SORLA-FIX Consortium

CSF sSORL1 levels as a potential biomarker for identifying pathogenic SORL1 variants

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Aims, research questions and working hypothesis

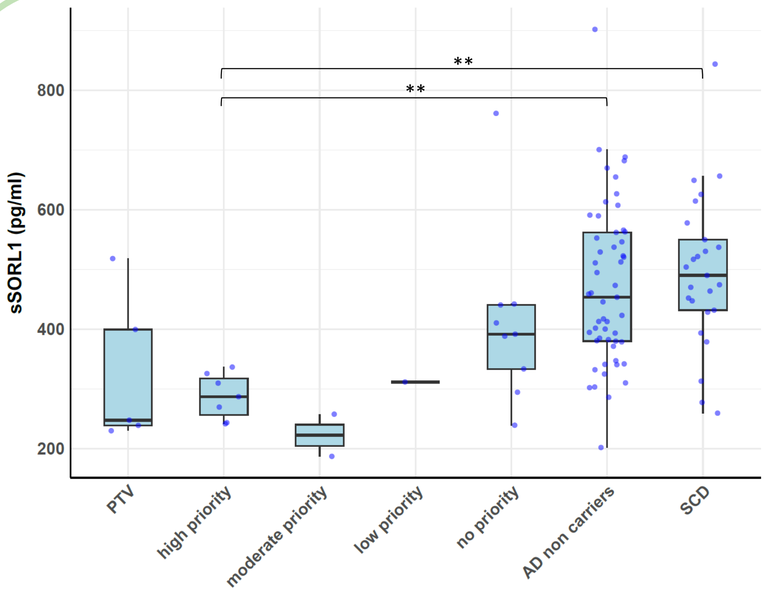
The SORL1 protein is critical to the endolysosomal pathway, a key process for maintaining brain health. It facilitates the trafficking of cargo from endosomes to the Golgi or cell surface. When functioning properly, SORL1 is cleaved at the cell surface in a soluble form, known as sSORL1. However, variants that strongly impair SORL1 disrupt its localization to the cell surface, resulting in reduced levels of sSORL1 in the interstitial fluid. We hypothesize that CSF sSORL1 levels are reflective of the pathogenicity of the variant, serving as an indicator of their impact on protein function.

Methods implemented by the consortium

•**Variant Identification and Prioritization**:
Used **Domain Mapping of Disease Mutations (DMDM)[1]** to identify SORL1 variants linked to Alzheimer's disease, categorizing them as high (HPV), moderate (MPV), low (LPV), no priority (NPV), or protein truncating (PTV).

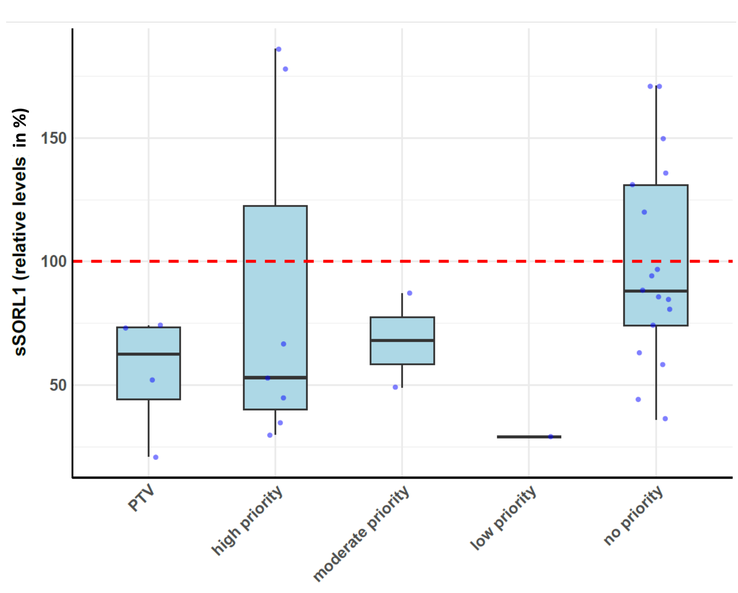
•**CSF and Protein Analysis**:
Measured **sSORL1 levels** in cerebrospinal fluid (CSF) using **ELISA**, comparing SCD controls (n=25), AD patients (n=43), and variant carriers (HPV n=7; MPV n=2; LPV n=1; NPV n=9; PTV n=5). Validated findings with **Western blotting** by determining the relative sSORL1 levels in a subset of carriers paired with controls.

•**Functional Validation**:
Conducted **in vitro studies** by transfecting cells with high-priority variants to confirm effects on sSORL1 shedding.



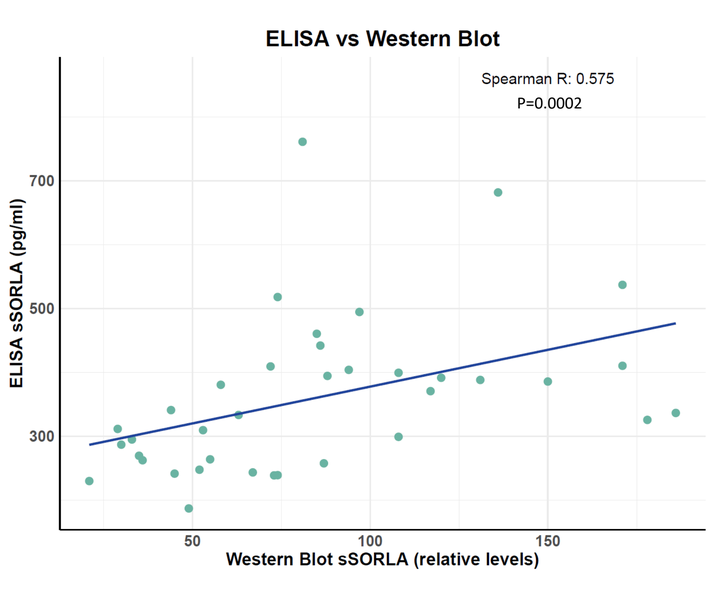
ELISA: sSORL1 levels in CSF between different priority groups

HPVs have significantly lower levels of sSORL1 compared to AD non carriers and SCD controls. PTVs and MPVs show a trend towards significance. LPV and NPV show no significant difference with the control groups.



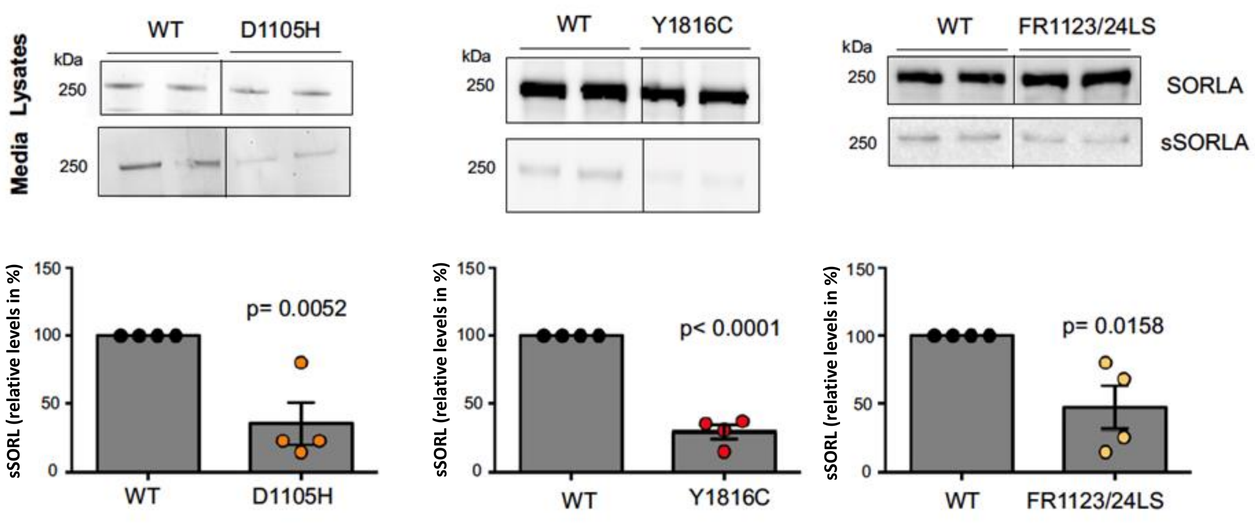
Western Blot: sSORL1 levels in CSF between different priority groups

No significant differences were observed between priority groups and the reference (100%). However, most carriers exhibited lower sSORL1 levels compared to matched controls, aligning with the ELISA results.



ELISA vs Western Blot

Despite differences in measuring absolute values (ELISA) and relative levels (Western Blot), a moderately strong correlation was observed between the two techniques.



Transfected cells containing high priority variants

Cells containing high priority variants secrete less sSORL1 compared to WT.

Significance, impact and future steps

These findings provide preliminary evidence that SORL1 variants impairing SORL1 function can be identified by their reduced levels in CSF. We will optimize this ELISA assay by analyzing CSF samples from an independent dataset of SORL1 variant carriers representing diverse priorities (HPV n=11, MPV n=10, LPV n=4, NPV n=3 and PTV n=4). When successful, use of this assay may support SORL1 variant pathogenicity in future clinical settings.

1. Andersen, O. M., et al. Relying on the relationship with known disease-causing variants in homologous proteins to predict pathogenicity of *SORL1* variants in Alzheimer's disease; BioRxiv.