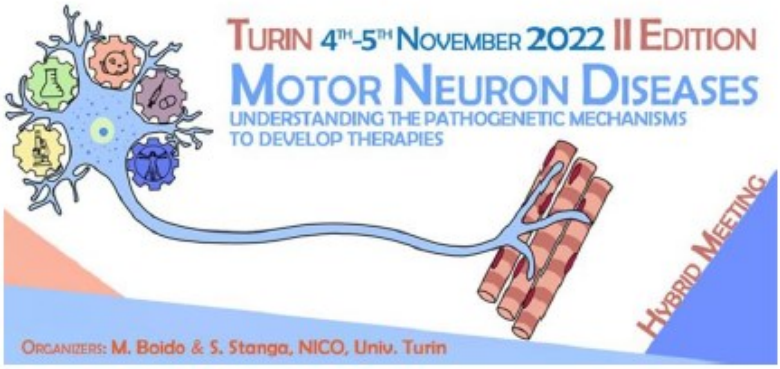
Under the patronage of



Organizers: Marina Boido & Serena Stanga

Contacts: marina.boido@unito.it & serena.stanga@unito.it

SUPPORTED BY:

Under the patronage of

ONE GENE, MANY PHENOTYPES: INVESTIGATING KIF5A-LINKED NEURODEGENERATION MECHANISMS

Cozzi M.[1], Tedesco B.[1,2], Cristofani R.[1], Ferrari V.[1], Casarotto E.[1], Chierichetti M.[1], Pramaggiore P.[1], Piccolella M.[1], Galbiati M.[1], Rusmini P.[1], Crippa V.[1], Gellera C.[2], Magri S.[2], Santangelo S.[3,4], Ratti A.[3,4], Taroni F.[2], Poletti A.[1]

[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy; [2]UOC Genetica Medica – Neurogenetica, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; [3]Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Milan, Italy; [4]Dipartimento di Neurologia e Stroke Unit – Laboratorio di Neuroscienze, IRCCS Istituto Auxologico Italiano, Milan, Italy

KIF5A is a neuron-specific kinesin involved in anterograde axonal transport. It comprises an Nterminal motor domain for ATP-dependent microtubule binding, a coiled-coil stalk for dimerization, and a tail domain for cargo/adaptor binding and autoinhibition. Mutations targeting the three KIF5A domains are associated with distinct motor neuron diseases (MNDs) – including spastic paraplegia, Charcot-Marie-Tooth disease, and amyotrophic lateral sclerosis (ALS) – but the molecular mechanisms underpinning such phenotypic heterogeneity are not fully understood yet. Our aim is to functionally characterize four KIF5A mutants (R17Q, R280C, R864X, N999Vfs*39) to gain insight into KIF5A-related neurodegeneration. Upon overexpression in NSC-34 cells, the R864X and N999Vfs*39 mutants displayed abnormal distribution by preferentially localizing within neurites instead of being diffused in the whole cytoplasm like wild-type (WT) KIF5A. Such pattern is consistent with impaired KIF5A autoinhibition, respectively depending on loss or alteration of the tail domain for the two mutants. More in detail, the N999Vfs*39 variant formed p62-positive puncta, while R864X KIF5A was diffused within neurites. Notably, both mutants also showed limited colocalization with mitochondria, whose axonal transport relies on KIF5A, and sequestered WT KIF5A within cell protrusions. Cycloheximide chase in SH-SY5Y cells evidenced shorter half-life for the R17Q and N999Vfs*39 mutants compared to WT KIF5A, hinting at altered protein turnover. In line with this observation, proteasomal blockage induced R17Q and N999Vfs*39 KIF5A accumulation into detergent-insoluble inclusions, indicating that the two mutants are preferentially degraded by the ubiquitin-proteasome system and that they may form harmful aggregates upon proteostasis impairment. Interestingly, some features characterizing the ALS-associated N999Vfs*39 mutant are recapitulated by another frameshift variant, C975Vfs*73 KIF5A, linked to a severe neurodevelopmental disorder. Indeed, C975Vfs*73 KIF5A, too, was found to aggregate into large, detergent-insoluble, p62-positive inclusions sequestering WT KIF5A and to display limited colocalization with mitochondria. Together, our results suggest that both unique and shared pathogenetic mechanisms underlie KIF5Alinked MNDs.