

# **Molecular characterization of *HTT* and *ATXN3* transcripts in the context of their roles in the pathogenesis and as targets in therapies of polyglutamine diseases HD and SCA3**

Paweł Joachimiak

Polyglutamine diseases (polyQ) are a group of neurodegenerative disorders. They are caused by the expansion of a CAG repeat tract in the coding region of specific genes, which results in an expanded tract of glutamines in protein sequence. Due to an autosomal dominant inheritance pattern, most of polyQ disease patients carry two alleles of specific gene – normal allele (WT), and mutant allele (MUT). For most polyQ disorders, normal allele is characterized by the range of 5-30 CAG repeats, while mutant allele is usually possesses a tract of > 39 CAGs. Huntington's disease (HD) and spinocerebellar-ataxia type 3 (SCA3) are two best described polyQ diseases. Although, our knowledge about polyQ diseases is still rising, there is no efficient treatment for polyQ patients.

Generally, a mutant polyQ protein is recognized as the main pathogenic factor in polyQ diseases. In case of polyQ transcripts, it was observed that they are good therapeutic targets for strategies aimed at the downregulation of mutant polyQ genes. On the other hand, the exact role of mutant transcripts in pathogenic mechanisms in polyQ diseases is being unraveled. Hence, the main goal of my PhD thesis was to become acquainted with polyQ transcripts' characteristics which can affect both, the pathogenesis of polyQ diseases, and the design of therapeutic approaches.

At the beginning of my PhD studies I investigated therapeutic aspect related to polyQ disorders. I participated in unraveling the mechanism leading to allele-selective silencing of mutant polyQ genes, using a specific A2 oligonucleotide targeting CAG repeat tract located within huntingtin (*HTT*) transcript sequence. Obtained results allowed for indicating the occurrence of translation inhibition and transcript deadenylation, as well as for determining the role of AGO2, in the investigated mechanism. Next, I started exploring implications of mutant polyQ transcripts in the pathogenesis of polyQ diseases. I reviewed results from studies exploring polyadenylation and alternative polyadenylation (APA) processes in polyQ

transcripts, and I proposed future perspectives in this topic. Then, I developed an experimental approach designed for quantitative analysis of endogenous ataxin-3 (*ATXN3*) and *HTT* transcripts. This approach utilizes droplet digital PCR (ddPCR) technology and heterozygous SNP variants present in sequences of these transcripts, which allow for discrimination between WT and MUT transcripts. I used this approach for precise, accurate and quantitative determination of the WT/MUT allele ratio in a set of HD and SCA3 patient-derived cell lines, as well as in brain tissues derived from HD mouse model. My experiments demonstrated that neural differentiation can impact the WT/MUT allele ratio, as well as the total number of transcripts (WT+MUT) per cell. Mover, this approach was used to assess the efficacy of allele-selective therapeutic strategy, and to verify whether genome engineering techniques affect the expression of targeted alleles. To conclude, my research contributed to a better understanding of mutant polyQ transcripts in the context of both, the pathogenesis of polyQ disorders, and as molecular targets in therapeutic strategies.