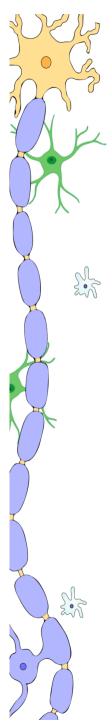




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## **POSTER P-A14**

## VCP mutants cause lysosomal alterations and autophagy induction in ALS-neuronal model

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Valosin Containing Protein (VCP) is an ATPase protein that has a key role in various pathways critical for the maintenance of cellular homeostasis and vitality. In particular, VCP is involved in the Protein Quality Control System. Indeed, VCP-mutants have been correlated to different proteinopathies as IBMPFD and ALS. The presence of VCP mutations has been associated with ubiquitin inclusions, TDP-43 mislocalization and aggregation, and abnormal vacuoles. To date VCP-mutants pathological mechanisms are still controversial. Thus, we decided to better define VPC-mutants pathological mechanisms in an ALS-model overexpressing VCP WT, VCP R155H, and VCP R191Q in NSC-34, a motor neuron mouse immortalized cell line.

Firstly, we determined that VCP-mutants form insoluble aggregates in this neuronal model. In addition, we observed that the presence of VCP-mutants triggers significant lysosomal alterations in morphology, size, activity, and membrane breakage. Lysosomal alterations have been described to induce cell toxicity and death. To remove damaged lysosomes and therefore to maintain cell vitality, cells activate different mechanisms like autophagy induction. Thus, we analysed LC3 conversion and p62 accumulation, markers of autophagic flux, to determine if the presence of VCP-mutants triggered activation of the autophagic flux. Data showed that VCPmutants were correlated with an activation of the autophagic flux. Moreover, we determined that the activation of the autophagic flux was specifically regulated by TFE3 calcineurin-dependent dephosphorylation and activation. Calcineurin is a calcium-dependent phosphatase that could be activated by lysosomal leakage supporting a correlation between VCP-mutants lysosomal damage and autophagy activation. In addition, we excluded the involvement of TFEB in this pathway. Together these data suggest that lysosomal damage and leakage induced by VCPmutants activate calcineurin which in turn mediates TFE3 dephosphorylation and nuclear translocation inducing autophagy. In support of this, we found that VCPmutants enhanced insoluble protein-aggregates with a specific dependency on the autophagic pathway.