Structural variation in amyloid-β fibrils from Alzheimer’s disease clinical subtypes

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Aggregation of amyloid-β peptides into fibrils or other self-assembled states is central to the pathogenesis of Alzheimer’s disease. Fibrils formed in vitro by 40- and 42-residue amyloid-β peptides (Aβ40 and Aβ42) are polymorphic, with variations in molecular structure that depend on fibril growth conditions1–12. Recent experiments1,13–16 suggest that variations in amyloid-β fibril structure in vivo may correlate with variations in Alzheimer’s disease phenotype, in analogy to distinct prion strains that are associated with different clinical and pathological phenotypes17–19. Here we investigate correlations between structural variation and Alzheimer’s disease phenotype using solid-state nuclear magnetic resonance (ssNMR) measurements on Aβ40 and Aβ42 fibrils prepared by seeded growth from extracts of Alzheimer’s disease brain cortex. We compared two atypical Alzheimer’s disease clinical subtypes—the rapidly progressive form (r-AD) and the posterior cortical atrophy variant (PCA-AD)—with a typical prolonged-duration form (t-AD). On the basis of ssNMR data from 37 cortical tissue samples from 18 individuals, we find that a single Aβ40 fibril structure is most abundant in samples from patients with t-AD and PCA-AD, whereas Aβ40 fibrils from r-AD samples exhibit a significantly greater proportion of additional structures. Data for Aβ42 fibrils indicate structural heterogeneity in most samples from all patient categories, with at least two prevalent structures. These results demonstrate the existence of a specific predominant Aβ40 fibril structure in t-AD and PCA-AD, suggest that r-AD may relate to additional fibril structures and indicate that there is a qualitative difference between Aβ40 and Aβ42 aggregates in the brain tissue of patients with Alzheimer’s disease.

There is a range of evidence that amyloid-β fibril polymorphism may correlate with variations in clinical and pathological features of Alzheimer’s disease. First, Aβ40 fibrils with different molecular structures exhibit different levels of toxicity in primary neuronal cell cultures1. Second, patterns of amyloid deposition in transgenic mice, induced by exogenous amyloid-β-containing biological material, vary with the source of this material13,14. Third, the size and composition of amyloid plaques induced in transgenic mice by synthetic Aβ42 fibrils depend on the morphology and growth conditions of these fibrils15. Fourth, the size distributions and resistance to chemical denaturation of Aβ42 aggregates in brain tissue differ between patients with rapidly progressing and slowly progressing Alzheimer’s disease16. Improved characterization of the structures of neurotoxic amyloid-β assemblies in Alzheimer’s disease and of correlations between structure and disease phenotype would have a major impact on our understanding of pathogenesis, on the development of appropriate diagnostic and therapeutic biomarkers, and on drug development.

Data from ssNMR are particularly sensitive to structural variations, allowing 2D 13C–13C and 15N–13C ssNMR spectra to be used as ‘fingerprints’ of specific fibril polymorphs1,12,21. Since ssNMR requires milligram-scale quantities of isotopically labelled fibrils, we amplified and labelled structures in brain tissue by seeded growth, using brain tissue as the source of fibril seeds, as described previously4. In prion diseases, different strains produce different durations of illness, preferentially target different brain regions and are associated with conformational differences in disease-related prion proteins17–19,22. By analogy, we selected tissue samples from patients from two unusual Alzheimer’s disease subtypes: PCA-AD, which is associated with disruption of visual processing23; and r-AD, in which neurodegeneration occurs within months and which clinically resembles Creutzfeldt–Jakob disease24. We also included tissue from three individuals who died without dementia but who were nevertheless found to have amyloid-β deposition at autopsy.

We prepared Aβ40 and Aβ42 fibrils separately by seeded growth from amyloid-enriched cortical extracts. Transmission electron microscope (TEM) images were acquired and ssNMR measurements attempted for all fibril samples, although in some cases the signal-to-noise ratios in ssNMR data were insufficient for acquisition or subsequent analysis of 2D spectra. Table 1 summarizes the tissue samples, patient categories, and ssNMR measurements. Examples of TEM images and full sets of 2D spectra are given in Extended Data Figs 1–3. Control experiments using cortical extracts from a patient without Alzheimer’s disease or substantial amyloid-β deposition are described in the Supplementary Discussion and Extended Data Fig. 4.

Figure 1 shows representative data for Aβ40 fibrils. In TEM images (Fig. 1a and Extended Data Fig. 1), certain fibrils exhibit modulations of their apparent width with a period of 107 ± 20 nm (mean ± s.d. from 65 measurements). This fibril morphology may be more abundant in images of Aβ40 fibrils derived from t-AD and PCA-AD tissue samples. However, quantification of the relative populations of fibril polymorphs from the TEM images is not possible because only a small subset of the fibrils can be visualized clearly. By contrast, all fibrils contribute to the 2D ssNMR spectra. Most 2D spectra of brain-seeded Aβ40 fibrils contain the same set of strong cross-peak signals, indicated by assignments to the isotopically labelled residues in Fig. 1b, c, although spectra of certain samples contain additional cross-peak signals with varying intensities.

Figure 2 shows representative data for Aβ42 fibrils. TEM images do not show a single morphology that is clearly predominant within any of the tissue categories (Fig. 2a). The 2D spectra of most samples do not show a single set of cross-peak signals from the isotopically labelled residues (Fig. 2b, c).

We analysed the 2D ssNMR spectra by two independent methods, both intended to be objective and devoid of assumptions about the nature of the fibril structures or structural variations. Only 2D spectra with adequate signal-to-noise ratios (see Supplementary Methods) were included in these analyses. In the first method, we compared each 2D spectrum in a given set (13C–13C or 15N–13C, Aβ40 or Aβ42) with all other 2D spectra in the same set. Comparisons were quantified by pairwise root mean square deviation (r.m.s.d.) values between 2D spectra, calculated as described in the Supplementary Methods. For 2D 13C–13C and 15N–13C spectra of Aβ40 fibrils, plots in Fig. 3 indicate that r.m.s.d. values among spectra from t-AD and PCA-AD...
samples are relatively small (blue shades) in most cases, whereas r.m.s.d. values between spectra from r-AD samples and spectra from either t-AD or PCA-AD samples are relatively large (red shades). Thus, t-AD and PCA-AD spectra are similar to one other, but contrast sharply with r-AD spectra, which have larger variability. Statistics are summarized in Extended Data Table 1a. Mean r.m.s.d. values among t-AD spectra are not significantly different from mean r.m.s.d. values between t-AD spectra and PCA-AD spectra. However, mean r.m.s.d. values among t-AD or PCA-AD spectra are significantly smaller than mean r.m.s.d. values between t-AD and r-AD spectra or between PCA-AD and r-AD spectra (P < 0.001, Welch’s t-test). In addition, for 2D $^{15}$N–$^{13}$C spectra of Aβ40 fibrils, the mean r.m.s.d. value among PCA-AD spectra is significantly smaller than the mean r.m.s.d. value among t-AD spectra, the mean r.m.s.d. value among PCA-AD spectra is significantly smaller than the mean r.m.s.d. value among r-AD spectra, and the mean r.m.s.d. value among t-AD spectra is significantly smaller than the mean r.m.s.d. value among r-AD spectra (P < 0.001).

For 2D spectra of Aβ42 fibrils, plots in Fig. 3 do not show clear patterns, and no statistically significant differences among mean r.m.s.d. values are identified.

In the second method of analysis, we used singular value decomposition to determine principal component spectra$^{25}$ for each set of 2D spectra (see Supplementary Methods). For each set, the number $N$ of principal component spectra equals the number of experimental 2D spectra. The experimental spectra within each set, including both cross-peak signals and random noise, can be represented exactly as linear combinations of the principal component spectra (Extended Data Figs 5 and 6), with coefficients $C_k$ ($k = 1, 2, \ldots, N$). As shown in Fig. 4 and Extended Data Table 1b, mean values of $C_2$ for 13C–13C and 15N–13C spectra of Aβ40 fibrils derived from r-AD tissue are significantly larger than the corresponding mean values of $C_2$ for both t-AD spectra and PCA-AD spectra (P < 0.01). This indicates that the 2D spectra of Aβ42 fibrils derived from r-AD tissue differ more strongly from the average 2D spectra than do spectra of Aβ40 fibrils derived from t-AD

### Table 1 | Summary of brain tissue samples and 2D ssNMR data

| Patient | Gender | Age at clinical onset$^*$ | Clinical duration | Sample$^†$ | Cortical region | Aβ40 $^{13}$C–$^{13}$C | Aβ40 $^{15}$N–$^{13}$C | Aβ42 $^{13}$C–$^{13}$C | Aβ42 $^{15}$N–$^{13}$C |
|---------|--------|--------------------------|------------------|------------|----------------|---------------------|---------------------|---------------------|---------------------|
| t-AD1   | Female | 65                       | 11 yr            | t-AD1f     | Frontal        | X                   | X                   | X                   | X                   |
|         |        |                          |                  | t-AD1o     | Occipital      | X                   | X                   | w                   | n                   |
|         |        |                          |                  | t-AD1p     | Parietal       | X                   | X                   | X                   | X                   |
|         |        |                          |                  | t-AD2f     | Frontal        | X                   | X                   | X                   | X                   |
| t-AD2   | Male   | 64                       | 13 yr            | t-AD2o     | Occipital      | w                   | w                   | X                   | X                   |
|         |        |                          |                  | t-AD2p     | Parietal       | X                   | X                   | w                   | n                   |
|         |        |                          |                  | t-AD3f     | Frontal        | X                   | X                   | X                   | X                   |
|         |        |                          |                  | t-AD3o     | Occipital      | X                   | X                   | X                   | X                   |
| t-AD3   | Male   | 57                       | 7 yr             | t-AD3o'    | Occipital      | X                   | X                   | n                   | n                   |
|         |        |                          |                  | t-AD3p     | Parietal       | X                   | w                   | X                   | X                   |
|         |        |                          |                  | t-AD3p'    | Parietal       | X                   | n                   | n                   | n                   |
| t-AD4   | Female | 51                       | 11 yr            | t-AD4f     | Frontal        | X                   | X                   | w                   | n                   |
| t-AD5   | Female | 65                       | 5 yr             | t-AD5f     | Frontal        | X                   | X                   | w                   | n                   |
| t-AD6   | Female | 58                       | 21 yr            | t-AD6f     | Frontal        | X                   | X                   | w                   | w                   |
|         |        |                          |                  | PCA1f      | Frontal        | X                   | w                   | X                   | X                   |
| PCA-AD1 | Male   | 55                       | 9 yr             | PCA1o      | Occipital      | X                   | X                   | X                   | X                   |
|         |        |                          |                  | PCA1p      | Parietal       | X                   | n                   | X                   | X                   |
|         |        |                          |                  | PCA2f      | Frontal        | X                   | X                   | X                   | X                   |
| PCA-AD2 | Male   | 56                       | 6 yr             | PCA2o      | Occipital      | X                   | X                   | X                   | w                   |
|         |        |                          |                  | PCA2p      | Parietal       | X                   | w                   | n                   | n                   |
|         |        |                          |                  | PCA3f      | Frontal        | X                   | X                   | X                   | w                   |
| PCA-AD3 | Male   | 58                       | 10 yr            | PCA3o      | Occipital      | X                   | w                   | X                   | X                   |
|         |        |                          |                  | PCA3p      | Parietal       | X                   | w                   | n                   | n                   |
| r-AD1   | Male   | 79                       | 3 mth            | r-AD1f     | Frontal        | X                   | X                   | w                   | w                   |
|         |        |                          |                  | r-AD1p     | Parietal       | X                   | X                   | w                   | n                   |
|         |        |                          |                  | r-AD2f     | Frontal        | w                   | X                   | X                   | X                   |
|         |        |                          |                  | r-AD2f'    | Frontal        | X                   | X                   | n                   | n                   |
| r-AD2   | Male   | 83                       | 6 mth            | r-AD2o     | Occipital      | X                   | X                   | n                   | n                   |
|         |        |                          |                  | r-AD2p     | Parietal       | X                   | X                   | X                   | X                   |
|         |        |                          |                  | r-AD2p'    | Parietal       | X                   | X                   | n                   | n                   |
| r-AD3   | Female | 73                       | 8 mth            | r-AD3f     | Frontal        | w                   | X                   | X                   | X                   |
| r-AD4   | Female | 74                       | 18 mth           | r-AD4f     | Frontal        | X                   | X                   | w                   | n                   |
| r-AD5   | Female | 66                       | 21 mth           | r-AD5f     | Frontal        | X                   | X                   | w                   | n                   |
| r-AD6   | Female | 65                       | 18 mth           | r-AD6f     | Frontal        | w                   | n                   | n                   | n                   |
| ND1     | Female | 73                       | –                | ND1f       | Frontal        | X                   | X                   | w                   | X                   |
| ND2     | Male   | 88                       | –                | ND2f       | Frontal        | X                   | w                   | w                   | n                   |
| ND3     | Male   | 83                       | –                | ND3f       | Frontal        | w                   | w                   | n                   | n                   |

$^*$For non-dementia (ND) control samples, age at death.

$^†$Samples t-AD3o', t-AD3p', r-AD2f', and r-AD2p' are separate cortical tissue samples from the same patients and brain regions as t-AD3o, t-AD3p, r-AD2f, and r-AD2p, respectively.

n, data not obtained; w, data obtained, but low signal-to-noise; X, data obtained.
and PCA-AD tissue. For Aβ42 fibrils, differences in the mean values of Cα are not significant.

An alternative analysis of the 2D 15N–13C spectra, which the spectra were fit with a fixed number of cross-peaks at fixed chemical-shift positions, is described in the Supplementary Methods and Extended Data Fig. 7. According to this analysis, on average, the predominant Aβ40 fibril structure typically accounts for approximately 80% of the cross-peak signal volume in 2D 15N–13C spectra of Aβ40 fibrils derived from t-AD and PCA-AD tissue, and approximately 65% in 2D 15N–13C spectra of Aβ40 fibrils derived from r-AD tissue. We compare 15N and 13C chemical shifts in our spectra of brain-seeded fibrils with previously reported chemical shifts for Aβ40 and Aβ42 fibrils1,2,4,6,7,26–28 in the Supplementary Discussion and Extended Data Fig. 8.

We draw the following main conclusions from these ssNMR experiments: (i) although brain-seeded Aβ340 fibrils can be polymorphic, a single structure is the most abundant in the cortical tissue of most patients with t-AD and PCA-AD; (ii) polymorphism of Aβ40 fibrils is more pronounced in r-AD samples than it is in t-AD and PCA-AD samples; and (iii) brain-seeded Aβ42 fibrils are generally more structurally heterogeneous than brain-seeded Aβ40 fibrils, lacking a clearly predominant structure even in t-AD and PCA-AD samples. Although the small number of samples prevents us from drawing definite conclusions regarding fibril structures in the brain tissue of patients without dementia that have amyloid-β fibrils, we have not observed ssNMR signals that are unique to fibrils derived from patients without dementia.

The 2D ssNMR spectra of the predominant Aβ40 fibril structure, along with the fibril morphology in Fig. 1a, match data for Aβ40 fibrils from one of two patients with Alzheimer’s disease described previously4. This patient, called ‘patient 2’, had t-AD. By contrast, Aβ40 fibrils from ‘patient 1’ exhibited distinctive ssNMR signals that are not present in any of the measurements described above (Extended Data Fig. 8). The clinical history of patient 1 was also different, including an initial diagnosis of Lewy body dementia rather than Alzheimer’s disease. Thus, it remains possible that the Aβ40 fibril structure of patient 1, for which a full molecular model was developed4, is associated with a specific Alzheimer’s disease variant that we have not examined here.

The fact that we cannot distinguish between amyloid-β fibrils from t-AD and PCA-AD tissue suggests that phenotypic differences between these two categories arise from factors other than fibril structure, perhaps including genetic factors or differences in non-fibrillar amyloid-β assemblies. The greater polymorphism seen in Aβ40 fibrils from r-AD tissue, indicated by 2D ssNMR spectra, may mean that one or more fibril structures that are prevalent in r-AD tissue have enhanced neurotoxicity, through either direct or indirect mechanisms. Alternatively, in r-AD samples, fibrils could reside in the tissue for a shorter period than in t-AD and PCA-AD samples, potentially allowing fibrils with a greater range of thermodynamic stabilities or resistance to degradation to be present at autopsy.

The greater overall heterogeneity of Aβ42 fibrils seeded with t-AD and PCA-AD extract, compared to the corresponding Aβ40 fibrils, is conceivably a consequence of imperfect amplification of Aβ42 fibril structures from brain tissue under our experimental conditions, possibly due to a stronger tendency of the more hydrophobic Aβ2 peptide to form non-fibrillar aggregates spontaneously, or to the lower

Figure 1 | Representative TEM images and 2D ssNMR spectra of brain-seeded Aβ40 fibrils. a, Images of negatively-stained fibrils derived from t-AD3f, PCA1o, and r-AD1f tissue, recorded 4 h after initiation of seeded fibril growth (representative of 37 fibril samples). Single-headed arrows indicate the periodic modulation of apparent fibril width in a common Aβ40 fibril morphology. Double-headed arrows indicate an additional morphology. b, Aliphatic regions of 2D 13C–13C spectra of the same samples (colour-coded as in a), with assignments of cross-peak signals to isotopically labelled residues shown in the 2D spectrum of t-AD3f fibrils. Aβ40 was uniformly 15N–13C-labelled at residues F19, V24, G25, S26, A30, L34 and M35. Contour levels increase by successive factors of 1.3. Shown on the right are 1D slices at 21.0 p.p.m. and 53.5 p.p.m., with double-headed arrows indicating signals that arise from the less common fibril structures. c, The 2D 15N–13C spectra of the same samples, with assignments of the predominant cross-peak signals shown in the 2D spectrum of t-AD3f fibrils and assignments of additional signals (with asterisks) shown in the 2D spectrum of r-AD1f fibrils. Contour levels increase by successive factors of 1.3. Shown on the right are 1D slices at 112.7 p.p.m. and 121.0 p.p.m., with double-headed arrows indicating signals that arise from the less common fibril structures.
abundance of Aβ42 fibrils in most of our tissue samples (Extended Data Table 2). However, 2D ssNMR spectra of a control sample, in which Aβ42 fibrils were grown in the presence of extract from occipital tissue that lacked detectable fibril seeds, show surprisingly sharp cross-peak signals that indicate a roughly 70% population of a single structure (Supplementary Discussion and Extended Data Fig. 4).

Thus, the greater structural heterogeneity of Alzheimer’s disease brain-seeded Aβ42 fibrils in our experiments most probably indicates greater heterogeneity of the fibril seeds within the cortical tissue. Recent research indicates that there are differences in the structural heterogeneity and sizes of Aβ42 aggregates, but not Aβ40 aggregates, in r-AD and t-AD brain tissue10.

![Representative TEM images and 2D ssNMR spectra of brain-seeded Aβ42 fibrils.](image1)

**Figure 2** | Representative TEM images and 2D ssNMR spectra of brain-seeded Aβ42 fibrils. a. Images of negatively-stained fibrils derived from t-AD3p, PCA1p, and r-AD2p tissue (representative of 33 fibril samples). b. The 2D 13C–13C spectra of the same samples (colour-coded as in a), with assignments of cross-peak signals to isotopically labelled residues shown in the 2D spectrum of t-AD3p fibrils. Aβ42 was uniformly 13C-labelled at F19, G25, A30, I31, L34, and M35. Shown on the right are 1D slices at 27.6 p.p.m. and 53.5 p.p.m. c. The 2D 15N–13C spectra of the same samples as in a, with assignments of the cross-peak signals shown in the 2D spectrum of t-AD3p fibrils. Two 13C–15N–13C cross-peaks with similar intensities are observed for A30 and 13I, with and without asterisks, indicating similar populations of two distinct fibril structures. Shown on the right are 1D slices at 119.5 p.p.m. and 127.1 p.p.m.

![Pairwise differences among 2D ssNMR spectra of brain-seeded Aβ40 and Aβ42 fibrils.](image2)

**Figure 3** | Pairwise differences among 2D ssNMR spectra of brain-seeded Aβ40 and Aβ42 fibrils. r.m.s.d. values are displayed on the colour scales shown to the right of each plot, with blue and red shades indicating relatively similar and dissimilar spectra, respectively. r.m.s.d. plots for Aβ40 fibrils indicate that fibrils derived from t-AD and PCA-AD tissue have similar 2D spectra in most cases, whereas greater differences are observed in spectra of Aβ40 fibrils derived from r-AD tissue. For Aβ42 fibrils, correlations between r.m.s.d. values and tissue categories are not observed. Statistical analyses are summarized in Extended Data Table 1a. Shades above white represent significant differences between 2D spectra. Samples prefixed with ND are from patients without dementia.
The experiments described above represent the first use, to our knowledge, of ssNMR to screen multiple tissue samples for variations in amyloid fibril structure. Similar approaches can be applied to other amyloid diseases, in which related phenomena exist. However, goals for future work are to develop a full structural model for the predominant Aβ40 polymorph identified here and to determine whether structurally distinct Aβ40 polymorphs can consistently seed different patterns of amyloid-β pathology in suitable animal models.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions W.Q., J.-X.L., and R.T. designed experiments, including selection of tissue samples, development of protocols for preparation of brain-seeded fibrils, and selection of ssNMR measurements. W.Q., J.-X.L., and R.T. prepared fibril samples and acquired TEM images and ssNMR data. W.-M.Y. synthesized isotopically labelled peptides and performed ELISA measurements. W.Q. and R.T. analysed ssNMR data. J.C. and R.T. wrote the manuscript, with contributions from all other authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to R.T. (robertty@mail.nih.gov) or J.C. (jc@prion.ucl.ac.uk).
Extended Data Figure 1 | Additional TEM images of brain-seeded fibrils. a, TEM grids were prepared 4 h after the addition of solubilized Aβ40 or Aβ42 to sonicated brain extract and were negatively stained with uranyl acetate. Collagen fibrils in the extract (40–100 nm width, with characteristic transverse bands) appear in some images. Material with an amorphous appearance are non-fibrillar, non-Aβ components of the brain extract. Yellow arrows indicate Aβ40 fibrils with an apparent width modulation, attributable to an approximately periodic twisting of the fibril structure about the fibril growth direction. TEM images of all 37 brain-seeded Aβ40 and all 33 Aβ42 fibril samples are available at http://dx.doi.org/10.17632/whgp9r7tkd.1. b, Histogram of distances between width minima for Aβ40 fibrils with apparent width modulation. The Gaussian fit to this histogram (red curve) has a mean value of 107.2 nm (n = 65) and a full-width-at-half-maximum of 46.1 nm.
Extended Data Figure 2 | 2D ssNMR spectra of brain-seeded Aβ40 fibrils. a, 2D 13C–13C spectra of fibrils seeded with extract from t-AD, PCA-AD, r-AD, or non-dementia (ND) tissue. Aliphatic regions are shown, with 15 contour levels (increasing by successive factors of 1.3, and with the highest contour at the maximum signal in each 2D spectrum). b, 2D 15N–13C spectra of fibrils seeded with extract from t-AD, PCA-AD, r-AD, or non-dementia tissue. Regions containing intra-residue 15N–13C α cross-peaks are shown, with 11 contour levels (increasing by successive factors of 1.3, with the highest contour at the maximum signal in each spectrum). 15N–13C3 cross-peaks from L34 appear in some spectra. Positions of cross-peaks from the predominant Aβ40 fibril structure are indicated by colour-coded circles (F19, blue; V24, cyan; G25, pink; S26, orange; A30, purple; I31, red; L34, green; M35, magenta). Only 2D spectra that were included in the analyses in Figs. 3 and 4 are shown. The full set of 42 2D 13C–13C spectra and 40 2D 15N–13C spectra, including those with lower signal-to-noise ratios, controls, and technical replicates, is available on-line at http://dx.doi.org/10.17632/tbp45pm92x.1.
Extended Data Figure 3 | 2D ssNMR spectra of brain-seeded Aβ42 fibrils. a, 2D ¹³C–¹³C spectra of fibrils seeded with extract from t-AD, PCA-AD, r-AD, or non-dementia (ND) tissue. Aliphatic regions are shown, with 15 contour levels (increasing by successive factors of 1.3, and with the highest contour at the maximum signal in each spectrum). b, 2D ¹⁵N–¹³C spectra of fibrils seeded with extract from t-AD, PCA-AD, r-AD, or non-dementia tissue. Regions containing intra-residue ¹⁵N–¹³C{α} cross-peaks are shown, with 11 contour levels (increasing by successive factors of 1.3, with the highest contour at the maximum signal in each spectrum). Only 2D spectra that were included in the analyses in Figs. 3 and 4 are shown. The full set of 33 2D ¹³C–¹³C spectra and 23 2D ¹⁵N–¹³C spectra, including those with lower signal-to-noise, controls, and technical replicates, is available at http://dx.doi.org/10.17632/tbp45pm92x.1.
Extended Data Figure 4 | Control experiments using cortical tissue without Aβ deposits. a, Comparison of TEM images of control tissue extract and r-AD2p′ extract after incubation for 4 h with solubilized Aβ40, under conditions identical to those that led to fibrils shown in Extended Data Fig. 1a. Fibrils associated with brain material were abundant on the TEM grid of the r-AD2p′-seeded sample, but were not observed in an extensive search over the TEM grid of the control sample. b, TEM images of control tissue extract and r-AD2p′ extract after incubation for 4 h with solubilized Aβ42, under conditions identical to those that led to fibrils shown in Extended Data Fig. 1b. Fibrils associated with brain material were abundant on the TEM grid of the r-AD2p′-seeded sample, but were not observed in an extensive search over the TEM grid of the control sample. c, 2D 13C–13C and 15N–13C spectra of Aβ40 fibrils (blue) and Aβ42 fibrils (red) that developed in control samples after 168 h or 48 h incubation, respectively, followed by 24 h intermittent sonication (see Supplementary Methods) and a further 72 h of additional incubation. Contour levels increase by successive factors of 1.4. d, r.m.s.d. values between 2D spectra of control fibrils and 2D spectra of AD brain-seeded fibrils, with dashed lines at values corresponding to white shades in Fig. 3. Occipital tissue of a female who died from cardiac arrest at age 86 was used as a control.
Extended Data Figure 5 | Principal component analyses of 2D $^{13}\text{C}-^{13}\text{C}$ and $^{15}\text{N}-^{13}\text{C}$ ssNMR spectra of brain-seeded Aβ₄₀ fibrils. a, The first five principal components (PC1–PC5) of the 32 experimental 2D $^{13}\text{C}-^{13}\text{C}$ spectra shown as contour plots, with positive contours in blue and negative contours in red. Principal component spectra were obtained by singular-value decomposition of the experimental spectra, considering only the aliphatic region and excluding points within 5 p.p.m. of the diagonal. Contour levels increase (or decrease, in the case of negative contours) by successive factors of 1.5. b, Experimental 2D $^{13}\text{C}-^{13}\text{C}$ spectrum of t-AD4f Aβ₄₀ fibrils (left) and 2D spectrum constructed as a linear combination of PC1–PC5 (right, with coefficients of PC1–PC5 shown in parentheses). c, Experimental 2D $^{13}\text{C}-^{13}\text{C}$ spectrum of r-AD1f Aβ₄₀ fibrils (left) and 2D spectrum constructed as a linear combination of PC1–PC5 (right). d, The first five principal components of the 29 experimental 2D $^{15}\text{N}-^{13}\text{C}$ spectra. e, Experimental 2D $^{15}\text{N}-^{13}\text{C}$ spectrum of PCA2p Aβ₄₀ fibrils (left) and 2D spectrum constructed as a linear combination of PC1–PC5 (right). f, Experimental 2D $^{15}\text{N}-^{13}\text{C}$ spectrum of r-AD2o Aβ₄₀ fibrils (left) and 2D spectrum constructed as a linear combination of PC1–PC5 (right).
Extended Data Figure 6 | Principal component analyses of 2D $^{13}$C–$^{13}$C and $^{15}$N–$^{13}$C ssNMR spectra of brain-seeded Aβ42 fibrils. a, The first five principal components (PC1–PC5) of the 17 experimental 2D $^{13}$C–$^{13}$C spectra, plotted as in Extended Data Fig. 5. b, Experimental 2D $^{13}$C–$^{13}$C spectrum of t-AD1p Aβ42 fibrils (left) and 2D spectrum constructed as a linear combination of PC1–PC5 (right, with coefficients of PC1–PC5 shown in parentheses). c, Experimental 2D $^{13}$C–$^{13}$C spectrum of r-AD2f Aβ42 fibrils (left) and 2D spectrum constructed as a linear combination of PC1–PC5 (right). d, The first five principal components of the 15 experimental 2D $^{15}$N–$^{13}$C spectra. e, Experimental 2D $^{15}$N–$^{13}$C spectrum of t-AD3f Aβ42 fibrils (left) and 2D spectrum constructed as a linear combination of PC1–PC5 (right). f, Experimental 2D $^{15}$N–$^{13}$C spectrum of r-AD2p Aβ42 fibrils (left) and 2D spectrum constructed as a linear combination of PC1–PC5 (right).
Extended Data Figure 7 | Analysis of 2D $^{15}$N–$^{13}$C ssNMR spectra of brain-seeded fibrils by fitting with cross-peaks at fixed chemical-shift positions. a, Examples of 2D spectra (of the 29 Aβ40 and 15 Aβ42 spectra with adequate signal-to-noise ratios presented in Table 1), with fitted cross-peak positions indicated by crosses. Red and blue crosses indicate cross-peaks for chemical shift sets 'a' and 'b', respectively (see Supplementary Methods, Supplementary Discussion and Extended Data Fig. 8). Cyan crosses indicate additional cross-peaks. Contour levels increase by successive factors of 1.4.

b, Pairwise differences among fitted cross-peak volumes for spectra of Aβ40 fibrils (left) and Aβ42 fibrils (right), with colour scales representing r.m.s.d. values. Total cross-peak volumes in each spectrum were normalized before calculation of r.m.s.d. values. Results from this cross-peak-fitting analysis are similar to those in Fig. 3, in which the same experimental data were analysed by direct comparisons of signal amplitudes in 2D spectra without fitting the signals with cross-peaks at specific positions.

c, Fractions of the total fitted cross-peak volumes at 'a' and 'b' chemical shifts, with mean values indicated by horizontal bars. For Aβ40, mean values of 'a' volumes in spectra of t-AD ($n = 12$) or PCA-AD ($n = 6$) samples are significantly greater than the mean value ($n = 10$) in spectra of r-AD samples ($P < 0.02$, Welch's t-test; $P < 0.02$, Mann–Whitney–Wilcoxon test).
Extended Data Figure 8 | Comparisons of ssNMR chemical shifts of brain-seeded Aβ40 and Aβ42 fibrils with previously reported chemical shifts. a, 15N and 13C chemical shifts (p.p.m.) from spectra of brain-seeded samples in Table 1 (grouped into sets 'a', 'b', and so on, based on correlations of the corresponding signal amplitudes over multiple 2D spectra) are compared with chemical shifts from previous ssNMR studies of Aβ40 and Aβ42 fibrils, as deposited in the Biological Magnetic Resonance Bank (http://www.bmrb.wisc.edu/) with the indicated BMRB accession numbers. b, Differences in chemical shift after adjustments of chemical shift referencing in each set to make the average 13Cα shifts and the average 15N shifts equal in all sets.

| Aβ40 fibrils with two-fold symmetry, prepared in vitro, Protein Data Bank (PDB) files 2LM2 and 2LM0. | Aβ40 fibrils with three-fold symmetry, prepared in vitro, PDB file 1LMQ and 2LM0. | Brain-seeded Aβ40 fibrils, PDB file 2LNX. | "E231" Aβ40 fibrils, PDB file 2ENX. | "E231N" Aβ40 fibrils with an antiparallel β-sheet structure, PDB file 2L0E. | Aβ42 fibrils prepared in vitro, PDB file 2A2Q. | Aβ42 fibrils prepared in vitro, PDB file 2MKU. | Aβ42 fibrils prepared in vitro, PDB file 5K5C. |
|---|---|---|---|---|---|---|---|
| Aβ40 fibrils with two-fold symmetry, prepared in vitro, Protein Data Bank (PDB) files 2LM2 and 2LM0. | Aβ40 fibrils with three-fold symmetry, prepared in vitro, PDB file 1LMQ and 2LM0. | Brain-seeded Aβ40 fibrils, PDB file 2LNX. | "E231" Aβ40 fibrils, PDB file 2ENX. | "E231N" Aβ40 fibrils with an antiparallel β-sheet structure, PDB file 2L0E. | Aβ42 fibrils prepared in vitro, PDB file 2A2Q. | Aβ42 fibrils prepared in vitro, PDB file 2MKU. | Aβ42 fibrils prepared in vitro, PDB file 5K5C. |

Extended Data Figure 8 | Comparisons of ssNMR chemical shifts of brain-seeded Aβ40 and Aβ42 fibrils with previously reported chemical shifts. a, 15N and 13C chemical shifts (p.p.m.) from spectra of brain-seeded samples in Table 1 (grouped into sets 'a', 'b', and so on, based on correlations of the corresponding signal amplitudes over multiple 2D spectra) are compared with chemical shifts from previous ssNMR studies of Aβ40 and Aβ42 fibrils, as deposited in the Biological Magnetic Resonance Bank (http://www.bmrb.wisc.edu/) with the indicated BMRB accession numbers. b, Differences in chemical shift after adjustments of chemical shift referencing in each set to make the average 13Cα shifts and the average 15N shifts equal in all sets.

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Extended Data Table 1 | Statistical significance of analyses in Figs. 3 and 4

**a**

| 2D ssNMR data | t-AD: t-AD vs. t-AD/PCA-AD | t-AD: t-AD vs. t-AD/r-AD | PCA-AD: PCA-AD vs. PCA-AD/AD/PCA-AD | t-AD: t-AD vs. PCA-AD | t-AD: t-AD vs. t-AD/r-AD | PCA-AD: PCA-AD vs. PCA-AD/AD/PCA-AD | PCA-AD: PCA-AD vs. PCA-AD/r-AD |
|---------------|---------------------------|-------------------------|-------------------------------------|-----------------------|------------------------|-------------------------------------|--------------------------------|
|               |                           |                         |                                     |                       |                        |                                     |                                 |
| Alf-40 ^13^C-1^3^C | mean RMSD values          | 0.246,0,255             | 0.246,0,308                         | 0.246,0,248           | 0.246,0,299            | 0.246,0,299                         | 0.246,0,299                        |
|                | degrees of freedom        | 182                     | 147                                 | 50                    | 54                     | 40                                  | 60                               |
|                | p-value                   | 0.45                    | <0.001                              | <0.001                | 0.91                   | 0.005                               | 0.026                            |
|               |                           |                         |                                     |                       |                        |                                     |                                 |
| Alf-40 ^13^N-1^3^C | mean RMSD values          | 0.299,0,262             | 0.298,0,399                         | 0.220,0,381           | 0.299,0,220            | 0.299,0,396                         | 0.220,0,396                        |
|                | degrees of freedom        | 134                     | 165                                 | 67                    | 53                     | 84                                  | 57                               |
|                | p-value                   | 0.032                   | <0.001                              | <0.001                | <0.001                 | <0.001                              | <0.001                           |
|               |                           |                         |                                     |                       |                        |                                     |                                 |
| Alf-42 ^13^C-1^3^C | mean RMSD values          | 0.428,0,465             | 0.428,0,464                         | 0.404,0,431           | 0.428,0,404            | 0.428,0,552                         | 0.404,0,552                        |
|                | degrees of freedom        | 37                      | 39                                  | 38                    | 35                     | 2                                    | 2                                |
|                | p-value                   | 0.46                    | 0.50                                | 0.56                  | 0.65                   | 0.63                                 | 0.57                             |
|               |                           |                         |                                     |                       |                        |                                     |                                 |
| Alf-42 ^13^N-1^3^C | mean RMSD values          | 0.317,0,381             | 0.317,0,380                         | 0.387,0,373           | 0.317,0,387            | 0.317,0,437                         | 0.387,0,437                        |
|                | degrees of freedom        | 46                      | 36                                  | 12                    | 8                      | 2                                    | 3                                |
|                | p-value                   | 0.11                    | 0.20                                | 0.85                  | 0.29                   | 0.012                               | 0.77                             |

**b**

| 2D ssNMR data | t-AD: PCA-AD | t-AD: r-AD | PCA-AD: PCA-AD | PCA-AD: r-AD |
|---------------|--------------|------------|----------------|--------------|
|               | mean C_2 values | -1.15,-1.60 | -1.15,-4.35   | -1.60,-4.35  |
| Alf-40 ^13^C-1^3^C | t-statistic | 0.37       | -4.16         | -3.98        |
|                | degrees of freedom | 15         | 12            | 14           |
|                | p-value | 0.72       | 0.0012        | 0.0013       |
|               | mean C_2 values | -4.56,-7.47 | -4.56,-16.53 | -7.47,-16.53 |
| Alf-40 ^13^N-1^3^C | t-statistic | 0.714      | -3.38         | -3.58        |
|                | degrees of freedom | 10         | 12            | 13           |
|                | p-value | 0.49       | 0.0056        | 0.0032       |
|               | mean C_2 values | -3.07,1.85  | -3.07,-1.80   | 2.85,1.80    |
| Alf-42 ^13^C-1^3^C | t-statistic | -1.93      | -0.82         | 0.19         |
|                | degrees of freedom | 10         | 3             | 2            |
|                | p-value | 0.082      | 0.47          | 0.06         |
|               | mean C_2 values | 8.32,-8.08  | 8.32,-7.33    | -8.08,-7.33  |
| Alf-42 ^13^N-1^3^C | t-statistic | 1.76       | 1.06          | -0.05        |
|                | degrees of freedom | 8          | 3             | 3            |
|                | p-value | 0.12       | 0.37          | 0.96         |

a. From Fig. 3, mean r.m.s.d. values for pairs of 2D spectra within a given tissue category are compared with mean r.m.s.d. values between tissue categories or within a different tissue category. The significance of differences in mean r.m.s.d. values is assessed by Welch’s t-test (two-sided). b. From Fig. 4, mean values of coefficients of the second principal component in 2D spectra (C_2) from three tissue categories are compared. The significance of differences in mean C_2 values is assessed by Welch’s t-test (two-sided).
Extended Data Table 2 | Quantification by ELISA of Aβ40/Aβ42 molar ratios in amyloid-enriched brain extracts

| sample  | Aβ40/Aβ42 ratio |
|---------|-----------------|
| t-AD1o  | 2.4             |
| t-AD2f  | 1.4             |
| t-AD2o  | 1.0             |
| t-AD2p  | 1.5             |
| t-AD3f  | 3.4             |
| t-AD3o  | 3.8             |
| t-AD3p  | 0.7             |
| PCA1o   | 0.5             |
| PCA1p   | 6.7             |
| PCA2f   | 2.2             |
| PCA2o   | 4.5             |
| PCA3f   | 3.2             |
| PCA3o   | 1.8             |
| PCA3p   | 2.1             |
| r-AD1f  | 3.1             |
| r-AD1p  | 4.5             |
| r-AD2f  | 4.1             |
| r-AD2o  | 1.2             |
| r-AD2p  | 1.9             |
| r-AD3f  | 0.6             |

Estimated uncertainties are approximately 20%.