THE ROLE OF SPLICING FACTOR SRSF6 IN INCOMPLETE SPLICING OF THE *HTT* TRANSCRIPT

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Background:
Huntington's disease (HD) is caused by an expanded CAG repeat in exon 1 of the *HTT* gene. In models of HD, it has been shown that an expanded CAG repeat in *HTT* causes premature termination of *HTT* RNA during transcription; this occurs by a process called incomplete splicing. Incompletely spliced *HTT* (*HTTexon1*) includes exon 1 of the coding region of the *HTT* gene, as well as a 5' region of intron 1, which is non-coding. *HTTexon1* encodes a truncated exon 1 HTT protein, which is implicated in HD pathogenesis. Although the precise RNA processing mechanism of *Httexon1* is unknown, splicing factor SRSF6 has been shown to co-precipitate with transcripts containing *Htt* intron 1 in HD mice.

Aim:
To elucidate the role of splicing factor SRSF6 in incomplete splicing of *Htt* in HD mice.

Methods:
Heterozygous *Srsf6* knock out mice (*Srsf6*$^{+/-}$*)* were generated by CRISPR/Cas9. Characterisation of *Srsf6*$^{+/-}$ mice was undertaken by quantitative RT-PCR and western blotting. Viability of homozygous *Srsf6* knock out (*Srsf6*$^{-/-}$) mice was examined by inbreeding of *Srsf6*$^{+/-}$ mice. To assess whether decreasing levels of SRSF6 modulates levels of incomplete splicing, *Srsf6*$^{+/-}$ mice were bred to HD knock in mice (*zQ175*) and tissues were collected at 2 months of age. Levels of incompletely spliced *Httexon1* were measured by Quantigene, a gene expression assay.

Results:
Srsf6$^{-/-}$ homozygotes were embryonic lethal, limiting us to the use of *Srsf6*$^{+/-}$ mice only. In *Srsf6*$^{+/-}$ heterozygotes, *Srsf6* mRNA was decreased by 50% in several brain and peripheral regions, and SRSF6 protein was decreased by 70% in mouse brain compared to wild type mice. However, heterozygosity for *Srsf6* knock out did not modulate the level on incomplete splicing in zQ175 mice.

Conclusion:
Ablation of a single *Srsf6* allele did not reduce levels of incomplete splicing in HD mice and therefore, further *Srsf6* knock down may be required. Accordingly, mouse embryonic fibroblasts (MEFs) have been generated and *Httexon1* levels will be measured in HD MEFs after further *Srsf6* knockdown by RNA interference.