# SESSION II - NEURODEGENERATION AND NEUROPROTECTION

## α-SYNUCLEIN OLIGOMERS IN SKIN BIOPSY AS BIOMARKER FOR PARKINSON'S DISEASE

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The pathological hallmark of Parkinson's disease (PD) is α-Synuclein inclusion formation in the brain areas affected by neurodegeneration. PD is now considered as a multisystemic disorder and α-Synuclein-related pathology is also present in the peripheral nervous system, that could be exploited to unravel novel disease-related mechanisms. α-Synuclein oligomers have recently been indicated as 'a new hope' in the search of a reliable biomarker for synucleinopathies, including PD and multiple system atrophy. The oligomeric species of α-Synuclein consist in small aggregates of the protein, which occur in the early stage of the pathology, preceding and probably triggering the formation of the fibrillar conformation present in Lewy bodies. In the present study we explored α-Synuclein oligomers using the proximity ligation assay (PLA), an innovative approach to detect *in situ* protein interactions, in the peripheral nervous system by focusing on skin biopsies. We conducted a comparative analysis in a cohort of PD patients (n=38) and healthy subjects (n=29), including a subgroup of monozygotic twins discordant for the disease (n=19). In this case-control study, we observed previously undetected α-Synuclein oligomers within synaptic terminals of autonomic fibers in skin biopsies and proposed a method for their quantification, namely the PLA score. This score was found to have good sensitivity (82%), specificity (86%) and positive predictive value (89%). Intriguingly, although no difference in median values was detected between consecutive healthy controls and healthy twins, the prevalence of healthy subjects positive for PLA score was significantly greater in twins than in the consecutive cohort (47% *vs* 14%). This suggests that genetic predisposition is important, but not sufficient, in the aetiology of the disease and strengthens the contribution of environmental factors. All these finding endorse the hypothesis that α-Synuclein oligomers could be used as a reliable diagnostic biomarker for PD. Furthermore, this important starting point opened the way to investigate the molecular mechanisms involved in triggering α-Synuclein oligomerization and aggregation, including cytoskeletal remodeling in the peripheral and central nervous system.

## PROTECTIVE EFFECTS OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE IN AN *IN VITRO* MODEL OF ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of upper and lower motor neurons (MNs). Not all MNs are susceptible to degeneration in ALS: in fact neurons of the oculomotor nucleus, controlling eye movements, are more resistant as compared to hypoglossal nucleus MNs. The analysis of *post mortem* samples from ALS patients has shown a differential genomic pattern between the two nuclei. Among identified genes, adenylate cyclase activating polypeptide 1 (ADCYAP1) gene, encoding for pituitary adenylate cyclase-activating polypeptide (PACAP), was found overexpressed in the oculomotor *vs* hypoglossal nucleus, suggesting that the peptide could exerts a role on MNs in ALS. In the present study, we investigated the potential ability of PACAP to counteract MNs degeneration, by using a motor neuron like hybrid cell line (NSC 34) expressing human superoxide dismutase (SOD1) G93A mutation, as an *in vitro* model of ALS. Our results showed that PACAP promotes cell viability following serum deprivation, *via* EGFR transactivation mediated by protein kinase A stimulation. Furthermore, PACAP significantly decreased hypoxia-induced mutant SOD1 accumulation by modulating the autophagy process through the activation of the MAPK/ERK survival signaling pathway. Overall, our data demonstrated that PACAP exerts a protective role in MNs during ALS progression, suggesting that the different vulnerability of some cranial nerve motor nuclei could be due to differential expression of PACAP and its receptors in MNs.

## EPIGENETIC MODULATION IN SOD1(G93A) ALS MICE

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ALS is a neurodegenerative disease that affects motor neurons (MNs). Transcriptional dysfunction which involves a defect in histone homeostasis has recently been implicated in MN degeneration. Histone homeostasis strongly depends on the activity of histone deacetylases (HDACs). These enzymes, which includes an important group known as sirtuins (SIRT) have been implicated in cellular processes such as cell death. Recent studies from our lab have demonstrated that the combination of two epigenetic drugs, MS-275 (which inhibits HDACs) and Resveratrol (an activator of the AMP-activated kinase (AMPK)-sirtuin 1 pathway) provided neuroprotective effects and improved motor performance in ALS mice. However, MS-275 is currently not approved for clinical trials. Several studies have indicated that Valproate, another pharmacological inhibitor of HDACs, improves cell survival by promoting histone acetylation, gene transcription and protein synthesis in cancer and ischemic stroke, and is currently been used in clinical trials. To improve the translational power of this approach, the overall aim of this study was to investigate the efficacy of MS-275 replacement with