Mismatch in Mice and Men

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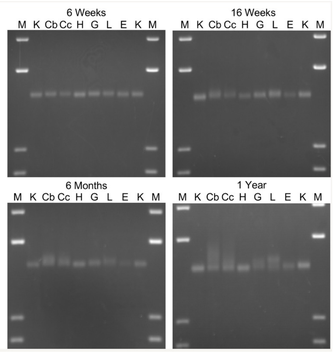
Introduction

Friedreich ataxia (FA) is a progressive degenerative neuromuscular disorder caused by expansion of a repetitive region of DNA. Following transcription, structures tend to form that recruit mismatch repair (MMR) protein complexes. Recognition of these structures is accomplished by a Mut-S complex, followed by recruitment of a Mut-L complex containing endonuclease activity to cut the mismatched region. During the repair process, nucleotides can be added resulting in expansion. Most DNA repeat expansion disorders appear to share a common mechanism of expansion involving these two complexes.

Expansion of the repeat sequence throughout the lifespan of a patient with Friedreich ataxia results in disease onset once a repeat number threshold is surpassed. Patients frequently present with progressive ataxia, diabetes mellitus, and cardiomyopathy. Expansion rates have been found to be tissue specific within a patient, with the heart expanding more rapidly. Friedreich ataxia is most often fatal due to heart failure caused by cardiomyopathy. Preventing expansion within the heart of Friedreich ataxia patients is a priority to prolong their lifespan.

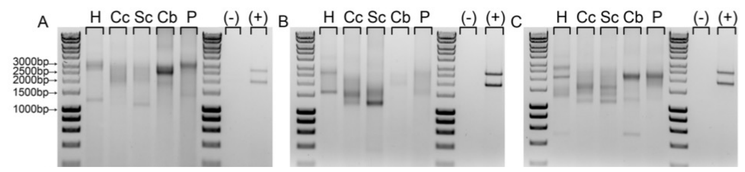
Transgenic mice are frequently used as a model to study diseases including Friedreich ataxia. Repeat expansion has been observed to be tissue specific within both humans and the mouse model. Our lab has observed that the repeats within the heart of most mice do not expand. In order to develop therapeutics for Friedreich ataxia, the mechanism of expansion within the heart must be understood. This experiment aims to compare the differences in mismatch repair protein expression in cardiac tissue taken from transgenic Friedreich mice with other expansion positive tissue within the same mouse.

Figure 1: Tissue Specific Expansion in FA Transgenic Mice



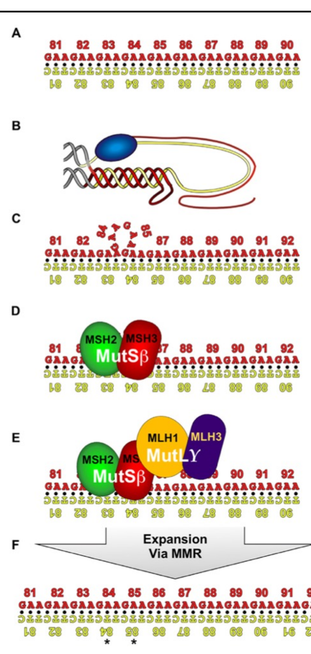
PCR amplification of genomic DNA isolated from Friedreich ataxia transgenic (FA Tg) mouse kidney (K), cerebellum (Cb), cerebral cortex (Cc), heart (H), gastrocnemius (G), liver (L), and ear (E). It was observed that the heart expanded less over the course of 1 year than other tissues.

Figure 2: Tissue Specific Expansion in Human FA Patients[1]



PCR amplification of genomic DNA isolated from the heart (H), cerebral cortex (Cc), spinal cord (Sc), cerebellum (Cb), and pancreas (P) of three FA patients. (-) represents template control. (+) represents positive control of expansion positive fibroblast of an unrelated FA patient. It was observed that the heart, cerebellum, and pancreas demonstrated significant expansion.

Figure 3: Mismatch Repair Mediated Expansion[2]



A. Trinucleotide tandem repeat region within intron of *frataxin* gene

B. Transcription results in formation of RNA•DNA hybrid

C. Reannealing following transcription results in small loop formation of 3-12 nucleotides

D. Loop is recognized by Mut-S DNA mismatch repair complex

E. Recruitment of Mut-L complex with endonuclease activity

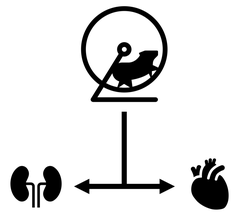
F. Resultant expansion of trinucleotide tandem repeat region

Objectives

- Determine DNA MMR proteins present within expansion positive (kidney) and expansion negative (cardiac) tissue of FA Tg Mice
- Compare these to DNA MMR proteins present in normal human cardiac tissue utilizing Western blot analysis

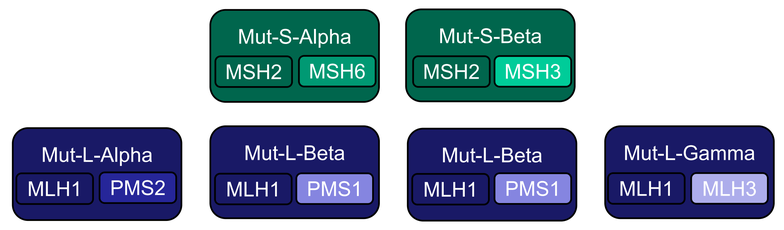
Methods

Protein Extraction



Mouse heart and kidney tissue were collected from Tg FA mice following dissection. Kidney tissue was used as an expansion positive sample to compare to cardiac tissue. Samples were placed in Laemmli buffer, blended, boiled, and sheared using an insulin syringe.

Western Blot Analysis



Mismatch Repair Complex Protein Subunits

Mouse 1° Ab

Western blot analysis was performed using mouse primary antibodies specific to human MMR proteins. Secondary antibody application resulted in binding of endogenous mouse IgG heavy and light chains preventing visualization of MMR proteins.

Rabbit 1° Ab

The Western blot was attempted using rabbit primary antibodies specific to human MMR proteins. Cross-reactivity was not observed.

Mouse 1° Ab

IgG heavy and light chains were trimmed from the gel prior to incubation with the primary and secondary antibodies. Cross-reactivity was not observed.

Conclusion and Next Steps

- Acquisition of mouse MMR protein-specific primary antibodies for Western blot analysis
- Western blot analysis of normal human cardiac tissue for comparison to mouse data

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References:
[1]: Long A, Napierala JS, Polak U, Hauser L, Koeppen AH, Lynch DR, Napierala M. Somatic instability of the expanded GAA repeats in Friedreich's ataxia. PLoS One. 2017 Dec 19;12(12):e0189990. doi: 10.1371/journal.pone.0189990. PMID: 29261783; PMCID: PMC5736210.
[2]: Halabi A, Fuselier KTB, Grabczyk E. GAA•TTC repeat expansion in human cells is mediated by mismatch repair complex MutLγ and depends upon the endonuclease domain in MLH3 isoform one. Nucleic Acids Res. 2018 May 4;46(8):4022-4032. doi: 10.1093/nar/gky143. PMID: 29529236; PMCID: PMC5934671.