matters

Analysis of $A\beta_{x-42}/A\beta_{x-40}$ and Tau upon siRNA-mediated downregulation of APOE, BIN1, PICALM, CLU, PRNP and CST3 in wildtype mouse primary neurons

Gabriele Siegel, Lawrence Rajendran

Systems and Cell Biology of Neurodegeneration, IREM, University of Zurich, Schlieren Campus, 8952 Schlieren (Zurich), Switzerland

Correspondence
gabriele.siegel@bli.uzh.ch
rajendran@bli.uzh.ch

Disciplines
Cell Biology
Neurobiology

Keywords
GWAS
Alzheimer'S Disease
Abeta
Tau
Alzgenes

Type of Observation
Standalone

Type of Link
Confirmatory data (published elsewhere)

Submitted Nov 24, 2017
Published May 15, 2018

Full Open Access
Supported by the Velux Foundation, the University of Zurich, and the EPFL School of Life Sciences.

Abstract

Alzheimer's disease (AD) is the most common form of dementia in the elderly. It is a progressive neurodegenerative disorder that is characterized by the abundant presence of cerebral β-amyloid (Aβ) plaques and neurofibrillary Tau tangles. The rare, early-onset AD is caused by mutations mainly within either the amyloid precursor protein (APP) or Presenilins 1 or 2 (PS1 or PS2), the catalytic subunits of the γ-secretase complex. These mutations either increase overall Aβ production or specifically alter γ-secretase mediated processing towards an increased production ratio of $A\beta_{42}$:$A\beta_{40}$. For late-onset AD, however, which accounts for the vast majority of AD cases, the exact mechanisms by which the disease is caused are not known. While genome-wide association studies (GWAS) have identified certain genetic risk factors associated with late-onset AD, the mechanisms through which they contribute to the pathogenesis are still elusive. Previously, using a HeLa cell model of Aβ production, it was shown that, in contrast to the early-onset AD causing mutations, knockdown of late-onset AD susceptibility genes did not specifically affect the $A\beta_{42}$:$A\beta_{40}$ ratio [1]. To validate these findings in a neuronal setting without any overexpression of APP, here we re-addressed the role of six late-onset AD risk genes (APOE, BIN1, PICALM, CLU, PRNP and CST3) in the regulation of γ-secretase mediated APP processing in wild-type mouse primary neurons by analyzing $A\beta_{x-40}$ and $A\beta_{x-42}$. In addition, we extended the analysis by also including measurements of total Tau protein and phosphorylation of Tau at Threonine 231, a non-physiological phosphorylation site that is associated with AD. The siRNA-mediated knockdown of the studied LOAD risk genes neither affected the $A\beta_{x-42}$:$A\beta_{x-40}$ ratio nor altered the levels of total Tau or phospho(Thr231)-Tau. Our results thus show that acute downregulation of these genes in wild-type mouse primary neurons does not significantly impact on γ-secretase mediated APP processing nor Tau homeostasis or Tau phosphorylation.

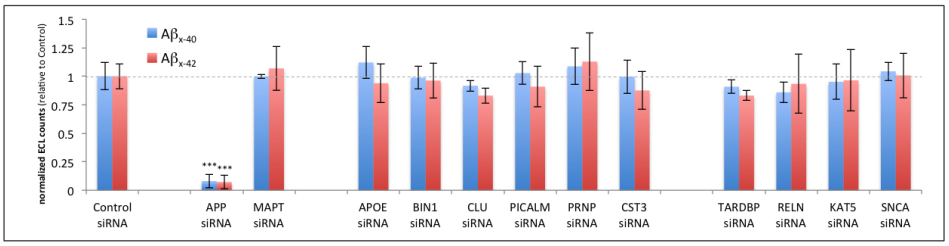
Introduction

The brains of Alzheimer's disease (AD) patients show widespread formation of extracellular senile plaques composed of aggregated β-amyloid (Aβ) peptides as well as intraneuronal aggregates of misfolded Tau protein, so-called neurofibrillary tangles. According to the onset of disease one can distinguish two types of AD: a rare, early-onset AD (EOAD) with an onset before 65 years of age, and the common, late-onset AD (LOAD) with an onset after 65 years of age. EOAD is caused by a specific set of highly penetrant mutations, which affect almost exclusively either the amyloid precursor protein (APP) or the catalytically active Presenilin subunit of the Aβ-producing γ-secretase complex. These mutations enhance the overall production of Aβ peptides and/or increase the ratio of the aggregation prone and neurotoxic $A\beta_{42}$ over the common, shorter $A\beta_{40}$. The genetic contribution to LOAD, on the other hand, is not very well understood. Inheritance patterns within families point to multifactorial inheritance of LOAD, involving both genetic and environmental factors. Initial linkage analysis studies and GWAS analyses have identified a number of susceptibility genes for LOAD (Alzgenes), the majority of which having only small effect sizes on disease risk [2]. The only exception is the APOE ε4 locus which increases LOAD risk 2- to 4-fold in individuals with one copy of the allele and 8-to 15-fold in individuals carrying two copies [3] [4] [5] [6] [7].

While an increased production of Aβ and/or an increased Aβ 42:40 ratio have been acknowledged as the driving pathogenic mechanism in EOAD, the etiology of LOAD appears to be much more complex and it is still not absolutely clear if Aβ accumulation plays an active causative role in the initial pathogenesis of the disease. Also the mechanisms that drive Aβ accumulation appear to be different in LOAD and EOAD. While some studies showed effects of LOAD risk genes on Aβ production, most of the data suggest that Aβ accumulation in the brains of LOAD patients results from impaired Aβ clearance rather than an increased production as seen in EOAD [8] [9]. For example APOE, which is the strongest genetic risk factor for LOAD, was shown to bind to Aβ and mediate its clearance across the blood brain barrier (BBB) or its endocytosis into brain cells for lysosomal degradation [10] [11]. Similarly, also CLU and PICALM had been shown to enhance the transport of Aβ across the blood-brain barrier and the blood-cerebrospinal fluid barrier [12] [13] [14] [15]. In line with the notion that LOAD genes do not specifically affect Aβ production, a recent study did not detect specific effects of the knockdowns of several LOAD susceptibility genes on Aβ levels or the 42:40 ratio in a HeLa cell APP overexpression system [1].

Objective

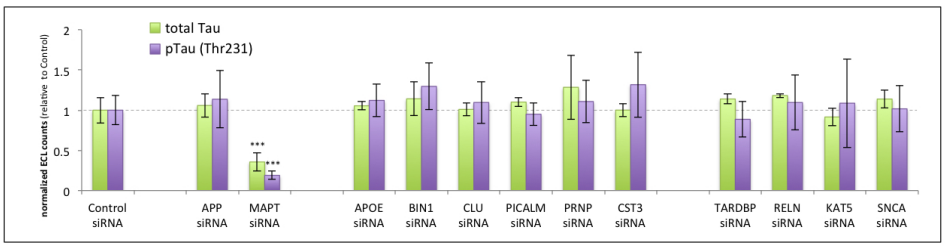
We wanted to study the effect of acute downregulation of selected LOAD risk genes on γ-secretase mediated processing of endogenous APP and on levels of endogenous total Tau and phospho-Tau (pThr231) in wild-type neurons. The selected risk genes comprised four high ranking Alzgenes (Apolipoprotein E [APOE], Bridging integrator 1 [BIN1], Phosphatidylinositol binding clathrin assembly protein [PICALM], Clusterin [CLU] and two LOAD risk genes with weaker association (Prion protein [PRNP] and Cystatin C [CST3]).



	knockdown efficiency (mRNA)
APP	91±3%
MAPT	88±4%
APOE	72±9%
BIN1	71±5%
CLU	75±14%
PICALM	82±2%
PrnP	81±7%
CST3	87±4%
TARDBP	55±6%
RELN	77±5%
KAT5	61±2%
SNCA	80±5%

sample size: APP, BIN1, CLU, PICALM, PRNP, CST3, KAT5 and SNCA: n=4; MAPT, APOE, TARDBP and RELN: n=3
student's t-test: *** $p < 0.01$

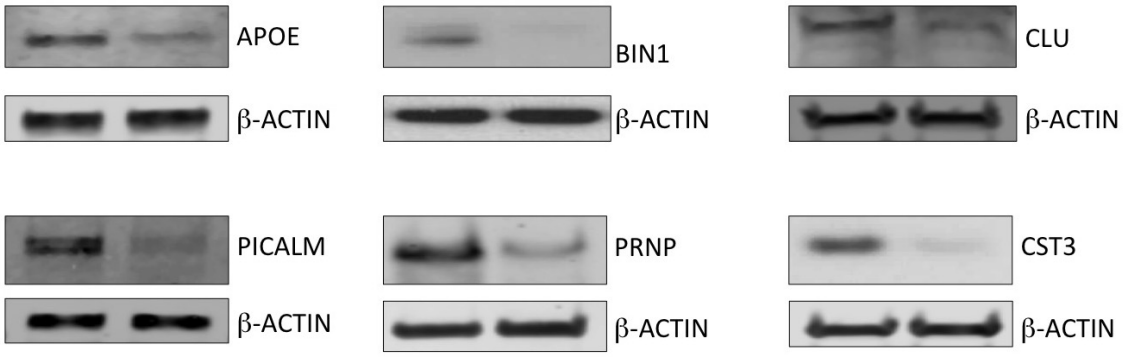
a



	knockdown efficiency (mRNA)
APP	=91±4%
MAPT	=88±4%
APOE	=72±9%
BIN1	=65±9%
CLU	=62±5%
PICALM	=81±2%
PRNP	=78±12%
CST3	=84±7%
TARDBP	=47±4%
RELN	=61±17%
KAT5	=60±1%
SNCA	=75±5%

sample size: n=3
student's t-test: *** $p < 0.01$

b



c

Figure Legend

Figure 1. Knockdown of APOE, BIN1, CLU, PICALM, PRNP or CST3 in wildtype mouse primary neurons does not alter levels of secreted Aβx-40 and Aβx-42 nor levels of total Tau and phospho(Thr231)-Tau.

4DIV wild-type mouse primary neurons were transfected with the indicated siRNAs and cultured until 8DIV. Levels of secreted $A\beta_{x-40}$ and $A\beta_{x-42}$ and levels of total Tau and phosphoThr231-Tau were measured by an electrochemiluminescence (ECL) assay.

(A) Left panel: Analysis of relative $A\beta_{x-40}$ and $A\beta_{x-42}$ levels in 24 h conditioned medium that was collected from 7DIV until 8DIV. Results are displayed as average ± STDEV (n=3). Statistical significance was calculated by student's t-test for comparison of each experimental condition to the control siRNA condition (*** p <0.001). Right panel: mRNA knockdown efficiencies as analyzed by real-time PCR.

(B) Left panel: Analysis of relative levels of total Tau and phosphoThr231-Tau in the protein fraction of whole cell lysates from 8DIV neuronal cultures. Results are displayed as average ± STDEV (n=3). Statistical significance was calculated by student's t-test for comparison of each experimental condition to the control siRNA condition (*** p <0.001). Right panel: mRNA knockdown efficiencies as analyzed by real-time PCR.

(C) Representative Western Blots for analysis of knockdown efficiency on protein level for the tested LOAD risk genes. APOE, BIN1, CLU, PICALM and PRNP levels were analyzed from cell lysates. CST3 levels were analyzed from 24h conditioned medium.

Results & Discussion

In order to study the role of selected LOAD risk genes on γ-secretase mediated processing of endogenous APP and on levels of endogenous total Tau and phospho-Tau in neurons, we performed individual siRNA-mediated gene knockdowns in primary neuronal cultures prepared from embryonic wildtype mice. We chose a set of four high ranking Alzgenes (APOE, BIN1, CLU, PICALM) and two risk genes with weaker association (PRNP and CST3) which were readily detectable at mRNA level in the primary cultures and amenable to gene knockdown by RNAi. As negative control, we transfected a pool of non-targeting siRNAs. In addition, we chose four non-AD control genes (TARDBP: TAR-DNA-binding protein 43, RELN: Reelin, KAT5: Lysine acetyltransferase 5 and SNCA: α-synuclein) that are implicated in other neurodegenerative diseases and neurological conditions including amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTD), schizophrenia, autism, multiple sclerosis (MS) and Parkinson's disease (PD). As positive controls, we transfected siRNAs that target either APP or MAPT. Knockdown-efficiencies of all candidate and control genes were assessed at the mRNA level by real-time PCR in each experimental round. In addition, knockdown of candidate genes at the protein level were confirmed by Western Blot analysis. For the simultaneous detection of both $A\beta_{x-40}$ and $A\beta_{x-42}$ or phospho(Thr231)-Tau and total Tau, we used specific multiplex electrochemiluminescence assays for analysis of the

conditioned neuronal culture medium or the recovered protein fraction of the whole cell lysate respectively. Knockdown of the positive control APP led to the expected reduction of both $A\beta_{x-40}$ and $A\beta_{x-42}$ without altering Tau levels (Fig.1A) while transfection of MAPT siRNA significantly lowered the signal of total and phospho (Thr231)-Tau without affecting Aβ (Fig.1B), thus proving the functionality of the RNAi approach and the validity of the assays.

Silencing any of the tested Alzgenes did not affect secreted $A\beta_{x-40}$ and $A\beta_{x-42}$ or the $A\beta_{x-42}$:$A\beta_{x-40}$ ratio (Fig.1A) nor did it alter total Tau and phospho(Thr231)-Tau levels (Fig.1B). Our results suggest that, in wildtype mouse primary cortical/hippocampal neurons, acute knockdown of the studied LOAD risk genes does not crucially impact on γ-secretase mediated APP processing nor on total Tau levels or Tau(Thr231)-phosphorylation. Susceptibility to AD through these genes might involve effects on APP processing or Tau that build up only over a longer time or only at a later stage, when neurons have already undergone substantial aging. Alternatively, these genes might play a role in mediating Aβ toxicity to neurons, or alter Aβ clearance pathways by non-neuronal cell types, or modulate Aβ aggregation. Indeed, experimental evidence for multiple of these possible mechanisms have been provided by previous studies for several of the herein studied susceptibility genes. For example, in addition to the aforementioned roles of APOE, CLU and PICALM in facilitating Aβ clearance, APOE lipoproteins were shown to regulate the association of oligomeric Aβ with synapses [16], CLU and CST3 were shown to inhibit Aβ aggregation [17] [18] [19] [20] and PRNP was shown to play a role in mediating the deleterious effects of Aβ on synaptic transmission and learning and memory [21] [22] [23] [24] [25] [26] [27], though this finding was challenged by at least three other groups [28] [29] [30]. Importantly, while in our study the knockdown of the tested genes in mouse primary neurons altered neither $A\beta_{x-40}$ and $A\beta_{x-42}$ nor total Tau and phospho(Thr231)-Tau levels, a number of previous studies using different systems reported effects on Aβ or Tau pathology for some of the tested genes. APOE, for example, was shown to regulate neuronal Aβ production by modulating APP recycling [31] or APP transcription [32] when applied extracellularly. It is conceivable that the reason why we did not observe any effects of APOE knockdown on $A\beta_{x-40}$ and $A\beta_{x-42}$ is because it is expressed mainly by astrocytes and produced only at low amounts by primary neuronal cultures as used for our study. For BIN1, conflicting results have been reported on its role in Aβ regulation: while depletion of BIN1 in neurons resulted in a slight decrease in secreted $A\beta_{40}$ with a concomitant increase in intracellular $A\beta_{40}$ and $A\beta_{42}$ in one study [33], increased levels of secreted Aβ were observed by another group [34] and no effects were detected in neuroblastoma cells in a third study [35]. Similarly, also for PICALM the observed effects on Aβ are diverging: in one study, PICALM was reported to promote Aβ generation in cells and to accelerate Aβ pathology in APP transgenic mice [36] whereas another study observed no effect on cellular Aβ production [37]. As for PRNP, a direct role was proposed in the regulation of Aβ production through inhibition of β-secretase-mediated cleavage of APP [38]. In our study, neither $A\beta_{x-40}$ nor $A\beta_{x-42}$ levels changed upon knockdown of BIN1, PICALM or PRNP in mouse primary neurons. Our results thus suggest that the overall APP processing by γ-secretase is not altered by downregulation of either of these genes in young cultured wild-type neurons. Since the assay used in our analysis was chosen so as to detect all $A\beta_{x-40}$ and $A\beta_{x-42}$ species produced by γ-secretase, comprising full-length Aβ and the shorter Aβ' and p3 peptides [39], our data do not necessarily challenge earlier findings on altered full-length Aβ levels. Regarding Tau pathology, only for BIN1 a link has been reported previously: BIN1 expression was shown to correlate with the amount of neurofibrillary tangles (NFT) or total-Tau/phospho-Tau in AD patients in two studies [40] [41] and a direct interaction between BIN1 and Tau was suggested by another [42]. However, no significant overlap between BIN1 and neurofibrillary tangles was seen in a more recent study [43]. Previously reported effects of experimentally altered BIN1 expression on Tau pathology have been controversial too. While loss of BIN1 was shown to reduce Tau mediated neurodegeneration in Drosophila [42], it reduced the propagation of Tau pathology in an *in vitro* system [44]. We did not observe a significant effect of BIN1 downregulation on wildtype total Tau or pTau(Thr231) levels in our study. Effects on Tau pathology progression as seen in the earlier studies might possibly

require a disease context as provided by the presence of Tau mutations in these studies.

Conclusions

Our results show that acute downregulation of the studied LOAD risk genes in mouse primary neurons does not significantly alter γ-secretase mediated APP processing, Tau levels or Tau(Thr231)-phosphorylation. Susceptibility to AD through these genes might be conferred through other mechanisms, for example modulation of Aβ clearance or Aβ aggregation/toxicity as it has been proposed by some of the studies discussed above and/or Aβ and Tau-independent signaling pathways that directly affect neuronal function.

Limitations

There are three important limitations to our study:
1.) The *in vitro* character of the study: The simple intercellular connections that are formed in a primary neuronal culture do not allow to model the complex interactions that occur in an intact brain. Any effects that depend on the network activity within or between certain brain areas can therefore not be assessed by our model.
2.) The limited time frame during which we assessed the role of the studied Alzgenes: Knockdowns were performed for about 72 h. Any effects on APP processing or Tau that build up only over a longer period, as it might be the case in AD, would not be detected in our system.
3.) The age of the primary neurons: Primary neuronal cultures were prepared from embryonic neurons, thus neurons in their very early developmental stage. LOAD, however, is a disease of the aged brain. The expression profiles of the tested Alzgenes and their interaction partners might be very different in young and old neurons.
We are now planning to study the role of the respective risk gene variants in neurons derived from human induced pluripotent stem cells (iPSCs) which have been modified using CRISPR/Cas9 technology for targeted genome editing to introduce the respective LOAD associated genetic polymorphisms. This will not only allow us to assess long-term effects of the respective risk variants on APP processing and Tau homeostasis but also to test whether these genes would have any roles in neuronal physiology and function in an Aβ and Tau independent manner.

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201803000014.

Funding Statement

L.R. was supported by the Swiss National Science Foundation grants (Sinergia and Core Interdisciplinary grants), by the Velux Foundation, the Novartis Foundation grant, the Cure Alzheimer Foundation, the Hurka Stiftung, the Bangerter Stiftung, the Baugarten Stiftung and the Synapsis Foundation. G.S. was supported by an EMBO long-term fellowship (ALTF 668-2011) and the University of Zurich's Forschungskredit (K-82033-02-01).

Acknowledgements

We thank the lab members for their stimulating discussions.

Ethics Statement

All animal experiments were done according to the guidelines of and approved by the veterinary office of the Canton of Zürich, Switzerland.

Citations

[1] Bali et al. "Role of genes linked to sporadic Alzheimer's disease risk in the production of beta-amyloid peptides". In: *Proc Natl Acad Sci U S A* 109.38 (2012), pp. 15307–11. URL: https://www.ncbi.nlm.nih.gov/pubmed/22949636.

[2] Bertram et al. "Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database". In: *Nat Genet* 39.1 (2007), pp. 17–23. URL: https://www.ncbi.nlm.nih.gov/pubmed/17192785.

[3] Ashford et al. "Non-familial Alzheimer's disease is mainly due to genetic factors". In: *J Alzheimers Dis* 4.3 (2002), pp. 169–77. URL: https://www.ncbi.nlm.nih.gov/pubmed/12226536.

[4] Ertekin-Taner and N. "Genetics of Alzheimer's disease: a centennial review". In: *Neurol Clin* 25.3 (2007), pp. 611–67, v. URL: https://www.ncbi.nlm.nih.gov/pubmed/17659183.

[5] Ertekin-Taner and N. "Genetics of Alzheimer disease in the pre- and post-GWAS era". In: *Alzheimers Res Ther* 2.1 (2010), p. 3. URL: https://www.ncbi.nlm.nih.gov/pubmed/20236449.

[6] Gatz et al. "Role of genes and environments for explaining Alzheimer disease". In: *Arch Gen Psychiatry* 63.2 (2006), pp. 168–74. URL: https://www.ncbi.nlm.nih.gov/pubmed/16461860.

[7] Livingston Gill et al. "Dementia prevention, intervention, and care". In: *The Lancet* 390.10113 (2017), pp. 2673–2734. DOI: 10.1016/s0140-6736(17)31363-6. URL: https://doi.org/10.1016/s0140-6736(17)31363-6.

[8] Mawuenyega et al. "Decreased clearance of CNS beta-amyloid in Alzheimer's disease". In: *Science* 330.6012 (2010), p. 1774. URL: https://www.ncbi.nlm.nih.gov/pubmed/21148344.

[9] Weller et al. "Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease". In: *Brain Pathol* 18.2 (2008), pp. 253–66. URL: https://www.ncbi.nlm.nih.gov/pubmed/18363936.

[10] Gong et al. "Apolipoprotein E (ApoE) isoform-dependent lipid release from astrocytes prepared from human ApoE3 and ApoE4 knock-in mice". In: *J Biol Chem* 277.33 (2002), pp. 29919–26. URL: https://www.ncbi.nlm.nih.gov/pubmed/12042316.

[11] Deane et al. "apoE isoform-specific disruption of amyloid beta peptide clearance from mouse brain". In: *J Clin Invest* 118.12 (2008), pp. 4002–13. URL: https://www.ncbi.nlm.nih.gov/pubmed/19033669.

[12] Bell et al. "Transport pathways for clearance of human Alzheimer's amyloid beta-peptide and apolipoproteins E and J in the mouse central nervous system". In: *J Cereb Blood Flow Metab* 27.5 (2007), pp. 909–18. URL: https://www.ncbi.nlm.nih.gov/pubmed/17077814.

[13] Merino-Zamorano et al. "Modulation of Amyloid-beta1-40 Transport by ApoA1 and ApoJ Across an in vitro Model of the Blood-Brain Barrier". In: *J Alzheimers Dis* 53.2 (2016), pp. 677–91. URL: https://www.ncbi.nlm.nih.gov/pubmed/27232214.

[14] Zlokovic et al. "Glycoprotein 330/megalin: probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer disease amyloid beta at the blood-brain and blood-cerebrospinal fluid barriers". In: *Proc Natl Acad Sci U S A* 93.9 (1996), pp. 4229–34. URL: https://www.ncbi.nlm.nih.gov/pubmed/8633046.

[15] Zhao et al. "Central role for PICALM in amyloid-beta blood-brain barrier transcytosis and clearance". In: *Nat Neurosci* 18.7 (2015), pp. 978–87. URL: https://www.ncbi.nlm.nih.gov/pubmed/26005850.

[16] Koffie et al. "Apolipoprotein E4 effects in Alzheimer's disease are mediated by synaptotoxic oligomeric amyloid-beta". In: *Brain* 135.Pt 7 (2012), pp. 2155–68. URL: https://www.ncbi.nlm.nih.gov/pubmed/22637583.

[17] Beeg et al. "Clusterin Binds to Abeta1-42 Oligomers with High Affinity and Interferes with Peptide Aggregation by Inhibiting Primary and Secondary Nucleation". In: *J Biol Chem* 291.13 (2016), pp. 6958–66. URL: https://www.ncbi.nlm.nih.gov/pubmed/26884339.

[18] Sastre et al. "Binding of cystatin C to Alzheimer's amyloid beta inhibits in vitro amyloid fibril formation". In: *Neurobiol Aging* 25.8 (2004), pp. 1033–43. URL: https://www.ncbi.nlm.nih.gov/pubmed/15212828.

[19] Selenica et al. "Cystatin C reduces the in vitro formation of soluble Abeta1-42 oligomers and protofibrils". In: *Scand J Clin Lab Invest* 67.2 (2007), pp. 179–90. URL: https://www.ncbi.nlm.nih.gov/pubmed/17365997.

[20] Tizon et al. "Cystatin C protects neuronal cells from amyloid-beta-induced toxicity". In: *J Alzheimers Dis* 19.3 (2010), pp. 885–94. URL: https://www.ncbi.nlm.nih.gov/pubmed/20157244.

[21] Barry et al. "Alzheimer's disease brain-derived amyloid-beta-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein". In: *J Neurosci* 31.20 (2011), pp. 7259–63. URL: https://www.ncbi.nlm.nih.gov/pubmed/21593310.

[22] Freir et al. "Interaction between prion protein and toxic amyloid beta assemblies can be therapeutically targeted at multiple sites". In: *Nat Commun* 2 (2011), p. 336. URL: https://www.ncbi.nlm.nih.gov/pubmed/21654636.

[23] Gimbel et al. "Memory impairment in transgenic Alzheimer mice requires cellular prion protein". In: *J Neurosci* 30.18 (2010), pp. 6367–74. URL: https://www.ncbi.nlm.nih.gov/pubmed/20445063.

[24] Hu et al. "mGlu5 receptors and cellular prion protein mediate amyloid-beta-facilitated synaptic long-term depression in vivo". In: *Nat Commun* 5 (2014), p. 3374. URL: https://www.ncbi.nlm.nih.gov/pubmed/24594908.

[25] Klyubin et al. "Peripheral administration of a humanized anti-PrP antibody blocks Alzheimer's disease Abeta synaptotoxicity". In: *J Neurosci* 34.18 (2014), pp. 6140–5. URL: https://www.ncbi.nlm.nih.gov/pubmed/24790184.

[26] Lauren et al. "Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers". In: *Nature* 457.7233 (2009), pp. 1128–32. URL: https://www.ncbi.nlm.nih.gov/pubmed/19242475.

[27] Um et al. "Alzheimer amyloid-beta oligomer bound to postsynaptic prion protein activates Fyn to impair neurons". In: *Nat Neurosci* 15.9 (2012), pp. 1227–35. URL: https://www.ncbi.nlm.nih.gov/pubmed/22820466.

[28] Balducci et al. "Synthetic amyloid-beta oligomers impair long-term memory independently of cellular prion protein". In: *Proc Natl Acad Sci U S A* 107.5 (2010), pp. 2295–300. URL: https://www.ncbi.nlm.nih.gov/pubmed/20133875.

[29] Calella et al. "Prion protein and Abeta-related synaptic toxicity impairment". In: *EMBO Mol Med* 2.8 (2010), pp. 306–14. URL: https://www.ncbi.nlm.nih.gov/pubmed/20665634.

[30] Kessels et al. "The prion protein as a receptor for amyloid-beta". In: *Nature* 466.7308 (2010), E3–4, E3–4. URL: https://www.ncbi.nlm.nih.gov/pubmed/20703260.

[31] Ye et al. "Apolipoprotein (apo) E4 enhances amyloid beta peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target". In: *Proc Natl Acad Sci U S A* 102.51 (2005), pp. 18700–5. URL: https://www.ncbi.nlm.nih.gov/pubmed/16344478.

[32] Huang et al. “ApoE2, ApoE3, and ApoE4 Differentially Stimulate APP Transcription and Abeta Secretion”. In: *Cell* 168.3 (2017), 427–441 e21. URL: https://www.ncbi.nlm.nih.gov/pubmed/28111074.

[33] Ubelmann et al. “Bin1 and CD2AP polarise the endocytic generation of beta-amyloid”. In: *EMBO Rep* 18.1 (2017), pp. 102–122. URL: https://www.ncbi.nlm.nih.gov/pubmed/27895104.

[34] Miyagawa et al. “BIN1 regulates BACE1 intracellular trafficking and amyloid-beta production”. In: *Hum Mol Genet* 25.14 (2016), pp. 2948–2958. URL: https://www.ncbi.nlm.nih.gov/pubmed/27179792.

[35] Glennon et al. “BIN1 is decreased in sporadic but not familial Alzheimer’s disease or in aging”. In: *PLoS One* 8.10 (2013), e78806. URL: https://www.ncbi.nlm.nih.gov/pubmed/24205320.

[36] Xiao et al. “Role of phosphatidylinositol clathrin assembly lymphoid-myeloid leukemia (PICALM) in intracellular amyloid precursor protein (APP) processing and amyloid plaque pathogenesis”. In: *J Biol Chem* 287.25 (2012), pp. 21279–89. URL: https://www.ncbi.nlm.nih.gov/pubmed/22539346.

[37] Wu et al. “The clathrin assembly protein AP180 regulates the generation of amyloid-beta peptide”. In: *Biochem Biophys Res Commun* 385.2 (2009), pp. 247–50. URL: https://www.ncbi.nlm.nih.gov/pubmed/19450545.

[38] Parkin et al. “Cellular prion protein regulates beta-secretase cleavage of the Alzheimer’s amyloid precursor protein”. In: *Proc Natl Acad Sci U S A* 104.26 (2007), pp. 11062–7. URL: https://www.ncbi.nlm.nih.gov/pubmed/17573534.

[39] Siegel Gabriele et al. “The Alzheimer’s Disease γ-Secretase Generates Higher 42:40 Ratios for β-Amyloid Than for p3 Peptides”. In: *Cell Reports* 19.10 (2017), pp. 1967–1976. DOI: 10.1016/j.celrep.2017.05.034. URL: https://doi.org/10.1016/j.celrep.2017.05.034.

[40] Holler et al. “Bridging integrator 1 (BIN1) protein expression increases in the Alzheimer’s disease brain and correlates with neurofibrillary tangle pathology”. In: *J Alzheimers Dis* 42.4 (2014), pp. 1221–7. URL: https://www.ncbi.nlm.nih.gov/pubmed/25024306.

[41] Wang et al. “Bridging Integrator 1 (BIN1) Genotypes Mediate Alzheimer’s Disease Risk by Altering Neuronal Degeneration”. In: *J Alzheimers Dis* 52.1 (2016), pp. 179–90. URL: https://www.ncbi.nlm.nih.gov/pubmed/27003210.

[42] Chapuis et al. “Increased expression of BIN1 mediates Alzheimer genetic risk by modulating tau pathology”. In: *Mol Psychiatry* 18.11 (2013), pp. 1225–34. URL: https://www.ncbi.nlm.nih.gov/pubmed/23399914.

[43] De Rossi Pierre et al. “BIN1 localization is distinct from Tau tangles in Alzheimer’s disease”. In: *Matters* (2017). DOI: 10.19185/matters.201611000018. URL: https://doi.org/10.19185/matters.201611000018.

[44] Calafate et al. “Loss of Bin1 Promotes the Propagation of Tau Pathology”. In: *Cell Rep* 17.4 (2016), pp. 931–940. URL: https://www.ncbi.nlm.nih.gov/pubmed/27760323.