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PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) GENETIC DELETION ATTENUATES AMYLOID-β PATHOLOGY, NEUROINFLAMMATION AND IMPROVES COGNITIVE FUNCTIONS IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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Background: PCSK9 may be involved in the pathogenesis of Alzheimer's disease (AD) beyond its well-established plasma cholesterol-regulating activity, although the underlying mechanisms are not fully understood. Consistently, we found **elevated PCSK9 levels in the cerebrospinal fluid of AD** patients[1] and recently demonstrated *in vitro* that **PCSK9 impairs HDL-mediated cholesterol transport** from astrocytes to neurons and **enhances amyloid β (Aβ)-induced neurotoxicity**[2].

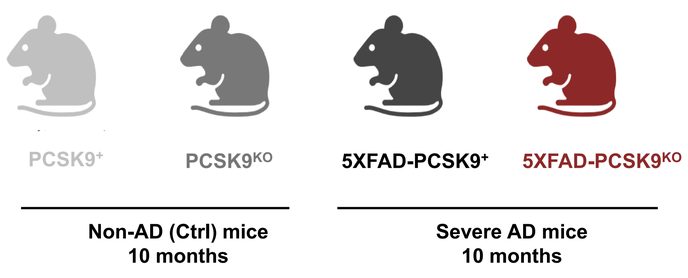
Aim: to investigate the influence of PCSK9 on **cognitive performances**, **Aβ plaque deposition**, **neuroinflammation**, **brain cholesterol** and its key **oxidative metabolites** in a mouse model of severe AD (5XFAD^{het} mice).

MAIN FINDINGS:

Present data evidence a protective role of PCSK9 genic deletion in 5XFAD^{het} mice against AD-related cognitive impairment, which is associated with reduced Aβ cerebral deposition, attenuated neuroinflammation and a partial restoration of brain cholesterol concentration, thus providing the bases to identify PCSK9 as a valuable pharmacological target for the development of novel therapeutic strategies for Alzheimer's Disease.

Methods

We generated a mouse model of severe AD in which PCSK9 was genetically ablated (n=12), compared with AD mice expressing PCSK9 (n=15) and with WT mice expressing (n=14) or not expressing PCSK9 (n=10).



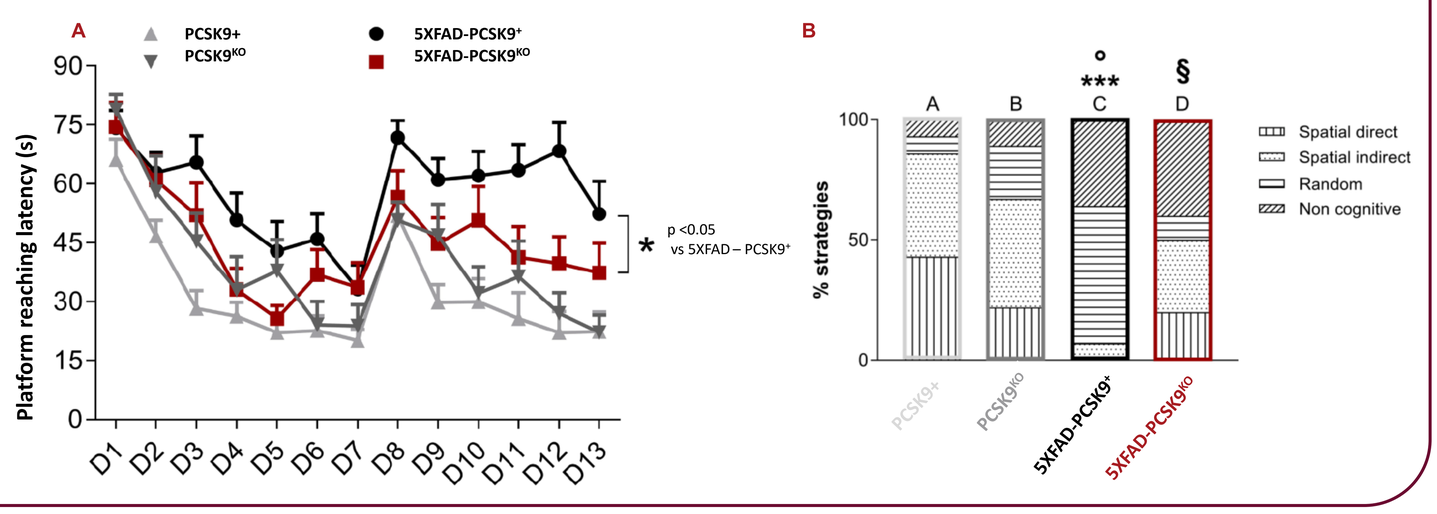
At 10 months of age we assessed **cognitive performances** (spatial learning and memory using the Morris Water Maze test), hippocampal and cortical **Aβ burden** (ThioflavinS$^+$ staining), **microglial and astrocytic reactivity** (IBA1$^+$ and GFAP$^+$, respectively; IHC), and **cerebral cholesterol** and **hydroxysterols** content (fluorometric and LC-MS/MS analyses).

Results (I):

PCSK9 genetic ablation in AD mice improves spatial-reference learning and memory

5xFAD-PCSK9$^+$ mice (black) are characterised by a worse learning curve (platform reaching latency, **Fig. 1A**) compared to Ctrl mice (grey), whereas 5xFAD-PCSK9^{ko} mice (red) show a **significantly reduced latency** compared to 5xFAD-PCSK9$^+$ mice ($p<0.05$), with values similar to those of Ctrl mice. Occupancy plots confirm the development of a **quadrant- and platform position-specific preference** by 5XFAD-PCSK9^{Ko} mice compared to 5XFAD-PCSK9$^+$ (**Fig. 1B**).

Figure 1



Results (II):

PCSK9 genetic ablation in AD mice attenuates cortico-hippocampal neuroinflammation

5xFAD-PCSK9$^+$ show a significant increase in cortico-hippocampal IBA1 ir, an index of microgliosis, compared to Ctrl mice, which is **significantly counteracted by the loss of PCSK9 in 5XFAD mice** (**Fig. 3A-B**). Similarly, also GFAP ir, an index of astrocyte reactivity, is increased in 5XFAD-PCSK9$^+$ mice compared to Ctrl mice, and is overall **counteracted by the absence of PCSK9**, reaching a statistically significant difference only in the hippocampal corpus callosum (**Fig. 4A-B**)

Figure 3

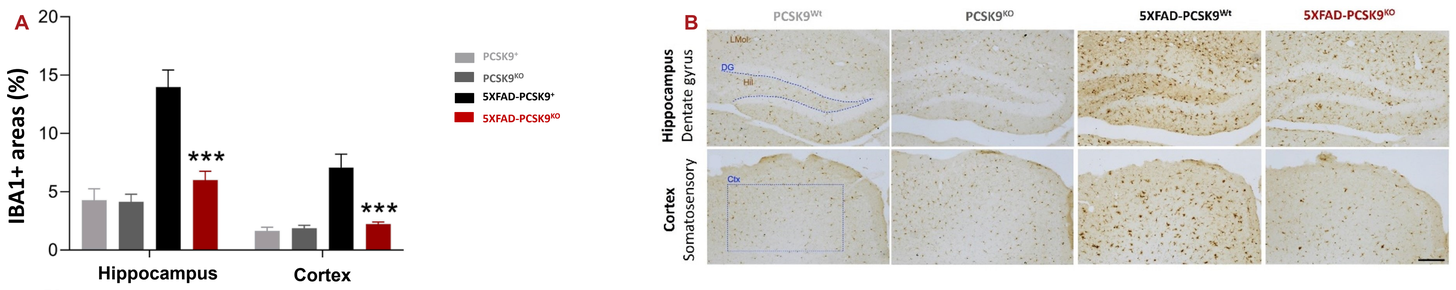
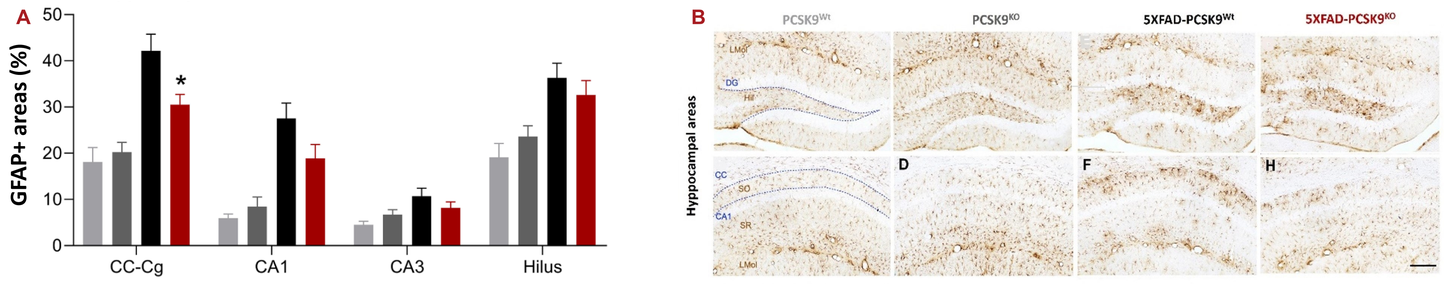


Figure 4

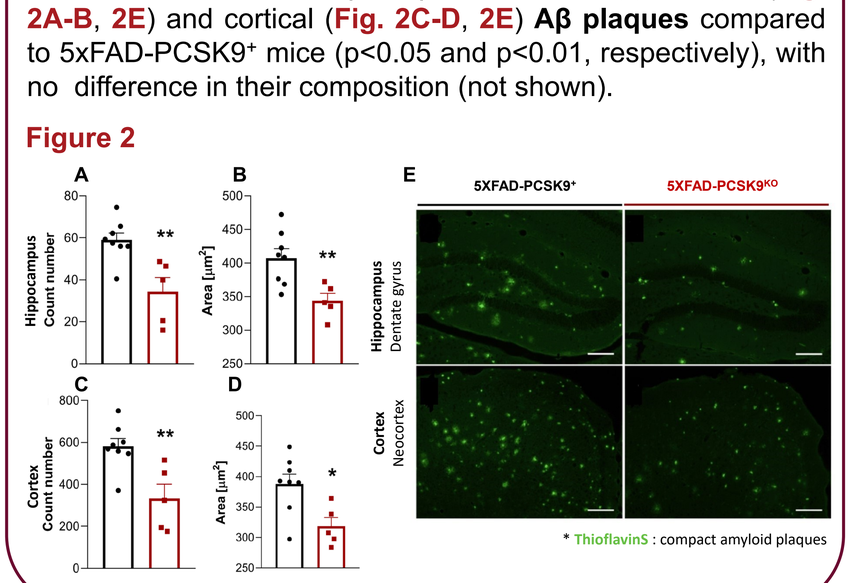


Results (III):

PCSK9 genetic ablation in AD mice reduces cortico-hippocampal Aβ burden

5xFAD-PCSK9^{ko} mice are characterised by a significant reduction in the **number (-40%) and area** of hippocampal (**Fig. 2A-B**, **2E**) and cortical (**Fig. 2C-D**, **2E**) **Aβ plaques** compared to 5xFAD-PCSK9$^+$ mice ($p<0.05$ and $p<0.01$, respectively), with no difference in their composition (not shown).

Figure 2



* ThioflavinS : compact amyloid plaques

Results (IV):

PCSK9 genetic ablation in AD mice partially restores brain cholesterol concentration

Brain cholesterol concentration is similar in Ctrl groups, regardless of PCSK9 expression, whereas **5XFAD-PCSK9$^+$ mice have significant lower brain cholesterol** ($p<0.01$ vs Ctrls). This reduction is **partially reversed in 5XFAD-PCSK9^{KO} mice** (**Table 1**). The most relevant bioactive cholesterol metabolites, 24-, 25- and 27-HC, were quantified in the brain, observing comparable brain 24- and 27-HC levels between the four experimental groups, while 25-HC was not detectable.

Table 1

Group	CHOLESTEROL	OXYSTEROLS		
	µg/mg cerebral tissue	24-OHC [ng/ml]	25-OHC [ng/ml]	27-OHC [ng/ml]
PCSK9+	1.86 ± 0.58	2.67 ± 0.89	n.d.	2.10 ± 0.80
PCSK9KO	1.35 ± 0.77	3.08 ± 0.74	n.d.	2.32 ± 1.82
5XFAD-PCSK9+	**0.95 ± 0.42 ****	3.45 ± 1.59	n.d.	1.95 ± 1.96
5XFAD-PCSK9KO	1.18 ± 0.57	2.87 ± 1.84	n.d.	1.86 ± 1.21

[1]Zimetti F et al., (2017). *Increased PCSK9 cerebrospinal fluid concentrations in Alzheimer's disease*. Journal of Alzheimer's Disease;
[2]Papotti B et al., (2022). *PCSK9 Affects Astrocyte Cholesterol Metabolism and Reduces Neuron Cholesterol Supplying In Vitro: Potential Implications in Alzheimer's Disease*. Int J Mol Sci

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