

Multi network-based analysis reveal sex-dependent plasma protein co-expression linked to Alzheimer’s genetic risk

Alba Fernández-Bonet¹; Patricia Genius^{1,2}; Blanca Rodríguez-Fernández^{1,2}; Armand González-Escalante^{1,2}; Eugenia Domingo-Güell^{1,2}; Gerard Temprano-Sagrera^{1,3}; Marta del Campo^{1,2}; Marc Suárez-Calvet^{1,4}; Arcadi Navarro^{1,5}; Betty Tijms^{6,7}; Natalia Vilor-Tejedor^{1,3}; Federica Anastasi^{1,2,6,7}

¹BarcelonaBeta Brain Research Center (BBRC), Pasqual Maragall Foundation, Barcelona, Spain. ²Hospital del Mar Research Institute, Barcelona, Spain. ³Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, the Netherlands. ⁴Servei de Neurologia, Hospital del Mar, Barcelona, Spain. ⁵Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain. ⁶Alzheimer’s Center Amsterdam, Amsterdam University Medical Center, Location VUmc, Amsterdam, the Netherlands. ⁷Amsterdam Neuroscience, Neurodegeneration, Amsterdam, the Netherlands.

Background

- Late-onset **Alzheimer’s disease** (AD) has a complex and **highly polygenic architecture** extending beyond the ***APOE* locus**, yet the molecular mechanisms linking this genetic risk to AD pathophysiology are not fully understood [1].
- The **preclinical stage of AD** provides a critical window to investigate **molecular alterations associated with AD genetic risk** prior to cognitive decline [2].
- Network-based plasma proteomic analysis** can identify **modules of correlated plasma proteins**, enabling the study of systemic molecular signatures in an accessible biofluid [3].
- Although **sex is a key modifier of AD pathogenesis**, its role in shaping these plasma proteomic signatures of AD polygenic risk remains poorly characterized [4].

Aim

To identify **sex-dependent plasma proteomic networks** associated with **Alzheimer’s disease polygenic risk beyond *APOE*-ε4** in cognitively unimpaired adults.

Methods

I. Study sample

ALFA+ cohort (*n* = 436)

alfastudy
ALZHEIMER FAMILIES

Plasma proteomics
SomaScan v4.1 7k
n = 7,289

Genetics
Illumina Neurochip
PRS-AD

Age: 57 [53–61]
Women: 60.3%
Aβ+: 33.7%
APOE-ε4: 55.2%

III. Statistical analysis*

- Main effect models**
Module Eigenprotein ~ **Sex** + Age + Education
Module Eigenprotein ~ **PRS-AD** + Age + Sex
Module Eigenprotein ~ **PRS-AD_{noAPOE}** + Age + Sex
Module Eigenprotein ~ ***APOE*-ε4** + Age + Sex
- Interaction analyses**
Module Eigenprotein ~ **Sex** × **PRS-AD** + Age
Module Eigenprotein ~ **Sex** × **PRS-AD_{noAPOE}** + Age
Module Eigenprotein ~ **Sex** × ***APOE*-ε4** + Age
- Sex-stratified analyses**
for modules showing nominally significant interactions

*Multiple-testing correction: FDR

II. WGCNA network construction

- Quality control and scaling.**
- Selection of soft-thresholding power (β) to approximate scale-free network topology.**

Scale-free topology model fit (R²)

Mean connectivity

β = 8

- Adjacency and TOM matrix construction to measure shared protein connectivity (corType = "bicor", networkType = "signed").**

$$s_{i,j}^{\text{signed}} = 0.5 + 0.5 \text{Cor}(i, j)$$
$$a_{i,j}^{\text{signed}} = (s_{i,j}^{\text{signed}})^{\beta}$$

- Module detection by dynamic tree cutting (deepSplit = 4, minModuleSize = 10).**
- Module merging and eigenprotein calculation (mergeCutHeight = 0.15).**
- Module-trait association and hub proteins identification (kME).**

Results

WGCNA identified **19 plasma protein modules** (M1–M19) (Figure 1).

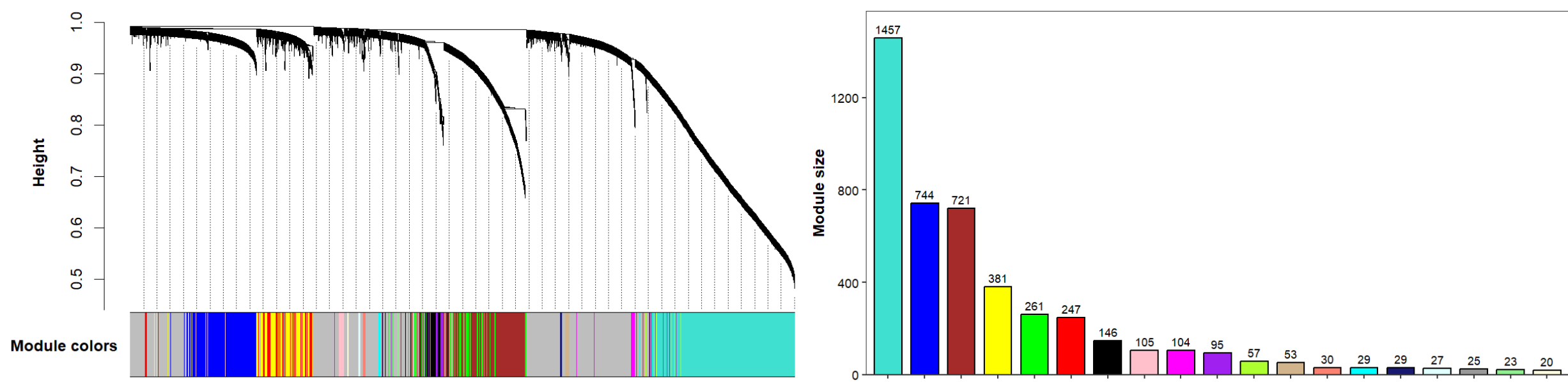


Figure 1. WGCNA protein modules (M1–M19). Cluster dendrogram generated by unsupervised hierarchical clustering of all measured plasma proteins based on topological overlap, with modules identified by dynamic branch cutting and indicated by the color bar (left). Bar plot showing the number of proteins assigned to each WGCNA module (right).

Main effect models (Figure 2)

- Seven modules** showed **sex-associated** expression patterns.
- M12** was negatively associated with both **PRS-AD** and ***APOE*-ε4** carriership.

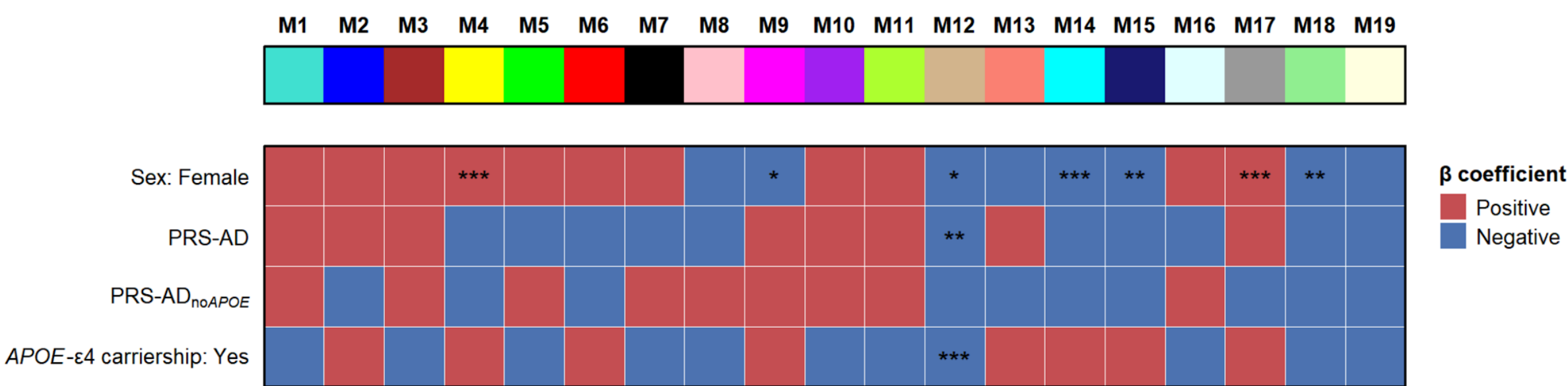


Figure 2. Sex- and genetic risk-dependent associations between plasma protein co-expression modules and Alzheimer’s disease (AD) genetic risk. Heatmap showing β coefficients from linear regression models (adjusted for age and years of education, and for sex when applicable) testing associations between module eigenproteins (M1–M19; top color bar indicates WGCNA module identity) and sex, AD polygenic risk score (PRS-AD), PRS-AD excluding the *APOE* region (PRS-AD_{noAPOE}), and *APOE*-ε4 carriership. Red and blue denote positive and negative associations, respectively. *Significance*: **p*<0.05, ***p*_{FDR}<0.05, ****p*_{FDR}<0.0001.

Sex modifies the relationship between AD genetic risk and plasma proteomic networks (Figure 3)

- Four modules** showed **sex-dependent** associations with AD genetic risk.
- M8** displayed **opposite associations in women and men**.
- M7, M10** and **M11** were negatively associated with ***APOE*-ε4** carriership only in **men**.

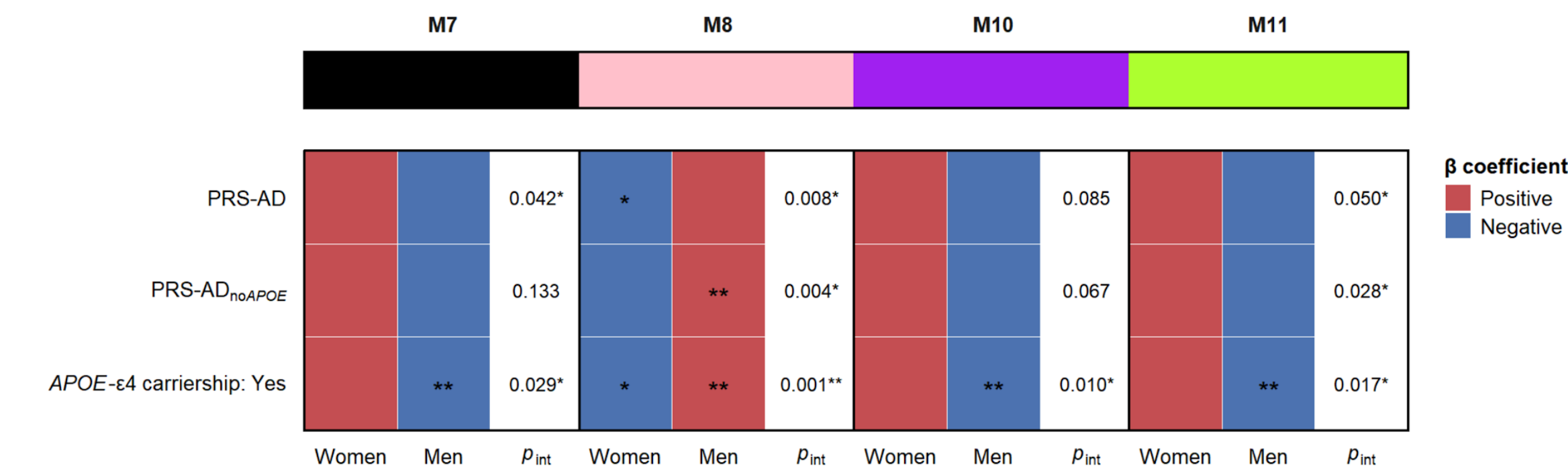


Figure 3. Sex-specific associations between plasma protein co-expression modules and Alzheimer’s disease (AD) genetic risk. Heatmap showing β coefficients from sex-stratified linear regression models (adjusted for age) testing associations between module eigenproteins and AD polygenic risk score (PRS-AD), PRS-AD excluding the *APOE* region (PRS-AD_{noAPOE}), and *APOE*-ε4 carriership in women and men. Analyses were restricted to modules showing nominally significant sex × AD genetic risk interactions (M7, M8, M10, M11; top color bar indicates WGCNA module identity). Red and blue denote positive and negative associations, respectively. *Significance*: **p*<0.05, ***p*_{FDR}<0.05, ****p*_{FDR}<0.0001. Nominal interaction *p* values are reported as *p*_{int}.

M8 included **105 proteins**, among which proteins involved in **synaptic function** (e.g., ARC) and **oxidative stress** (e.g., OGG1, PON2), previously implicated in AD pathophysiology (Figure 4).

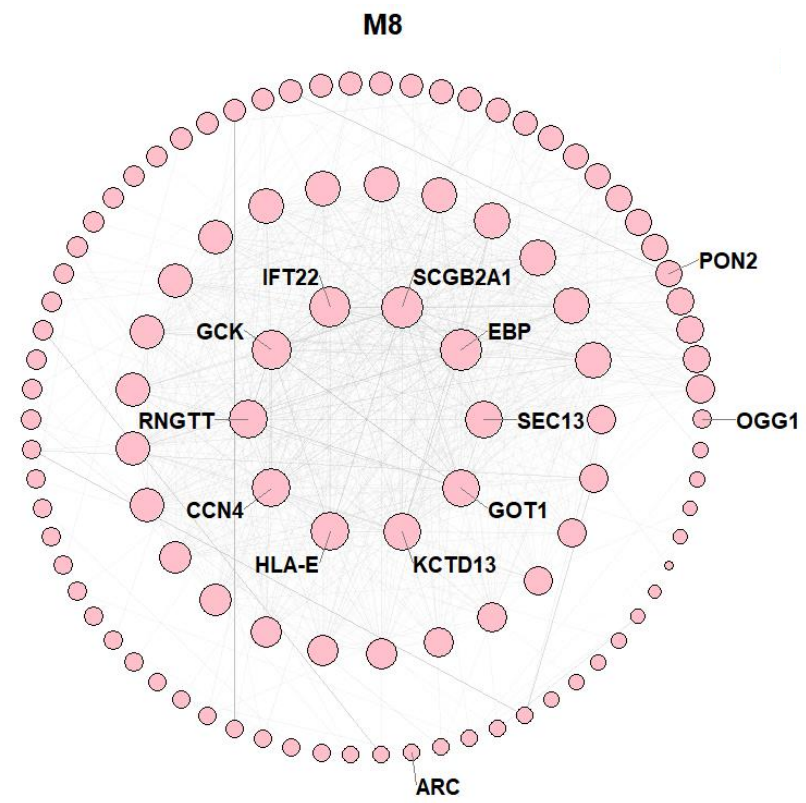


Figure 4. Top 10 hub proteins in AD genetic risk-associated WGCNA module M8 based on intramodular connectivity measured by kME. Nodes represent proteins and edges indicate connections between proteins, with a maximum of top 700 connections shown for each module.

Conclusions

- AD genetic risk is associated with coordinated plasma protein networks** in cognitively unimpaired adults.
- Four plasma protein networks showed sex-specific associations with AD genetic risk, indicating that **sex may influence the molecular pathways linking genetic susceptibility to early AD-related biology**.
- Sex modifies the association between plasma proteomic signatures and AD genetic risk **beyond the *APOE* region**, suggesting **sex-specific biological responses to inherited AD risk** and highlighting the importance of **sex-aware study design and prevention strategies in preclinical AD**.

References

[1] Lambert, J.C., Ramirez, A., Grenier-Boley, B., *et al.* Step by step: towards a better understanding of the genetic architecture of Alzheimer’s disease. *Mol Psychiatry* **28**, 2716–2727 (2023). [10.1038/s41380-023-02076-1](https://doi.org/10.1038/s41380-023-02076-1)
[2] Dubois, B., Hampel, H., Feldman, H. H., *et al.* Preclinical Alzheimer’s disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement* **12**(3), 292–323 (2016). [10.1016/j.jalz.2016.02.002](https://doi.org/10.1016/j.jalz.2016.02.002)
[3] Afshar, S., Dammer, E.B., Bian, S. *et al.* Plasma proteomic associations with Alzheimer’s disease endophenotypes. *Nat Aging* **5**, 2104–2124 (2025). [10.1038/s43587-025-00965-4](https://doi.org/10.1038/s43587-025-00965-4)
[4] Altmann, A., Tian, L., Henderson, V. W., *et al.* Sex modifies the *APOE*-related risk of developing Alzheimer’s disease. *Ann Neurol* **75**(4), 563–573 (2014). [10.1002/ana.24135](https://doi.org/10.1002/ana.24135)

Acknowledgements

We would like to express sincere gratitude to the participants of the ALFA Study and their families, whose commitment made this research possible. NV-T acknowledges support from the project PID2022-143106OA-I00, funded by MIN/AEI/10.13039/501100011033, with co-funding from FSE + and FEDER, EU, and received support from the Williams H. Gates Sr. AD Fellowship Cohort I from the Alzheimer’s Disease Data Initiative, and the Alzheimer’s Association, AD Strategic Fund (Project #VCID-UMD-26-1514428). FA receives funding from the BrightFocus Foundation Alzheimer Program Award A2025004F.



Alba Fernández-Bonet
Molecular Epidemiology and Data Science group
malba@barcelonabeta.org
github.com/GeneticNeuroStats
barcelonaBeta
Brain Research Center
www.barcelonabeta.org
Utrecht University
www.uu.nl