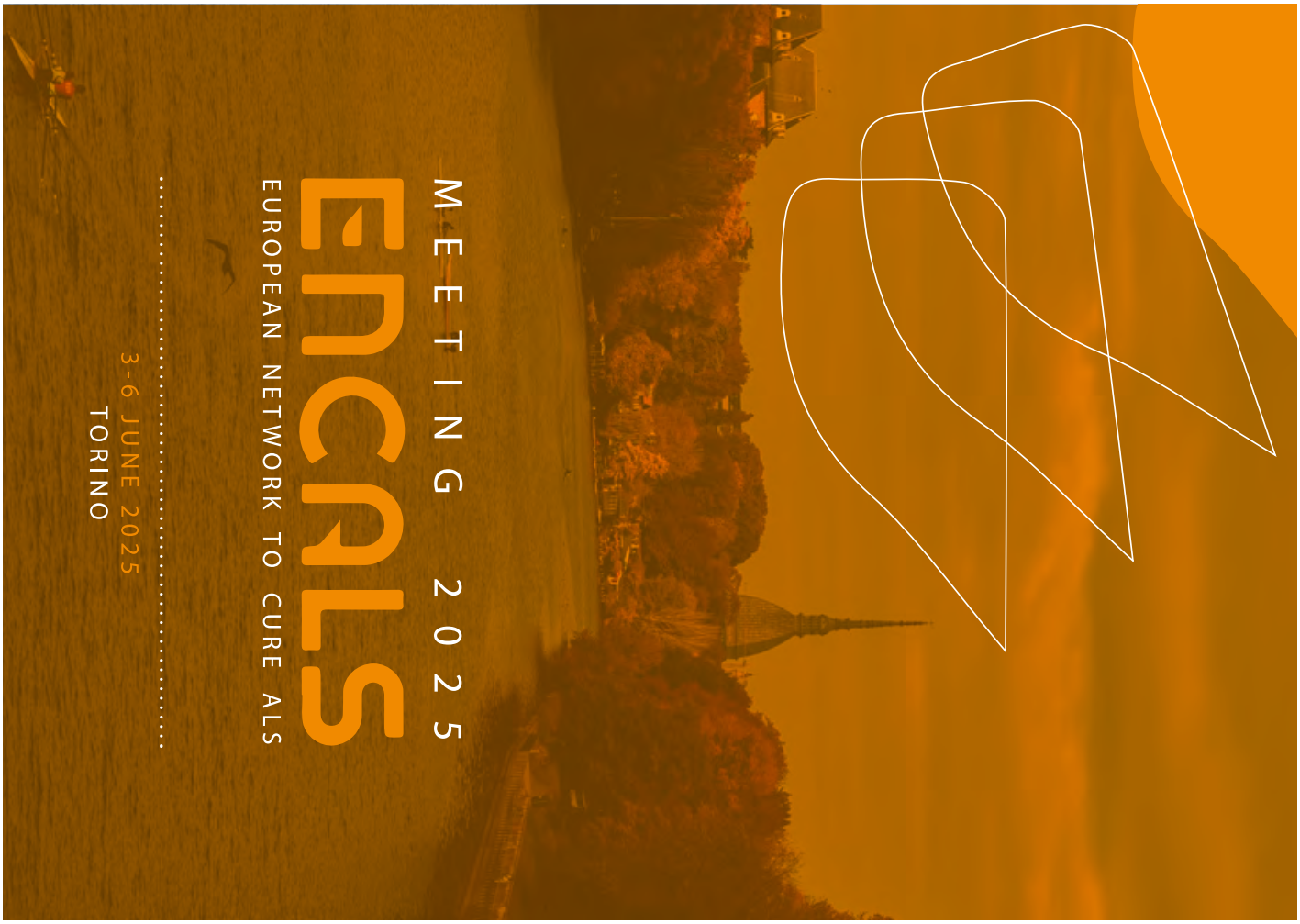

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25372, SEX-DEPENDENT CHAPERONE-ASSISTED SELECTIVE AUTOPHAGY (CASA) ACTIVITY IN ALS ASTROCYTES AND MICROGLIA

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that differentially affects males and females, and in which astrocytes and microglia have been shown to play a pivotal role in disease progression. Autophagy disfunction in both males and females is a common event in ALS, and alterations in CASA complex components, like HSPB8 and BAG3, have been previously described; however, it is unknown whether this system is impaired in a sex-dependent way in astrocytes and microglia, during ALS progression. Adult mouse primary spinal cord astrocytes (P120) RNAseq analyses identified 620 DEGs in females and 187 DEGs in males, including a reduced HSPB8 and HSPB6 expression in SOD1-G93A female primary astrocytes. CASA complex evaluation by qPCR in SOD1-G93A primary astrocytes (P120) showed increased HSPB8 and BAG3 mRNA expression in male mice, meanwhile in females, the opposite effect was observed. Interestingly, no alterations were detected in astrocytes isolated at P2. Quantification of p62 bodies in male astrocytes showed a higher number of p62 bodies per cell in WT versus G93A conditions, and autophagy inhibition through NH4Cl treatment drastically increased this effect. In females, a lower number of p62 bodies per cell was observed in WT versus G93A astrocytes. Moreover, we observed a lower number of p62 bodies in WT female astrocytes compared to males, meanwhile in G93A mice, a significant increase in p62 bodies per cell was observed. Analyses on LC3 puncta showed a decrease in the number of puncta per cell in G93A versus WT astrocytes in males, meanwhile no changes were detected between female samples. Finally, studies in microglia primary cultures, isolated from mouse spinal cord at P2 and stimulated with a cytokine cocktail, only showed a significant decrease in LC3 mRNA expression in G93A microglia, both in female and male samples, meanwhile at the protein level, a significant reduction in BAG3 expression was observed in female microglia, both in WT and G93A conditions. Altogether, data suggests that G93A male astrocytes increase the expression of proteins such as HSPB8 and BAG3, possibly boosting autophagy to counteract misfolded protein accumulation. On the other hand, female astrocytes possess a higher autophagy capacity than male astrocytes; however, this feature is reduced in presence of G93A ALS mutation. Acknowledgements: PRIN 2022 finanziato dall'Unione europea – Next Generation EU, componente M4C2, investimento 1.1 (n. P2022B5J32)