

## Commentary

# Modelling Alzheimer's disease in a dish: dissecting amyloid- $\beta$ metabolism in human neurons

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This scientific commentary refers to 'Inhibition of insulin-degrading enzyme in human neurons promotes amyloid- $\beta$  deposition' by Rowland et al. (<https://doi.org/10.1042/NS20230016>). Insulin-degrading enzyme (IDE) and neprilysin (NEP) have been proposed as two A $\beta$ -degrading enzymes supported by human genetics and *in vivo* data. Rowland et al. provide complementary evidence of a key role for IDE in A $\beta$  metabolism in human-induced pluripotent stem cell (iPSC)-derived cortical neurons.

## The value of a complementary mechanistic approach to manipulate A $\beta$ deposition

Alzheimer's disease (AD) is a devastating, progressive neurodegenerative disease characterised by the aggregation and deposition of A $\beta$  to form A $\beta$  plaques in the brain. This has been proposed as the initiation event triggering downstream biochemical and cellular dysfunction ultimately leading to the clinical phase of the disease. Although we are living in an exciting age with disease-modifying A $\beta$ -targeting immunotherapies reaching the clinic, the effects are modest and are associated with severe side effects in some patients. Whilst we may expect improvements to these treatments over time, there nevertheless remains an unmet need to substantially reduce the progression of neurodegeneration in AD, with a view to developing complementary approaches promoting A $\beta$  degradation.

## Previous evidence for IDE and NEP involvement in A $\beta$ metabolism

A $\beta$  clearance is mediated through proteolytic degradation, which is driven by the action of multiple proteases. Two such enzymes with a strong evidence base as being the key players involved are the zinc metalloproteases insulin-degrading enzyme (IDE) and neprilysin (NEP).

Human genetic studies have implicated IDE in late-onset AD [1] and age of AD onset [2]. Extracellular A $\beta$  has also been shown to be modulated by IDE-mediated proteolysis in primary rat cortical neurons [3], whilst IDE has been shown to be a major A $\beta$ -degrading enzyme *in vivo* with *Ide*<sup>-/-</sup> mice reported to have a higher load of endogenous A $\beta$ , albeit modest [4]. Additionally, IDE overexpression in APP transgenic mice lowered brain A $\beta$  levels abrogating A $\beta$  plaque formation [5].

There is also a wide range of literature supporting the role of NEP in A $\beta$  clearance. NEP levels and activity are reportedly lower in AD patient brain (reviewed in [6]) and there is human genetics evidence that variants at the *MME* locus (encoding NEP) increase AD risk in certain human populations [7,8]. Interestingly, AD risk is further increased if individuals simultaneously harbour risk variants at the *IDE* locus [9]. There is also substantial *in vivo* evidence for a role of NEP in A $\beta$  metabolism with mice lacking NEP exhibiting an increase in A $\beta$  [10]. Concurrently, overexpression of NEP in AD mouse models has been shown to lead to lower brain A $\beta$  levels, reduced plaques and increased survival [5,11–13].

Taken together, these and similar studies provide strong evidence for a role of IDE and NEP in A $\beta$  degradation. However, one question that remains unaddressed is whether these enzymes contribute to

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A $\beta$  metabolism specifically in human neurons, which would provide complementary data to the evidence base.

## IDE modulates A $\beta$ clearance in human iPSC-derived neurons

Rowland et al. [14] provide complementary evidence to extend this work with the authors demonstrating that IDE is the major contributor to A $\beta$  degradation in human-induced pluripotent stem cell (iPSC)-derived cortical neurons. They not only show this in human iPSC-derived neuronal lysates, but further validated these findings using an elegant 3D extracellular matrix (ECM) model with embedded human iPSC-derived cortical neurons, allowing the visualisation of A $\beta$  deposition with the resulting plaques showing similar immunological properties to deposits in human AD brain. This experimental iPSC model was derived from individuals without AD and without familial AD mutations and interestingly shows a low baseline level of plaque formation.

They provide convincing evidence that IDE inhibition induced by three different inhibitors with distinct mechanisms of action promoted A $\beta$  deposition in neurons derived from two independent iPSC lines. Considering the IDE inhibitors reduced A $\beta$  degradation by >65%, this clearly nominates IDE as the predominant A $\beta$ -degrading protease in this system, providing experimental support that enhancing IDE activity could be harnessed therapeutically as a complementary A $\beta$ -lowering approach in human neurons.

## No effect of NEP inhibition on A $\beta$ metabolism in human iPSC-derived neurons

Despite the strong evidence for a role of NEP in A $\beta$  metabolism from *in vivo* studies, Rowland et al. [14] show that NEP does not appear to have a major role in A $\beta$  degradation in iPSC-derived cortical neurons. Notably, NEP has been reported to robustly degrade disease-associated oligomeric forms of A $\beta$ 40 as well as oligomeric forms of A $\beta$ 42 *in vitro* [15]. Therefore, further work is warranted to investigate metabolism of longer, more aggregation prone A $\beta$  forms in the human cellular context, including using models more relevant to the disease context such as using iPSCs derived from aged AD patients or patients with familial AD mutations. Finally, it is well established that non-neuronal cell types provide significant contributions in neurodegeneration; thus, further work is, therefore, also needed to decipher A $\beta$  metabolism in more diverse human cellular contexts.

## Implications for therapeutic strategies

This study provides a human neuronal cellular context promoting the case for modulating IDE activity as a therapeutic strategy for AD and patients with cerebral  $\beta$ -amyloid angiopathy. Promoting A $\beta$  clearance via modulating endogenous metabolic pathways has the potential to clear build-up of abnormal pathological A $\beta$  assemblies and/or to slow the progress of pathological A $\beta$  templated misfolding and propagation via lowering levels of non-disease associated A $\beta$  substrate. The capacity to visualise and quantify A $\beta$  in this system provides a platform for investigating a wider scope of disease contexts and modulating factors for A $\beta$  lowering.

### Data Availability

NA

### Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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### CRedit Author Contribution

**Elizabeth Hill:** Writing—original draft, Writing—review & editing. **Thomas J. Cunningham:** Writing—original draft, Writing—review & editing.

### Abbreviations

AD, Alzheimer's disease; ECM, extracellular matrix; IDE, insulin-degrading enzyme; iPSC, induced pluripotent stem cell; NEP, neprilysin.

## References

- 1 Bertram, L., Blacker, D., Mullin, K., Keeney, D., Jones, J., Basu, S. et al. (2000) Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science (New York, NY)* **290**, 2302–2303, <https://doi.org/10.1126/science.290.5500.2302>
- 2 Li, Y.J., Scott, W.K., Hedges, D.J., Zhang, F., Gaskell, P.C., Nance, M.A. et al. (2002) Age at onset in two common neurodegenerative diseases is genetically controlled. *Am. J. Hum. Genet.* **70**, 985–993, <https://doi.org/10.1086/339815>
- 3 Vekrellis, K., Ye, Z., Qiu, W.Q., Walsh, D., Hartley, D., Chesneau, V. et al. (2000) Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J. Neurosci.: Off. J. Soc. Neurosci.* **20**, 1657–1665, <https://doi.org/10.1523/JNEUROSCI.20-05-01657.2000>
- 4 Farris, W., Mansourian, S., Chang, Y., Lindsley, L., Eckman, E.A., Frosch, M.P. et al. (2003) Insulin-degrading enzyme regulates the levels of insulin, amyloid  $\beta$ -protein, and the  $\beta$ -amyloid precursor protein intracellular domain in vivo. *Proc. Natl. Acad. Sci.* **100**, 4162–4167, <https://doi.org/10.1073/pnas.0230450100>
- 5 Leissring, M.A., Farris, W., Chang, A.Y., Walsh, D.M., Wu, X., Sun, X. et al. (2003) Enhanced proteolysis of  $\beta$ -amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. *Neuron* **40**, 1087–1093, [https://doi.org/10.1016/S0896-6273\(03\)00787-6](https://doi.org/10.1016/S0896-6273(03)00787-6)
- 6 Grimm, M.O., Mett, J., Stahlmann, C.P., Hauptenthal, V.J., Zimmer, V.C. and Hartmann, T. (2013) Neprilysin and A $\beta$  clearance: impact of the APP intracellular domain in NEP regulation and implications in Alzheimer's disease. *Front Aging Neurosci.* **5**, 98, <https://doi.org/10.3389/fnagi.2013.00098>
- 7 Wang, H.Z., Bi, R., Zhang, D.F., Li, G.D., Ma, X.H., Fang, Y. et al. (2016) Neprilysin confers genetic susceptibility to Alzheimer's disease in Han Chinese. *Mol. Neurobiol.* **53**, 4883–4892, <https://doi.org/10.1007/s12035-015-9411-z>
- 8 Helisalmi, S., Hiltunen, M., Vepsäläinen, S., Iivonen, S., Mannermaa, A., Lehtovirta, M. et al. (2004) Polymorphisms in neprilysin gene affect the risk of Alzheimer's disease in Finnish patients. *J. Neurol. Neurosurg. Psychiatry* **75**, 1746–1748, <https://doi.org/10.1136/jnnp.2004.036574>
- 9 Vepsäläinen, S., Helisalmi, S., Mannermaa, A., Pirttilä, T., Soininen, H. and Hiltunen, M. (2009) Combined risk effects of IDE and NEP gene variants on Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **80**, 1268–1270, <https://doi.org/10.1136/jnnp.2008.160002>
- 10 Iwata, N., Tsubuki, S., Takaki, Y., Shirotani, K., Lu, B., Gerard, N.P. et al. (2001) Metabolic regulation of brain A $\beta$  by neprilysin. *Science (New York, NY)* **292**, 1550–1552, <https://doi.org/10.1126/science.1059946>
- 11 Marr, R.A., Rockenstein, E., Mukherjee, A., Kindy, M.S., Hersh, L.B., Gage, F.H. et al. (2003) Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. *J. Neurosci.: Off. J. Soc. Neurosci.* **23**, 1992–1996, <https://doi.org/10.1523/JNEUROSCI.23-06-01992.2003>
- 12 Iwata, N., Mizukami, H., Shirotani, K., Takaki, Y., Muramatsu, S., Lu, B. et al. (2004) Presynaptic localization of neprilysin contributes to efficient clearance of amyloid-beta peptide in mouse brain. *J. Neurosci.: Off. J. Soc. Neurosci.* **24**, 991–998, <https://doi.org/10.1523/JNEUROSCI.4792-03.2004>
- 13 Poirier, R., Wolfer, D.P., Welzl, H., Tracy, J., Galsworthy, M.J., Nitsch, R.M. et al. (2006) Neuronal neprilysin overexpression is associated with attenuation of A $\beta$ -related spatial memory deficit. *Neurobiol. Dis.* **24**, 475–483, <https://doi.org/10.1016/j.nbd.2006.08.003>
- 14 Rowland, H.A., Moxon, S.R., Corbett, N.J., Hanson, K., Fisher, K., Kellett, K.A.B. et al. (2023) Inhibition of insulin-degrading enzyme in human neurons promotes amyloid- $\beta$  deposition. *Neuronal Signaling* NS20230016, <https://doi.org/10.1042/NS20230016>
- 15 Kanemitsu, H., Tomiyama, T. and Mori, H. (2003) Human neprilysin is capable of degrading amyloid beta peptide not only in the monomeric form but also the pathological oligomeric form. *Neurosci. Lett.* **350**, 113–116, [https://doi.org/10.1016/S0304-3940\(03\)00898-X](https://doi.org/10.1016/S0304-3940(03)00898-X)