# Behavioural assessment and brain imaging

# of new *Drosophila melanogaster* models

# for TDP-43 proteinopathies

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All over the world, tens of millions of people suffer from neurodegenerative diseases, a broad group of incurable syndromes characterized by neuronal loss and often leading to dementia. While the early causes of neurodegeneration remain poorly understood, aggregates of specific proteins, either within or around neurons, are common hallmarks of such disorders. Hence, protein dysregulation is widely regarded as one of the main initiators of neurotoxicity and, accordingly, certain illnesses are referred to as proteinopathies.

Despite having dissimilar clinical manifestations, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) belong to the same category of diseases, termed TDP-43 proteinopathies. Indeed, they share two central characteristics, namely nuclear depletion and cytoplasmic accumulation of the RNA-regulating protein TDP-43. Moreover, neuronal inclusions of this protein are not only found in ALS and FTD patients, but also in some cases of other neurological conditions, including Alzheimer’s disease. Consequently, TDP-43 is believed to play a major role in early neurodegeneration, through a mechanism that could be shared by different pathologies. Understanding the root of this mechanism could mean finding the key to treating a whole range of disorders, which is why research remains essential in this field.

There are numerous risk factors, at both genetic and environmental levels, that underly TDP-43 proteinopathy development, with ageing being the commonest. In most cases, the protein acquires its pathogenic potential without being mutated, hypothetically because of an intrinsically disordered region located in its C-terminal domain. Nevertheless, a number of mutations have been identified in patients and are likely to enhance the propensity of the protein to misfold and aggregate, as the vast majority of these occur within the C-terminus.

In this study, we established new humanized *Drosophila melanogaster* models to investigate how pathogenic TDP-43 species affect brain integrity, cognition, and behaviour. We used the CRISPR-Cas9 knock-in system to replace the fly orthologous gene, *TBPH*, with the human one, *TARDBP*. In order to study the effects of disease-associated mutations, we created one line expressing the wildtype variant of human TDP-43, and two lines expressing the following mutant forms: TDP-43 Q331K and TDP-43 R361T.

To assess the cognitive abilities of these models, we performed classical conditioning assays, which revealed learning or short-term memory defects in all three humanized lines. Moreover, results from immunofluorescence brain analyses suggested that TDP-43 expression may disrupt synaptic connectivity or neural development. However, no significant phenotypic differences were observed between the line expressing wildtype TDP-43 and the two lines expressing mutant variants. Finally, we took preliminary steps in the establishment of a novel assay for single-fly locomotion tracking. Overall, our new *Drosophila* models have promising potential for the investigation of human TDP-43 pathogenicity and could allow to refine our understanding of some of the earliest mechanisms underlying neurodegeneration in various illnesses.

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