Original Research: Open Access

Comparative Characterization Profile of Transgenic Mouse Models of Alzheimer´s Disease

Iván Carrera[1,*], Lucía Fernández-Novoa[1], Oscar Teijido[2], Carolina Sampedro[1], Silvia Seoane[1], Madepalli Lakshmana[3] and Ramón Cacabelos[2]

[1]Department of Health Biotechnology, EuroEspes Biotechnology, 15165 - Corunna, Spain

[2]EuroEspes Biomedical Research Center, Institute for CNS Disorders and Genomic Medicine, 15166 - Corunna, Spain

[3]Section of Neurobiology, Torrey Pines Institute for Molecular Studies, 34987 - Port Saint Lucie, Florida, USA

ABSTRACT

Alzheimer's disease (AD) presents amyloid plaques as one of its earliest and most characteristic hallmarks, occurring up to 20 years before clinical diagnosis. Their precise role in the onset of AD is still being investigated but they appear to be an effective biomarker of its pre clinical stages. These plaques seem to be part of the neurodegeneration process in AD that leads to the progressive deterioration of glia and neurons in specific brain regions. Working upon the hypothesis that the biological response to neurodegeneration induces the development and massive generation of pathological hallmarks in the brain, AD-related hallmark immune-expression was analyzed in transverse brain sections of two transgenic mouse models. A newly-generated triple-transgenic mouse (APP/BIN1/COPS5) was compared to double-transgenic mice (APP/PS1) using immunohistochemistry detection methods. A comparison of disease-specific hallmark changes and neuropathological biomarkers throughout disease evolution revealed a different hallmark pattern in the two models, providing novel insight into the development of AD pathology. This study presents for the first time an age-related comparative pattern of the neuropathological framework of AD in transgenic mouse models, key to understanding the genetic-specific targets for immunotherapy and neuronal protection.

INTRODUCTION

In AD, soluble oligomers of amyloid β (Aβ) are believed to be one of the major causes of synaptic degeneration and cognitive dysfunction in patients in the early stages of AD [1]. This hypothesis is based primarily on experimental studies demonstrating that Aβ oligomers impair normal synaptic plasticity [2] and memory [2,3] and cause loss of synapses when applied exogenously to rat cerebral ventricle, cultured brain slices, or dissociated neurons[4]. Moreover, several studies have previously supported this evidence by demonstrating a direct correlation between levels of soluble oligomers of Aβ and synaptic and cognitive impairment in humans [5] as well as animalmodels of AD[6]. AD is a heterogeneous and complex disorder in which hundreds of genes distributed across the human genome might be involved in close cooperation with environmental factors and epigenetic phenomena, leading to the neurodegeneration process that characterizes this disease [7-12]. In patients, the clinical detection of amyloid plaques is currently based on positron emission tomography (PET) imaging with three radioactive agents recently approved by the Food and Drug Administration (FDA)[13]. However, PET presents a low spatial resolution that inhibits the visualization of individual plaques, while in animal models; PET studies have provided controversial results [14]. In particular, some studies successfully detected amyloid progression in APP23 [15] and 5xFAD [16] mice while other studies failed to detect these amyloidotic changes [17,18]. Other imaging technologies have also been developed to detect amyloid plaques in animals, such as optical or two-photon imaging. Although the Two-photon imaging can detect individual amyloid plaques at very high resolution (1 μm), its limitation is the impossibility of recording large images of the whole brain [19].

Corresponding author: Ivan Carrera, Department of Health Biotechnology, EuroEspes Biotechnology, 15165 - Corunna, Spain, Email: biotecnologiasalud@ebiotec.com

In the past decades, the gene-targeted technology applied to generate specific transgenic mice has proven to be crucial for modeling the main hallmarks of AD neuropathology, although no mouse model fully recapitulates its entire neuropathological spectrum [20]. At the present time, numerous models have successfully replicated amyloid plaque deposition, generally by inducing high levels of APP overexpression. Moreover, the inclusion of a mutant PS1 allele can increase the deposition rate of this amyloid plaque deposition as well as exacerbating its severity [21]. However, the majority of AD models have developed one hallmark pathological lesion that has been insufficient to trigger the development of the other signature lesion. Consequently, to develop the manifestation of both plaques and tangles in the same model has required the introduction of multiple transgenes into the same mouse, which has generally been achieved by crossing several independent transgenic lines, or alternatively, by microinjecting pathological protein into the brains of single-transgenic mice [22,23].

Here we describe the comparative development of the main AD hallmark in a novel triple-transgenic model (APP/BIN1/COPS5) and double-transgenic model (APP/PS1). We report that (to our knowledge) this is the first comparative profile between these two robust transgenic models in AD-affected brain regions. The 3×Tg-AD mice develop extracellular Aβ deposits prior to those observed in 2xTg-AD mice, consistent with the amyloid cascade hypothesis. Although both mice exhibited deficits in synaptic plasticity, the severity of the neuropathological degeneration is more severe and earlier in onset in the 3×Tg-AD mice. This study will be useful for addressing the impact of 3×Tg-AD mice as a new powerful AD model by recapitulating the early-onset neurodegenerative effects of Aβ deposits.

MATERIALS AND METHODS

Mouse models

The double-transgenic mice B6C3F1/J (APPswe/PS1dE9), expressing a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-ΔE9), both directed toward central nervous system (CNS) neurons, exhibit Aβ plaques in the hippocampus andcortex beginning at 6months of age (Jackson Laboratory, Bar Harbor, ME).

The triple-transgenic 3xTg-AD mice (APP/BIN1/COPS5), which overexpress the Swedish mutation of APP (human amyloid precursor protein) together with BIN1 (bridging integrator 1, AMPH2) and COPS5 (COP9 constitutive photomorphogenic homolog subunit 5, Jab1), individually generated in Dr. Lakshmana´s lab and backcross-bred in our laboratory, closely mimicthe human brain pathology. DNA constructs and transgenic generation proceedings have been described previously [24,25] and were sequence-verified

prior to breeding the transgenic colony. All experimental procedures were performed in accordance with the guidelines established by the European Communities Council Directive (86/609/EEC), EU Directive 2010/63/EU, and Spanish Royal Decree 1201/2005 on animal experimentation,and were approved by the Ethics Committee of the EuroEspes Biotechnology Research Centre (Permit number: EE/2015-184).

Experiment Design

Double- (APP/PS1) and triple- (BIN1/COPS5/APP) transgenic mice of 0-1, 6, and 12 months of age were used during experimentation and then sacrificed, together with wild-type mice used as control groups. Double- and triple-transgenic mice were randomly divided into these 5 experimental groups by age (**Figure 1**), as follows: Group A (0-1 months of age) was formed by 15 mice (12 transgenic and 3 wild-type mice); group B (6 months of age), formed by 15 mice (12 transgenic and 3 wild-type mice); and group C (12 months of age), formed by 9 mice (6 transgenic and 3 wild-type mice).

Immunohistochemistry

Immunohistochemical Aβ hallmarks were analyzed by using the methods described, exactly as previously published [26-29]. In summary, parallel transverse sections (12-14 µm) from the left half of the brain were obtained by cryostat and pretreated with H_2O_2 in phosphate-buffered saline at room temperature for 15 minutes, to eliminate endogenous peroxidase. They were then rinsed twice in 0.05M Trizma buffered saline (TBS) containing 0.1% Tween-20 at pH 7.4 (TBS-T) for 10 minutes each, pretreated with blocking avidin/biotin kit (Vector) and then incubated overnight with the primary antibodies (Millipore; 1/1000). The sections were successively rinsed in TBS-T, incubated in goat IgG anti-rat (Millipore) or goat IgG anti-mouse (Sigma), depending on the primary antibody, for 1 hour, rinsed in TBS-T, and then incubated for 30 minutes in ABC kit system (Vectastain; Vector). The labeling was revealed by incubating sections with 3,3-diaminobenzidine (Sigma) with chromogen and hydrogen peroxide as oxidant. In several adjacent sections, negative controls performed by omitting the primary, secondary, or tertiary antibodies showed no immunostaining. Images were visualized using a microscope (Olympus BX50) and digitized using a digital camera (DP-10; Olympus). The photomicrographs were adjusted for brightness and contrast with Corel Photo-Paint (Corel 11, Ottawa, Canada) and figure images were composed using Corel Draw.

RESULTS

Histopathological comparison of Aβ deposits in AD transgenic mice

Immunohistochemical data obtained from mouse brain analysis at 0-1, 6, and 12 months of age showed that APP,

Bin1 and COPS5 expressing genes promote the severity of Aβ plaque deposits throughout the first age of development (**Figure 1A-F**). Results indicated that, when compared with double-(APP/PS1) transgenic mice, the triple-transgenic mice (APP/Bin1/COPS5) showed Aβ deposits at very early stages of development (0-1 month of age). Results also showed that the plaque burden density observed in affected brain regions of triple-transgenic mice was notably increased, compared with double-transgenic mice of the same age (**Figure 1A-F).** As shown in **figure 1**, all the triple-transgenic mice showed a progressive accumulation of Aβ deposits in the brain, while in APP/PS1 Tg mice, cerebral Aβ plaques were only observed at 6 months of age.
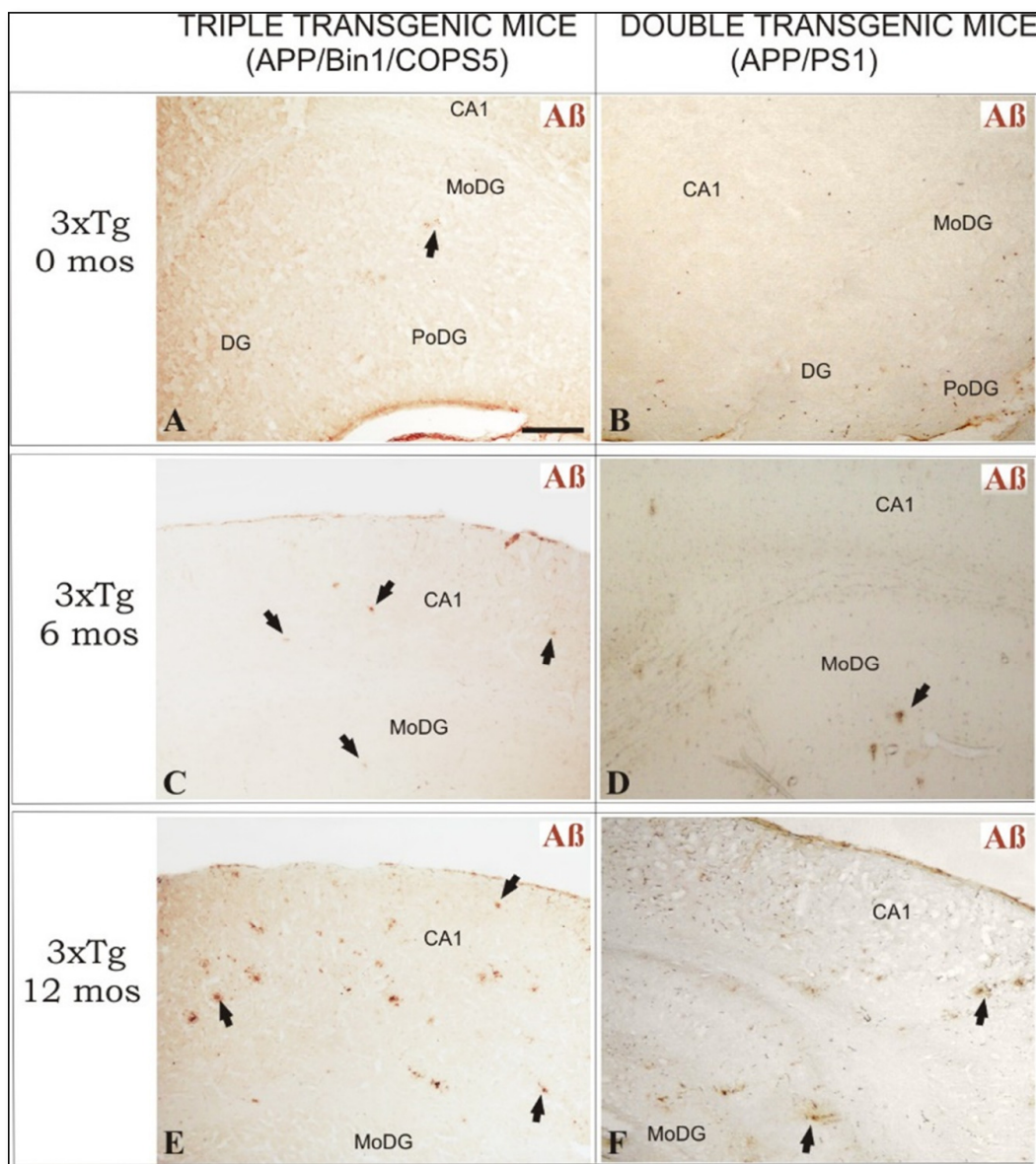

Figure 1. Progressive accumulation of Aβ deposition in transgenic AD mouse brains. Comparative photomicrographs showing representative cortical and hippocampal brain sections of 0-, 6- and 12-month-old transgenic mice immunostained for Aβ. Images are shown according to the age group and transgenic type. A-B, Images showing incipient Aβ-immunoreactive plaques in the dentate gyrus of 3xTg-AD mice but absent in 2xTg-AD mice. C-D, Some incipient Aβ-immunoreactive plaques observed in the external layers of the neocortex and dentate gyrus of 3xTg-AD mice, while a few incipient plaques appear in the dentate gyrus of 2xTg-Ad mice. E-F, A large density of Aβ-immunoreactive plaques are present in the neocortical layers, in larger numbers than in the same region of 2xTg-AD mice. Scale bar: 100 µm.

Aβ plaques in transgenic models of 0-1 months of age

Aβ immunoreactive plaques were first detectable in neocortical regions and subsequently in CA1 pyramidal neurons in newborn triple-transgenic micebetween 0-1 month of age. Aβ deposits first became apparent in the hippocampus (**Figure 1A)** and were consistently evident by the end of the first month. Also, by this point in time, Aβ deposits were apparent in the frontal cortex, suggesting that there is an age-related and regional brain correlation to Aβ deposition in these 3×Tg-AD mice. A detailed examination of the brain tissues at higher magnification showed that, over the course of 0-1 months, the 3×Tg-AD mice exhibited the typical incipient pattern of fibrillar amyloid accumulation, primarily in the form of small sparse deposits (**Figure 1A**). However, no immunoreactivity was observed in any brain region of the newborn double-transgenic mice between 0-1 month of age (**Figure 1B).** The main affected areas were completely devoid of Aβ deposits, and no other related hallmarks were identified in these 2xTg-AD mouse models.

Aβ plaques in transgenic models of 4-6 months of age

The incipient Aβ deposits observedbetween 4 and 6 months of age (**Figure 1C-D**) in the neocortex of 2xTg-AD mice (APP/PS1) presented a simple oligomer structure mainly located at the inner layers of the cortex and dentate gyrus (**Figure 1D**). Although these Aβ deposits were sparse and low in density they already formed a conspicuous hallmark in the 2xTg-AD mouse brain regions. However, 3x-Tg-AD mice from 4 to 6 months of age exhibited a large density of Aβ immunoreactive plaques with a dense core (**Figure 1C**), although with a sparse structure. These deposits were Type 1-like plaques formed by aggregates of weakly Aβ-immunoreactive material with a reticular appearance (**Figure 1C).** The Aβ deposits in both models were located in the cortex and dentate gyrus, although in different densities in each model.

Aβ plaques in transgenic models of 12 months of age

Aβ plaques in triple-transgenic mouse brains were observed in great density in the neocortical layers and dentate gyrus, showing a more complex structure than in early stages (**Figure 1E**). The morphological structure of these Aβ plaques still resembles the Type-1-like morphological classification that has been described as a mesh of stained fibrils with a larger area, although they showed a more conspicuous and enlarged deposition area. In double-transgenic mice, however, the plaques were detected in similar density in both affected regions (**Figure 1F**), cortical and hippocampal layers of mouse brain sections, and their density and dimensions were not comparable to those observed in triple models.

DISCUSSION

The aim of this study was to describe the comparative development of the main AD hallmark in a novel triple-transgenic model (APP/BIN1/COPS5) and a double-transgenic model (APP/PS1).The Aβ peptides are believed to play a crucial role in AD neuropathology [30,31], mainly in the loss of cognitive function in AD patients. Consistent with results obtained in previous studies [32,33], our present results showed a great difference in Aβ production and deposition in the hippocampus of triple-transgenic mice (APP/BIN1/COPS5), when compared with double-transgenic mice (APP/PS1) of the same age. Recently, the 3×Tg mouse model for AD, which displays both Aβ and tau hallmark accumulation, was used to study pathological changes in AD. Though long-term wheel running was shown to enhance neuroprotection in 3×Tg-AD mice, the traditional markers of AD neuropathology were not altered [34,35]. However, the rotarod test lasting for 11 months increased neurogenesis at 20 months of age in 3×Tg-AD mice [35], while 6 months of rotarod testing reduced oxidative stress and improved synaptic function in the 7-month-old 3×Tg-AD mice [34]. The mouse strain differences observed in the present study may play a crucial role in these divergent results. According to a previous study, Aβ plaques can be detected in the cortex and hippocampus of double-transgenic mice (APP/PS1) as early as 4-6 months of age, and amyloid plaque burden increases with age [36]. Several lines of evidence indicate that through the non-amyloidogenic α-secretase pathway, APP protein is cleaved to produce the sAPPα fragment [37], which is beneficial for neuronal survival [38,39], whereas through the amyloidogenic α-secretase pathway, APP protein is cleaved to form neurotoxic Aβ, which is involved in AD pathogenesis. APP processing can be modulated by different mechanisms, including but not limited to an altered APP expression, as well as expression/activity of secretase involved in APP processing. In the current study, we observed a significant difference in the expression of soluble oligomers of amyloid β (Aβ) between the double- and triple-transgenic mouse strains. Accordingly, we propose that the genetic combination of APP, Bin1 and COPS5 AD-related genes may modulate APP-processing through the changes in α-secretase and α-secretase activity. Previous genome-wide association studies have demonstrated that these specific genes have been identified as the most associated loci for the neurodegenerative process of AD. Therefore, it is known that BIN1 undergoes complex alternative splicing to generate multiple isoforms with diverse functions in multiple cellular processes including endocytosis, membrane remodeling and the potential for a role of BIN1 in the membrane remodeling that accompanies the process of myelination [40]. Moreover, BIN1 increases cellular BACE1 levels through impaired endosomal trafficking and reduces BACE1 lysosomal degradation, resulting in increased Aβ production [41]. On the other hand, Wang and colleagues [42] demonstrated previously that COPS5 regulates Aβ generation in neuronal cell lines in a RanBP9-

dependent manner, since by 12 months, COPS5 overexpression in APΔE9 mice (APΔE9/COPS5-Tg) significantly increased $A\beta_{40-42}$ levels in the cortex and hippocampus. They have also proved that COPS5 robustly increased Aβ generation, followed by increased soluble APP-β (sAPP-β) and decreased soluble-APP-α (sAPP-α) levels. In particular, they observed that down-regulation of COPS5 by siRNAs reduced Aβ generation, implying that endogenous COPS5 regulates Aβ generation. Finally, COPS5 levels were significantly increased in AD brains and in APΔE9 transgenic mice, and overexpression of COPS5 strongly increased RanBP9 protein levels by increasing its half-life. Taken together, these studies suggest that COPS5 increases Aβ generation by inducing APP processing and Aβ generation by stabilizing RanBP9 protein levels [42].

In the present study, we have demonstrated clear differences in the appearance, structure, density and amount of Aβ deposits between the two transgenic mouse models evaluated. Numerous lines of transgenic mice are available for AD research, and many variables, including the number and choice of transgenes, the promoters used, the background strain and the sex of the animals, affect the pathology expressed by different mouse lines [43]. Moreover, the structures of Aβ deposits also vary markedly depending on the genetic factors mentioned above. Tg2576 mice reportedly already exhibit increased Aβ plaque deposits at 9 months of age [44]. However, in the present study, sparse fibrillar deposition became visible at 0-1 months of age in triple-transgenic mice, although their staining intensity was much weaker than that observed in later developmental stages. Overall, development of Aβ deposits in triple-transgenic mice was surprisingly early and fast, and therefore this model appears crucial for amyloid imaging studies. In double-transgenic mice (APPswe-PS1dE9), incipient Aβ deposition was visible at 4-6 months of age and at 9 months in high immunohistochemical intensity. In triple-transgenic mice, Aβ deposition was fast during brain development, although it was more diffuse than fibrillar Aβ. In double-transgenic mice with mutations in both APP and PS1, the deletion of PS1 exon 9 reportedly results in PS1 gain of function and the occurrence of large, homogeneous plaques that are only slightly congophilic stained [45]. Severe Aβ deposition was observed in the triple transgenic mice of 6 months of age, a finding that does support the use of this model for brain neuropathological reference region-based analysis.

CONCLUSION

In conclusion, our present findings demonstrate that the combination of APP, Bin1 and COPS5 genes plays a crucial role in the onset of neurodegenerative AD hallmarks, particularly in the early development of Aβ plaques. This study shows that the insertion of the three AD-related genes involved in the development of Aβ pathogenesis improves hallmark expression in mice and reduces the time for their appearance in the affected brain regions of neocortex and hippocampus. When compared with double-transgenic mice (APP/PS1), this triple-transgenic model showed an early onset of the Aβ developmental pattern, leading to a more robust AD animal model. Taken together, our results indicate that APP, Bin1 and COPS5 may acceleratethe onset of the amyloidogenic pathway and modulate theprocessing of APP deposition in the mouse brain. However, additional studies are required to address fully the potential ofthis new triple-transgenic mouse modelin the preclinical study of AD-like symptoms and pathology.

REFERENCES

1. Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. Science 298: 789-791.

2. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al. (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 14: 837-842´.

3. Lesne´ S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, et al. (2006) A specific amyloid-β protein assembly in the brain impairsmemory. Nature 440: 352-357.

4. Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL (2007) Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamatereceptor-dependent signaling pathway. J Neurosci 27: 2866 - 2875.

5. Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE, et al. (2003) Alzheimer's disease-affected brain: presence of oligomeric Aβ ligands (ADDLs) suggests a molecular basis for reversible memoryloss. Proc Natl Acad Sci USA 100: 10417-10422.

6. Matsuyama S, Teraoka R, Mori H, Tomiyama T (2007) Inverse correlation between amyloid precursor protein and synaptic plasticity in transgenicmice. Neuroreport 18: 1083-1087.

7. Cacabelos R, Torrellas C (2014) Epigenetic drug discovery for Alzheimer's disease. Expert Opin Drug Discov 9: 1059-86.

8. Cacabelos R (2008) Pharmacogenomics in Alzheimer's disease. Methods Mol Biol 448: 213-357.

9. Cacabelos R, Martinez R, Fernandez-Novoa L, Carril JC, Lombardi V, Carrera I, et al. (2012) Genomics of Dementia: APOE- and CYP2D6-Related Pharmacogenetics. Int J Alzheimers Dis 518901.

10. Cacabelos R, Torrellas C, Teijido O, Carril JC (2016) Pharmacogenetic considerations in the treatment of Alzheimer's disease. Pharmacogenomics 17: 1041-1074.

11. Cacabelos R, Fernández-Novoa L, Lombardi V, Kubota Y, Takeda M (2005) Molecular genetics of Alzheimer's disease and aging. Methods Find. Exp Clin Pharmacol 27: 1-573.

12. Cacabelos R, Cacabelos P, Torrellas C, Tellado I, Carril JC (2014) Pharmacogenomics of Alzheimer's disease: novel therapeutic strategies for drug development. Methods Mol Biol 1175: 323-556.

13. Morris E, et al. (2016) Diagnostic accuracy of (18) F amyloid PET tracers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Eur J Nucl Med Mol Imaging 43: 374-385.

14. Dudeffant C, Vandesquille M, Herbert K, Garin CM, Alves S, Blanchard V, et al. (2017) Contrast-enhanced MR microscopy of amyloid plaques infive mouse models of amyloidosis and in human Alzheimer's disease brains. Sci Rep 7: 4955.

15. Snellman A, et al. (2013) Longitudinal amyloid imaging in mouse brain with 11C-PIB: comparison of APP23, Tg2576, and APPswe-PS1dE9 mouse models of Alzheimer disease. J Nucl Med Off Publ Soc Nucl Med 54: 1434-1441.

16. Rojas S, Herance JR, Gispert JD, Abad S, Torrent E, et al. (2013) In vivo evaluation of amyloid deposition and brain glucose metabolism of 5XFAD mice using positronemission tomography. Neurobiol Aging 34: 1790-1798.

17. Kuntner C, et al. (2009) Limitations of small animal PET imaging with [18F] FDDNP and FDG for quantitative studies in a transgenicmouse model of Alzheimer's disease. Mol. Imaging Biol. MIB Off. Publ. Acad. Mol. Imaging 11: 236-240.

18. Poisnel G, et al. (2012) PET imaging with [18F]AV-45 in an APP/PS1-21 murine model of amyloid plaque deposition. Neurobiol Aging 33: 2561-2571.

19. Delatour B, Dhenain M (2008) Diagnosis of Alzheimer's disease: the contribution of preclinical research. Bull Acad Natl Med 192: 345-360

20. Wong PC, Cai H, Borchelt DR, Price DL (2002) Genetically engineered mouse models of neurodegenerative diseases. Nat Neurosci 5: 633-639.

21. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, et al. (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39: 409-421.

22. Gotz, J, Chen, F, van Dorpe, J, Nitsch RM (2001) Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science 293: 1491-95.

23. Lewis J, Dickson D, Lin W, Chisholm L, Corral A, et al. (2001) Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science 293: 1487-1491.

24. Wang H, Dey D, Carrera I, Minond D, Bianchi E, Xu S, Lakshmana MK (2013) COPS5 (Jab1) protein increases β site processing of amyloid precursor protein and amyloid β peptide generation by stabilizing RanBP9 protein levels. J Biol Chem 288: 26668-26677.

25. Wang R, Wang H, Carrera I, Xu S, Lakshmana MK (2015) COPS5 protein overexpression increases amyloid plaque burden, decreases spinophilin-immunoreactive puncta, and exacerbates learning and memory deficits in the mouse brain. J Biol Chem 290: 9299-9309.

26. Carrera I, Etcheverría I, Fernández-Novoa L, Lombardi VR, Lakshmana MK, Cacabelos R, et al. (2015) A comparative evaluation of a novel vaccine in APP/PS1 mouse models of Alzheimer's disease. Biomed Res Int 807146.

27. Carrera I, Etcheverría I, Li Y, Fernández-Novoa L, Lombardi V, Vigo C, et al. (2013) Immunocytochemical characterization of Alzheimer disease hallmarks in APP/PS1 transgenic micetreated with a new anti-amyloid-β vaccine. Biomed Res Int 709145.

28. Carrera I, Etcheverría I, Fernández-Novoa L, Lombardi V, Cacabelos R, Vigo C (2012) Vaccine Development to Treat Alzheimer's Disease Neuropathology in APP/PS1Transgenic Mice. Int J Alzheimers Dis 376138.

29. Carrera I, Fernández-Novoa L, Vigo C, Cacabelos R (2016) Sphingosine-1-phosphate (S1P)-Containing Niosomes as Vehicle to Deliver Amyloid-β in a Novel Alzheimer´s Disease Vaccine. J Genomic Med Pharmacogenomics 1: 225-232.

30. Apelt J, Schliebs R (2001) Beta-amyloid-induced glial expression of both pro- and anti-inflammatory cytokines in cerebral cortex of aged transgenic Tg2576 micewith Alzheimer plaque pathology. Brain Res 894: 21-30.

31. García-Mesa Y, López-Ramos JC, Giménez-Llort L, Revilla S, Guerra R, Gruart A, et al. (2011) Physical exercise protects against Alzheimer's disease in 3 × Tg-AD mice. J Alzheimer's Dis 24: 421-454.

32. Blanchard V, Moussaoui S, Czech C, Touchet N, Bonici B, Planche M, et al. (2003) Time sequence of maturation of dystrophic neurites associated with Abetadepositsin APP/PS1 transgenic mice. Exp Neurol 184: 247-263.

33. Radde R, Bolmont T, Kaeser SA, Coomaraswamy J, Lindau D, Stoltze L, et al. (2006) Abeta42-driven cerebral amyloidosis in transgenic mice reveals early androbust pathology. EMBO Rep 7: 940-946.

34. Götz J, Chen F, van Dorpe J, Nitsch RM (2001) Formation of neurofibrillary tan-gles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science 293: 1491-1495.

35. Marlatt MW, Potter MC, Bayer TA, van Praag H, Lucassen PJ (2013) Prolonged run-ning, not fluoxetine treatment, increases neurogenesis, but does not alterneuropathology, in the 3 × Tg mouse model of Alzheimer's disease. Curr Topics Behav Neurosci 15: 313-340.

36. Garcia-Alloza M, Robbins EM, Zhang-Nunes SX, Purcell SM, Betensky RA, Raju S, et al. (2006) Characterization of the amyloid deposition in the

APPswe/PS1dE9 mouse model of Alzheimer's disease. Neurobiol Dis 24: 516-524.

37. Esch FS, Keim PS, Beattie EC, Blacher RW, Culwell AR, Oltersdorf T, et al. (1990) Cleavage of amyloid beta peptide during constitutive processing of its precursor. Science 248: 1122-1124.

38. Mattson MP, Barger SW, Furukawa K, Bruce AJ, Wyss-Coray T, Mark RJ, et al. (1997) Cellular signaling roles of TGF beta, TNF alpha and beta APP in brain injury responses and Alzheimer's disease. Brain Res Brain Res Rev 23: 47-61.

39. Wallace WC, Akar CA, Lyons WE (1997) Amyloid precursor protein potentiates the neurotrophic activity of NGF. Brain Res Mol Brain Res 52: 201-212.

40. De Rossi P, Buggia-Prévot V, Clayton BL, Vasquez JB, van Sanford C, et al. (2016) Predominant expression of Alzheimer's disease-associated BIN1 in mature oligodendrocytes and localization to white matter tracts. Mol Neurodegener 11: 59.

41. Miyagawa T, Ebinuma I, Morohashi Y, Hori Y, Young Chang M, et al. (2016) BIN1 regulates BACE1 intracellular trafficking and amyloid-β production. Hum Mol Genet 25: 2948-2958.

42. Wang H, Dey D, Carrera I, et al. (2013) COPS5 (Jab1) increases β-site processing of amyloid precursor protein and Aβ generation by stabilizing RanBP9 protein levels. J Biol Chem. 288: 2668-277.

43. Duyckaerts C, Potier MC, Delatour B (2008) Alzheimer disease models and human neuropathology: similarities and differences. Acta Neuropathol 115: 5-38.

44. Hsiao K, Chapman P, Nilsen S, et al. (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science 274: 99-102.

45. Garcia-Alloza M, Robbins EM, Zhang-Nunes SX, et al. (2006) Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. Neurobiol Dis 24: 516-524.