Published in final edited form as:

Neurobiol Dis. 2009 September ; 35(3): 352–358. doi:10.1016/j.nbd.2009.05.024.

Soluble Fibrillar Oligomer Levels are Elevated in Alzheimer's Disease Brain and Correlate with Cognitive Dysfunction

Jennifer L. Tomic¹, Anna Pensalfini¹, Elizabeth Head^{2,*}, and Charles G. Glabe¹

¹Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697, USA.

²Department of Neurology and Institute for Brain Aging and Dementia, University of California, Irvine, CA 92697, USA.

Abstract

Recent evidence has suggested a role for soluble oligomeric A β species in the pathology of Alzheimer's disease (AD). Fibrillar plaque deposits are present in non-demented individuals and levels of soluble A β correlate better with cognitive dysfunction in AD and transgenic mouse models. We have previously reported that there are at least two conformationally distinct types of A β oligomers: prefibrillar oligomers that are kinetic intermediates in fibril assembly reactions and are specifically recognized by A11 antibody and fibrillar oligomers that may represent fibril seeds or small pieces of fibrils and are recognized by a fibril specific antibody, OC. We have examined the levels of these two types of oligomers in the PBS soluble fraction of brain tissue from control cases, cases with senile degenerative changes (SDC) and AD patients. We found that the levels of soluble fibrillar oligomers detected by OC antibody are significantly elevated in multiple brain regions of AD patients. The elevated fibrillar oligomer levels were found not to be an artifact of tissue homogenization, nor a result of increased A β or APP levels. The concentration of fibrillar oligomers in adjacent brain regions of the same patient can vary widely and were not detected in post mortem cerebrospinal fluid. In contrast, the level of prefibrillar oligomers are variable in both AD and age matched controls, indicating that they are not correlated with cognitive dysfunction and suggesting that they precede dementia in AD. Significant correlations were found between the levels of fibrillar oligomers and cognitive decline (MMSE scores) as well as the neuropathological hallmarks of AD. These results indicate that fibrillar oligomers may play a key role in the pathology of AD and may be a new target for diagnostic and therapeutic development.

Keywords

Alzheimer; A β ; amyloid oligomers; cognitive dysfunction; dementia; conformation dependent antibodies; fibrillar oligomers; prefibrillar oligomers; annular protofibrils

© 2009 Elsevier Inc. All rights reserved.

Address correspondence to: Dr. Charles Glabe, Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, CA 92697-3900, Email: cglabe@uci.edu, Phone: 949 824-6081, FAX: 949 824-8551.

*Current Address: Department of Molecular and Biomedical Pharmacology, Sanders-Brown Center on Aging, 203 Sanders-Brown Building, 800 South Limestone St., Lexington, KY, 40536

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

The genetics of inherited forms of Alzheimer's disease (AD) provides strong evidence for a causal role of A β , especially the faster aggregating A β 42, in AD (Hardy, 2006; Tanzi and Bertram, 2005). However, while some groups have reported a clear correlation between the number of A β plaques and dementia severity in support of this hypothesis (Blessed et al., 1968; Cummings and Cotman, 1995), others found no such correlation (Bennett et al., 1993; McKee et al., 1991; Terry et al., 1991). Additionally, individuals with normal cognitive function have been observed that have A β plaque loads that either meet or exceed the criteria for AD diagnosis (Katzman et al., 1988; Dickson et al., 1992). These observations suggest that fibrillar A β may not be the primary toxic assembly state responsible for the pathology of AD. Subsequent studies of human subjects have revealed a positive correlation between soluble A β levels and the severity of dementia (Lue et al., 1999; McLean et al., 1999; Wang et al., 1999), further indicating a role for soluble oligomeric species in the pathology of AD. Additional *in vitro* studies have reported that not only are soluble oligomers and protofibrils toxic to neuronal cell cultures (Hartley et al., 1999; Lambert et al., 1998), but that they are more toxic than fibrils (Dahlgren et al., 2002; Kaye et al., 2003).

The particular type of oligomer that is most closely associated with AD is a subject of considerable interest. Oligomers have been distinguished on the basis of size and elevated levels of approximately 56 kDa A β oligomers (ADDLs) have been identified in human brain (Gong et al., 2003) and a 56 kDa oligomer (A β *56) has been reported that is correlated with cognitive dysfunction in transgenic mouse models (Lesne et al., 2006). Soluble dimers have been purified from human AD brain that inhibit LTP and cause cognitive dysfunction in rats (Shankar et al., 2008). Another way of classifying oligomers is on the basis of their underlying structure, using conformation dependent antibodies (Barghorn et al., 2005; Kaye et al., 2007; Kaye et al., 2003; Kaye et al., 2009; Lambert et al., 2001). These antibodies are specific for aggregated forms of A β and show low or no immunoreactivity against monomeric A β . A11, OC and α APF recognize generic epitopes that are specifically associated with prefibrillar oligomers (A11) (Kaye et al., 2003), fibrils or fibrillar oligomers (OC) (Kaye et al., 2007) and annular protofibrils or pores (α APF) (Kaye et al., 2009). M94 and 5598 antisera show a significant preference for aggregated A β as compared to monomer (Barghorn et al., 2005; Lambert et al., 2001). We have previously reported the presence of prefibrillar oligomers in AD patient's brain using the anti-oligomer antibody A-11 (Kaye et al., 2003) and M94 and 5598 detect oligomers in AD brain (Barghorn et al., 2005; Lambert et al., 2001). Recently, we have reported the development of two new polyclonal antisera that detect unique generic epitopes associated with specific amyloid assembly states. OC recognizes a conformation dependent epitope specific to amyloid fibrils as well as 100,000 $\times$ G soluble fibrillar oligomers (Kaye et al., 2007) and α APF recognizes annular protofibrils, which are ring-like structures that resemble membrane pores (Kaye et al., 2009). It is not yet clear how the levels of oligomers detected by A11, OC and α APF are correlated with dementia severity in AD and how these antibodies compare to other conformation dependent antibodies, M94 and 5598 (Barghorn et al., 2005). In this report, we have analyzed levels of distinct types of oligomers that are present in the PBS soluble extracts of human brain samples from normal control cases, cases with degenerative changes but insufficient for a diagnosis of AD and patients with AD. The results demonstrate that soluble fibrillar oligomers that are recognized by the OC antibody are significantly increased in AD patients as compared to A11 and α APF positive oligomers that are also elevated in some age matched control brains.

Methods and Materials

Patient Brain Tissue Selection

Frozen brain tissue was obtained from the Institute for Brain Aging and Dementia and was collected from individuals enrolled in the Alzheimer Disease Research Center. Subjects enrolled in the ADRC were given the Mini Mental State Examination (MMSE) and scores from this test were used for correlative studies. As a standard protocol for ADRC autopsy cases, Braak & Braak neurofibrillary tangle and plaque staging was completed (Braak and Braak, 1991). We chose to examine tissues from the frontal cortex (Brodmann's Area's 4, 9 and 11), hippocampus, entorhinal cortex, transentorhinal cortex, cerebellum and the olfactory bulb based on the progression of β -amyloid deposition over the course of the disease as described by Thal et. al. (Thal et al., 2002). We selected cases that would represent the spectrum of Braak neurofibrillary tangle staging (Braak and Braak, 1991) seen in AD progression and Table 1 lists the clinical and pathological details of the cases used in this study. Patients were matched for age, gender and post-mortem interval (PMI). There were a total of 12 cases used, 5 were clinically normal, 1 was mildly cognitively impaired and 6 were demented. In terms of neuropathology, 4 showed little or no AD pathology, 2 showed mild AD pathology but insufficient for a diagnosis of AD (senile degenerative changes – SDC) and 5 had AD pathology.

Human brain sample preparation

Frozen tissues were weighted, diced and homogenized in a 4:1 volume of freshly prepared ice-cold PBS, 0.02% NaN_3 , pH 7.4 with protease inhibitor cocktail (PBS volume/brain wet weight). The samples were ultracentrifuged at $100,000 \times G$ for 1 hr at 4°C . The PBS soluble fraction was collected, aliquoted and stored at -80°C for future testing. The insoluble pellet was resuspended in 150 mM NaCl, 10 mM Tris, 2% Triton X-100, 0.02% NaN_3 , pH 8.0 with protease inhibitor cocktail and sonicated. Samples were then incubated on ice for 1 hr prior to being ultracentrifuged at $100,000 \times G$ for 1 hr at 40°C . The resultant Triton X-100 soluble fraction was aliquoted and stored at -80°C for future testing. The Triton X-100 insoluble pellet was resuspended in PBS, 0.02% NaN_3 , pH 7.4 with protease inhibitor cocktail, sonicated and diluted with PBS to equal protein concentrations based on the BCA Protein Assay (Thermo Scientific, Rockford, IL). Finally, the Triton X-100 insoluble pellet insoluble pellet was solubilized into 8 M urea, 50 mM Tris, pH 7.6 and incubated overnight at room temperature. The total protein concentration of all samples was determined by the BCA assay.

Dot Blot Assay

PBS soluble or urea-treated Triton X-100 insoluble samples were spotted onto nitrocellulose membrane (Whatman 10 402 495), and allowed to air dry. The total protein amount spotted onto the membrane for the PBS soluble and Triton X-100 insoluble fractions was 5.2 μg and 17.0 μg , respectively. Non-reactive sites were blocked with 10% Milk in low-Tween TBS-T, (20 mM Tris, 137 mM NaCl, 0.01% Tween 20 pH 7.6) for one hour at room temperature with shaking. Following three 5-minute washes, the blot was incubated overnight at 40°C in primary antibody (OC 1:10,000, A11 1:2000, αAPF 1:5,000). After washing, the blot was incubated with goat- α -rabbit HRP conjugated secondary antibody (Jackson Immuno Research 111-035&delhyphen;003, 1:12000) for 1 hr at room temperature, washed again and detected with ECL (Amersham, RPN2106). The membrane was then exposed to film (Denville, E-3012) and the film developed by the Kodak M35A X-OMAT Processor. Additional dot blots were also performed using anti-A β 17–24 (Covance, 4G8 1:10000), anti-N-terminal of amyloid precursor protein (APP) (Chemicon, 22C11 1:5000) followed by incubation with goat- α -mouse HRP conjugated secondary antibody (Jackson Immuno Research 115-035–146, 1:12000) and ECL detection. As a control for brain tissue preparation, the neuronal marker α -synaptophysin was detected in the Triton X-100 soluble fractions using anti- α -synaptophysin (Calbiochem,

1:1000), followed by incubation with goat- α -mouse HRP conjugated secondary antibody (Jackson Immuno Research 115-035–146, 1:12000) and ECL detection.

Quantitation of fibrillar oligomers and statistical analysis

Films were scanned and the sample dots quantitated using the freely available Java image processing program (ImageJ). The results, expressed as ng/ μ l, were calculated using a reference curve based on the reactivity of synthetic A β prefibrillar oligomers, fibrillar oligomers and annular protofibrils prepared as previously described (Kayed et al., 2007; Kaye et al., 2003; Kaye et al., 2009), taken as standards. The detection limit is approximately 1 ng/ μ l of A β for prefibrillar oligomers, fibrillar oligomers and annular protofibrils. Non reactive sites of the film were also scanned and used to subtract the background for the sample dots. All statistical analyses were performed using GraphPad Instat Version 3.0 (GraphPad Software, San Diego CA). Values were expressed as means $\pm$ standard deviation (SD) and are the result of four independent experiments. To compare oligomer immunoreactivity between AD patients and non-AD patients, one-way analysis of variance (ANOVA) followed by Bonferroni's t-test was performed. A probability value less than 0.05 was accepted as statistically significant. Spearman correlations were calculated to determine relationships between oligomer reactivity with dementia severity, as measured by the Mini Mental Status Examination (Folstein et al., 1975), and neuropathology, as determined by Braak and Braak staging (Baner et al., 1993; Braak and Braak, 1991; Kaye et al., 2003).

Results

Fibrillar Oligomers are Elevated in AD Brain

To examine the levels of soluble oligomers in human brain, PBS lysates of tissue samples from 8 different brain regions were prepared as described above and centrifuged at $100,000 \times G$ for 1 hr to separate the soluble supernatant and insoluble pellet fractions. Samples were obtained from 12 different cases: patients with AD, cases with senile degenerative changes (SDC –i.e. AD pathology insufficient for a diagnosis of disease) and age matched controls. The clinical and neuropathological diagnoses of the cases used in the study are listed in Table I. The fractions were analyzed by dot blot and ELISA assays, but since the signal was higher for the dot blot samples (ELISA data not shown) the levels of oligomers was determined by quantitation of the dot blot images (Fig. 1). The levels of $100,000 \times G$ soluble fibrillar oligomers detected with the fibril specific OC antibody were elevated in AD brain compared to normal controls and patients with senile degenerative changes (Figure 1a). The level of fibrillar oligomers was conspicuously higher in the olfactory bulb of normal controls and SDC samples compared to the other regions of the brain, suggesting that higher levels of fibrillar oligomers may first appear in the olfactory bulb. The levels of soluble fibrillar oligomers is also variable between different regions of the same brain, indicating that they do not diffuse far and suggesting that they may be produced locally and either retained or degraded in the same location. Fibrillar oligomer levels were below the limit of detection in AD lumbar cerebrospinal fluid samples using the dot blot assay (data not shown).

Brain tissue from patients with severe AD is known to contain fewer neurons and more astrocytes in areas particularly vulnerable to AD pathology. Therefore, the blots were also probed for α -synaptophysin which is a typical neuronal protein whose levels have been shown to be reduced in the brains of AD subjects (Masliah et al., 2001). As expected, soluble fibrillar oligomer levels were also found to be significantly elevated when normalized to α -synaptophysin reactivity (Fig S1).

The PBS soluble fraction was prepared by homogenizing brain tissue samples and since many samples in this group have insoluble amyloid plaques, this raises the possibility that the soluble

fibrillar oligomers may be an artifact of the homogenization process by breaking up the fibrillar amyloid plaques. To exclude this possibility we compared the soluble, fibrillar oligomer levels and the insoluble fibril levels in the same tissue samples by dot blot analysis. As with the PBS soluble fraction (Figure 1a), all AD patients demonstrated strong immunoreactivity to the OC antibody in the Triton X-100 insoluble fraction (Figure 1d). However, two patients (4 and 5,) who were not demented at death with mild neuropathology demonstrated strong OC positive immunoreactivity in the insoluble fraction while no OC immunoreactivity was present in the PBS soluble fraction. The lack of fibrillar oligomers in the PBS soluble fractions of these two patients that contain large amounts of insoluble fibrils argues that the soluble fibrillar oligomers present in the AD patients of Figure 1a are not an artifact of the homogenization process. With few exceptions, the levels of insoluble fibrils are low or undetectable in the olfactory bulb and cerebellum. Detectable levels of soluble fibrillar oligomers are observed in the cerebellum of two AD samples (11, 12) that contain no detectable insoluble fibrils, which indicates that the presence of insoluble fibrils is not necessary for the observation of soluble fibrillar oligomers.

Prefibrillar oligomer and annular protofibrils levels are not elevated in AD brains

Prefibrillar oligomers and annular protofibrils are structurally and immunologically distinct from fibrillar oligomers, although the sizes of these oligomers broadly overlap (Kayed et al., 2007; Kaye et al., 2003; Kaye et al., 2009). The levels of prefibrillar oligomers and annular protofibrils were determined in the same samples using A11 and α APF antibodies (Fig. 1b, c). Although A11 positive prefibrillar and α APF positive annular protofibrils were detected in the samples, no difference was observed in AD brain and normal controls and SDC samples. As observed for fibrillar oligomers, the levels of prefibrillar oligomers and annular protofibrils are variable among different brain regions, indicating that they do not diffuse ideally. In particular, the levels of prefibrillar oligomers are low in the olfactory bulb compared to other brain regions. We also examined the levels of A β in the brain samples using 4G8, which recognizes residues 17–28 of A β (Fig. 1e). Unlike previous reports (Lue et al., 1999; McLean et al., 1999), we did not observe a statistically significant difference in the levels of soluble A β in AD, SDC and control samples although the methods and antibodies used here are different than those of the earlier reports. We used the level of soluble APP (sAPP) detected by 22C11 as a loading control to confirm equal amounts of protein were loaded in each sample (Fig. 1f).

Oligomer levels were quantified from dot blots in quadruplicate, using synthetic A β fibrillar oligomers, prefibrillar oligomers and annular protofibrils as standards. The quantitative analysis confirms that the elevation of soluble fibrillar oligomer levels in some of the brain regions is highly statistically significant (Fig. 2). Soluble fibrillar oligomer levels are elevated approximately 2–5 fold in all regions of the brain examined, except olfactory bulb. The difference between soluble fibrillar oligomer levels in AD and the other groups is highly statistically significant in the transentorhinal cortex, entorhinal cortex and hippocampus ($P < 0.001$), and significant ($P < 0.05$) in the frontal cortex. Surprisingly, soluble fibrillar oligomer levels are also elevated in the cerebellum ($P < 0.01$), a region that is believed to be spared until late in the disease.

Soluble Fibrillar Oligomers Correlate with Cognitive Dysfunction and Pathology

We examined whether the amounts of soluble fibrillar oligomers correlated with quantitative measures of cognitive dysfunction as measured by the MMSE (Folstein et. al., 1975) and neuropathological indices of amyloid plaque and tau tangle staging (Fig. 3a). We found that oligomer levels are positively correlated with cognitive decline. As shown in Table 2, Spearman correlation analysis revealed significant correlations between MMSE score and soluble fibrillar oligomer levels in the transentorhinal cortex, entorhinal cortex, Brodmann's Area 4, Brodmann's Area 9, Brodmann's Area 11, and cerebellum. Levels of soluble fibrillar

oligomers hippocampus ($r = -0.61$, $p = 0.11$) neared, but did not reach, significance, whereas no significant difference in the olfactory bulb was found ($r = 0.20$, $p = 0.58$). Significant correlations were present between soluble fibrillar oligomer levels and plaque stage (Fig. 3b and Table 2) in the transentorhinal cortex, entorhinal cortex, hippocampus, Brodmann's Area 4 and Brodmann's Area 9, and Brodmann's Area 11. The cerebellum ($r = 0.57$, $p = 0.08$) neared, but did not reach significance, while the olfactory bulb ($r = -0.14$, $p = 0.70$) did not demonstrate significant correlations with extent of soluble fibrillar oligomers. Finally, significant correlations were also observed between soluble fibrillar oligomers and tangle stage (Figure 3c and Table 2) in all brain regions except olfactory bulb that failed to reach significance ($r = -0.46$, $p = 0.18$). This may indicate that soluble fibrillar oligomers are involved with initiating tau pathology, as has been suggested recently by Oddo et. al. (2007). Together, these data indicate that increased levels of soluble fibrillar oligomers are associated with increased levels of dementia and pathological hallmarks of AD, thus suggesting a potential role for the involvement of soluble fibrillar oligomers in the pathogenesis of AD.

Discussion

The availability of specific antibodies that can recognize prefibrillar oligomers (A11), fibrils and fibrillar oligomers (OC) and annular protofibrils (aAPF) has allowed a detailed analysis of the levels and distributions of these distinct types of soluble oligomers in AD in individuals with plaque and tangle pathology but without dementia and nondemented brains with low or undetectable levels of pathology. We found significantly elevated levels of soluble fibrillar oligomers in AD patients compared to SDC and controls. Elevated soluble fibrillar oligomers were present in all frontal cortex regions tested, as well as the hippocampus, entorhinal cortex, transentorhinal cortex and cerebellum. With the exception of the cerebellum, each of these regions is known to develop neuropathology over the course of AD progression. We determined that the elevated levels of fibrillar oligomers were not an artifact created by homogenization of plaque-containing tissue because regions of control and SDC tissue that have high levels of insoluble fibrillar plaques did not have correspondingly high level of soluble fibrillar oligomers. Further, the soluble fibrillar oligomers were found not to be a product of increased expression of APP or A β . Having ruled these possibilities out, we can be confident that the fibrillar oligomers reported here are indeed a distinct structure and aggregation state present primarily in AD brain.

The only brain region examined that did not exhibit elevated levels of soluble fibrillar oligomers in AD was the olfactory bulb. The olfactory bulb fibrillar oligomer levels are elevated in SDC and age matched normal control brain, compared to other regions of the same brain. Neurons in the olfactory bulb are known to degenerate in AD and this is associated with the focal accumulation of A β (Struble and Clark, 1992). The elevation of fibrillar oligomers in the olfactory bulb is potentially interesting for two reasons. The first is that olfactory function declines with age overall (Schiffman, 1997), and overall high levels of soluble fibrillar oligomers may represent an aging phenomenon. Thus, one extension of the current study would be to examine younger individuals with the prediction that levels of oligomers would be low or undetectable. Second, there is evidence that a loss of smell may be an early indicator of incipient AD and is detectable in individuals that go on to develop mild cognitive impairments (Gilbert and Murphy, 2004; Wilson et al., 2007). The potential correlation between olfactory bulb fibrillar oligomer levels and olfactory deficits warrants further investigation.

The finding that soluble fibrillar oligomer levels are elevated in cerebellum is interesting because this brain region is not known to be functionally impaired in AD. Amyloid plaques have been reported in the cerebellum of AD patients, although this region does not exhibit neurofibrillary tangle pathology (Braak et al., 1989). Lacor et al. have also reported elevated levels of A β oligomers in AD brain, but the levels were not statistically significantly elevated

compared to controls (Lacor et al., 2004). The reasons for the discrepancy between the significantly elevated levels we observe are not entirely clear, but could be due to differences in the selectivity of the antibodies used. If soluble fibrillar oligomers are causally related to neuronal dysfunction, the fact that their levels are elevated in the cerebellum suggests that cerebellar neurons may be less susceptible to their toxic activity. This is consistent with previous reports of the resistance of cerebellar neurons to aggregated A β in vitro (Ueda et al., 1994).

We also found an inverse correlation between soluble fibrillar oligomers and MMSE score. With increasing levels of fibrillar oligomers in brain regions associated with intact memory function, decreased cognitive function was evident. We also found significant correlations between fibrillar oligomer levels and the neuropathological hallmarks of AD, amyloid plaques and tau tangles. Considering the variable reports of both a lack of correlation between insoluble fibrillar aggregates or a high correlation with cognitive dysfunction, the correlation of soluble fibrillar oligomers we observe in the current study to the clinical and neuropathological progression of AD points to this species as being potentially a key factor involved with neurodegeneration and thus a potential therapeutic target.

In contrast, we found that prefibrillar oligomer and annular protofibril levels do not correlate with cognitive dysfunction in AD brain. We previously reported that A11 positive prefibrillar oligomers are detected in human AD brain (Kayed et al., 2003), but the quantitative analysis conducted here indicate that they are also found in age-matched non demented control brain. Prefibrillar oligomers are toxic in vitro (Demuro et al., 2005; Kaye et al., 2003), so if they are intrinsically toxic, this suggests that something that is present in brains of non-demented individuals may modulate their toxicity. Since prefibrillar oligomers are kinetic intermediates in fibril formation (Kayed et al., 2003), it is possible that their conformational conversion to fibrillar oligomers is important for toxicity and AD pathogenesis. Prefibrillar oligomers appear to be precursors for annular protofibril levels. Since prefibrillar oligomer levels are elevated in age matched control brain, it is perhaps not surprising that annular protofibril levels are also not correlated with AD.

In summary, soluble fibrillar oligomers are specifically correlated with pathology and cognitive dysfunction in AD brain. Soluble fibrillar oligomers may represent a target for the development of immunological reagents or small molecules that specifically inhibit their formation or toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This work was supported by a grant from the NIH AG00538 and grants from the Cure Alzheimer Fund and The Larry L. Hillblom Foundation.

References

- Bancher C, Braak H, Fischer P, Jellinger KA. Neuropathological staging of Alzheimer lesions and intellectual status in Alzheimer's and Parkinson's disease patients. *Neurosci Lett* 1993;162:179–182. [PubMed: 8121624]
- Barghorn S, Nimmrich V, Striebing A, Krantz C, Keller P, Janson B, Bahr M, Schmidt M, Bitner RS, Harlan J, et al. Globular amyloid beta-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. *J Neurochem* 2005;95:834–847. [PubMed: 16135089]

- Bennett DA, Cochran EJ, Saper CB, Leverenz JB, Gilley DW, Wilson RS. Pathological changes in frontal cortex from biopsy to autopsy in Alzheimer's disease. *Neurobiol Aging* 1993;14:589–596. [PubMed: 8295661]
- Blessed G, Tomlinson BE, Roth M. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *Br J Psychiatry* 1968;114:797–811. [PubMed: 5662937]
- Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol (Berl)* 1991;82:239–259. [PubMed: 1759558]
- Braak H, Braak E, Bohl J, Lang W. Alzheimer's disease: amyloid plaques in the cerebellum. *J Neurol Sci* 1989;93:277–287. [PubMed: 2556503]
- Cummings BJ, Cotman CW. Image analysis of beta-amyloid load in Alzheimer's disease and relation to dementia severity. *Lancet* 1995;346:1524–1528. [PubMed: 7491048]
- Dahlgren KN, Manelli AM, Stine WB Jr, Baker LK, Krafft GA, LaDu MJ. Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem* 2002;277:32046–32053. [PubMed: 12058030]
- Demuro A, Mina E, Kaye R, Milton SC, Parker I, Glabe CG. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J Biol Chem* 2005;280:17294–17300. [PubMed: 15722360]
- Dickson DW, Crystal HA, Mattiace LA, Masur DM, Blau AD, Davies P, Yen SH, Aronson MK. Identification of normal and pathological aging in prospectively studied nondemented elderly humans. *Neurobiol Aging* 1992;13:179–189. [PubMed: 1311804]
- Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189–198. [PubMed: 1202204]
- Gilbert PE, Murphy C. Differences between recognition memory and remote memory for olfactory and visual stimuli in nondemented elderly individuals genetically at risk for Alzheimer's disease. *Exp Gerontol* 2004;39:433–441. [PubMed: 15036403]
- Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE, Krafft GA, Klein WL. Alzheimer's disease-affected brain: presence of oligomeric A beta ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proc Natl Acad Sci U S A* 2003;100:10417–10422. [PubMed: 12925731]
- Hardy J. Has the amyloid cascade hypothesis for Alzheimer's disease been proved? *Curr Alzheimer Res* 2006;3:71–73. [PubMed: 16472206]
- Hartley DM, Walsh DM, Ye CP, Diehl T, Vasquez S, Vassilev PM, Teplow DB, Selkoe DJ. Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *Journal of Neuroscience* 1999;19:8876–8884. [PubMed: 10516307]
- Katzman R, Terry R, DeTeresa R, Brown T, Davies P, Fuld P, Renbing X, Peck A. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann Neurol* 1988;23:138–144. [PubMed: 2897823]
- Kayed R, Head E, Sarsoza F, Saing T, Cotman CW, Necula M, Margol L, Wu J, Breydo L, Thompson JL, et al. Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers. *Mol Neurodegener* 2007;2:1–11. [PubMed: 17224059]
- Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 2003;300:486–489. [PubMed: 12702875]
- Kayed R, Pensalfini A, Margol L, Sokolov Y, Sarsoza F, Head E, Hall JE, Glabe C. Annular protofibrils are a structurally and functionally distinct type of amyloid oligomer. *J Biol Chem*. 2009In press
- Lacor PN, Buniel MC, Chang L, Fernandez SJ, Gong Y, Viola KL, Lambert MP, Velasco PT, Bigio EH, Finch CE, et al. Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *J Neurosci* 2004;24:10191–10200. [PubMed: 15537891]
- Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, et al. Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proceedings of the National Academy of Sciences of the United States of America* 1998;95:6448–6453. [PubMed: 9600986]

- Lambert MP, Viola KL, Chromy BA, Chang L, Morgan TE, Yu J, Venton DL, Krafft GA, Finch CE, Klein WL. Vaccination with soluble Abeta oligomers generates toxicity-neutralizing antibodies. *J Neurochem* 2001;79:595–605. [PubMed: 11701763]
- Lesne S, Koh MT, Kotilinek L, Kaye R, Glabe CG, Yang A, Gallagher M, Ashe KH. A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* 2006;440:352–357. [PubMed: 16541076]
- Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *American Journal of Pathology* 1999;155:853–862. [PubMed: 10487842]
- Masliah E, Mallory M, Alford M, DeTeresa R, Hansen LA, McKeel DW Jr, Morris JC. Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. *Neurology* 2001;56:127–129. [PubMed: 11148253]
- McKee AC, Kosik KS, Kowall NW. Neuritic pathology and dementia in Alzheimer's disease. *Ann Neurol* 1991;30:156–165. [PubMed: 1910274]
- McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Annals of Neurology* 1999;46:860–866. [PubMed: 10589538]
- Schiffman SS. Taste and smell losses in normal aging and disease. *JAMA* 1997;278:1357–1362. [PubMed: 9343468]
- Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 2008;14:837–842. [PubMed: 18568035]
- Struble RG, Clark HB. Olfactory bulb lesions in Alzheimer's disease. *Neurobiol Aging* 1992;13:469–473. [PubMed: 1508297]
- Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell* 2005;120:545–555. [PubMed: 15734686]
- Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol* 1991;30:572–580. [PubMed: 1789684]
- Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 2002;58:1791–1800. [PubMed: 12084879]
- Ueda K, Fukui Y, Kageyama H. Amyloid beta protein-induced neuronal cell death: neurotoxic properties of aggregated amyloid beta protein. *Brain Res* 1994;639:240–244. [PubMed: 7515762]
- Wang J, Dickson DW, Trojanowski JQ, Lee VM. The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. *Experimental Neurology* 1999;158:328–337. [PubMed: 10415140]
- Wilson RS, Schneider JA, Arnold SE, Tang Y, Boyle PA, Bennett DA. Olfactory identification and incidence of mild cognitive impairment in older age. *Arch Gen Psychiatry* 2007;64:802–808. [PubMed: 17606814]

Figure 1. Soluble Oligomer levels in AD Brain

A. Dot blot analysis of PBS soluble fraction with the anti-fibril antibody (OC). PBS soluble fractions from the transentorhinal cortex (TEC), entorhinal cortex (EC), hippocampus (HIP), Brodmann's Area 4 (B4), Brodmann's Area 9 (B9), Brodmann's Area 11 (B11), olfactory bulb (OLF) and cerebellum (CBL) were spotted onto nitrocellulose membrane and reacted with the OC antibody. The most prominent immunoreactivity was found in all brain regions of AD cases while reduced or no immunoreactivity was present in normal controls or cases with senile degenerative changes (SDC). The signal present in these cases indicates the presence of soluble fibrillar oligomers. **B, C.** Dot blot analysis of prefibrillar oligomers with A11 antibody and annular protofibrils with αAPF antibody, respectively. Although prefibrillar oligomer levels

are low in the olfactory bulb, no differences in the levels were observed in AD, SDC and normal controls. **D.** The $100,000 \times G$ insoluble fractions were solubilized with 8M urea, 50 mM Tris pH 7.4 and spotted onto nitrocellulose membrane for detection with OC antibody. Prominent immunoreactivity was found in all AD cases as well as two cases (4 and 5) with mild neuropathology. The presence of immunoreactivity in the insoluble fraction of these two cases and the lack of immunoreactivity in the PBS fractions of the same cases (**A**) indicates that the soluble fibrillar oligomers present in Figure 1a are not an artifact of homogenization. **E, F.** Dot blot analysis of PBS soluble fraction with 4G8 (anti-A β) and 22C11 (anti-APP) antibodies, respectively. The levels of total soluble A β do not differ significantly between AD, SDC and normal controls. The uniform distribution of 22C11 immunoreactivity indicates that equal amounts of lysate were spotted. X indicates that this sample was not available for that case.

Figure 2. Fibrillar oligomers are elevated in AD brain

Quantitative analysis of the dot blot data in Figure 1, showing human brain sample reactivity with **A** OC, **B** A11, and **C**. α APF antibodies. **A**. Fibrillar oligomer levels were significantly higher in AD cases (AD, red bars) compared to normal (N, blue bars) and SDC (SDC, green bars) subjects in all the brain regions examined except OLF. **B.**, **C**. No significant difference in A11 and α APF reactivity between normal, SDC and AD samples were found. Human brain sample reactivity is expressed as ng/μl on the basis of a standard curve obtained using synthetic fibrillar oligomer, prefibrillar oligomer and annular protofibrillar preparations. Values are the mean $\pm$ SD of four independent experiments (* $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$).

Figure 3. Soluble fibrillar oligomer levels in AD brain correlate with cognitive decline and neuropathology

A. OC immunoreactivity was compared to the MMSE, a measure of cognitive dysfunction. Significant inverse correlations were determined for TEC (black diamond), EC (red square), HIP (blue triangle), B4 (green square), B9 (yellow circle) and CBL (light blue cross).

B. OC immunoreactivity was compared to the plaque stage and significant correlations between fibrillar oligomer levels and amyloid pathology were found in TEC, EC, HIP, B4 and B9.

C. OC immunoreactivity was compared to the tangle stage and significant correlations between fibrillar oligomer levels and tau pathology were found in TEC, EC, HIP, B4, B9, B11 (purple dash) and CBL. The *r* and *p* values are reported in Table 2.

Table 1
Patient Demographics

Patient Number	Clinical Diagnosis *	Neuropath. Diagnosis *	Sex	Tau Tangle Stage	A β Plaque Stage	Post Mortem Index (Hours)	Age at Death	MMSE Score
1	N/A	Normal	M	I	A	4.9	71	N/A
2	Control	Normal	F	I	0	4.5	87	N/A
3	CIND	Normal	M	II	0	6.4	81	27
4	Control	Normal	F	II	0	5.0	88	29
5	Control	SDC	F	III	A	5.8	84.8	29
6	MCI/AD	SDC	M	III	0	6.3	84.5	22
7	DLB	AD/DLB	F	IV	B	5.4	77	0
8	Possible AD	AD	M	IV	C	4.5	77.5	19
9	Possible AD	AD	M	V	B	5.0	81	12
10	Probable AD	AD	F	V	B	4.3	81	0
11	Possible AD	AD	F	VI	B	6.6	82	4
12	Possible AD	AD	M	VI	C	3.5	83.2	0

* CIND – cognitively impaired not demented
MCI – mild cognitive impairment
DLB – diffuse Lowy Body Disease
AD – Alzheimer disease
SDC – Senile degenerative changes

Table 2
Soluble Fibrillar Oligomers Correlate with Cognitive Decline and Pathology

	MMSE			Plaque Stage			Tangle Stage		
	r	r ²	p	r	r ²	p	r	r ²	p
TEC	-0.72	0.52	0.01	0.90	0.80	0.00	0.86	0.74	0.00
EC	-0.78	0.61	0.00	0.72	0.52	0.01	0.69	0.48	0.01
HIP	-0.61	0.38	0.11	0.92	0.84	0.00	0.76	0.58	0.05
B4	-0.67	0.45	0.02	0.70	0.49	0.02	0.71	0.51	0.01
B9	-0.58	0.34	0.02	0.70	0.49	0.01	0.59	0.35	0.04
B11	-0.65	0.43	0.02	0.66	0.43	0.02	0.72	0.52	0.01
OLF	0.20	0.04	0.58	-0.14	0.02	0.70	-0.46	0.21	0.18
CBL	-0.63	0.39	0.05	0.57	0.33	0.08	0.78	0.61	0.01

Correlation coefficient (Spearman r), coefficient of determination (r²) and significant values (p) are reported; significant values are highlighted in grey.