

ALZHEIMER'S DISEASE

Exploring the origins of nucleation

An approach called deep mutational scanning is improving our understanding of amyloid beta aggregation.

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Related research article Seuma M, Faure A, Badia M, Lehner B, Bolognesi B. 2021. The genetic landscape for amyloid beta fibril nucleation accurately discriminates familial Alzheimer's disease mutations. *eLife* 10:e63364. doi: [10.7554/eLife.63364](https://doi.org/10.7554/eLife.63364)

Alzheimer's disease is a progressive neurodegenerative disease that initially compromises memory and ultimately stops people from being able to perform everyday tasks. The exact causes of the disease are still unknown, but genetic, lifestyle and environmental factors could all play a role, with age being the strongest risk factor for developing the condition. The lack of treatment to halt or slow down the progression Alzheimer's disease makes it one of the greatest challenges of our times.

Alzheimer's disease begins decades before the appearance of clinical symptoms. It is thought that changes in the metabolism of a peptide called amyloid beta (A β) lead to its misfolding. Although the details are not fully understood, scientists propose that the accumulation of these misfolded molecules in the brain triggers a series of molecular events which, in turn, lead to neuroinflammation, the aggregation of tau proteins in neurons, and eventually, neuronal death (*Selkoe and Hardy, 2016*).

Preventing the build-up of A β has been explored as a potential therapeutic approach. However, designing strategies that specifically target the formation of neurotoxic A β , rather than the aggregation of A β in general, requires a detailed understanding (in terms of both mechanisms and kinetics) of how the protein misfolds and how this is connected to the disease (*Yang et al., 2017; Brinkmalm et al., 2019*). Now, in eLife, Benedetta Bolognesi (IBEC in Barcelona), Ben Lehner (the Center for Genomic Regulation, also in Barcelona) and colleagues – including Mireia Seuma as first author, Andre Faure and Marta Badia – report new insights into how mutations affect the initial nucleation of A β aggregates (*Seuma et al., 2021*).

The researchers used an approach called deep mutational scanning (*Gray et al., 2019*) to generate a library of 468 single and 14,015 double mutant A β variants to see how the mutations affect the ability of A β to form new aggregates. They focused on the first step of this process, known as nucleation, and then determined the relationship between amino acid sequence and the nucleation rate of A β 42 (a form of A β that contains 42 amino acids) (*Figure 1*).

Seuma et al. found that mutations that reduce nucleation are clustered in the hydrophobic part of A β 42 (the C-terminal), while those that increase this process are located in the N-terminal part, which is hydrophilic. This suggests that A β is organized in a modular manner, with the N- and C-terminal parts having different roles in nucleation and aggregation. Intriguingly, such a modularity is also reflected in the

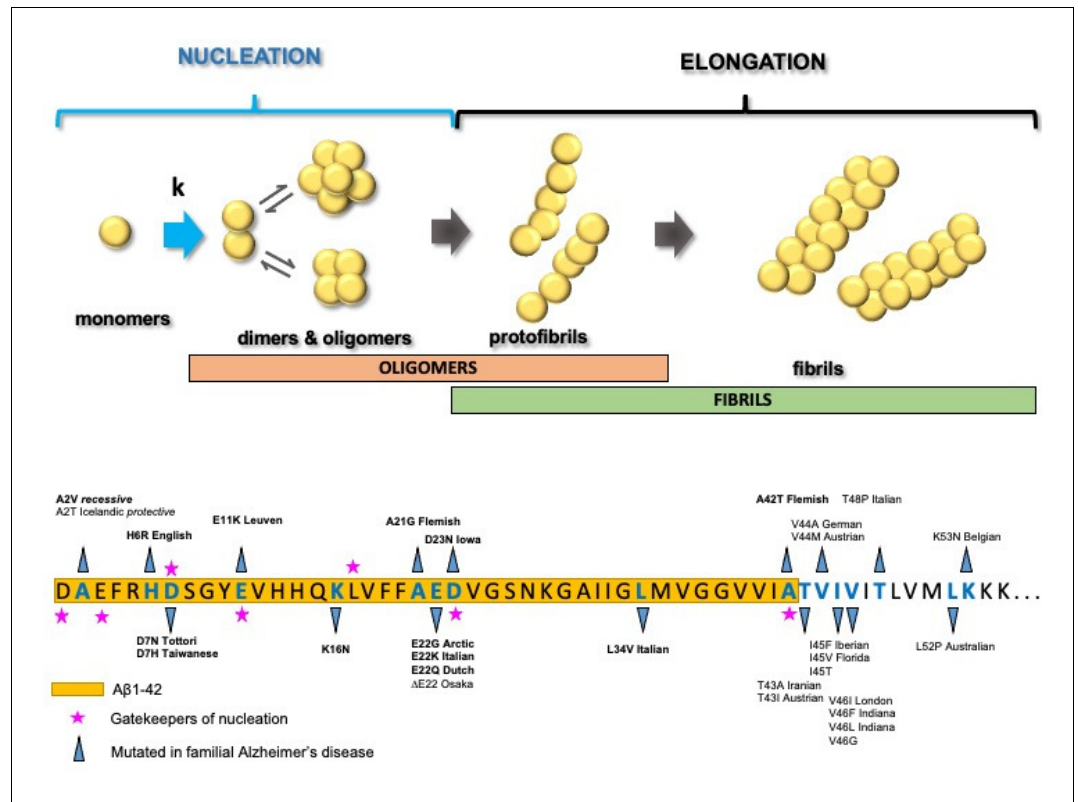


Figure 1. Amyloid beta peptide and Alzheimer's disease. It is thought that Alzheimer's disease is caused by amyloid beta ($A\beta$) (yellow circles; top) first forming dimers and then oligomers in a process called nucleation, with the oligomers then going on to build protofibrils and fibrils. Aggregated $A\beta$ then deposits in amyloid plaques, which are a pathological hallmark of Alzheimer's disease. Familial Alzheimer's disease is hereditary and is marked by an unusually early onset of symptoms. Several genes have been linked to this form of the disease, including the gene for the amyloid precursor protein (*APP*). Certain amino acid residues in $A\beta$ are called 'gatekeepers' of nucleation (magenta stars; bottom) because they prevent nucleation. The mutations indicated (blue arrows), affecting amino acid residues highlighted in blue, cause familial Alzheimer's disease or protect (A2T) against it. Pathogenic mutations in *APP* are shown in text (the numbering corresponds to the positions with $A\beta$). The sequence of amino acids shown here is from the N-terminal of $A\beta_{42}$. Seuma et al. found that dominant pathogenic mutations within the $A\beta_{42}$ peptide (indicated in bold text; bottom) all display increased rate of nucleation.

distribution of pathogenic mutations in the $A\beta$ precursor called *APP* (Figure 1). These mutations have been linked to familial Alzheimer's disease, a rare genetic form presenting a much earlier onset (people usually develop symptoms in their thirties, forties and fifties).

Seuma et al. further identified five negatively charged residues in $A\beta_{42}$, which acted as 'gatekeepers', preventing the peptides from sticking to each other (Rousseau et al., 2006). Mutations at these sites frequently resulted in increased nucleation. This raises several questions: could the gatekeepers of nucleation prevent neurodegeneration; and do mutations that promote nucleation prime the system for the onset of Alzheimer's disease?

To investigate this further, Seuma et al. studied the nucleation rates for 12 mutations in $A\beta_{42}$, which have been linked to familial Alzheimer's disease. The mutations all resulted in increased nucleation rates, although the rates did not correlate with the severity of the disease (as reflected by the age at disease onset). These results suggest that $A\beta$ nucleation plays a key role in the formation of neurotoxic aggregates, but other factors are also involved.

It is worth noting that the reservoir of $A\beta$ in the brain contains a heterogeneous mixture of peptides of varying lengths, generated by the sequential cleavage of *APP*. Pathogenic mutations in both *APP* and the enzymes responsible for its cleavage favor the production of longer $A\beta$ peptides (Szaruga et al., 2017). Importantly

a large number of mutations linked to familial Alzheimer's disease do not change the amino acid sequence of A β 42, but affect the composition of A β profiles by shifting them towards longer peptides (Szaruga *et al.*, 2015). Whether these pathogenic changes in peptide length (and hydrophobicity) lead to an increase in nucleation, and how this is related to the clinical phenotypes of Alzheimer's disease, warrant further investigation.

Disentangling the complex pathogenesis of Alzheimer's disease will likely require the interrogation of various pathogenic variants under controlled conditions and the plotting of biochemical and cellular data against clinical severity of the condition. The study by Seuma *et al.* certainly shows that the nucleation assay is a valuable tool for such investigations.

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References

Brinkmalm G, Hong W, Wang Z, Liu W, O'Malley TT, Sun X, Frosch MP, Selkoe DJ, Portelius E, Zetterberg

- H, Blennow K, Walsh DM. 2019. Identification of neurotoxic cross-linked amyloid- β dimers in the Alzheimer's brain. *Brain* **142**:1441–1457. DOI: <https://doi.org/10.1093/brain/awz066>, PMID: 31032851
- Gray VE, Sitko K, Kameni FZN, Williamson M, Stephany JJ, Hasle N, Fowler DM. 2019. Elucidating the molecular determinants of a β aggregation with deep mutational scanning. *G3: Genes, Genomes, Genetics* **9**:3683–3689. DOI: <https://doi.org/10.1534/g3.119.400535>
- Rousseau F, Serrano L, Schymkowitz JW. 2006. How evolutionary pressure against protein aggregation shaped chaperone specificity. *Journal of Molecular Biology* **355**:1037–1047. DOI: <https://doi.org/10.1016/j.jmb.2005.11.035>, PMID: 16359707
- Selkoe DJ, Hardy J. 2016. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Molecular Medicine* **8**:595–608. DOI: <https://doi.org/10.15252/emmm.201606210>, PMID: 27025652
- Seuma M, Faure A, Badia M, Lehner B, Bolognesi B. 2021. The genetic landscape for amyloid beta fibril nucleation accurately discriminates familial Alzheimer's disease mutations. *eLife* **10**:e63364. DOI: <https://doi.org/10.7554/eLife.63364>, PMID: 33522485
- Szaruga M, Veugelen S, Benurwar M, Lismont S, Sepulveda-Falla D, Lleo A, Ryan NS, Lashley T, Fox NC, Murayama S, Gijzen H, De Strooper B, Chávez-Gutiérrez L. 2015. Qualitative changes in human γ -secretase underlie familial Alzheimer's disease. *Journal of Experimental Medicine* **212**:2003–2013. DOI: <https://doi.org/10.1084/jem.20150892>
- Szaruga M, Munteanu B, Lismont S, Veugelen S, Horré K, Mercken M, Saido TC, Ryan NS, De Vos T, Savvides SN, Gallardo R, Schymkowitz J, Rousseau F, Fox NC, Hopf C, De Strooper B, Chávez-Gutiérrez L. 2017. Alzheimer's-causing mutations shift A β length by destabilizing γ -secretase-A β n interactions. *Cell* **170**:443–456. DOI: <https://doi.org/10.1016/j.cell.2017.07.004>, PMID: 28753424
- Yang T, Li S, Xu H, Walsh DM, Selkoe DJ. 2017. Large soluble oligomers of amyloid β -Protein from Alzheimer brain are far less neuroactive than the smaller oligomers to which they dissociate. *The Journal of Neuroscience* **37**:152–163. DOI: <https://doi.org/10.1523/JNEUROSCI.1698-16.2016>, PMID: 28053038