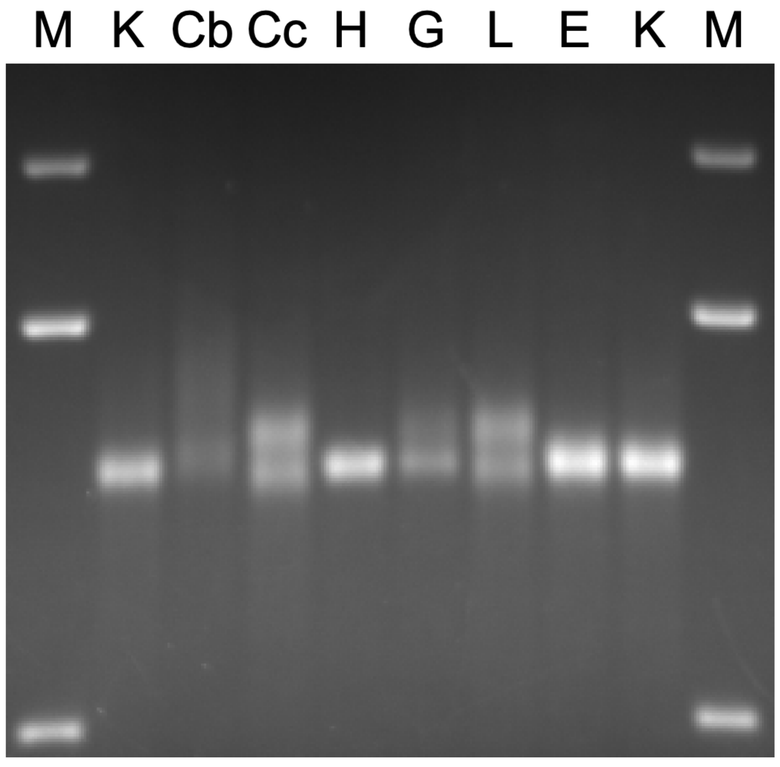
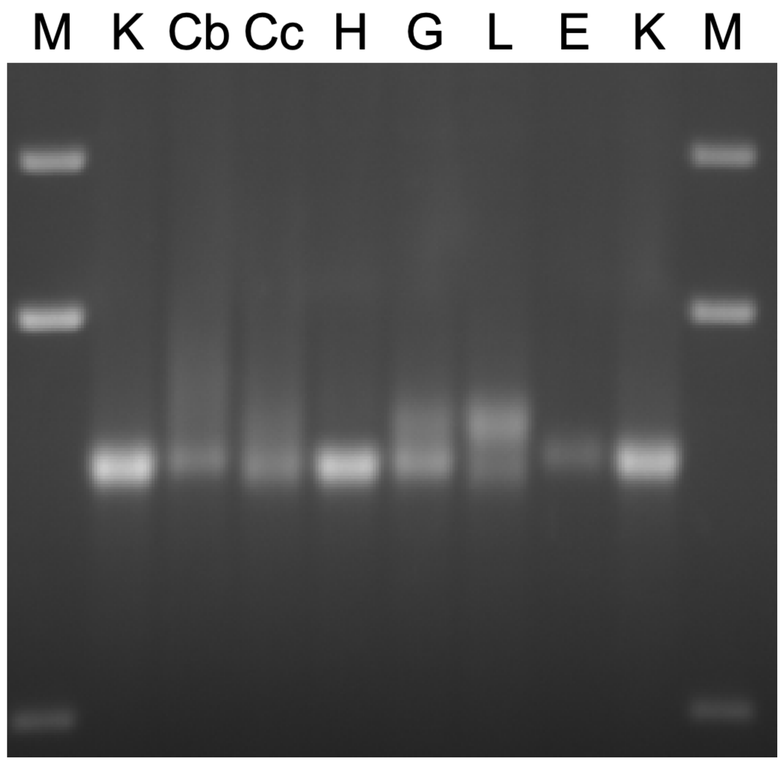


Wild Type



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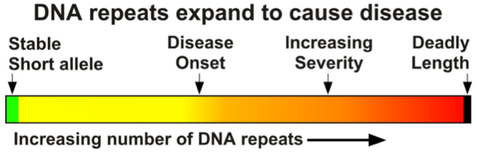

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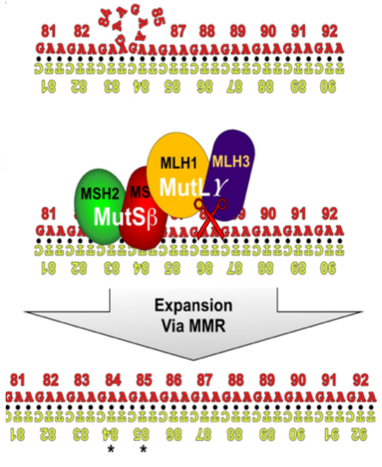

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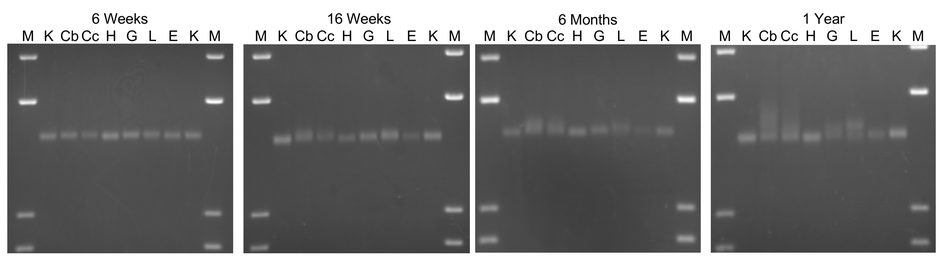

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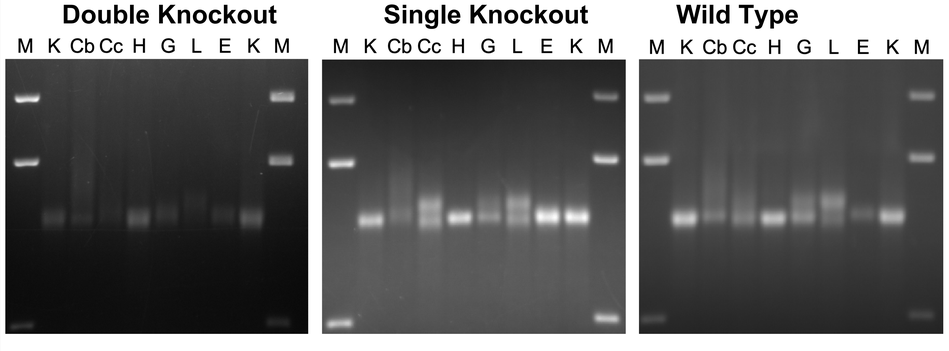

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