Modulating ataxin-3 abundance using a novel high-throughput drug testing pipeline

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**ABSTRACT:**

Neurodegenerative diseases are similar in proteinopathy in which the accumulation of toxic protein has a higher propensity to form into insoluble aggregates. Due to this, it has been largely disputed whether the aggregate or the toxic protein itself lead to the degeneration of neurons. Machado-Joseph disease (MJD) is one of these neurodegenerative diseases caused by a trinucleotide expansion of CAG in the ATXN3 gene and in turn produces polyglutamine expanded ataxin-3 protein. Several studies are currently investigating modulation of ataxin-3 abundance as a treatment for MJD.

Our lab generated a transgenic zebrafish model of MJD with the intention to understand the pathogenesis of disease and investigate potential therapeutic candidates (Watchon et al., 2017).Characterising this model displayed a variety of disease phenotypes such as ataxin-3 positive aggregates, ataxin-3 cleavage products, and a swimming phenotype (Watchon et al., 2017; Robinson et al., 2021a) We have used these zebrafish to test the therapeutic potential of various small compounds (Watchon et al., 2017; Robinson et al., 2021a, 2021b; Watchon et al., 2021, Watchon et al., 2023; Watchon et al., 2024a, 2024b). However, when compared to other studies, small compound testing in our zebrafish model is relatively time consuming due to the techniques used.

Therefore, we aimed to generate a novel method utilising our MJD zebrafish model to test an array of small compounds in a high-throughput manner. Our zebrafish model expresses an enhanced green fluorescence protein (EGFP) tagged to human ataxin-3 with a polyglutamine length of 84Q representing mutant ataxin-3. We hypothesised that compounds that modulate ataxin-3 levels can be detected via alterations to the abundance of EGFP. This was measured using fluorescent microscopy and microplate reading. We initially tested this method with compounds known to increase or decrease ataxin-3 levels, identified via increased/decreased EGFP levels, in both imaging and microplate reading of treated MJD zebrafish. We also confirmed ataxin-3 and EGFP abundance via western blotting.

Using the above readout technique, we next tested an array of small compounds extracted from natural sources in a high-throughput manner (~500) using a multi-well plate. We determined positive ‘hits’ as decreasing or increasing EGFP levels by 30% in both microplate reading and fluorescent microscopy. Positive ‘hits’ were then tested again on the MJD zebrafish for swimming analysis and protein abundance via western blotting. This method for testing small compounds in our MJD zebrafish model is a faster and less biased approach for discovering a potential therapeutic for MJD patients.

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