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Pathogenic properties of Alzheimer's β -amyloid identified from structure–property patient–phenotype correlations†

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β -Amyloid ($A\beta$) plays a central role in Alzheimer's disease (AD), but the specific molecular mechanism and associated structures remain unknown. We compiled patient data for carriers of genetic variants of $A\beta$ that cause AD and correlated these data against chemical properties for 56 mutant conformations derived from four published experimental conformations of $A\beta$ of variable structure and chemical environment. Disease onset of variants is significantly ($p \sim 0.006$) correlated to hydrophobic surfaces of disordered conformations (2LFM), whereas structured conformations yielded no correlations. Correlation also applied ($p < 0.03$) to *in vitro* steady-state $A\beta$ levels. We conclude that disordered monomers are likely to be pathogenically important in contrast to structured conformations and that hydrophobic surface correlates with pathogenesis. This first established correlation between clinical and chemical data suggests that specific exposed, disordered monomers are viable targets for AD therapy.

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Introduction

The major neurodegenerative disease Alzheimer's disease (AD) is characterized by impaired memory, gradual decline of cognitive abilities, and personality changes.^{1–5} It mostly occurs sporadically with no apparent inheritance,⁶ a broad clinical spectrum,³ and with age as the largest among many risk factors of small, but cumulative significance.^{1,7} Genetically inherited AD accounts for ~5% of all cases and causes particularly severe, early-onset familial AD (FAD), most notably due to mutations in the enigmatic, transmembrane Amyloid Precursor Protein (APP) or in presenilin (PSEN), which forms part of the γ -secretase complex that degrades APP.^{8,9}

The central hallmark of AD is extracellular deposits of senile plaques consisting of β -amyloid ($A\beta$) peptides, which are proteolytic cleavage products of APP found in the membranes

of cells and organelles.^{1,10} For this reason and because of the FAD genetic risk factors relating to APP or its processing, $A\beta$ is thought to play a central role in AD *viz.* the “Amyloid Cascade Hypothesis”, *i.e.* that overload of $A\beta$ causes disease.¹¹

However, neurodegeneration and cognitive decline are not generally correlated with plaque $A\beta$ levels,¹² and some genetic risk factors such as PSEN1 variants actually reduce total $A\beta$ levels.¹³ Consequently, focus has changed towards longer $A\beta$ isoforms such as $A\beta_{42}$ and in particular the ratio between long and short isoforms (notably $A\beta_{42}/A\beta_{40}$) and small soluble oligomers of $A\beta$ as the perceived toxic species^{14,15} that damage neuronal cells^{14,16} and tend to follow disease progression.¹⁷

Polypeptide misfolding due to exposure of hydrophobic protein parts is considered a major driving force behind processes leading to oligomers and fibrils.^{18,19} Given its role in Alzheimer's Disease, during the last decades, structural and dynamic studies of $A\beta$ species have emerged,^{20,21} including mature $A\beta$ fibrils,^{20,22} protofibrils,²³ oligomers²⁴ and various polymorphisms.^{21,25} These species generally contain substantial β -sheet character typical of aggregates and fibrillar peptide deposits. Despite the renewed focus on small soluble forms of $A\beta$, soluble $A\beta$ monomers, which form critical intermediates that are precursors of $A\beta$ misfolding,^{26–29} have not been comprehensively studied, partly because these monomers, in contrast to aggregates, are disordered in solution with little β -sheet and only some α -helix character.³⁰

The neurotoxicity of $A\beta$ is thought to be highly conformation and environment dependent³¹ and, as specifically utilized in the present work, conformations and hydrophobic

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† Electronic supplementary information (ESI) available: Fig. S1–S4: wild type and mutant structural conformation for $A\beta_{42}$ and $A\beta_{40}$; Fig. S6: effect of age of onset for wild type and A2T variant; Fig. S7–S12: scatter graph plots for correlation between computed and experimental properties; Tables S1–S8: computed structural properties for wild type and mutant $A\beta_{42}$ and $A\beta_{40}$; Tables S9 and S10: normalized data for t_{onset} and total $A\beta$ where $A\beta$ levels are from two sources; Tables S11–14: data for correlating t_{onset} with computed properties of wild type and mutant for $A\beta_{42}$ and $A\beta_{40}$; Table S15: total helix percentage calculated for WT and mutant $A\beta_{40}$ and $A\beta_{42}$; Tables S16–S19: data for correlating total $A\beta$ with computed properties of wild type and mutant $A\beta_{42}$ and $A\beta_{40}$. See DOI: 10.1039/c4dt03122a

exposure is highly environment-dependent both for fibrils^{28,32,33} and monomers, as evident in NMR structures.^{34,35} Conformation and size-dependent hydrophobic properties may determine the pathogenicity of small A β_{42} oligomers rather than the presence of β -sheet aggregates.^{36,37} Previous NMR³⁸ and computational³⁹ studies have shown that the hydrophobicity arising from central and C-terminal regions of A β is responsible for formation of an extended β -sheet conformation with a connecting turn between them. This “hairpin” topology is suggested to resemble A β in their fibril forms.⁴⁰

Despite this, the conformations, sizes, and specific pathogenic molecular modes of action remain unknown, partly because patient data cannot be correlated directly to these forms *in vivo*.^{13,41} Thus, identifying the causative molecular species and chemical properties of A β remains a primary priority.

We report here the analysis of compiled data for patients carrying genetic variants of A β known to cause disease, correlated against robust measures of hydrophobic surface areas and other chemical properties derived from four different experimental structures of wild-type (WT) A β monomers, two for A β_{42} and two for A β_{40} . These four structures mimic variable *in vivo* environments by representing a spectrum from 100% water (2LFM⁴²), *via* 20% and 70% water (1IYT,³⁵ 1Z0Q⁴³) to a water-micelle like environment representative of membrane-

interacting species (1BA4³⁴). They also represent a hierarchy from little structure (2LFM and to some extent 1BA4) to more structured (1IYT, 1Z0Q).

Importantly, we establish a correlation between patient age of disease onset (t_{onset} , in years) and hydrophobic exposure in the disordered conformations (in particular 2LFM), but not the more structured conformations. This indicates that hydrophobic exposure is a pathogenically relevant property for disordered amyloid species, but not for the more structured amyloids. The statistical significance of this observation is unlikely to be coincidental (confidence level >99%) and thus implies that structural features resembling the 2LFM conformation are related to disease, *e.g.* as the relevant precursor properties leading to oligomers in AD. These findings suggest a change of focus towards controlling disordered and exposed monomer species in AD, with the 2LFM structure providing the template for such structure-based design.

Results and discussion

15 known missense mutations and one deletion (E693 Δ , corresponding to E22 Δ in A β numbering) within the A β_{42} region of APP are associated with FAD or cerebral amyloid angiopathy (Fig. 1A and 1B). A β_{40} is represented by only

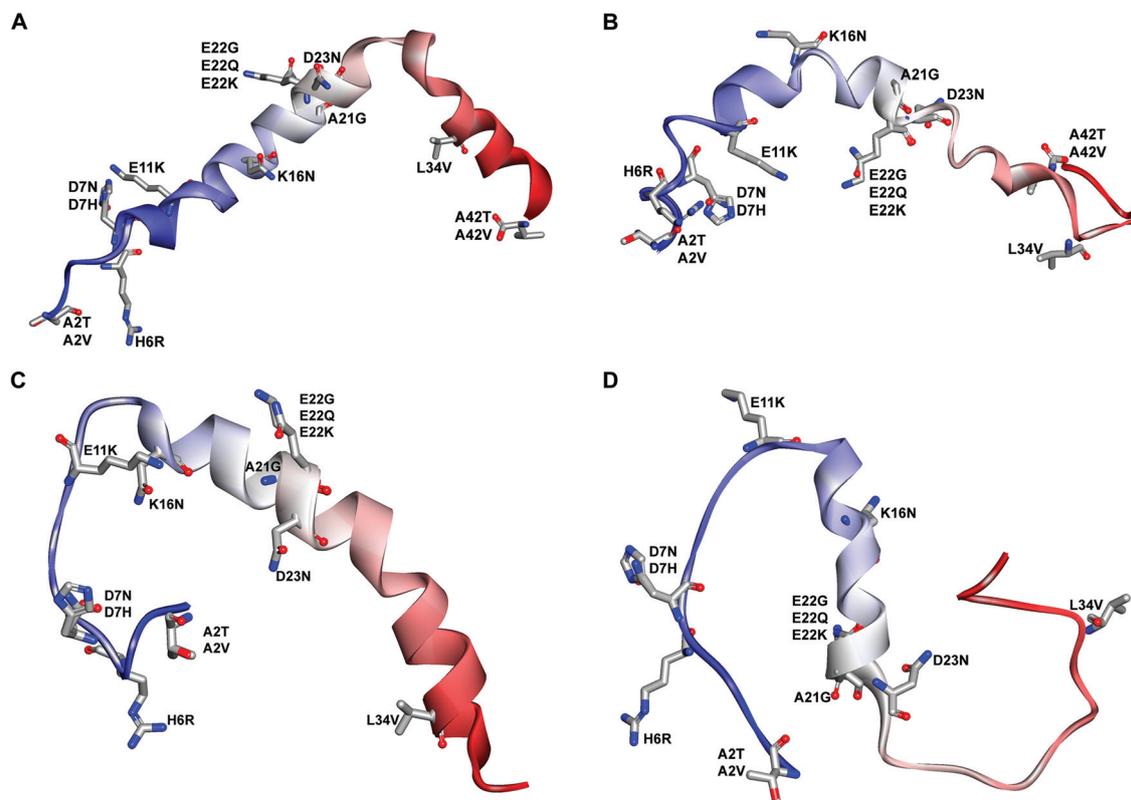


Fig. 1 Wild-type and mutant structures of A β_{42} and A β_{40} . (A, B) The NMR structure of wild-type A β_{42} (PDB codes 1IYT and 1Z0Q) and their 15 superimposed computational structures of A β_{42} mutants. (C, D) The NMR structure of wild-type A β_{40} (PDB codes 1BA4 and 2LFM) and their 13 superimposed structures of A β_{40} mutants. The mutant residues are shown in stick-model. The peptide's secondary structure is shown with blue N-terminus and red C-terminus (the figure was generated using Discovery Studio (DS) 4.0 visualizer).

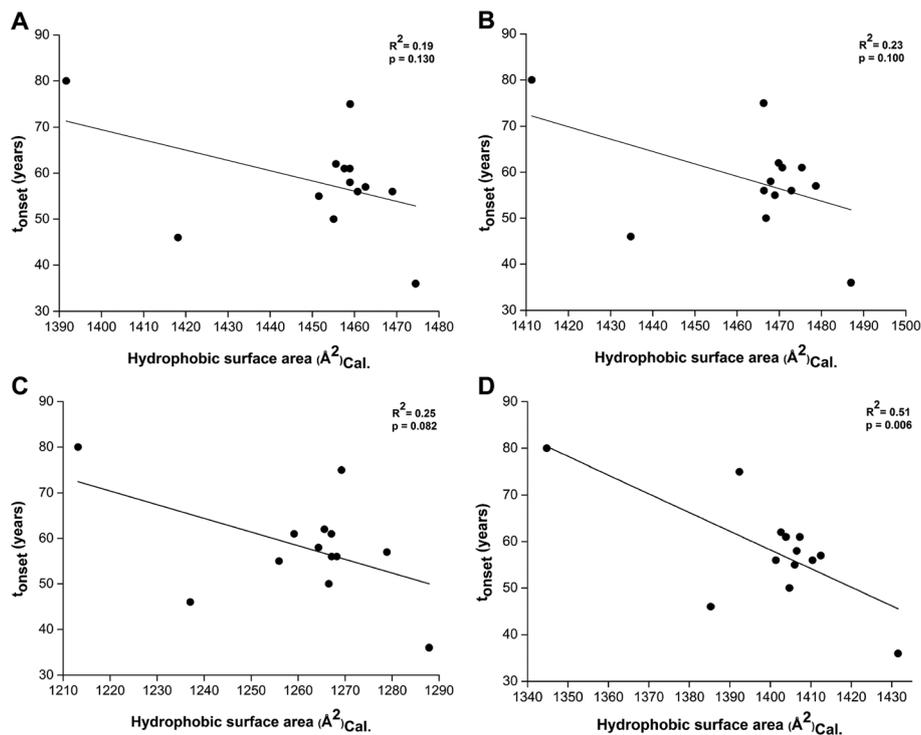


Fig. 2 Correlation between t_{onset} and calculated hydrophobic surface for WT and mutant $A\beta_{40}$ and $A\beta_{42}$. (A) vs. 1YT WT and mutants $A\beta_{42}$. (B) vs. 1Z0Q WT and mutants $A\beta_{42}$. (C) vs. 1BA4 WT and mutants $A\beta_{40}$. (D) vs. 2LFM WT and mutants $A\beta_{40}$.

13 mutations (Fig. 1C and 1D) since two variations occur at position 42.

56 mutant structures were derived from the four initial structures of $A\beta_{42}$ (1YT and 1Z0Q) and $A\beta_{40}$ (1BA4 and 2LFM) (Fig. S1–S4†). To preserve the experimental time-averaged conformational properties, the original backbone and non-mutated side-chain conformations were preserved during modeling.⁴⁴ Hydrogen atoms were added using the CHARMM force field⁴⁵ and protonation states for ionizable residues were manually verified for all 60 (four wild-type and 56 mutant) structures before analysis. The mutant conformations were constructed as described in the Material and methods section.

From the experimentally derived structures, we computed the total solvent accessible surface area (SAS) and the hydrophobic and hydrophilic surfaces for all WT and mutant structures. To account for method dependence, we used four different methods (see Materials and methods section) to compute SAS for each structure individually (Tables S1–S4†). All methods give very similar results, except for one K16N-1BA4 $A\beta_{40}$ mutation (Fig. S5†), which has minor effect on the overall correlation. To further test result sensitivity, we correlated total SAS from Discovery Studio 4.0 and StrucTools (Fig. S7†) for each structure. Strong correlations between the two sets of data for the same structure were observed (Fig. S7†).

We further calculated the hydrophobic and hydrophilic surface areas for all structures (Tables S5–S8†). We tested the structural sensitivity by correlating hydrophilic and hydrophobic surfaces of each pair of structures (1YT vs. 1Z0Q; $A\beta_{42}$

and 1BA4 vs. 2LFM; $A\beta_{40}$) (Fig. S8†). These correlations show significant similarities as expected for the same peptides but also clear indications of conformational differences, with $R^2 \sim 0.77\text{--}0.96$, $p < 0.001$ (Fig. S8A–8D†). From these sensitivity tests, we thus find that the applied methodology produces robust data for analysis.

For the studied genetic variants, we collected all relevant, available data on patient t_{onset} and total $A\beta$ levels observed for a given variant in cell studies (Tables S9 and S10†). Patient t_{onset} was not significantly correlated with total SAS or hydrophilic surfaces of $A\beta_{42}$ and $A\beta_{40}$ (Fig. S9 and S10; Tables S11–S14†) but was significantly correlated to hydrophobic surface of the disordered structures (Fig. 2). Correlation of t_{onset} to hydrophobic surface was strongly conformation-dependent: 1YT gave $R^2 = 0.19$; $p = 0.13$ (Fig. 2A), 1Z0Q gave $R^2 = 0.23$; $p = 0.10$ (Fig. 2B), 1BA4 had $R^2 = 0.25$; $p = 0.08$ (Fig. 2C), and 2LFM had $R^2 = 0.51$; $p = 0.006$; (Fig. 2D). Importantly, the correlation follows a trend of structure: To substantiate this, we calculated the helical percentage for the 60 structures (Table S15†). The structure 2LFM that showed strong statistically significant correlation to t_{onset} contains the least helix character (22.5%).

Thus, our analysis shows that among the two major isoforms of amyloid in the brain, only disordered structural features represented by 2LFM have pathogenic properties, and these relate to exposure of hydrophobic surface. Surface compositions of $A\beta$ have previously been suggested to be determinants of $A\beta$ -induced neurotoxicity in AD progression.^{46,47} As seen, hydrophobic exposure can explain real patient data from

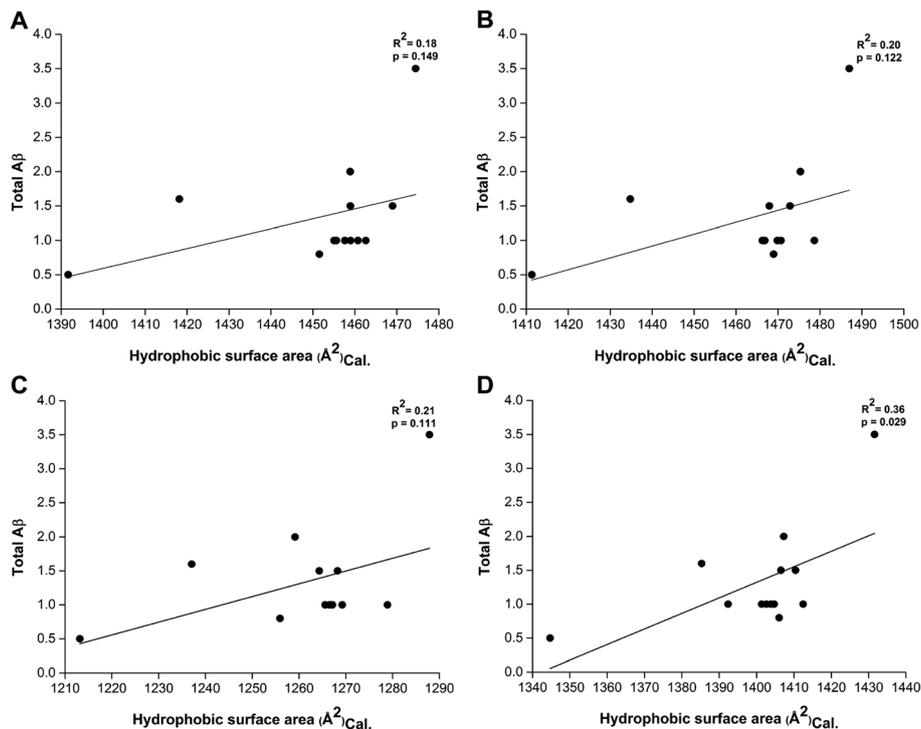


Fig. 3 Correlation between total Aβ levels and calculated hydrophobic surface for WT and mutant Aβ₄₀ and Aβ₄₂. (A) vs. 11YT WT and mutants Aβ₄₂. (B) vs. 1Z0Q WT and mutants Aβ₄₂. (C) vs. 1BA4 WT and mutants Aβ₄₀. (D) vs. 2LFM WT and mutants Aβ₄₀.

one specific, disordered conformation of the peptide, providing the first statistically significant link (at the 99% significance level) between clinical and chemical data in this field. The significance suggests that, despite well-known heterogeneities in lab protocols of the original experiments, upon proper normalization of data to their respective wild-type values, the probability that the presently identified correlations are coincidental is smaller than 1%.

Our observed relation between exposed hydrophobic surface and patient disease onset is consistent with previous mutagenesis studies that focused on the C-terminal of both Aβ₄₀ and Aβ₄₂: These studies found that improved hydrophobic exposure and interaction between putative β-sheets increase the stability of the hairpin structure.⁴⁸ The oligomerization of monomers likely occurs by disorder, exposure, and concomitant α-to-β transitions.

Aβ-induced cytotoxicity has been the subject of intense research. Recently Aβ's ability to mediate cytotoxicity *via* membrane disruption has received particular notice.⁴⁹ The mechanism of Aβ interaction with membranes remains to be further elucidated, but recent insight suggests that cell surface interactions are a prerequisite for cytotoxicity.^{50,51} Such a membrane-association mechanism is consistent with the exposed hydrophobic conformations identified as pathogenic in our work.

Finally, we also correlated *in vitro* total steady-state Aβ levels for the variants against chemical properties (Tables S16–S19†). Interestingly, except for the hydrophobic surface (Fig. 3), again the studied properties yielded insignificant or no correlation

with Aβ levels (Fig. S11 and S12†). Only the hydrophobic surface area of the least structured (22.5% helix) 2LFM conformation (Fig. 3D) was significantly correlated with steady-state levels at the 97% confidence level ($R^2 = 0.36$; $p = 0.03$). This suggests that the same chemical properties that relate to pathogenicity of the monomer (*i.e.* hydrophobic exposure) also relate to increased steady-state amyloid levels. Thus, hydrophobic exposure in the fully solvated disordered monomer is related to amyloid buildup in genetic variants, whereas other conformations did not relate to amyloid buildup.

Concluding remarks

In conclusion, we have established the first significant link between chemical properties of specific amyloid structures and real Alzheimer patient data at the 99% confidence level ($p \sim 0.006$) and identified particular disordered monomers (the 2LFM conformations) as related to pathogenesis. The correlation of both amyloid steady-state levels and disease onset to the same chemical property of disordered amyloids may explain why amyloid levels have been mistaken for pathogenic in earlier work leading to the classical “cascade” hypotheses. In fact, our analysis suggests that mainly the hydrophobic surface relates to disease.

The identified changes are caused by genetic variation in the Aβ variants studied in this work, but could conceivably be caused by post-translational modifications such as metal binding, phosphorylation, or oxidation, in the major sporadic

forms of the disease,⁵² providing perspectives for unifying the two forms. It has not escaped our attention that metal binding would immediately reduce the net negative charge from -3 to -1 and increase hydrophobicity of the amyloids, providing a “sporadic” counterpart to the familial AD mechanism described in the present work. Also, the identification of 2LFM-like features as potentially pathogenic provides perspectives for molecular targeting of specific disordered amyloid species in line with recent work in this direction.^{53,54}

The statistically significant correlations obtained in this study suggest that the heterogeneity in various lab protocols is not a major issue; and similar approaches could be utilized to rationalize relationships between structure and clinical phenotype for related disorders, in particular as patient data become more generally available.

Material and methods

Starting structural models and properties calculation

Initial coordinates of solution NMR WT structures for $A\beta_{42}$ (PDB: 1IYT³⁵ and 1Z0Q⁴³) and $A\beta_{40}$ (PDB: 1BA4³⁴ and 2LFM⁴²) were extracted from the RCSB Protein Data Bank (<http://www.rcsb.org/pdb/>). To mimic the physiological states, protonation states for ionizable residues in both $A\beta_{42}$ and $A\beta_{40}$ peptides were applied as suitable for a physiological pH, with both arginine and lysine residues modeled as positively charged and glutamic and aspartic acids as deprotonated. Histidine residues were modeled as neutral with only the δ nitrogen protonated. The N and C termini were chosen to be protonated and deprotonated, respectively, in both $A\beta_{42}$ and $A\beta_{40}$ peptides and employed as starting structure for mutant modeling.

Mutant modeling

The mutant structures were constructed by *in silico* by mutating the wild-type (WT) structure⁴⁴ using the protein design extension in Discovery Studio 4.0 (DS 4.0, Accelrys Inc., San Diego, CA).⁵⁵ Side-chain replacement and modeling were carried out with the routine “Build mutant” of MODELLER 9v9.⁵⁶ $A\beta_{42}$ embodied a total of 15 point mutations whereas $A\beta_{40}$ contains 13 point mutations linked to familial Alzheimer’s disease (FAD). A total of 10 conformations of each mutant were generated from four initial WT structures of $A\beta_{42}$ (1IYT and 1Z0Q) and $A\beta_{40}$ (1BA4 and 2LFM). In total, 560 mutant structures were created computationally for the 56 point mutations in $A\beta_{42}$ and $A\beta_{40}$.

Subsequently, these 560 structures were evaluated using DOPE (Discrete Optimized Protein Energy) scoring functions and PDF (Probability Density Function) total energy and finally 56 mutant structures were selected based on the lowest DOPE score and PDF total energy. DOPE score of a protein equates conformational energy which measures the relative stability of a conformation with respect to other conformations of the same protein. PDF Total Energy is the sum of the scoring function value of all homology-derived pseudo-energy terms and stereo chemical pseudo-energy terms. In total, 60 $A\beta_{42}$ and

$A\beta_{40}$ structures (4 WT structures, 56 mutant structures) were subjected to further analysis.

Solvent accessibility and helical percentage calculation

Total solvent accessibility surface (\AA^2) for all the 60 structures were calculated using DS 4.0 and other web-based tools; Get area,⁵⁷ StrucTools (<http://helixweb.nih.gov/structbio.html>) and MobyL.^{58,59} In addition to total solvent accessibility surface area, hydrophobic and hydrophilic solvent accessibility surface were also predicted using DS 4.0 for WT and mutant $A\beta_{42}$ and $A\beta_{40}$ structures. Furthermore, secondary structure analysis was carried for all the structures. Structural features such as helical geometry and percentage were calculated using PROMOTIF,⁶⁰ available on the SWISS-MODEL web server.

Pre-processing of biochemical and clinical of data

Biochemical and patient data on disease onset (t_{onset}) and total steady-state $A\beta$ levels measured during expression studies were collected from published data in original articles^{61–75} and from the recently updated AD & FTD Mutation Database (<http://www.molgen.ua.ac.be/ADMutations/>).

Total $A\beta$ steady-state levels were obtained by adding the reported $A\beta_{40}$ and $A\beta_{42}$ levels, which make up close to 100% of the isoforms. To put numbers from different experiments on the same scale, $A\beta$ levels for WT were normalized to one. Experimental data for $A\beta$ levels were extracted from Jonsson *et al.*⁶² (A2T, A2V vs. WT), Chen *et al.*⁶⁶ (D7H), Nilsberth *et al.*⁶¹ (E22G, E22K, E22Q, A21G), Zhou *et al.*⁶⁷ (E11K). Considering heterogeneous data for the A2V mutant, we also compared data from Jonsson *et al.*⁶² which differs from Di Fede *et al.*,⁶³ and data from Van Nostrand *et al.*⁶⁹ differ from data from Nilsberth *et al.*⁶¹

“Time of onset” of WT and A2T variant carriers

Except WT and A2T disease onsets (t_{onset}), all other t_{onset} data are from the original sources. To enable an inclusion of the normal and protective phenotypes in the analysis, t_{onset} for WT carriers and A2T variant carriers were added as proxy numbers. According to United Nations Population Division 2010 report, the life expectancy of populations in developed regions is estimated to be 77.1 years. Appreciating the early-onset nature of the genetic risk factors, t_{onset} for WT should be higher than the average life span of the population from which (developed regions) clinical data on variants has been collected.

To test the sensitivity of the correlations to the choice of WT and A2T age of onset, this should reflect a normal healthy population average, a value of 75, 77 and 80 years for WT and also the A2T protective variant may increase longevity, and thus a value of 75, 77, 78, 80 and 83 years were considered. We tested the correlations of t_{onset} against 2LFM wild type and mutant hydrophobic surfaces using three different values for WT (75, 77 and 80 years) and five different values for A2T (75, 77, 78, 80 and 83 years).

These correlations are shown in Fig. S6† and it can be seen that these alternate numbers used to test the choice of WT

and A2T age of onset have no significant effect on the overall correlation (Fig. S6†). However, the United Nations Population Division has estimated that the life expectancy of populations in developed regions would increase to around 80 years towards 2015. Thus, $t_{\text{onset}} = 75$ for WT and $t_{\text{onset}} = 80$ for A2T were used throughout the paper. We conclude that reasonable choices of t_{onset} for WT and A2T consistent with their normal and protective phenotypes do not change the conclusions of our work, providing an external validation of the significance of our conclusions.

Statistical treatment of data

In the present study, correlation coefficients (R^2) and the p -values for linear correlations were used to identify statistically significant relationships. Statistical significance of all potential relations between clinical phenotypes (time of disease onset and total A β) and computed properties (total SAS, hydrophobic and hydrophilic surfaces) of WT and A β variants were examined. To this end, we have considered values of $R^2 > 0.3$ and $p < 0.05$ significant enough for discussion.

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Notes and references

- 1 K. P. Kepp, *Chem. Rev.*, 2012, **112**, 5193–5239.
- 2 M. Eisenstein, *Nature*, 2011, **475**, S20–S22.
- 3 G. M. McKhann, D. S. Knopman, H. Chertkow, B. T. Hyman, C. R. Jack Jr., C. H. Kawas, W. E. Klunk, W. J. Koroshetz, J. J. Manly, R. Mayeux, R. C. Mohs, J. C. Morris, M. N. Rossor, P. Scheltens, M. C. Carrillo, B. Thies, S. Weintraub and C. H. Phelps, *Alzheimer's Dementia*, 2011, **7**, 263–269.
- 4 S. Ayton, P. Lei and A. I. Bush, *Free Radical Biol. Med.*, 2013, **62**, 76–89.
- 5 A. Rauk, *Chem. Soc. Rev.*, 2009, **38**, 2698–2715.
- 6 R. J. Guerreiro, D. R. Gustafson and J. Hardy, *Neurobiol. Aging*, 2012, **33**, 437–456.
- 7 D. E. Barnes and K. Yaffe, *Lancet Neurol.*, 2011, **10**, 819–828.
- 8 E. Levy-Lahad, W. Wasco, P. Poorkaj, D. M. Romano, J. Oshima, W. H. Pettingell, C. E. Yu, P. D. Jondro, S. D. Schmidt, K. Wang, *et al.*, *Science*, 1995, **269**, 973–977.
- 9 L. Chávez-Gutiérrez, L. Bammens, I. Benilova, A. Vandersteen, M. Benurwar, M. Borgers, S. Lismont, L. Zhou, S. Van Cleynenbreugel, H. Esselmann, J. Wiltfang, L. Serneels, E. Karran, H. Gijzen, J. Schymkowitz, F. Rousseau, K. Broersen and B. De Strooper, *EMBO J.*, 2012, **31**, 2261–2274.
- 10 B. Schneider, D. Prvulovic, V. Oertel-Knochel, C. Knochel, B. Reinke, M. Grexa, B. Weber and H. Hampel, *Prog. Neurobiol.*, 2011, **95**, 703–717.
- 11 J. A. Hardy and G. A. Higgins, *Science*, 1992, **256**, 184–185.
- 12 G. M. Shankar and D. M. Walsh, *Mol. Neurodegener.*, 2009, **4**, 48.
- 13 E. Karran, M. Mercken and B. De Strooper, *Nat. Rev. Drug Discovery*, 2011, **10**, 698–712.
- 14 R. Kaye, E. Head, J. L. Thompson, T. M. McIntire, S. C. Milton, C. W. Cotman and C. G. Glabe, *Science*, 2003, **300**, 486–489.
- 15 J. P. Cleary, D. M. Walsh, J. J. Hofmeister, G. M. Shankar, M. A. Kuskowski, D. J. Selkoe and K. H. Ashe, *Nat. Neurosci.*, 2005, **8**, 79–84.
- 16 S. Lesne, M. T. Koh, L. Kotilinek, R. Kaye, C. G. Glabe, A. Yang, M. Gallagher and K. H. Ashe, *Nature*, 2006, **440**, 352–357.
- 17 J. V. Rushworth and N. M. Hooper, *Int. J. Alzheimer's Dis.*, 2011, **2011**, 603052.
- 18 S. Ohnishi and K. Takano, *Cell. Mol. Life Sci.*, 2004, **61**, 511–524.
- 19 E. Hubin, N. A. van Nuland, K. Broersen and K. Pauwels, *Cell. Mol. Life Sci.*, 2014, **71**, 3507–3521.
- 20 A. K. Paravastu, R. D. Leapman, W. M. Yau and R. Tycko, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 18349–18354.
- 21 M. Fandrich, M. Schmidt and N. Grigorieff, *Trends Biochem. Sci.*, 2011, **36**, 338–345.
- 22 I. Bertini, L. Gonnelli, C. Luchinat, J. Mao and A. Nesi, *J. Am. Chem. Soc.*, 2011, **133**, 16013–16022.
- 23 H. A. Scheidt, I. Morgado, S. Rothemund, D. Huster and M. Fandrich, *Angew. Chem., Int. Ed.*, 2011, **50**, 2837–2840.
- 24 M. Ahmed, J. Davis, D. Aucoin, T. Sato, S. Ahuja, S. Aimoto, J. I. Elliott, W. E. Van Nostrand and S. O. Smith, *Nat. Struct. Mol. Biol.*, 2010, **17**, 561–567.
- 25 J. P. Colletier, A. Laganowsky, M. Landau, M. Zhao, A. B. Soriaga, L. Goldschmidt, D. Flot, D. Cascio, M. R. Sawaya and D. Eisenberg, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 16938–16943.
- 26 A. Jan, O. Adolfsson, I. Allaman, A. L. Buccarello, P. J. Magistretti, A. Pfeifer, A. Muhs and H. A. Lashuel, *J. Biol. Chem.*, 2011, **286**, 8585–8596.
- 27 E. Hellstrand, B. Boland, D. M. Walsh and S. Linse, *ACS Chem. Neurosci.*, 2010, **1**, 13–18.
- 28 Y. Miller, B. Ma and R. Nussinov, *Chem. Rev.*, 2010, **110**, 4820–4838.
- 29 S. Nag, B. Sarkar, A. Bandyopadhyay, B. Sahoo, V. K. Sreenivasan, M. Kombrabail, C. Muralidharan and S. Maiti, *J. Biol. Chem.*, 2011, **286**, 13827–13833.
- 30 D. J. Hayne, S. Lim and P. S. Donnelly, *Chem. Soc. Rev.*, 2014, **43**, 6701–6715.
- 31 A. Deshpande, E. Mina, C. Glabe and J. Busciglio, *J. Neurosci.*, 2006, **26**, 6011–6018.
- 32 R. M. Murphy, *Annu. Rev. Biomed. Eng.*, 2002, **4**, 155–174.
- 33 R. J. Morris, K. Eden, R. Yarwood, L. Jourdain, R. J. Allen and C. E. Macphee, *Nat. Commun.*, 2013, **4**, 1891.

- 34 M. Coles, W. Bicknell, A. A. Watson, D. P. Fairlie and D. J. Craik, *Biochemistry*, 1998, **37**, 11064–11077.
- 35 O. Crescenzi, S. Tomaselli, R. Guerrini, S. Salvadori, A. M. D'Ursi, P. A. Temussi and D. Picone, *Eur. J. Biochem.*, 2002, **269**, 5642–5648.
- 36 S. A. Kotler, P. Walsh, J. R. Brender and A. Ramamoorthy, *Chem. Soc. Rev.*, 2014, **43**, 6692–6700.
- 37 R. Kaye and C. A. Lasagna-Reeves, *J. Alzheimers Dis.*, 2013, **33**, S67–S78.
- 38 L. M. Hou, H. Y. Shao, Y. B. Zhang, H. Li, N. K. Menon, E. B. Neuhaus, J. M. Brewer, I. J. L. Byeon, D. G. Ray, M. P. Vitek, T. Iwashita, R. A. Makula, A. B. Przybyla and M. G. Zagorski, *J. Am. Chem. Soc.*, 2004, **126**, 1992–2005.
- 39 S. Mitternacht, I. Staneva, T. Hard and A. Irback, *Proteins*, 2010, **78**, 2600–2608.
- 40 A. Sandberg, L. M. Luheshi, S. Sollvander, T. Pereira de Barros, B. Macao, T. P. Knowles, H. Biverstal, C. Lendel, F. Ekholm-Pettersson, A. Dubnovitsky, L. Lannfelt, C. M. Dobson and T. Hard, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 15595–15600.
- 41 D. Lambracht-Washington and R. N. Rosenberg, *Discov. Med.*, 2013, **15**, 319–326.
- 42 S. Vivekanandan, J. R. Brender, S. Y. Lee and A. Ramamoorthy, *Biochem. Biophys. Res. Commun.*, 2011, **411**, 312–316.
- 43 S. Tomaselli, V. Esposito, P. Vangone, N. A. van Nuland, A. M. Bonvin, R. Guerrini, T. Tancredi, P. A. Temussi and D. Picone, *ChemBioChem*, 2006, **7**, 257–267.
- 44 M. K. Tiwari, R. K. Singh, R. Singh, M. Jeya, H. Zhao and J. K. Lee, *J. Biol. Chem.*, 2012, **287**, 19429–19439.
- 45 B. R. Brooks, C. L. Brooks 3rd, A. D. Mackerell Jr., L. Nilsson, R. J. Petrella, B. Roux, Y. Won, G. Archontis, C. Bartels, S. Boresch, A. Caflisch, L. Caves, Q. Cui, A. R. Dinner, M. Feig, S. Fischer, J. Gao, M. Hodoscek, W. Im, K. Kuczera, T. Lazaridis, J. Ma, V. Ovchinnikov, E. Paci, R. W. Pastor, C. B. Post, J. Z. Pu, M. Schaefer, B. Tidore, R. M. Venable, H. L. Woodcock, X. Wu, W. Yang, D. M. York and M. Karplus, *J. Comput. Chem.*, 2009, **30**, 1545–1614.
- 46 Y. Yoshiike, T. Akagi and A. Takashima, *Biochemistry*, 2007, **46**, 9805–9812.
- 47 B. Moores, E. Drolle, S. J. Attwood, J. Simons and Z. Leonenko, *PLoS One*, 2011, **6**, e25954.
- 48 J. Krishnamoorthy, J. R. Brender, S. Vivekanandan, N. Jahr and A. Ramamoorthy, *J. Phys. Chem. B*, 2012, **116**, 13618–13623.
- 49 S. M. Butterfield and H. A. Lashuel, *Angew. Chem., Int. Ed.*, 2010, **49**, 5628–5654.
- 50 S. A. Kotler, P. Walsh, J. R. Brender and A. Ramamoorthy, *Chem. Soc. Rev.*, 2014, **43**, 6692–6700.
- 51 M. F. M. Sciacca, S. A. Kotler, J. R. Brender, J. Chen, D. K. Lee and A. Ramamoorthy, *Biophys. J.*, 2012, **103**, 702–710.
- 52 A. Rijal Upadhaya, I. Kosterin, S. Kumar, C. A. von Arnim, H. Yamaguchi, M. Fandrich, J. Walter and D. R. Thal, *Brain*, 2014, **137**, 887–903.
- 53 A. Ramamoorthy and M. H. Lim, *Biophys. J.*, 2013, **105**, 287–288.
- 54 S. Lee, X. Y. Zheng, J. Krishnamoorthy, M. G. Savelieff, H. M. Park, J. R. Brender, J. H. Kim, J. S. Derrick, A. Kochi, H. J. Lee, C. Kim, A. Ramamoorthy, M. T. Bowers and M. H. Lim, *J. Am. Chem. Soc.*, 2014, **136**, 299–310.
- 55 Accelrys Inc., 2013.
- 56 A. Sali and T. L. Blundell, *J. Mol. Biol.*, 1993, **234**, 779–815.
- 57 R. Fraczekiewicz and W. Braun, *J. Comput. Chem.*, 1998, **19**, 319–333.
- 58 C. Alland, F. Moreews, D. Boens, M. Carpentier, S. Chiusa, M. Lonquety, N. Renault, Y. Wong, H. Cantalloube, J. Chomilier, J. Hochez, J. Pothier, B. O. Villoutreix, J. F. Zagury and P. Tuffery, *Nucleic Acids Res.*, 2005, **33**, W44–W49.
- 59 B. Neron, H. Menager, C. Maufrais, N. Joly, J. Maupetit, S. Letort, S. Carrere, P. Tuffery and C. Letondal, *Bioinformatics*, 2009, **25**, 3005–3011.
- 60 E. G. Hutchinson and J. M. Thornton, *Protein Sci.*, 1996, **5**, 212–220.
- 61 C. Nilsberth, A. Westlind-Danielsson, C. B. Eckman, M. M. Condron, K. Axelman, C. Forsell, C. Stenh, J. Luthman, D. B. Teplow, S. G. Younkin, J. Naslund and L. Lannfelt, *Nat. Neurosci.*, 2001, **4**, 887–893.
- 62 T. Jonsson, J. K. Atwal, S. Steinberg, J. Snaedal, P. V. Jonsson, S. Bjornsson, H. Stefansson, P. Sulem, D. Gudbjartsson, J. Maloney, K. Hoyte, A. Gustafson, Y. Liu, Y. Lu, T. Bhangale, R. R. Graham, J. Huttenlocher, G. Bjornsdottir, O. A. Andreassen, E. G. Jonsson, A. Palotie, T. W. Behrens, O. T. Magnusson, A. Kong, U. Thorsteinsdottir, R. J. Watts and K. Stefansson, *Nature*, 2012, **488**, 96–99.
- 63 G. Di Fede, M. Catania, M. Morbin, G. Rossi, S. Suardi, G. Mazzoleni, M. Merlin, A. R. Giovagnoli, S. Prioni, A. Erbetta, C. Falcone, M. Gobbi, L. Colombo, A. Bastone, M. Beeg, C. Manzoni, B. Francescucci, A. Spagnoli, L. Cantu, E. Del Favero, E. Levy, M. Salmons and F. Tagliavini, *Science*, 2009, **323**, 1473–1477.
- 64 K. Ono, M. M. Condron and D. B. Teplow, *J. Biol. Chem.*, 2010, **285**, 23186–23197.
- 65 Y. Hori, T. Hashimoto, Y. Wakutani, K. Urakami, K. Nakashima, M. M. Condron, S. Tsubuki, T. C. Saido, D. B. Teplow and T. Iwatsubo, *J. Biol. Chem.*, 2007, **282**, 4916–4923.
- 66 W. T. Chen, C. J. Hong, Y. T. Lin, W. H. Chang, H. T. Huang, J. Y. Liao, Y. J. Chang, Y. F. Hsieh, C. Y. Cheng, H. C. Liu, Y. R. Chen and I. H. Cheng, *PLoS One*, 2012, **7**, e35807.
- 67 L. Zhou, N. Brouwers, I. Benilova, A. Vandersteen, M. Mercken, K. Van Laere, P. Van Damme, D. Demedts, F. Van Leuven, K. Sleegers, K. Broersen, C. Van Broeckhoven, R. Vandenberghe and B. De Strooper, *EMBO Mol. Med.*, 2011, **3**, 291–302.
- 68 D. Kaden, A. Harmeier, C. Weise, L. M. Munter, V. Althoff, B. R. Rost, P. W. Hildebrand, D. Schmitz, M. Schaefer,

- R. Lurz, S. Skodda, R. Yamamoto, S. Arlt, U. Finckh and G. Multhaup, *EMBO Mol. Med.*, 2012, **4**, 647–659.
- 69 W. E. Van Nostrand, J. P. Melchor, H. S. Cho, S. M. Greenberg and G. W. Rebeck, *J. Biol. Chem.*, 2001, **276**, 32860–32866.
- 70 L. Miravalle, T. Tokuda, R. Chiarle, G. Giaccone, O. Bugiani, F. Tagliavini, B. Frangione and J. Ghiso, *J. Biol. Chem.*, 2000, **275**, 27110–27116.
- 71 T. Tomiyama, T. Nagata, H. Shimada, R. Teraoka, A. Fukushima, H. Kanemitsu, H. Takuma, R. Kuwano, M. Imagawa, S. Ataka, Y. Wada, E. Yoshioka, T. Nishizaki, Y. Watanabe and H. Mori, *Ann. Neurol.*, 2008, **63**, 377–387.
- 72 L. Obici, A. Demarchi, G. de Rosa, V. Bellotti, S. Marciano, S. Donadei, E. Arbustini, G. Palladini, M. Diegoli, E. Genovese, G. Ferrari, S. Coverlizza and G. Merlini, *Ann. Neurol.*, 2005, **58**, 639–644.
- 73 D. A. Carter, E. Desmarais, M. Bellis, D. Champion, F. Clerget-Darpoux, A. Brice, Y. Agid, A. Jaillard-Serradt and J. Mallet, *Nat. Genet.*, 1992, **2**, 255–256.
- 74 C. T. Jones, S. Morris, C. M. Yates, A. Moffoot, C. Sharpe, D. J. Brock and D. St Clair, *Nat. Genet.*, 1992, **1**, 306–309.
- 75 Y. Tomidokoro, A. Rostagno, T. A. Neubert, Y. Lu, G. W. Rebeck, B. Frangione, S. M. Greenberg and J. Ghiso, *Am. J. Pathol.*, 2010, **176**, 1841–1854.