

# **Pathomechanisms of ALS8: Altered autophagy and defective RNA binding protein (RBP) homeostasis due to the VAPB P56S mutation**

*Running title: Defective autophagy and RBP homeostasis in ALS8*

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## Abstract

Mutations in RNA binding proteins (RBPs) and in genes regulating autophagy are frequent causes of familial amyotrophic lateral sclerosis (fALS). The P56S mutation in vesicle-associated membrane protein-associated protein B (VAPB) leads to fALS (ALS8) and spinal muscular atrophy (SMA). While VAPB is primarily involved in the unfolded protein response (UPR), vesicular trafficking and in initial steps of the autophagy pathway, the effect of mutant P56S-VAPB on autophagy regulation in connection with RBP homeostasis has not been explored yet. Examining the muscle biopsy of our index ALS8 patient of European origin revealed globular accumulations of VAPB aggregates co-localized with autophagy markers LC3 and p62 in partially atrophic and atrophic muscle fibres. In line with this skin fibroblasts obtained from the same patient showed accumulation of P56S-VAPB aggregates together with LC3 and p62. Detailed investigations of autophagic flux in cell culture models revealed that P56S-VAPB alters both initial and late steps of the autophagy pathway. Accordingly, electron microscopy complemented with live cell imaging highlighted the impaired fusion of accumulated autophagosomes with lysosomes in cells expressing P56S-VAPB. Consistent with these observations, neuropathological studies of the brain and spinal cord of P56S-VAPB transgenic mice revealed signs of neurodegeneration associated with altered protein quality control and defective autophagy. Autophagy and RBP homeostasis are interdependent, as demonstrated by the cytoplasmic mislocalization of several RBPs including pTDP-43, FUS, Matrin 3 which often sequestered with P56S-VAPB aggregates both in cell culture and in the muscle biopsy of the ALS8 patient. Further confirming the notion that aggregation of the RBPs proceeds through the stress granule (SG) pathway, we found persistent G3BP- and TIAR1-positive SGs in P56S-VAPB expressing cells as well as in the ALS8 patient muscle biopsy. We conclude that P56S-VAPB-ALS8 involves a cohesive pathomechanism of aberrant RBP homeostasis together with dysfunctional autophagy.

**Keywords:** ALS8, VAPB, Neurodegeneration

## References (selected)

1. A. L. Nishimura *et al.*, A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am J Hum Genet* **75**, 822-831 (2004).
2. A. D. Funke *et al.*, The p.P56S mutation in the VAPB gene is not due to a single founder: the first European case. *Clin Genet* **77**, 302-303 (2010).
3. K. Kanekura, I. Nishimoto, S. Aiso, M. Matsuoka, Characterization of amyotrophic lateral sclerosis-linked P56S mutation of vesicle-associated membrane protein-associated protein B (VAPB/ALS8). *J Biol Chem* **281**, 30223-30233 (2006).

4. H. Suzuki *et al.*, ALS-linked P56S-VAPB, an aggregated loss-of-function mutant of VAPB, predisposes motor neurons to ER stress-related death by inducing aggregation of co-expressed wild-type VAPB. *J Neurochem* **108**, 973-985 (2009).
5. Y. G. Zhao *et al.*, The ER Contact Proteins VAPA/B Interact with Multiple Autophagy Proteins to Modulate Autophagosome Biogenesis. *Curr Biol* **28**, 1234-1245 e1234 (2018).
6. M. Kuijpers *et al.*, Amyotrophic lateral sclerosis (ALS)-associated VAPB-P56S inclusions represent an ER quality control compartment. *Acta Neuropathol Commun* **1**, 24 (2013).
7. P. Gomez-Suaga, S. Paillusson, C. C. J. Miller, ER-mitochondria signaling regulates autophagy. *Autophagy*, 1-2 (2017).
8. E. Kabashi *et al.*, Investigating the contribution of VAPB/ALS8 loss of function in amyotrophic lateral sclerosis. *Hum Mol Genet* **22**, 2350-2360 (2013).