Neurobiology of Aging 118 (2022) 106–107

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging.org

Negative results

# Elevated levels of tripeptidyl peptidase 1 do not ameliorate pathogenesis in a mouse model of Alzheimer disease

David E. Sleat[a,b,π,*], Isabella Maita[c,d,π], Whitney Banach-Petrosky[a], Katherine E. Larrimore[a], Tonia Liu[c], Dana Cruz[e], Lukas Baker[e], Frederick R. Maxfield[e], Benjamin Samuels[c,d], Peter Lobel[a,b,*]

[a] Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ, USA
[b] Department of Biochemistry and Molecular Biology, Robert Wood Johnson Medical School, Rutgers Biomedical Health Sciences, Rutgers University, Piscataway, NJ, USA
[c] Behavioral and Systems Neuroscience, Department of Psychology, Rutgers University-New Brunswick, Piscataway, NJ, USA
[d] Neuroscience Graduate Program, Rutgers-Robert Wood Johnson Medical School, Piscataway, NJ, USA
[e] Department of Biochemistry, Weill Cornell Medical College, New York, NY, USA

ARTICLE INFO

*Article history:*
Received 17 March 2022
Revised 14 June 2022
Accepted 30 June 2022
Available online 7 July 2022

*Keywords:*
Tripeptidyl peptidase 1
Alzheimer disease
Amyloid beta degradation
Mouse models
Cognitive/behavioral deficits
Pathology

ABSTRACT

One potential therapeutic strategy for Alzheimer disease (AD) is to promote degradation of amyloid beta (A$\beta$) and we previously demonstrated that the lysosomal protease tripeptidyl peptidase 1 (TPP1) can degrade A$\beta$ fibrils in vitro. In this study, we tested the hypothesis that increasing levels of TPP1 might promote degradation of A$\beta$ under physiological conditions, slowing or preventing its accumulation in the brain with subsequent therapeutic benefits. We used 2 approaches to increase TPP1 activity in the brain of J20 mice, an AD model that accumulates A$\beta$ and exhibits cognitive defects: transgenic overexpression of TPP1 in the brain and a pharmacological approach employing administration of recombinant TPP1. While we clearly observed the expected AD phenotype of the J20 mice based on pathology and measurement of behavioral and cognitive defects, we found that elevation of TPP1 activity by either experimental approach failed to have any measurable beneficial effect on disease phenotype.

© 2022 Elsevier Inc. All rights reserved.

## Introduction

The precise relationship between plaque formation and clinical presentation in AD is unclear and remains controversial, but interventions that enhance clearance of A$\beta$ peptides and reduce plaque formation may represent potential therapeutic avenues (Boland et al., 2018; Sikanyika et al., 2019). However, the insoluble $\beta$-sheet structure of A$\beta$ makes these peptides resistant to proteolysis (Antzutkin et al., 2002; Petkova et al., 2004).

The lysosome utilizes a variety of acid hydrolases to degrade macromolecules, and lysosomal activities may play a role in the degradation of A$\beta$ (Van Acker et al., 2021). For example, dysregulation of the endosomal-lysosomal system in microglia is associated with A$\beta$ accumulation (Nixon, 2017), and microglia may play a role in scavenging and degrading A$\beta$ (Majumdar et al., 2008; Majumdar et al., 2007). In addition, there is evidence that defects in lysosomal degradation of A$\beta$ within neurons can lead to plaque accumulation (Lee et al., 2022).

A lysosomal protease, tripeptidyl peptidase 1 (TPP1), has been demonstrated to degrade A$\beta$ fibrils efficiently *in vitro* (Sole-Domenech et al., 2018). TPP1 is synthesized as a proenzyme and nascent TPP1 is autocatalytically activated by removal of its propiece upon delivery to the lysosome (Lin et al., 2001). The goal of this study was to investigate the potential role of TPP1 in degradation of A$\beta$ and AD pathogenesis under physiological conditions in a mouse model of AD.

## Methods

J20 transgenic AD mice express mutant human amyloid precursor protein and they display a robust disease phenotype (Webster et al., 2014). The aim of this study was to determine whether elevated levels of TPP1, achieved by genetic overexpres-

*Abbreviations:* AD, Alzheimer disease; A$\beta$, amyloid beta; TPP1, Tripeptidyl peptidase 1; fA$\beta$, fibrillar A$\beta$.

* Corresponding author
π These authors contributed equally

0197-4580/$ – see front matter © 2022 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.neurobiolaging.2022.06.012

sion of murine TPP1 or administration of recombinant human proTPP1 (which is taken up by receptor-mediated endocytosis and converted to the active form upon delivery to the lysosome), could ameliorate pathology and cognitive behavioral deficits in the J20 mouse. Behavioral methods used to evaluate AD progression included Morris Water Maze, Novel Object Recognition, and Contextual Fear Conditioning.

## Results

A transgenic mouse that uses the cytomegalovirus enhancer/chicken-actin-globin (CAG) promoter (Miyazaki et al., 1989) to overexpress murine TPP1 at ~10-fold higher than normal levels throughout the brain (Nemtsova et al., 2018) was crossed with the J20 mice to determine the effect of elevated brain TPP1 on AD phenotype. Given the constitutive nature of this promoter, we expected elevated expression of TPP1 in all cells of the brain and immunohistochemical analyses confirmed this to be the case. In addition, we specifically demonstrated over-expression in NeuN- and Iba1-positive neurons and glia, respectively. Behavioral assays clearly indicated cognitive deficits in J20 mice and pathology analyses indicated an accumulation of amyloid plaque in the hippocampus and cortex at ~7 months of age. However, overexpression of TPP1 had no significant effect on behavioral deficits or pathology in J20 mice.

Cellular dysfunction as a result of A$\beta$ accumulation could interfere with the trafficking of newly synthesized lysosomal proteins including transgenically-expressed TPP1. We, therefore, conducted a parallel study in which recombinant human TPP1 was administered directly to J20 mice with the rationale that endocytic delivery of exogenous TPP1 bypasses the cellular biosynthetic pathway, circumventing any potential A$\beta$-related trafficking defects. Starting at age ~6 months, J20 mice were treated once a week for four weeks with recombinant human TPP1 together with a peptide that promotes the passage of TPP1 across the blood-brain barrier (Meng et al., 2014). Administration of exogenous TPP1 had no significant effect on behavioral deficits or pathology in J20 mice compared to J20 mice treated with vehicle alone.

## Conclusion

Based on pathology and measurement of behavioral and cognitive defects, we found that elevation of TPP1 activity by either of the two experimental approaches had no measurable beneficial effect on the disease phenotype of the J20 AD mice.

## Disclosure statement

Peter Lobel and David E. Sleat have received royalty payments as Inventors on Patent 8029781 “Methods of treating a deficiency of functional tripeptidyl peptidase I (CLN2) protein,” which is licensed to BioMarin Pharmaceutical Inc. Other authors have declared that no conflicts of interest exist.

## CRediT authorship contribution statement

**David E. Sleat:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Isabella Maita:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Whitney Banach-Petrosky:** Investigation, Writing – review & editing. **Katherine E. Larrimore:** Investigation, Writing – review & editing. **Tonia Liu:** Investigation, Writing – review & editing. **Dana Cruz:** Investigation, Writing – review & editing. **Lukas Baker:** Investigation, Writing – review & editing. **Frederick R. Maxfield:** Writing – review & editing, Supervision, Funding acquisition. **Benjamin Samuels:** Methodology, Formal analysis, Writing – review & editing, Supervision. **Peter Lobel:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision, Funding acquisition.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2022.06.012.

## References


Antzutkin, O.N., Leapman, R.D., Balbach, J.J., Tycko, R., 2002. Supramolecular structural constraints on Alzheimer's beta-amyloid fibrils from electron microscopy and solid-state nuclear magnetic resonance. Biochemistry. 41 (51), 15436–15450.

Boland, B., Yu, W.H., Corti, O., Mollereau, B., Henriques, A., Bezard, E., Pastores, G.M., Rubinsztein, D.C., Nixon, R.A., Duchen, M.R., Mallucci, G.R., Kroemer, G., Levine, B., Eskelinen, E.L., Mochel, F., Spedding, M., Louis, C., Martin, O.R., Millan, M.J., 2018. Promoting the clearance of neurotoxic proteins in neurodegenerative disorders of ageing. Nat. Rev. Drug. Discov. 17 (9), 660–688.

Lee, J.H., Yang, D.S., Goulbourne, C.N., Im, E., Stavrides, P., Pensalfini, A., Chan, H., Bouchet-Marquis, C., Bleiwas, C., Berg, M.J., Huo, C., Peddy, J., Pawlik, M., Levy, E., Rao, M., Staufenbiel, M., Nixon, R.A., 2022. Faulty autolysosome acidification in Alzheimer's disease mouse models induces autophagic build-up of Abeta in neurons, yielding senile plaques. Nat. Neurosci. 25 (6), 688–701.

Lin, L., Sohar, I., Lackland, H., Lobel, P., 2001. The human CLN2 protein/tripeptidyl-peptidase I is a serine protease that autoactivates at acidic pH. J. Biol. Chem. 276 (3), 2249–2255.

Majumdar, A., Chung, H., Dolios, G., Wang, R., Asamoah, N., Lobel, P., Maxfield, F.R., 2008. Degradation of fibrillar forms of Alzheimer's amyloid beta-peptide by macrophages. Neurobiol. Aging. 29 (5), 707–715.

Majumdar, A., Cruz, D., Asamoah, N., Buxbaum, A., Sohar, I., Lobel, P., Maxfield, F.R., 2007. Activation of microglia acidifies lysosomes and leads to degradation of Alzheimer amyloid fibrils. Mol. Biol. Cell. 18 (4), 1490–1496.

Meng, Y., Sohar, I., Sleat, D.E., Richardson, J.R., Reuhl, K.R., Jenkins, R.B., Sarkar, G., Lobel, P., 2014. Effective intravenous therapy for neurodegenerative disease with a therapeutic enzyme and a peptide that mediates delivery to the brain. Mol. Ther. 22 (3), 547–553.

Miyazaki, J., Takaki, S., Araki, K., Tashiro, F., Tominaga, A., Takatsu, K., Yamamura, K., 1989. Expression vector system based on the chicken beta-actin promoter directs efficient production of interleukin-5. Gene. 79 (2), 269–277.

Nemtsova, Y., Wiseman, J.A., El-Banna, M., Lobel, P., Sleat, D.E., 2018. Inducible transgenic expression of tripeptidyl peptidase 1 in a mouse model of late-infantile neuronal ceroid lipofuscinosis. PLoS One. 13 (2), e0192286.

Nixon, R.A., 2017. Amyloid precursor protein and endosomal-lysosomal dysfunction in Alzheimer's disease: inseparable partners in a multifactorial disease. FASEB J. 31 (7), 2729–2743.

Petkova, A.T., Buntkowsky, G., Dyda, F., Leapman, R.D., Yau, W.M., Tycko, R., 2004. Solid state NMR reveals a pH-dependent antiparallel beta-sheet registry in fibrils formed by a beta-amyloid peptide. J. Mol. Biol. 335 (1), 247–260.

Sikanyika, N.L., Parkington, H.C., Smith, A.I., Kuruppu, S., 2019. Powering amyloid beta degrading enzymes: a possible therapy for Alzheimer's Disease. Neurochem. Res. 44 (6), 1289–1296.

Sole-Domenech, S., Rojas, A.V., Maisuradze, G.G., Scheraga, H.A., Lobel, P., Maxfield, F.R., 2018. Lysosomal enzyme tripeptidyl peptidase 1 destabilizes fibrillar Abeta by multiple endoproteolytic cleavages within the beta-sheet domain. Proc. Natl. Acad. Sci. U S A. 115 (7), 1493–1498.

Van Acker, Z.P., Perdok, A., Bretou, M., Annaert, W., 2021. The microglial lysosomal system in Alzheimer's disease: Guardian against proteinopathy. Ageing. Res. Rev. 71, 101444.

Webster, S.J., Bachstetter, A.D., Nelson, P.T., Schmitt, F.A., Van Eldik, L.J., 2014. Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. Front. Genet. 5, 88.