The heart of the matter: Cardiac specific discrepancies between human and mouse models of Friedreich Ataxia

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Introduction

- Friedreich Ataxia (FRDA) is a relentlessly progressive neurodegenerative disease characterized by progressive gait and limb ataxia, loss of vibratory and position sense, progressive motor weakness, diabetes mellitus, and hypertrophic cardiomyopathy.
- Hypertrophic cardiomyopathy is the cause of death for approximately 60% of patients.
- FRDA is the most common inherited ataxia and is part of a group of over 40 inherited disorders called repeat expansion diseases.
- Repeat expansion diseases are caused by the expansion of unstable tandem repeats in a gene to a pathogenic size.
- FRDA is caused by the somatic expansion of GAA•TTC trinucleotide repeats within the first intron of the frataxin (FXN) gene, which decrease FXN mRNA expression.
- Repeat tract length correlates with disease severity and inversely with age of onset.
- FRDA repeat expansion is tissue specific and presents in multiple organ systems. The greatest expansion bias and longest repeat tracts are observed in the heart and pancreas.
- Our lab has shown that the DNA mismatch repair (MMR) complexes MutSβ and MutLγ are part of a central DNA expansion mechanism shared by all DNA repeat expansion disorders.
- There is currently no treatment for FRDA or any other DNA repeat disorder, so our lab focuses on testing potential therapeutic targets that act on DNA expansion.
- We have found that in contrast to extensive somatic expansion in heart tissue of FRDA patients, there is no repeat expansion in the heart of FRDA mouse models.
- We predict that this lack of expansion in the mouse heart reflects a difference in some component of MMR between human and mouse hearts.

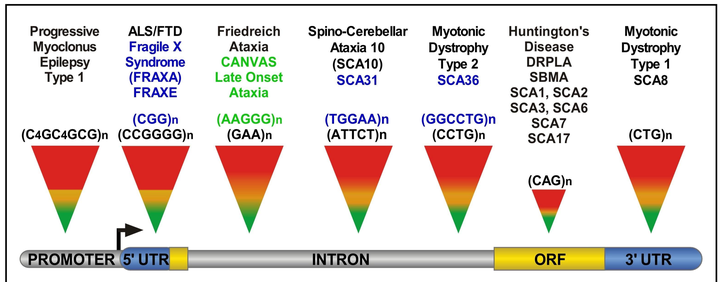

Figure 1. DNA Repeat Disorders. FRDA is part of a group of disorders called repeat expansion diseases. There are presently over 40 known repeat expansion diseases, several of which were newly discovered within the last year. There is currently no treatment or cure for any of these diseases.

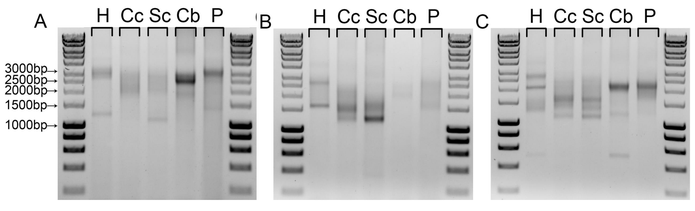

Figure 2. Repeat expansion in FRDA patients occurs in a tissue specific manner and presents in multiple organ systems. Genomic DNA from the heart (H), cerebral cortex (Cc), spinal cord (Sc), cerebellar cortex (Cb) and pancreas (P) tissues was extracted and the GAA repeats in the *FXN* locus were amplified by PCR. The results from three FRDA patients are shown as examples. The greatest expansion bias and longest repeat tracts are observed in the heart and pancreas. Figure adapted from Napierala et al.

Repeat expansion in mouse model

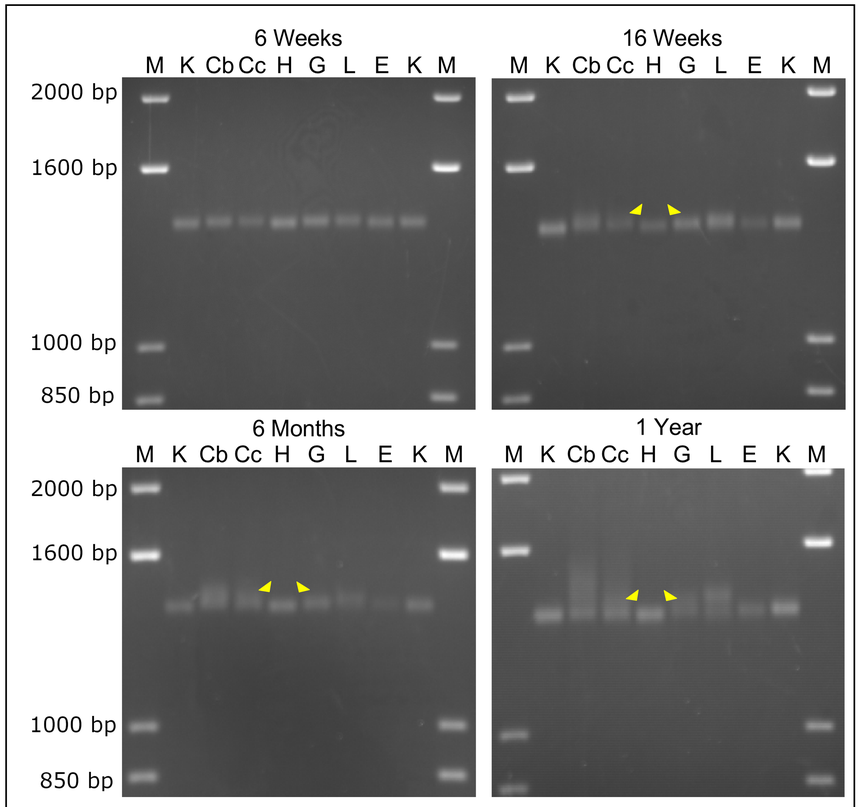

Figure 3. Tissue-specific and age-dependent expansion in the YG8s mouse model. PCR of GAA•TTC repeat size of representative YG8s mice at 6 weeks, 16 weeks, 6 months, and 1 year of age. Tissues analyzed include kidney (K), cerebellum (Cb), cerebral cortex (Cc), heart (H), gastrocnemius (G), liver (L), and ear (E). Arrows indicate lack of repeat expansion in heart tissue at 16 weeks, 6 months and 1 year of age for YG8 mice. Other tissues studied (cortex, cerebellum, liver, kidney, and gastrocnemius) all exhibit expansion. Figure adapted from L. Roy [Unpublished doctoral thesis].

Methods

- TK6 and HEK293 human cell lines and N2A mouse cells were grown on 50 mL plates with Dulbecco's Modified Eagle Medium with 10% FBS until plates reached 90% confluence.
- The cortex, cerebellum, and heart were dissected from a male YG8 mouse and frozen.
- Protein was extracted from both the human and mouse cell lines and the mouse tissue samples.
- SDS-PAGE on a 4-20% acrylamide gel was used to separate proteins by molecular weight.
- Coomassie Blue staining was used to confirm presence of protein and consistent protein loading.
- Antibodies for proteins critical to the MMR pathway either as part of MutS recognition complexes (MSH2, MSH3, MSH6), or MutL endonucleases (MLH1, PMS1, PMS2), were used to assess protein levels in the selected cell and tissue samples.

Results

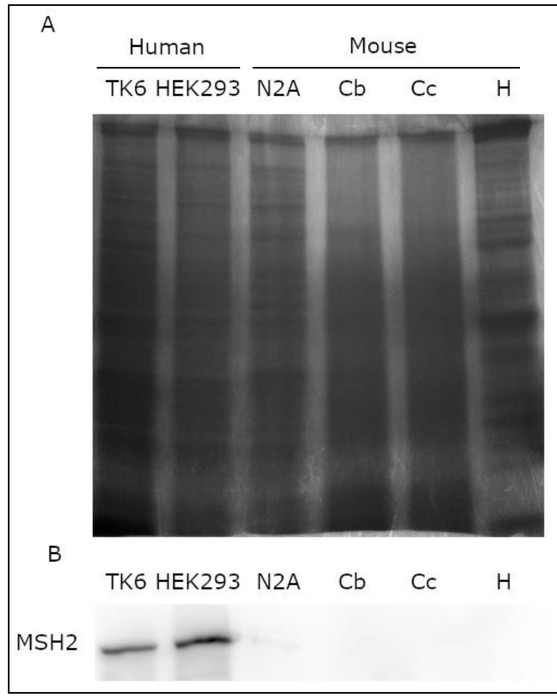

Figure 4. Protein extraction and assessment of MSH2 levels in human and mouse cell lines and YG8 mouse tissues. Cell lines analyzed include human TK6 and HEK293 cells and mouse N2A cells. Tissues analyzed include cerebellum (Cb), cerebral cortex (Cc), and heart (H). Mouse N2A cells and brain tissues are known to be positive for MSH2. Coomassie Blue staining detected sucessful extraction of protein from all samples (A). Western blotting detected presence of MSH2 in human cell controls, however not in the mouse N2A cell control or tissue samples (B).

Conclusions

- We successfully extracted protein from human and mouse cell lines, as well as from mouse cortex, cerebellum, and heart tissues.
- The MSH2 antibody we used was reported to work in both human and mouse samples. It worked on our human cell samples but did not on any of our mouse cell or tissue samples.
- We do not yet know if there are differences between components of the mismatch repair pathway in human and mouse hearts.
- Further testing is required to identify antibodies that will work in both human and mouse cells and tissues.
- Future work will complete our original plan to assess levels of proteins critical to the MMR pathway (MSH2, MSH3, MSH6, MLH1, PMS1, and PMS2) and determine if any of these key proteins are absent in the mouse heart, which may cause the lack of repeat expansion that we observed there.

References

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