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Structural insights into Arctic mutant (E22G) A β 40 fibrils using solid-state NMR and cryoEM

Introduction

Misfolded aggregates of amyloid- β (A β) peptide are a defining hallmark of Alzheimer's disease (AD).¹ While the 40-residue A β peptide (A β 40) is the most prevalent form in the brain, A β fibrils in amyloid plaques predominantly consist of the more aggregation-prone and cytotoxic 42-residue variant (A β 42).² The cytotoxicity of A β aggregates may be intricately linked to their structural properties, as different fibril structures have been associated with varying clinical and pathological features of AD. Hence, elucidating the structural characteristics of amyloid fibrils is critical for understanding the disease's molecular mechanisms and for developing therapeutic or preventive strategies.^{3,4}

Certain pathogenic mutations in the A β region of the amyloid precursor protein (APP) are linked to familial Alzheimer's disease (FAD). These mutations often accelerate A β aggregation, making the peptide more prone to fibrillogenesis, and in some cases, promote the formation of toxic oligomers.⁵ Several FAD-associated APP mutations cluster within residues 21–23 of the A β sequence, a hydrophobic region critical for fibril formation. Notable examples include the Flemish (A21G), Osaka (E22 Δ), and Arctic (E22G) variants.⁶ The Arctic (E22G) mutation promotes early-onset FAD by enhancing the assembly of A β into oligomers and fibrils.⁷ This mutation is unique in that it is the only known FAD-related mutation associated with clinical features typical of sporadic AD, unlike other mutations at position 22, such as the Italian (E22K) and Dutch (E22Q)

variants, which primarily induce cerebral amyloid angiopathy (CAA). E22G carriers exhibit classical AD pathology, including senile plaques, neurofibrillary tangles, progressive cognitive decline, and in some cases, cerebrovascular events.⁸ Interestingly, the amyloid plaques in Arctic FAD patients resemble cotton-wool plaques, characterized by a core rich in A β 40 and surrounded by a ring of both A β 42 and A β 40 fibrils, distinguishing them from the A β 42-enriched plaques commonly observed in sporadic AD.^{9,10}

The structural investigation of amyloid fibrils harboring FAD-related mutations has garnered significant interest, as these studies may shed light on how specific mutations drive the molecular onset of AD. Notably, fibril structures from Arctic mutant A β (E22G) are of particular interest, as they may provide insights into the mechanisms of sporadic AD. Previous studies using electron microscopy and solid-state NMR (SSNMR) have revealed at least five distinct fibrillar morphologies of E22G A β 40,^{11,12} with structural plasticity allowing the peptide to adopt both U-shaped and S-shaped β -sheet motifs¹³. More recent cryoEM studies have shown that E22G A β fibrils in human brains exhibit a tetrameric structure, with two pairs of protofilaments adopting U- and S-shaped β -sheet motifs within V12 – V40 and E11 – G37 residues respectively.¹⁴

Results and discussion

In this study, we developed a methodology to prepare a monomorphic E22G A β 40 fibril sample amenable to high-resolution structural analysis. Unlike the densely bundled fibrils observed in wild-type (WT) A β 40 and A β 42 fibrils, the E22G A β 40 fibrils formed in our preparations exhibited a less compact, twisted morphology with a uniform cross-over distance of 35 – 45 nm. It has been reported that E22G A β 40 has high propensity to form multiple forms of

fibrils (i.e., polymorphs), which have long hindered structural analysis of resultant fibrils. The fibrils prepared by our optimized method has a highly homogeneous twisted morphology with a uniform short cross-over distance of 35 – 45 nm. This morphological homogeneity facilitated structural elucidation using SSNMR and cryoEM, which revealed an atomic-resolution structure of the E22G A β 40 fibril. The structure comprised two A β molecules forming inner layers with slight distortions in C₂ symmetry, each adopting a unique W-shaped parallel β -sheet motif. While cryoEM data suggested the presence of additional β -sheet layers, these were not confirmed by SSNMR. Compared to previous structural studies on WT A β 40 and A β 42 fibrils, which exhibited U- and S-shaped motifs,^{15,16} the E22G A β 40 fibril presented here exhibits an extended conformation similar to the I-shaped A β 40 fibril polymorph¹⁷. This less compact fibrillar structure is consistent with the A β 40-rich cores observed in the cotton-wool plaques from Arctic FAD patients.¹⁸ Furthermore, the low Thioflavin-T (ThT) fluorescence associated with these fibrils supports their structural similarity to the A β 40-enriched plaques seen in Arctic mutation carriers,¹⁹ suggesting that the distinct structural features of the E22G A β 40 fibril may underpin the unique pathological characteristics of Arctic FAD.

We also investigated the misfolding kinetics of E22G and WT A β 40/A β 42 monomers, as well as the cross-seeding potential of E22G A β 40 fibrils. Our findings reveal that E22G A β 40 fibrils self-assemble more rapidly than WT A β 40 or A β 42 at physiologically relevant concentrations (A β 40 : β 42 ~9:1). Moreover, E22G A β 40 fibrils were able to cross-seed WT A β 40 and A β 42 fibril formation, suggesting that this mutation may facilitate pathological aggregation in a mixed A β environment.

Conclusion

Our results offer new insights into the structural properties of E22G A β 40 fibrils, highlighting their unique W-shaped β -sheet motif and less compact morphology. These findings suggest that the specific structure of the E22G A β 40 fibrils contributes to the distinct clinical and pathological features observed in Arctic FAD. Furthermore, the ability of E22G A β 40 fibrils to cross-seed WT A β fibrils provides a mechanistic explanation for their potential role in accelerating amyloid aggregation in the brain.

References

1. Selkoe, D. J. Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. *Nat Cell Biol* **6**, 1054–61 (2004).
2. Murphy, M. P. & LeVine, H. Alzheimer's disease and the amyloid-beta peptide. *J Alzheimers Dis* **19**, 311–23 (2010).
3. Petkova, A. T. *et al.* Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science* **307**, 262–5 (2005).
4. Di Fede, G. *et al.* Molecular subtypes of Alzheimer's disease. *Sci Rep* **8**, 3269 (2018).
5. Hatami, A., Monjaze, S., Milton, S. & Glabe, C. G. Familial Alzheimer's Disease Mutations within the Amyloid Precursor Protein Alter the Aggregation and Conformation of the Amyloid- β Peptide. *J Biol Chem* **292**, 3172–3185 (2017).
6. Gessel, M. M., Bernstein, S., Kemper, M., Teplow, D. B. & Bowers, M. T. Familial Alzheimer's disease mutations differentially alter amyloid β -protein oligomerization. *ACS Chem Neurosci* **3**, 909–18 (2012).
7. Nilsberth, C. *et al.* The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. *Nat Neurosci* **4**, 887–93 (2001).
8. Revesz, T. *et al.* Genetics and molecular pathogenesis of sporadic and hereditary cerebral amyloid angiopathies. *Acta Neuropathol* **118**, 115–30 (2009).
9. Kalimo, H. *et al.* The Arctic A β PP mutation leads to Alzheimer's disease pathology with highly variable topographic deposition of differentially truncated A β . *Acta Neuropathol Commun* **1**, 60 (2013).
10. Basun, H. *et al.* Clinical and neuropathological features of the arctic APP gene mutation causing early-onset Alzheimer disease. *Arch Neurol* **65**, 499–505 (2008).
11. Norlin, N. *et al.* Aggregation and fibril morphology of the Arctic mutation of Alzheimer's A β peptide by CD, TEM, STEM and in situ AFM. *J Struct Biol* **180**, 174–89 (2012).
12. Elkins, M. R. *et al.* Structural Polymorphism of Alzheimer's β -Amyloid Fibrils as Controlled by an E22 Switch: A Solid-State NMR Study. *J Am Chem Soc* **138**, 9840–52 (2016).
13. Yoo, B. K., Xiao, Y., McElheny, D. & Ishii, Y. E22G Pathogenic Mutation of β -Amyloid (A β) Enhances Misfolding of A β 40 by Unexpected Prion-like Cross Talk between A β 42 and A β 40. *J Am Chem Soc* **140**, 2781–2784 (2018).
14. Yang, Y. *et al.* Cryo-EM structures of amyloid- β filaments with the Arctic mutation (E22G) from human and mouse brains. *Acta Neuropathol* **145**, 325–333 (2023).
15. Petkova, A. T. *et al.* A structural model for Alzheimer's beta -amyloid fibrils based on experimental constraints from solid state NMR. *Proc Natl Acad Sci U S A* **99**, 16742–7 (2002).
16. Xiao, Y. *et al.* A β (1–42) fibril structure illuminates self-recognition and replication of amyloid in Alzheimer's disease. *Nat Struct Mol Biol* **22**, 499–505 (2015).
17. Ghosh, U., Thurber, K. R., Yau, W.-M. & Tycko, R. Molecular structure of a prevalent amyloid- β fibril polymorph from Alzheimer's disease brain tissue. *Proc Natl Acad Sci U S A* **118**, e2023089118 (2021).
18. Basun, H. *et al.* Clinical and neuropathological features of the arctic APP gene mutation causing early-onset Alzheimer disease. *Arch Neurol* **65**, 499–505 (2008).
19. Schöll, M. *et al.* Low PiB PET retention in presence of pathologic CSF biomarkers in Arctic APP mutation carriers. *Neurology* **79**, 229–36 (2012).