Extracellular Vesicles analysis in cerebrospinal fluid of transgenic pigs: a model for biomarker discovery in Amyotrophic Lateral Sclerosis

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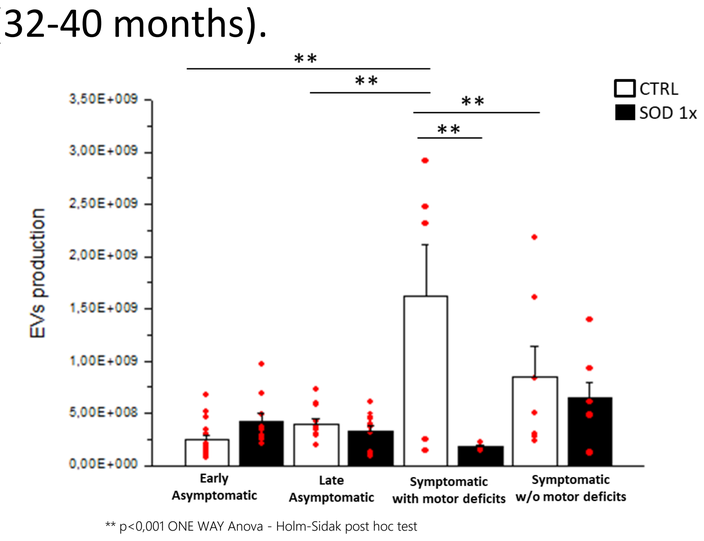
INTRODUCTION: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease gradually leading to paralysis of the whole body. Recently, a transgenic pig model expressing the human pathogenic superoxide dismutase SOD1 gene ($hSOD1^{G93A}$) has been generated [1] that displays behavioral, neurodegenerative and molecular features of ALS and is characterized by a quite long pre-symptomatic phase, offering the unique opportunity to investigate early ALS biomarkers.

AIM: Extracellular vesicles (EVs) contain markers of parental cells and represent a valuable source of biomarkers for neurodegenerative diseases. In this study we measured the concentration and size distribution of EVs present in the cerebrospinal fluid (CSF) of heterozygotes $hSOD1^{G93A}$ pigs [1] at different disease stages (early pre-symptomatic, late pre-symptomatic and symptomatic) and we characterized their cell origin.

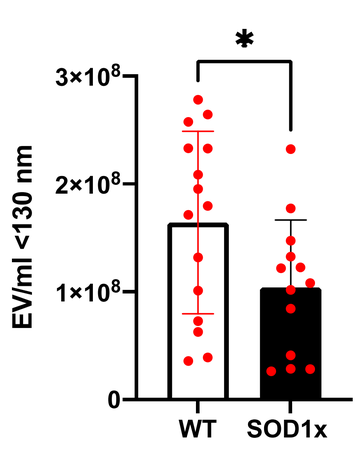
Animals analyzed in this study

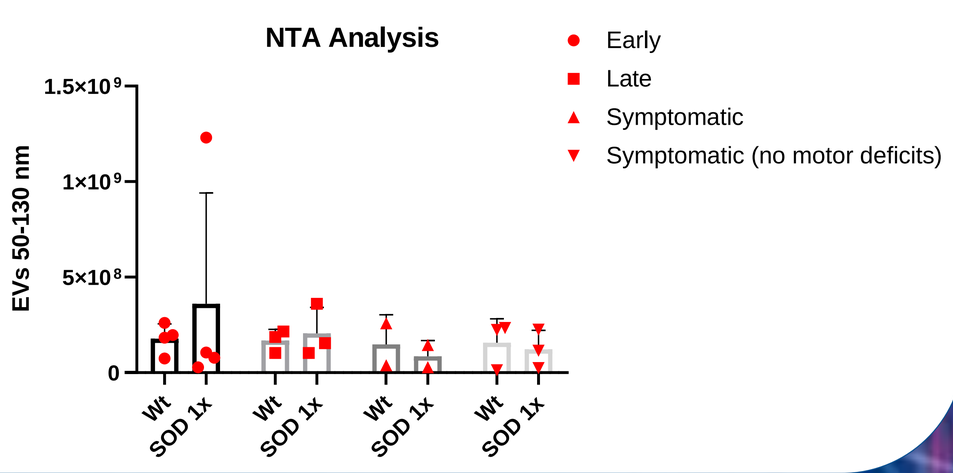
EARLY		LATE		SYMPTOMATIC with motor deficits		SYMPTOMATIC wi/o motor deficits	
WT	1X	WT	1X	WT	1X	WT	1X
405	448	187	200	91	20	787	26
406	451	306	283	25	23	24	21
358	359	188	282				
363	360						

TRPS analysis showed that the concentration of EVs increases with age in wild type but not in transgenic pigs, with a statistically significant difference at symptomatic stages (32-40 months).

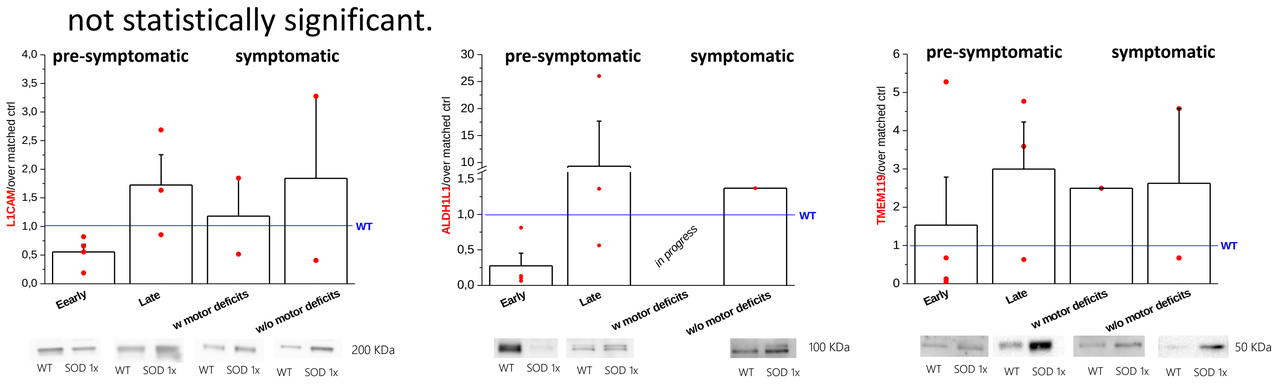


NTA analysis of the general populations showed that the concentration of EVs in the 50-130 nm range decreases in transgenic pigs, with a statistically significant difference

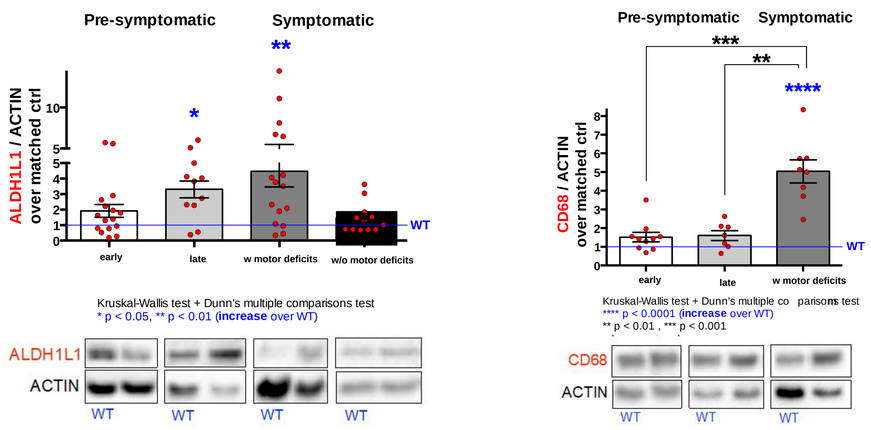




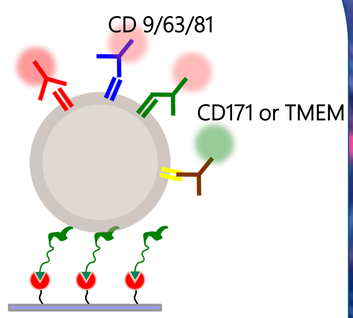
EVs were isolated by differential centrifugation from the CSF and analyzed by **western blot** for the neuronal marker **CD171/L1CAM**, the astrocyte marker **ADHL1**, the microglial marker **TMEM119**. We found a larger proportion of neuron-derived and astrocyte-derived EVs at late pre-symptomatic stages. Microglia-derived EVs tended to be more abundant at both late pre-symptomatic and symptomatic stages in $hSOD1^{G93A}$pigs, but the difference was not statistically significant.



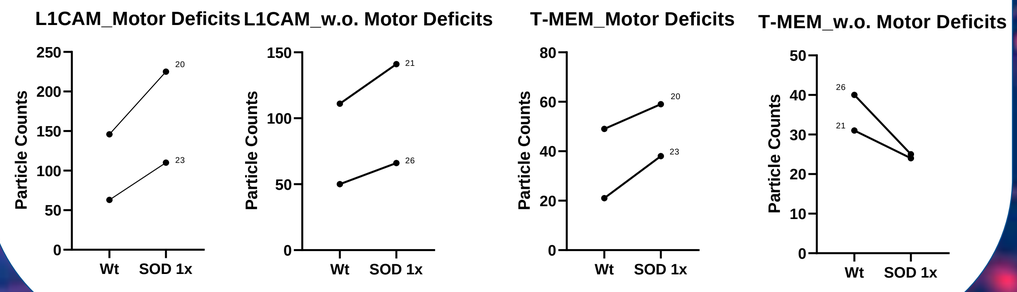
WB analysis of cervical tracts of the spinal cords revealed increased expression of the astrocytic marker ADHL1 in late presymptomatic and symptomatic pigs with motor deficits. **Thus, EV production reflects early astrocyte activation in ALS pigs and EVs-associated ADHL1 in the CSF may reprersent a possible early disease biomarker.**



Untreated CSF was analyzed by **peptide microarrays and ExoView** platform using chips spotted with a **membrane sensing peptide [2]** able to capture **small EVs** based on their membrane curvature.



Results of fluorescent co-localization of tetraspanins (Cy5) and CD171 or TMEM (Cy3)



References:
[1] Crociara, Pet al . Neurobiol. Dis. 2019, 124, 263–275.
[2] Gori A. et al Journal of Extracellular Vesicles , 2020, VOL. 9, 1751428

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