Study of MFG-E8 in experimental models of Cerebral Amyloid Angiopathy (CAA)

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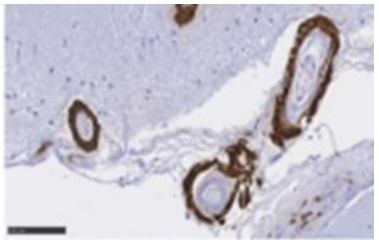
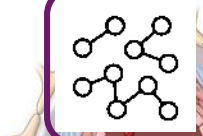
Cerebral Amyloid Angiopathy: Vascular ß-amyloid deposition

Cerebral Amyloid Angiopathy (CAA)



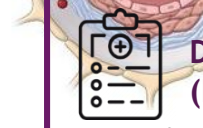
Physiological



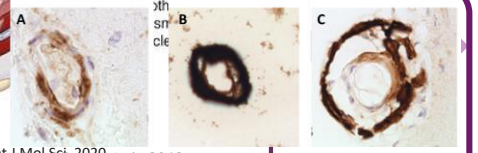
Pathological

No specific biologic/genetic markers for diagnosis

Boston criteria 2.0



Definite diagnosis is *post-mortem* (Histopathological confirmation)



Morrone CD et al. Potential Role of Venular Amyloid in Alzheimer's Disease Pathogenesis. Int J Mol Sci. 2020.

Charidimou et al., 2012

- Aß deposits in cerebral vessels
- Smooth

No effective treatments

Boston criteria 2.0

Clinical presentation ***Non-CAA biomarkers required***

- Spontaneous lobar intracerebral haemorrhage (ICH)
- Presence of small vessel disease markers
- Transient focal neurological episodes (TFNEs)
- Cognitive impairment (global cognition, perceptual speed, episodic memo

To allow a more accurate diagnosis and

To understand the pathophysiology

Charidimou et al., Brain 2017

Kozberg MG et al. International Journal of Stroke, 2021.

MFG-E8 (Lactadherin): A novel marker associated with CAA

Marazuela et al. acta neuropathol commun (2021) 9:154
https://doi.org/10.1186/s40478-021-01257-9

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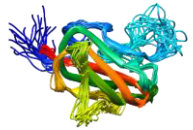
RESEARCH Open Access

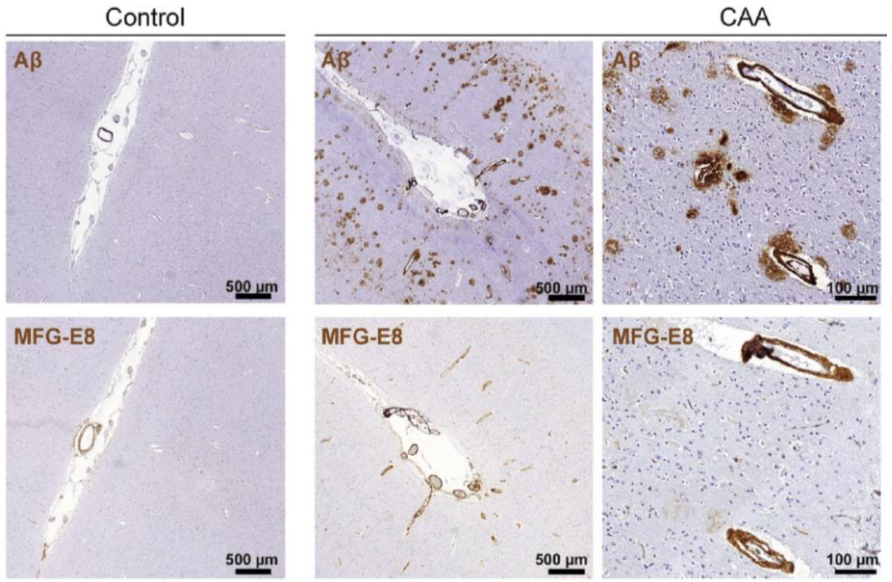
MFG-E8 (LACTADHERIN): a novel marker associated with cerebral amyloid angiopathy

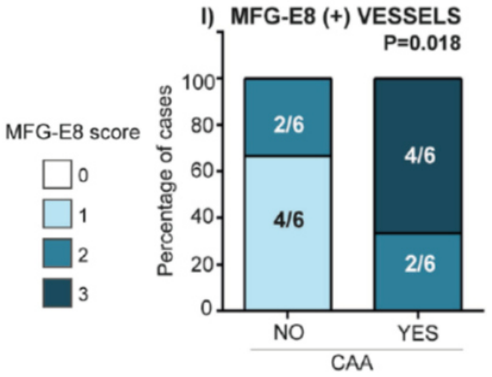
Paula Marazuela[1], Montse Solé[1], Anna Bonaterra-Pastra[1], Jesús Pizarro[1], Jessica Camacho[2], Elena Martínez-Sáez[2], H. Bea Kuiperij[3], Marcel M. Verbeek[3,4], Anna M. de Kort[3], Floris H. B. M. Schreuder[3], Catharina J. M. Klijn[3], Laura Castillo-Ribelles[5], Olalla Pancorbo[6], David Rodríguez-Luna[6], Francesc Pujadas[7], Pilar Delgado[1] and Mar Hernández-Guillamon[1*]

Milk fat globule-EGF factor 8 (MFG-E8) = Lactadherin

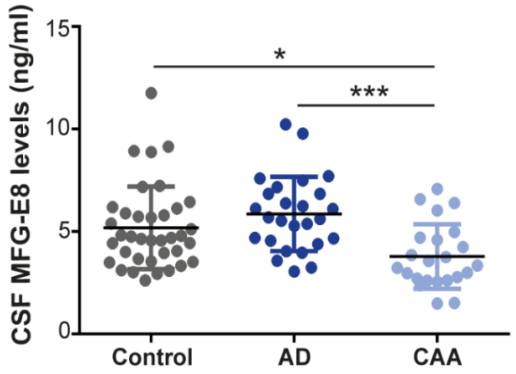
Glycoprotein expressed in epithelial cells, vascular smooth muscle cells, dendritic cells, etc. and associated with various physiological and pathological functions in the CNS, including phagocytosis of apoptotic cells, anti-inflammatory functions, and regulation of homeostasis.







Marazuela et al., Acta Neuropathol Commun2021



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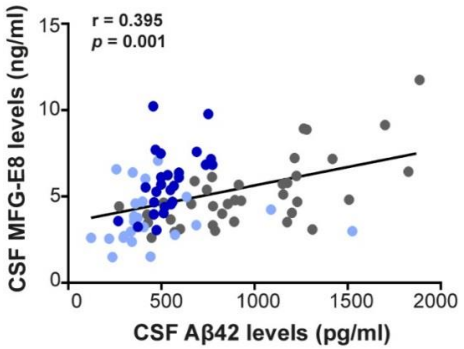


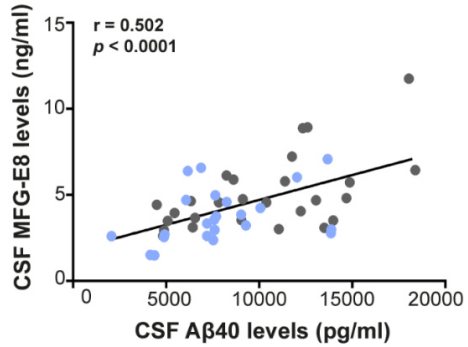


Table 1 Demographic characteristics and CSF parameters of healthy controls, CAA patients, and AD patients

	Control (n = 37)	CAA (n = 23)	AD (n = 26)	*p*-Value
Demographics				
Age, years, mean ± SD	63.8 ± 8.5	70.6 ± 7.8**	64.3 ± 7.3$	**0.004**
Sex (female), n (%)	11 (29.7%)	7 (30.4%)	14 (53.8%)	0.110
CSF parameters, pg/ml				
Aβ40, mean ± SD	10,187.9 ± 4009.1	7911.1 ± 3140.1	-	**0.032**
Aβ42, median (IQR)	895.1 (678–1225)	360 (317.5–462)***	514.6 (468.6–593.5)***	**< 0.001**
t-Tau, median (IQR)	231 (170–317)	403 (268–512.5)**	328.6 (183.4–395.3)	**0.002**
p-Tau, median (IQR)	28 (19–39)	45 (33.5–63.5)**	32.4 (18.1–39.6)$	**0.001**
MFG-E8, median (IQR)	4568.4 (3672.3–5898)	3345.5 (2661.8–4648.3)*	5655.6 (4552.6–6849.2)$$$	**< 0.001**

*CAA, Cerebral amyloid angiopathy; AD, Alzheimer's disease; CSF, cerebrospinal fluid; SD, standard deviation; IQR, interquartile range; -, not known. p-values below 0.05 are shown in bold: *p < 0.05 vs. the control group; **p < 0.01 vs. the control group; ***p < 0.001 vs. the control group; $p < 0.05 vs. the CAA group; $$$ p < 0.001 vs. the CAA group*

Hypothesis and Objectives

Hypothesis

Understanding the functional role of MFG-E8 may lead to:

- A new therapeutic opportunity to mitigate Aß-induced brain injury in CAA
- A better comprehension of cerebral ß-amyloidosis

The principal aim is to investigate the molecular implication of MFG-E8 in different CAA experimental models

In vitro

To analyze the modulation and localization of endogenous MFG-E8 induced by the treatment with Aß40-D peptide in HBVSMCs.

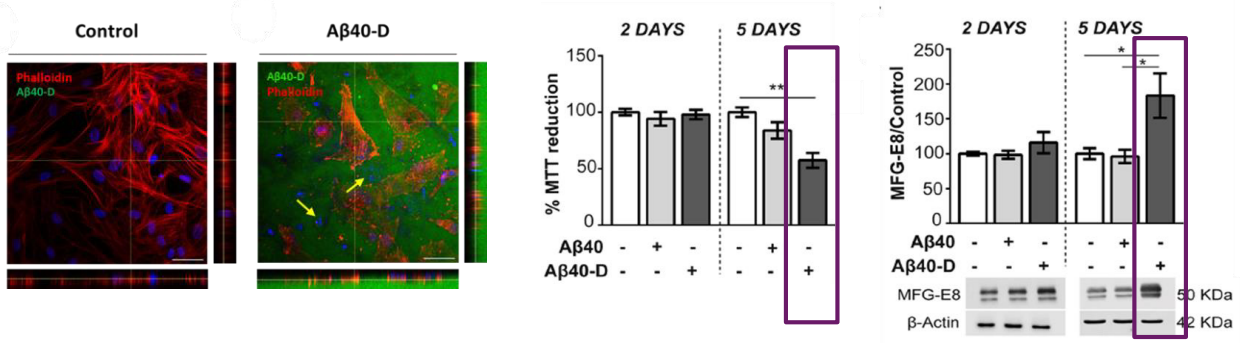
To study the impact of exogenous administration of recombinant human MFG-E8 protein on the cytotoxic effects of Aß40-D in HBVSMCs.

In vivo

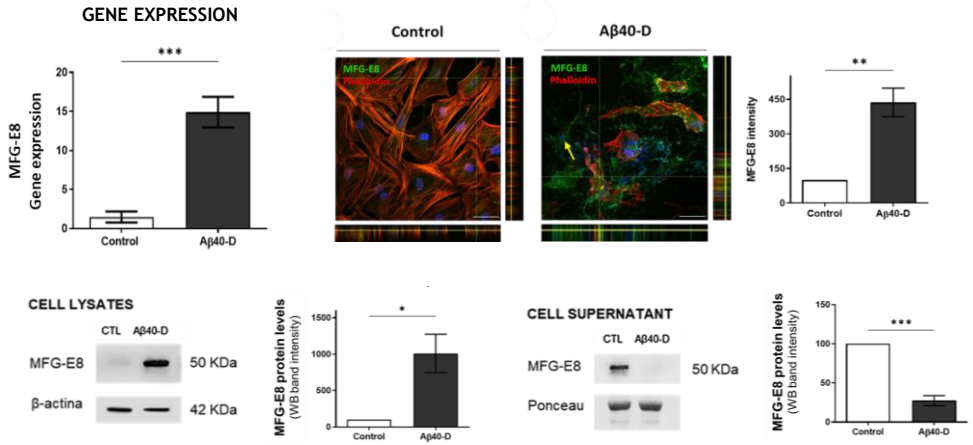
To analyse the expression and distribution of MFG-E8 in in vivo model of CAA (with the transgenic mouse model APP23), and its correlation with the expression of Aß.

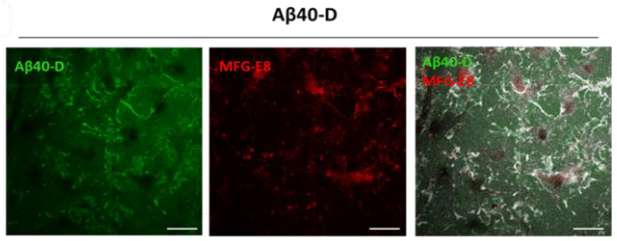
Results: In vitro

Human Brain Vascular Smooth muscle cells + Aβ40 WT/ Aβ40-Dutch (D) (E22Q)



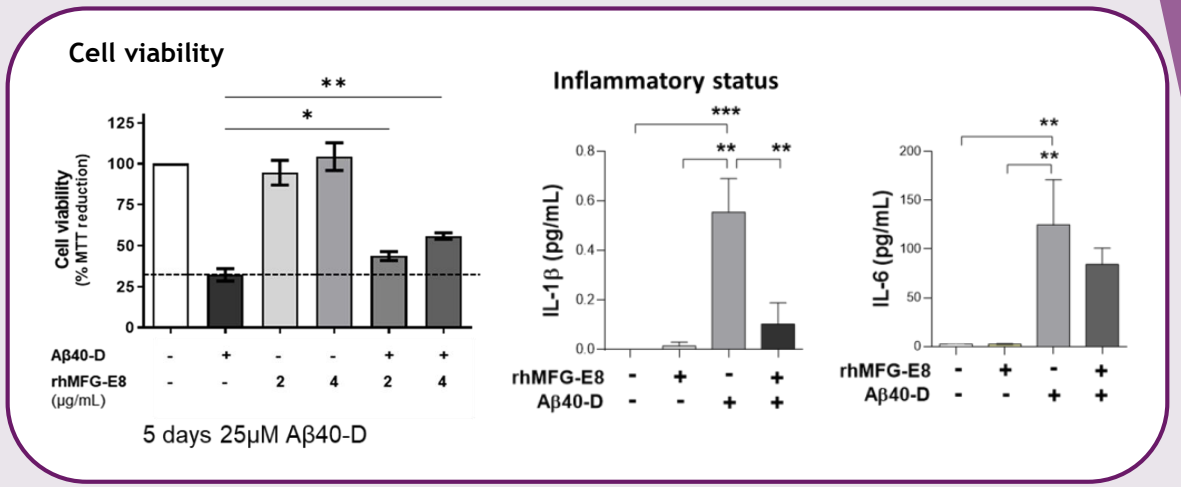
5 days 25 μM Aβ40-D



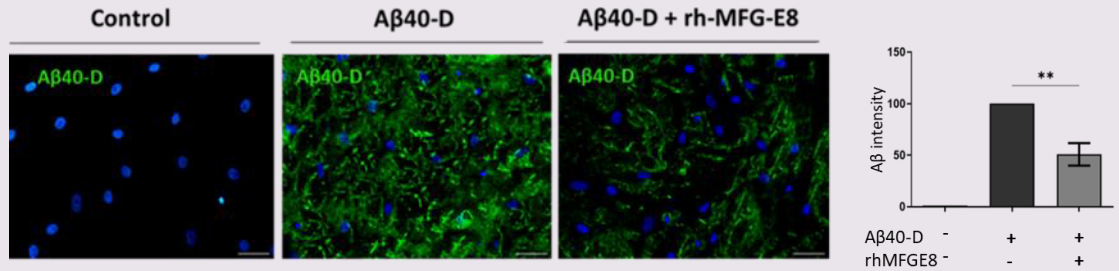


75% overlap between MFGE8 and Aβ on SMC

Recombinant human MFG-E8 treatment

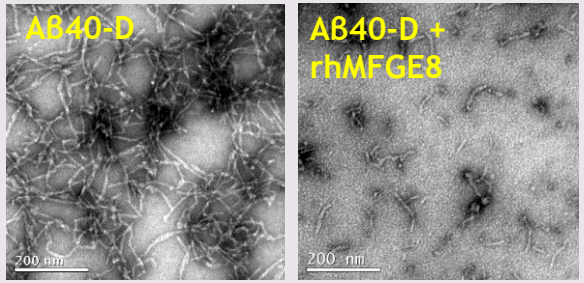


5 days 25μM Aβ40-D



5 days 25 μM Aβ40-D w/o 2 μg/ml rhMFGE8

TEM images

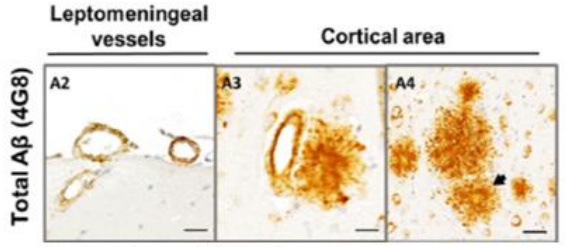


7 days 0.20 mM Aβ40-D + 20 μg/ml rhMFGE8 37°C

Results: In vivo

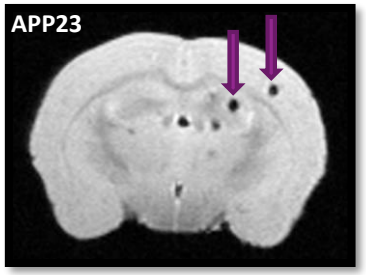
APP23

h-APP 751
Swedish mutation (K670N/M671L)

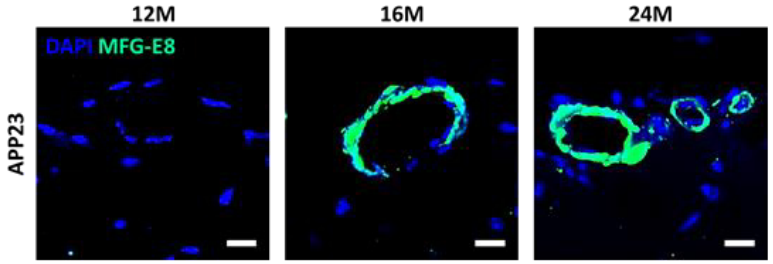


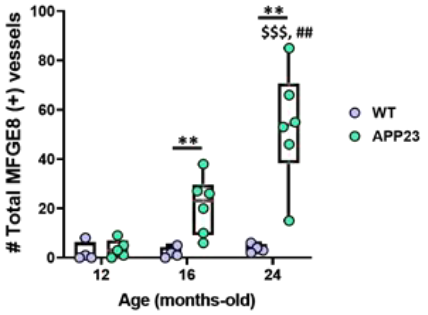
Predominant **Aβ40** deposits

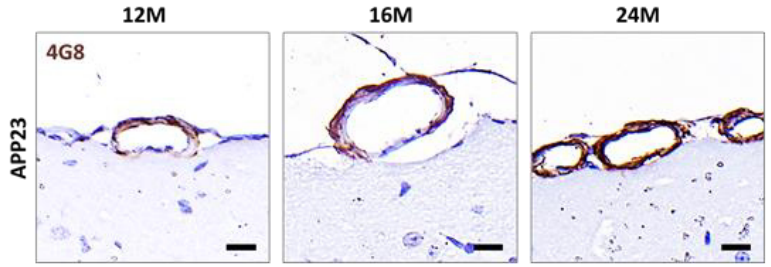
Vascular damage - Brain haemorrhages

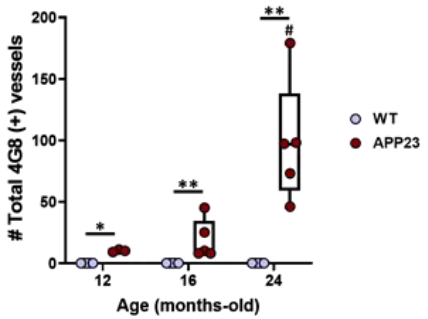


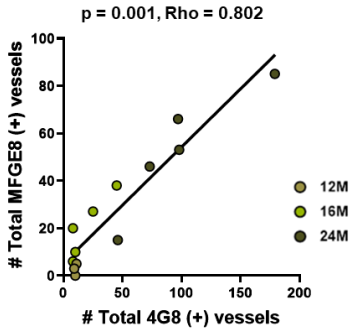
T2* MRI

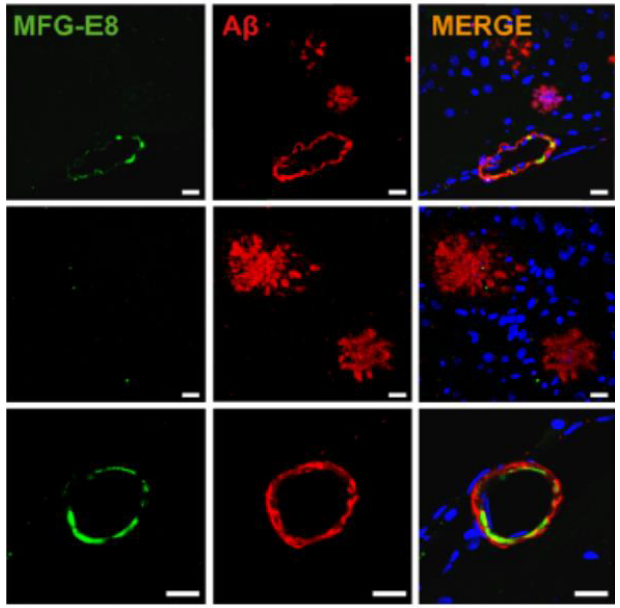


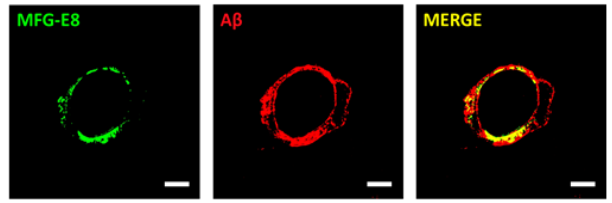




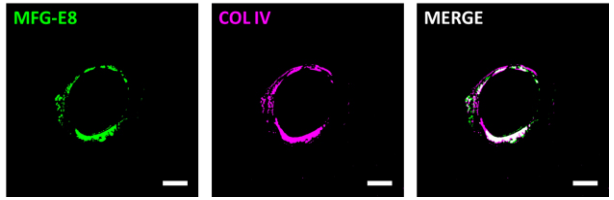








44.9±16.5% overlap between MFGE8 and Aß

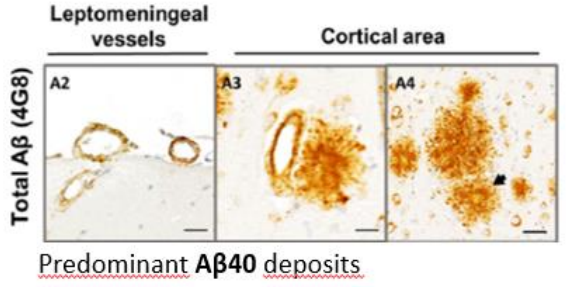


47.5±15.5% overlap between MFGE8 and COL IV

Amyloidosis transgenic mouse models

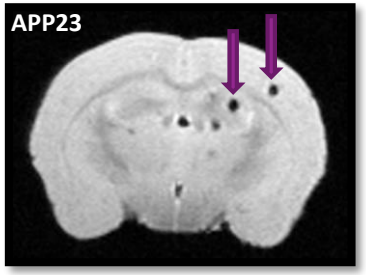
APP23

h-APP 751
Swedish mutation (K670N/M671L)



Predominant **Aβ40** deposits

Vascular damage - Brain haemorrhages



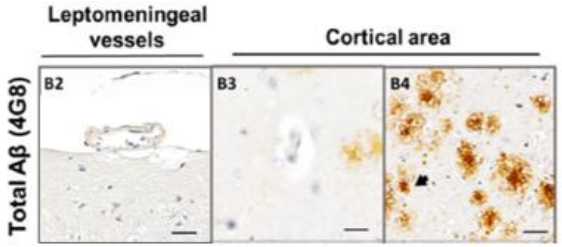
T2* MRI

5xFAD

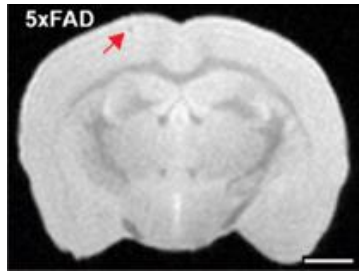
h-APP 695
Swedish (K670N/M671L)
Florida (I716V)
London (V717L)

+

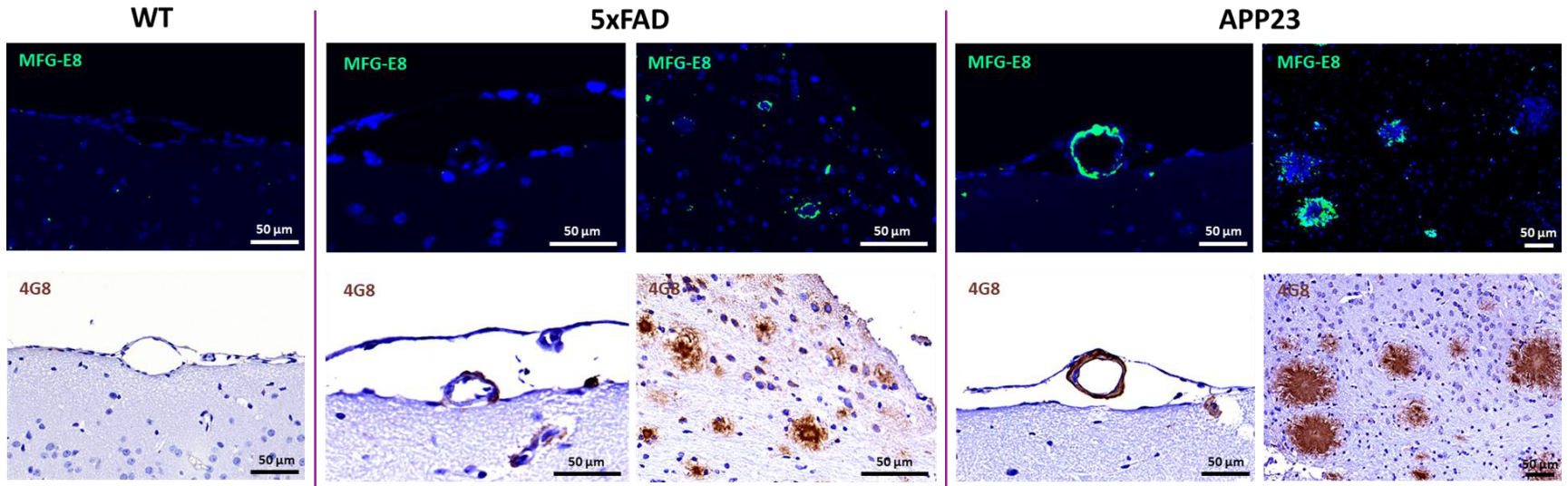
h-PS1
M145L
L286V

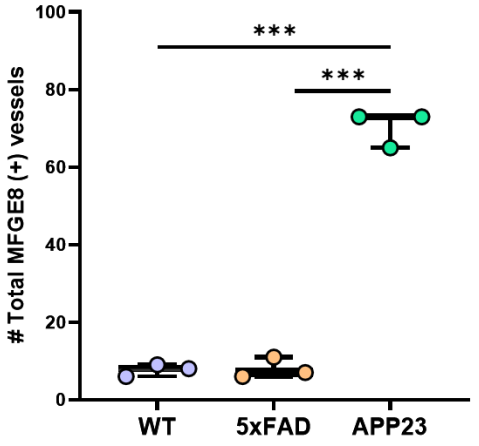


Predominant **Aβ42** deposits



T2* MRI





Conclusions

- MFG-E8 levels are increased in cerebral Aß-positive vessels but decreased in CSF from CAA patients.
- In cultured vascular smooth muscle cells, cytotoxic Aß peptide induces an increase of MFG-E8 expression accompanied by a decrease of the release of the protein in cell supernatants.
- The treatment with recombinant human MFG-E8 in vitro promotes a protective cellular effect, preventing the amyloid aggregation.
- MFG-E8 presence in brain vessels is associated with age and correlates with the vascular Aß accumulation in a transgenic mouse model of CAA.

Our findings demonstrate that MFG-E8 is closely related with CAA being a potential and specific marker.

Further investigations are needed to fully elucidate its role in CAA pathology and explore its potential role as a novel target for the development of therapeutic strategies.

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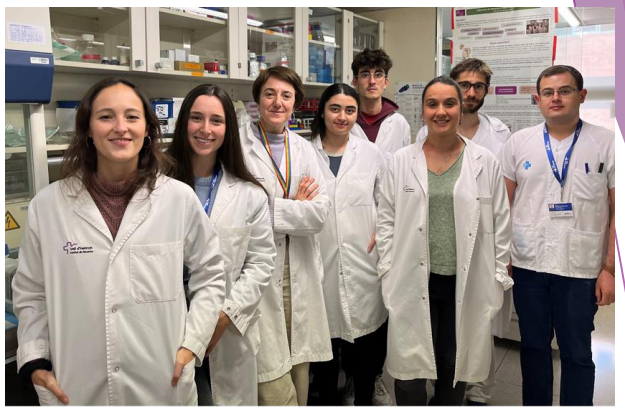

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