Encuentro de Investigación *Buscando sinergias*
Alicante | 7 de junio de 2022 Hospital General Universitario Dr. Balmis de Alicante. Salón de actos. De 10:00 h. a 13:30 h.

# Mecanismos moleculares y biomarcadores alterados en la enfermedad de Alzheimer

Javier Sáez Valero

ISABIAL/Instituto de Neurociencias-CSIC-UMH & CIBERNED (email: j.saez@umh.es)

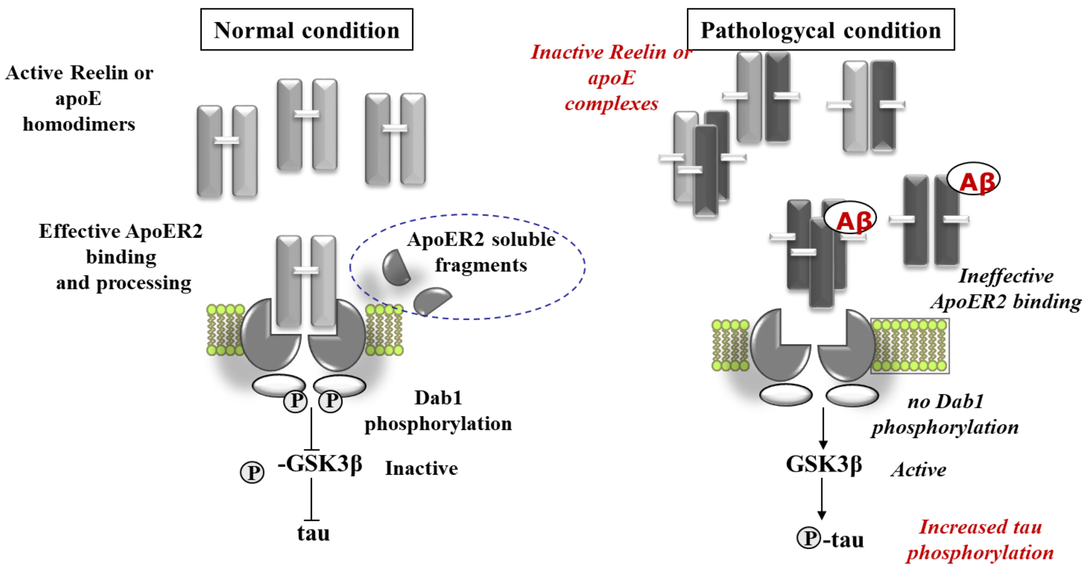
## *Current Research Interest*

- Relationship between **Aβ and presenilin-1 (PS1) with acetylcholinesterase**
- **ApoE** in Alzheimer's disease (AD)
  - * Influence of apoE in **ADAM10/α-secretase**
  - * Aβ and P-tau cross-talk, a role for **apoE/reelin/apoER2 signaling**?
- Mechanism behind the **failure in the therapy based in γ- and β-secretase inhibitors**
- New Alzheimer's **CSF biomarkers**:
  - * **Glycoforms of proteins**
  - * **Aβ related proteins** (secretases and APP proteolytic fragments)
- Neuronal and glia interplay: **Trem2 and SOCS3** microglial proteins.
- Prognostic biomarker in **COVID-19**, circulating levels of **ACE2** species, host receptor of the SARS-CoV-2 virus, as a **read-out of infection progression**.

## *Our expertise comprises*

i) **biochemical characterization of PTM for brain/CSF proteins**, **glycosylation and phosphorylation** analysis, characterization of **proteolytic processing**
ii) **characterization of ligand-receptor interaction** associated to signaling pathways
iii) assessment of **sustained inhibition of key enzymes** such as secretases.
iv) **cellular models**: **iPSC** (mutant *ADAM10*/*APOE* isogenic co-cultures)

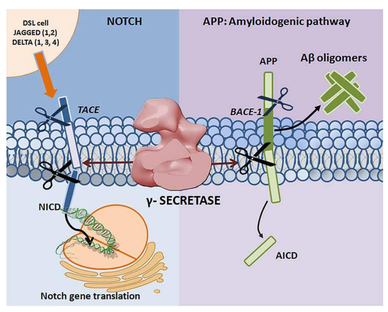
## Altered molecular mechanism: impaired apoE/reelin signaling, when more is less

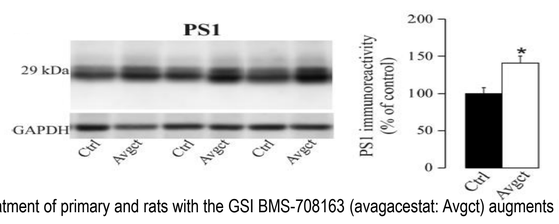



In the search for proteins that play a role in AD and that are related to the pathological hallmarks, we have studied reelin /apolipoprotein E signaling. Reelin signaling is involved in a cascade of cytoplasmic events that control tau phosphorylation and that regulate synaptic neurotransmission, plasticity, and memory. Both reelin expression and glycosylation are modulated by amyloid-β (Aβ), suggesting that the activity of reelin could be affected in AD and hence, its possible influence on this pathology should be taken into consideration. The levels of reelin in the brain of AD patients appear to be altered and interestingly, disrupted reelin signaling is associated with increased tau phosphorylation as well as with amyloid-β protein precursor processing. We demonstrated that Aβ increases reelin levels, but also disrupts reelin capacity to form homodimers, necessary to bind to its receptors, thereby compromising reelin signal transduction and possibly contributing to synaptic dysfunction in AD.

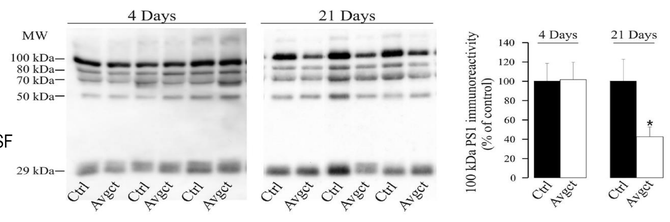
***Representative publications***:
***Proc Natl Acad Sci USA*** 103:5573-5578 **(2006)**; ***Neurobiol Dis*** 37:682-691 **(2010)**; ***PLoS One*** 8:e72297 **(2013)**; ***FASEB J*** 28:1543-54 **(2014)**; ***Sci Rep*** 6:31646 (**2016**); ***FASEB J*** 32:3536-3546 (**2018**); ***Clin Chem Acta*** 490:6-11 (**2019**); ***Alzheimers Res Ther*** 13:181 (**2021**)

## Inhibition of γ-secretase leads to an undesirable increase in PS1




Treatment of primary and rats with the GSI BMS-708163 (avagacestat: Avgct) augments PS1.

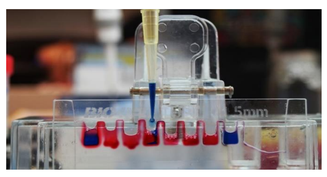


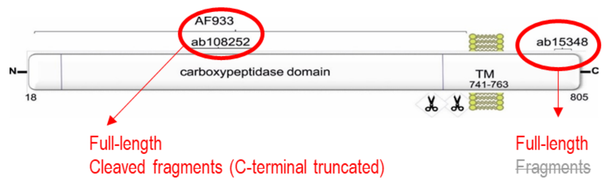

Effect of avagacestat (40 mg/kg, Avgct) on PS1 levels in CSF of rats treated for 4 and 21 days.

γ-Secretase inhibitors (GSIs) are potential therapeutic agents for AD (and cancer), however AD trials have proven disappointing. We demonstrated that GSI can provoke a rebound effect, elevating the levels of the catalytic γ-secretase subunit, PS1. We also demonstrated that PS1, can be detected as soluble heteromeric aggregates in the CSF.

***Representative publications***:
***Acta Neuropathol Commun*** 1:46 **(2013)**; ***Mol Neurodegener*** 11:66 (**2016**); ***Mol Neurobiol*** 6: 5047–5058 (**2018**)

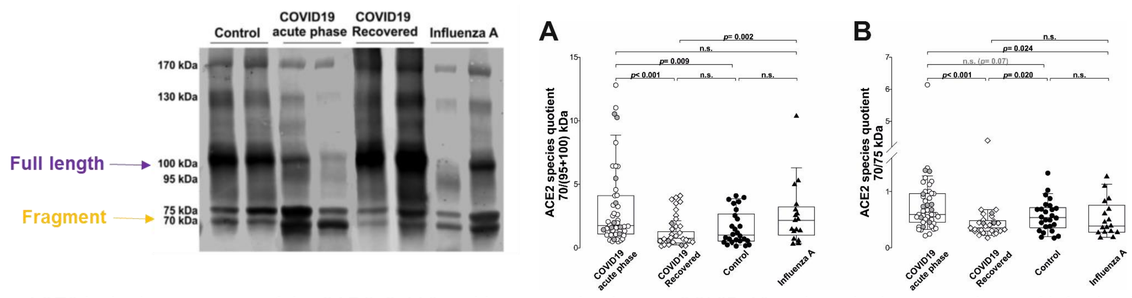
## Different species of a biomarker can display opposite trends: biochemical characterization and quantification





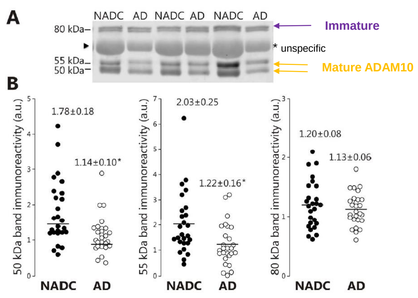

If full-length and truncated fragments a protein co-exist in a fluid, the western blot technique offers advantages over ELISA (or others), combining ectodomain and C-terminal antibodies. Different isoforms/glycoforms can also display opposite trends. COVID-19 patients in the acute phase of infection (n= 46) had significantly decreased levels of ACE2 full-length species, while a truncated 70 kDa form was marginally higher compared with non-disease controls

## Plasma ACE2 species are differentially altered in COVID-19 patients



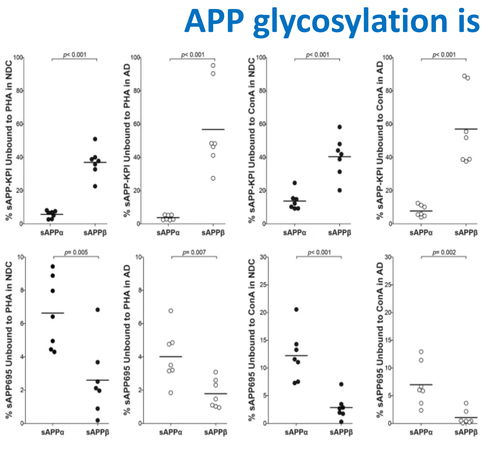

ACE2 is the host receptor of the SARS-CoV-2 and is present in plasma. COVID-19 patients in the acute phase of infection had significantly decreased levels of ACE2 full-length species, while a truncated fragment is marginally higher.

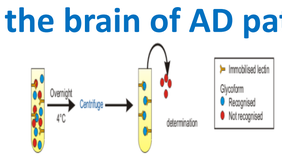
## Only mature ADAM10 levels are reduced in AD CSF




ADAM10, the α-secretase enzyme that processed APP in the non-amyloidogenic pathway precluding β-amyloid generation. ADAM10 is also present in CSF as several distinct species: an immature form retaining the prodomain (proADAM10, 80 kDa), a mature unprocessed full-length form (ADAM10f, 55 kDa) and a truncated large soluble form released from the membrane sADAM10, 50 kDa). Immunoblotting revealed a significant decrease in mature species, ADAM10f and sADAM10, in AD CSF, while immature proADAM10 levels remained unaltered.

## APP glycosylation is altered in the brain of AD patients




The amyloid precursor protein (APP) is a transmembrane glycoprotein that undergoes alternative proteolytic processing. Its processing through the amyloidogenic pathway originates a large sAPPβ ectodomain fragment and the β-amyloid peptide, while non-amyloidogenic processing generates sAPPα and shorter non-fibrillar fragments. Hence, measuring sAPPα and sAPPβ has been proposed as a means to identify imbalances between the amyloidogenic/non-amyloidogenic pathways. However, to date no consistent changes in these proteolytic fragments have been identified in AD individuals. The analysis of the lectin-binding differences between sAPPα and sAPPβ suggests that glycosylation dictates the proteolytic pathway for APP processing.

***Representative publications***:
***Mol Neurodegener*** 10:2 (**2015**); ***Sci Rep*** 7:2477 (**2017**); ***Mol Neurobiol*** 54(1):188-199(**2017**); ***J Neuroinflammation*** 15:213 (**2018**); ***Mol Neurobiol*** 56:8603-8616 (**2019**); ***Alzheimers Res Ther*** 12:139 (**2020**); ***Alzheimers Res Ther*** 12:96 (**2020**); ***FASEB J*** 35:e21745 (**2021**)

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