ENCALS

European Network to Cure ALS

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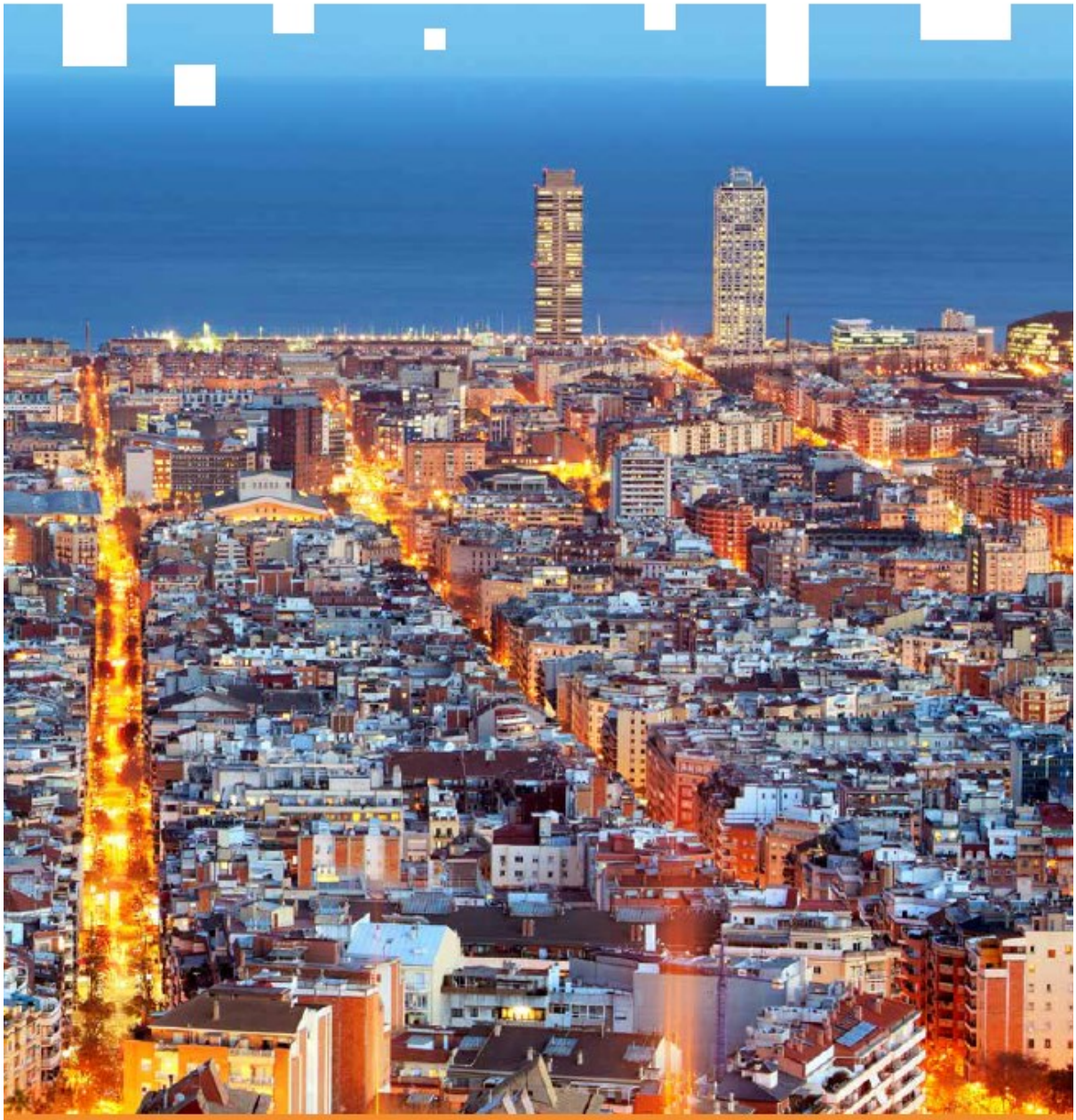

Poster List

https://www.encals.eu/meetings/barcelona/

71. Insights into KIF5A-related pathways to neurodegeneration

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KIF5A is a neuron-specific kinesin involved in anterograde axonal transport. It comprises a head domain for microtubule binding and ATP hydrolysis, a stalk domain for dimerization, and a tail domain for autoinhibition and cargo/adaptor binding. Mutations occurring in these KIF5A domains are associated with distinct neurodegenerative diseases (NDs), including ALS, but the bases of such genotype/phenotype heterogeneity still have to be fully elucidated. To investigate the molecular mechanisms underlying KIF5A-dependent NDs, we analysed the biochemical behaviour of five disease-associated KIF5A mutants (R17Q, R280C, R864X, N999VfsX39, C975VfsX73) affecting the different protein domains.

In NSC-34 cells, we overexpressed the ALS-linked N999VfsX39 and the Charcot-Marie-Tooth-related R864X mutants and found that they mainly localise within neurites instead of showing the diffused cytoplasmic distribution of overexpressed WT KIF5A. However, the two mutants differed, since R864X KIF5A was found to be diffused within neurites, while N999VfsX39 KIF5A formed p62-positive puncta. Notably, both mutants sequestered WT KIF5A within neurites and showed partial co-localisation with mitochondria, well-established KIF5A cargos.

In SH-SY5Y cells, cycloheximide chase evidenced a lower stability for the N999VfsX39 and the spastic paraplegia-associated R17Q mutants compared to WT KIF5A, hinting at an altered protein turnover. Accordingly, proteasomal blockage resulted in N999VfsX39 and R17Q KIF5A accumulation into detergent-insoluble inclusions, suggesting that these mutants are degraded by the ubiquitin-proteasome system and that proteostasis impairment might promote their deposition into aggregates.

Interestingly, the aberrant biochemical behaviours of N999VfsX39 KIF5A were recapitulated to a more severe extent by the novel C975VfsX73 variant, linked to neonatal intractable myoclonus (NEIMY) and sharing the last portion of its abnormal C-terminal tail with the ALS mutant. Indeed, C975VfsX73 KIF5A accumulated into large, p62-decorated inclusions that sequestered WT KIF5A and lacked interaction with mitochondria, highlighting a phenotypic similarity between the ALS- and NEIMY-related mutants.

Together, our results indicate that both unique and shared molecular mechanisms underpin KIF5A-dependent NDs.
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