Aim: To develop an experimental platform for in vivo investigation of candidate genetic modifiers of somatic CAG instability in Huntington’s disease.

Introduction: Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion within the huntingtin gene (HTT). Despite being a monogenic disorder, for which the mutation has been known for some time now, no cure or disease-modifying therapy is available, indicating that novel approaches are critical.

Somatic CAG repeat instability, characteristic of mutant HTT alleles, is inversely correlated with patient age of onset and may contribute to HD pathogenesis. This phenotype, common to other trinucleotide repeat disorders, was previously shown to be DNA mismatch repair (MMR) dependent. The DNA repair machinery was further implicated as a modifier of HD age of motor onset in a recent genome wide association study, underlining its promise as a relevant disease mechanism that could potentially be therapeutically targeted.

In this study, we are developing a CRISPR/Cas9-based approach that will enable the investigation of candidate genetic modifiers of HD age of onset as potential modifiers of somatic CAG repeat instability in a HD mouse model.

Methods: We have developed CRISPR reagents against known and candidate genetic modifiers of somatic CAG instability in Huntington’s disease. In preliminary experiments, we treated HD mice with CRISPR reagents against Mlh1 and investigated the level of gene editing achieved as well as the impact on liver CAG instability.

Results: We were able to significantly suppress the CAG expansion process in the liver of HD mice by knocking out the Mlh1 gene in our *in vivo* CRISPR platform. The efficiency achieved in modifying the instability phenotype makes us very confident that we will be able to test and validate additional candidate modifiers. To that end, we have already validated reagents for efficient knockout of a subset of known and candidate modifier genes and we have developed assays that will allow detailed characterization of gene editing at these sites.

Conclusion: We have successfully developed an *in vivo* CRISPR-Cas9-based platform that allows for knocking out genes of interest in the liver of adult mice, and consequently perturb the somatic CAG expansion process. We will next use this tool to test the role that candidate genes might play in that disease-relevant process. While the scope of this project was liver oriented, future work will also be aimed at targeting the striatum which is the main site of HD-related pathology.

References