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**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*).<sup>1</sup> 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.<sup>2,3</sup> This phenotype, common to other trinucleotide repeat disorders,<sup>4</sup> was previously shown to be DNA mismatch repair (MMR) dependent.<sup>5</sup> 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.<sup>6</sup>

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.

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Oncology & Molecular Biology Poster Session  
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## PS021

### Regulation of transcription factor MEF2C by RNA binding protein HuR



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**Aim:** We hypothesized that HuR RNA binding protein regulates MEF2C expression through association with MEF2C mRNA.

**Introduction:** MEF2C is earliest expressed member of the MADS-box super family during heart development. In the postnatal heart, decreased expression of MEF2C has been associated with myotonic dystrophy type 1 (DM1) heart disease. Hu proteins are known to regulate a wide range of gene expression by modulating mRNA's half-lives.

**Methods:** We use Human Fetal Cardiomyocyte cell line RL14. Cells are transfected with Superfect Transfection Reagent(Qiagen). And RNA Isolation performed by using RNeasy Plus Mini Kit. Real Time quantitative PCR (q-PCR) analysis performed using Fast SYBR Green Master Mix.

**Results:** Over expression of HuR in cardiomyocytes derived from primary human fetal ventricle increased MEF2C mRNA 47.3% ( $p = 0.01$ ). Knocking down of HuR by siRNA decreased MEF2C mRNA by 62% ( $p = 0.01$ ). RNA Immunoprecipitation showed HuR associated with MEF2C mRNA.

**Conclusion:** Our results suggest that RNA binding protein HuR associates with MEF2C mRNA in cardiomyocytes. And also HuR positively regulates MEF2C mRNA expression.

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## PS024

### The effect of prenatal Vitamin C deficiency on endochondral ossification in guinea pigs



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**Aim:** The aim of the research is to investigate the effect of prenatal vitamin C deficiency on endochondral ossification in guinea pigs.

**Introduction:** Vitamin C is an essential nutrient which inter alia enables the synthesis of collagen and therefore endochondral ossification. Throughout years a lot of research has been published investigating the exact role of vitamin C and the impairment developed due to its deficiency. However there is insufficient data about the effect of prenatal deficit of vitamin C on the developing bone structures.

**Methods:** The study encompassed 14 fertilized female albino guinea pigs. Their diet was comprised of vitamin C-free food and ad libitum water enriched with vitamin C. The 10th day of fertilization, experimental group was depleted of vitamin C. Deprivation lasted until the 50th day, after which the females were sacrificed and their fetuses were taken out. Forelegs of fetuses were fixed and dehydrated, after which they were embedded in paraffin and