The influence of the HTT exon 1 nucleotide sequence on exon 1 splicing: possible implications for Huntington's disease

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Background: Dysregulated pre-mRNA splicing is increasingly reported in neurodegenerative diseases, including Huntington's disease (HD). While HD pathogenesis has traditionally been attributed to mutant huntingtin (HTT) protein, incomplete splicing of HTT generates a truncated, highly toxic transcript (HTT1a) via cryptic polyadenylation in intron 1. CAG tract expansion is known to promote HTT1a production. However, the underlying regulatory mechanisms remain unclear. Here, we investigate how the nucleotide sequence of HTT exon 1 influences splicing efficiency and HTT1a formation, highlighting the need to integrate RNA biology into HD pathogenesis. Method: We leveraged an in-house developed mouse embryonic stem cell platform (HuntEx1), based on recombination-mediated cassette exchange (RMCE) that comprises 65 engineered cell lines, carrying distinct HTT exon 1 variants. This system enabled analysis in differentiated neurons. We combined this platform with mRNA structural predictions and RNA-binding protein (RBP) consensus site analysis to identify candidate cis-elements and trans-acting splicing factors regulating HTT exon 1 splicing. Results: Lines carrying human HTT exon 1 showed elevated HTT1a expression, which normalized when the human Proline-Rich Domain (PRD) flanking the CAG tract was replaced with the murine. This implicated the PRD as splicing regulatory element, potentially acting through modulation of RNA secondary structure. Free energy-based structural predictions showed that PRD sequence influences intramolecular pairing within HTT exon 1. RBP consensus site analysis identified SRSF7 as candidate PRD-binding factor. Targeted mutation of the predicted consensus site altered HTT1a expression, supporting a functional role for this region in splicing regulation. Conclusion: Our findings (Maffezzini, Iennaco et al, in press) demonstrate that the complex relationship between sequence, structure, and splicing factors recruitment at HTT exon 1 controls production of toxic HTT1a. Moreover, they also underline the importance of RNA biology in the HD field and suggest splicing as a mechanism to be carefully considered for the development of future therapeutic strategies.