Journal of Alzheimer's Disease 33 (2013) 923–928
DOI 10.3233/JAD-2012-121393
IOS Press

Short Communication

The ELISA-Measured Increase in Cerebrospinal Fluid Tau that Discriminates Alzheimer's Disease from other Neurodegenerative Disorders is not Attributable to Differential Recognition of Tau Assembly Forms

Seán T. O'Dowd[a,b], Mustafa T. Ardah[c], Per Johansson[d,e], Aleksey Lomakin[f], George B. Benedek[f], Kinley A. Roberts[b], Gemma Cummins[b], Omar M. El Agnaf[c], Johan Svensson[e,g], Henrik Zetterberg[h], Timothy Lynch[b] and Dominic M. Walsh[a,*]

[a]*Laboratory for Neurodegenerative Research, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Republic of Ireland*
[b]*Dublin Neurological Institute at the Mater Misericordiae University Hospital, Dublin, Republic of Ireland*
[c]*Faculty of Medicine and Health Sciences, Department of Biochemistry, United Arab Emirates University, Al Ain, United Arab Emirates*
[d]*Department of Neuropsychiatry, Skaraborg Hospital, Falköping, Sweden*
[e]*Department of Endocrinology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*
[f]*Department of Physics and Center for Material Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*
[g]*Department of Endocrinology, Skaraborg Hospital, Skövde, Sweden*
[h]*Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg and Mölndal, Sweden*

Accepted 13 September 2012

Abstract. Elevated cerebrospinal fluid concentrations of tau discriminate Alzheimer's disease from other neurodegenerative conditions. The reasons for this are unclear. While commercial assay kits are widely used to determine total-tau concentrations, little is known about their ability to detect different aggregation states of tau. We demonstrate that the leading commercial enzyme-linked immunosorbent assay reliably detects aggregated and monomeric tau and evinces good recovery of both species

*Correspondence to: Dominic M. Walsh, Laboratory for Neurodegenerative Research, Center for Neurologic Diseases, Harvard Institutes of Medicine (Room 921b), 77 Avenue Louis Pasteur, Boston, MA 02115, USA. Tel.: +1 617 5255059; Fax: +1 617 5255252; E-mail: dwalsh3@partners.org.

ISSN 1387-2877/13/$27.50

when added into cerebrospinal fluid. Hence, the disparity between total-tau levels encountered in Alzheimer's disease and other neurodegenerative conditions is not due to differential recognition of tau assembly forms or the extent of degeneration.

Keywords: Alzheimer's disease, cerebrospinal fluid, ELISA, tau

Supplementary data available online: http://www.j-alz.com/issues/33/vol33-4.html#supplementarydata01

Enzyme-linked immunosorbent assay (ELISA) determinations of total-tau (t-tau) and phospho-tau (p-tau) concentrations in cerebrospinal fluid (CSF) consistently discriminate Alzheimer's disease (AD) from normal controls and patients with other neurodegenerative conditions [1], such that the determination of CSF t-tau and p-tau has been included in the proposed revision to the NINCDS-ADRDA diagnostic criteria for AD [2]. However, CSF levels of t-tau and p-tau in other tauopathies have not been extensively studied and the available data are contradictory. In frontotemporal dementia (FTD), levels of t-tau have been reported as normal [3], increased [4], or decreased [5]. Studies on corticobasal degeneration (CBD) are compromised by small sample size and conflicting results [6, 7]. For progressive supranuclear palsy (PSP), the few studies undertaken indicate that t-tau levels are in the normal range [8, 9].

It is generally held that the elevation of t-tau in the CSF of patients with AD is a secondary phenomenon, reflective of neuronal death [10]. However, this ignores the fact that the extent of neurodegeneration in other tauopathies can be comparable or greater than that in AD. Consequently, we investigated the levels of t-tau and p-tau in CSF from a cohort of patients with well-characterized neurological disorders, some of which by brain imaging had extensive neurodegeneration. In agreement with prior studies, we found tau levels were elevated in AD patients relative to controls and other neurodegenerative diseases. One possible explanation why increased tau levels are not detected in non-AD neurodegeneration could be that ELISAs used to measure CSF tau differentially recognize different assembly states of tau. For instance, it has been demonstrated that certain ELISAs preferentially recognize specific assembly forms of aggregation-prone proteins [11, 12], thus we sought to determine if a total tau ELISA could detect aggregated and monomeric tau equally well. Thereafter we went on to apply this assay to the analysis of CSF from controls and individuals with various tauopathies.

CSF samples were obtained with approval of local ethics committees from 53 subjects. Patients were recruited prospectively from the Dublin Neurological Institute or from a memory clinic in Falköping. Controls were recruited contemporaneously from the local geographical area. Subjects were classified as neurologically normal ($n = 16$); AD ($n = 13$); other tauopathies (FTD, PSP, CBD) ($n = 17$), or α-synucleinopathy [idiopathic Parkinson's disease (IPD), multiple systems atrophy (MSA), or dementia with Lewy bodies (DLB)] ($n = 7$). In each case, a diagnosis was made according to validated criteria [13–19] by experienced neurologists. Two patients had FTDP-17-causing mutations on the *MAPT* gene. To eliminate cases of incipient AD from our non-AD groups we also measured CSF levels of $A\beta_{42}$ [20]. For further details see Supplementary Table 1.

CSF was collected from the L3/L4 interspace, centrifuged at 2500 *g* for 10 min at 4°C and the supernatant aliquoted, and stored at −80°C. Samples with an erythrocyte count >50/mm^3 were excluded. Total tau (t-tau), phospho-tau^{181} (p-tau), and $A\beta_{1\text{-}42}$ concentrations were measured using the Innotest® hTau Ag, Innotest® Phospho-tau$_{(181P)}$ (Innogenetics, Ghent, Belgium), and MSD Multi-Array® Human Abeta 42 (MSD, Gaithersburg, MD) ELISA kits. All samples were analyzed at least in duplicate and the inter-assay variation coefficient was <10%. Results are expressed as mean ± standard error, and inter-group differences were assessed using the Mann-Whitney *U*-test.

The human tau isoform, hTau 40, was expressed, purified, and aggregated as described previously [21] and the concentration determined by quantitative amino acid analysis (AAA). Aggregation was confirmed by Thioflavin-S binding assay and electron microscopy. Dynamic light scattering (DLS) confirmed that aggregates did not readily disassemble to monomer when diluted (Supplementary Figure 1; available online: http://www.j-alz.com/issues/33/vol33-4.html#supplementarydata01).

For comparison with the larger AD and control groups two broad diagnostic categories, namely "other tauopathies" (FTD, PSP, CBD) and "α-synucleinopathies" (IPD, DLB, and MSA) were used. AD patients had significantly higher t-tau levels

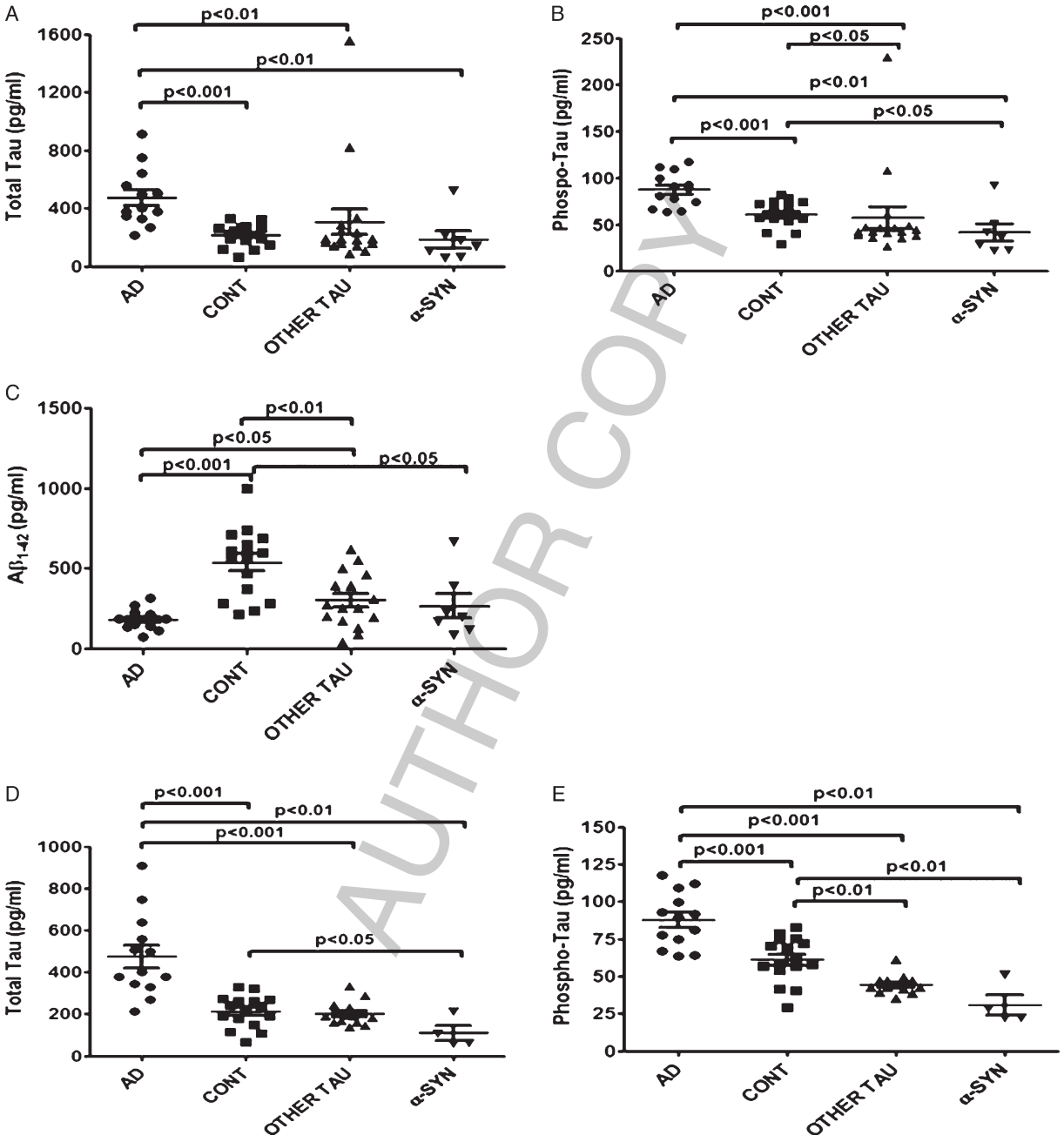


Fig. 1. Total- and phospho-tau[181] are elevated in CSF from patients with AD. Total-tau (A) and phospho-tau[181] (B) results were analyzed by grouped diagnoses where "Other Tau" refers to pooling of patients with FTD, PSP, and CBD into one diagnostic category and "α-Syn" refers to pooling of patients with IPD, DLB, and MSA into one diagnostic category. Mean values and standard errors are represented by a horizontal line and capped vertical line, respectively. The determination of CSF $A\beta_{1-42}$ levels (C) facilitated the exclusion of subjects which could have incipient AD. The exclusion of these subjects further enhanced the sensitivity of total-tau in distinguishing AD from other tauopathies (D), but did not significantly impact the phospho-tau results (E).

than both patients diagnosed with another tauopathy ($p < 0.01$) or an α-synucleinopathy ($p < 0.01$) (Fig. 1). Similarly, AD patients had significantly higher p-tau levels than controls ($p < 0.001$); other tauopathies ($p < 0.001$); and α-synucleinopathies ($p < 0.01$). Interestingly, the p-tau values in the groups of patients

with other tauopathies and α-synucleinopathies were significantly lower than the control cohort ($p < 0.05$). The fact that the control cohort was significantly older than both of these groups possibly explains this finding; nevertheless, it is remarkable that patients with certain neurodegenerative diseases had lower levels of a protein that supposedly leaks from dying neuronal axons than older normal subjects. In order to further secure the distinction between individuals with AD, all subjects with CSF $A\beta_{1\text{-}42}$ levels lower than the mean value of the AD group were excluded, lest they represent an incipient AD process [22]. This served to further increase the sensitivity of t-tau in distinguishing AD from other tauopathies ($p < 0.001$) (Fig. 1A, D).

Like certain prior studies, our findings indicate that t-tau and p-tau are elevated in AD compared to other neurodegenerative conditions [1, 9, 23]. The lack of elevation seen in non-AD conditions cannot be explained by length of disease course, nor by differences in the extent of neurodegeneration demonstrated radiologically. For example, one CBD patient had extensive bilateral fronto-parietal atrophy, yet her t-tau and p-tau levels were normal. Similarly, a patient with end-stage FTDP-17, who had notable bilateral temporal atrophy four years prior to CSF sampling, had normal t-tau and p-tau levels. By contrast, a number of patients with AD had minimal atrophy evident on MRI, yet both t-tau and p-tau were markedly elevated.

To address the issue of differential recognition of tau assemblies, aggregated and monomeric recombinant hTau 40 were used to assess the ability of the ELISA to detect these species. Specifically, solutions of aggregated and monomeric tau (with AAA-determined concentrations of 400 and 750 pg/ml, respectively) were estimated by ELISA to contain 411 ± 9 and 795 ± 5 pg/ml of aggregated tau and 385 ± 21 pg and 700 ± 23 pg/ml of monomeric tau respectively (Fig. 2D). Thus, the ELISA slightly overestimated the detection of aggregated tau and slightly underestimated the detection of tau monomer, but the two values deviated by less than 10%. We also investigated this issue by assessing how well the ELISA detected monomeric and aggregated tau when they were spiked into CSF. Sufficient aggregated tau was added into a CSF sample to raise the t-tau concentration from 105 pg/ml to 228 pg/ml, but the ELISA indicated a value of only 175 pg/ml. When the same CSF sample was similarly spiked with monomeric tau, ELISA determination produced a value of 179 pg/ml (Fig. 2E). Ordered protein aggregation, such as the assembly of monomeric tau into filaments, are highly concentration dependent processes, and below the critical concentration for aggregation, pre-formed aggregates are prone to disassemble [24]. However, using DLS we found that the average hydrodynamic radius of our tau aggregates (>100 nm) did not decrease when samples were diluted 400-fold and followed for 24 h (Supplementary Figure 1). Together, these results demonstrate that the Innotest® hTau Ag ELISA recognized monomeric and aggregated tau similarly well.

The absolute values for t-tau and p-tau determined for our AD and control cohorts were broadly in line with those determined in previous studies [25], and both assays readily discriminated between AD and controls ($p < 0.001$). T-tau and p-tau levels were significantly higher in the AD cohort than patients with other tauopathies ($p < 0.01$; $p < 0.001$ when possible incipient AD cases excluded) or α-synucleinopathies ($p < 0.01$). If, as has been previously assumed, the elevation of tau in AD CSF is simply a consequence of neuronal death, then one would expect that other diseases characterized by widespread neuronal loss would also exhibit an increase in tau. Why CSF tau is not similarly increased in non-AD tauopathies and in α-synucleinopathies could be explained in a number of ways. One possibility that we tested was that the assays employed do not detect all assembly forms of tau, such that if the tau liberated from dying neurons in non-AD neurodegeneration differed from the tau released in AD, then the ELISA may underestimate the amount of tau present. To our knowledge, this question has not been addressed previously and there is a dearth of literature relating to the precise nature of tau that commercial kits detect.

We tested the most widely used kit for measuring CSF tau, the Innotest® hTau Ag. The standard employed is a recombinant fusion protein, the aggregation state of which is not defined. Thus we assessed the performance of the assay using monomeric and aggregated tau. The assay reliably estimated the concentration of both samples and evinced good recovery when aggregated and monomeric tau were spiked into CSF. Since the t-tau assay detected both monomeric and aggregated tau, the disparity between t-tau levels encountered in AD and other neurodegenerative conditions cannot be ascribed to differential detection of assembly forms that could be present in different diseases. These findings are in line with burgeoning evidence that release of tau may not require cell death. Specifically, recent work has identified abnormal tau in exosomes from postmortem AD CSF [26] and that tau may be secreted from healthy human neurons by an exosome-independent mechanism [27]. Investigating non-conventional mechanisms for secretion of tau and

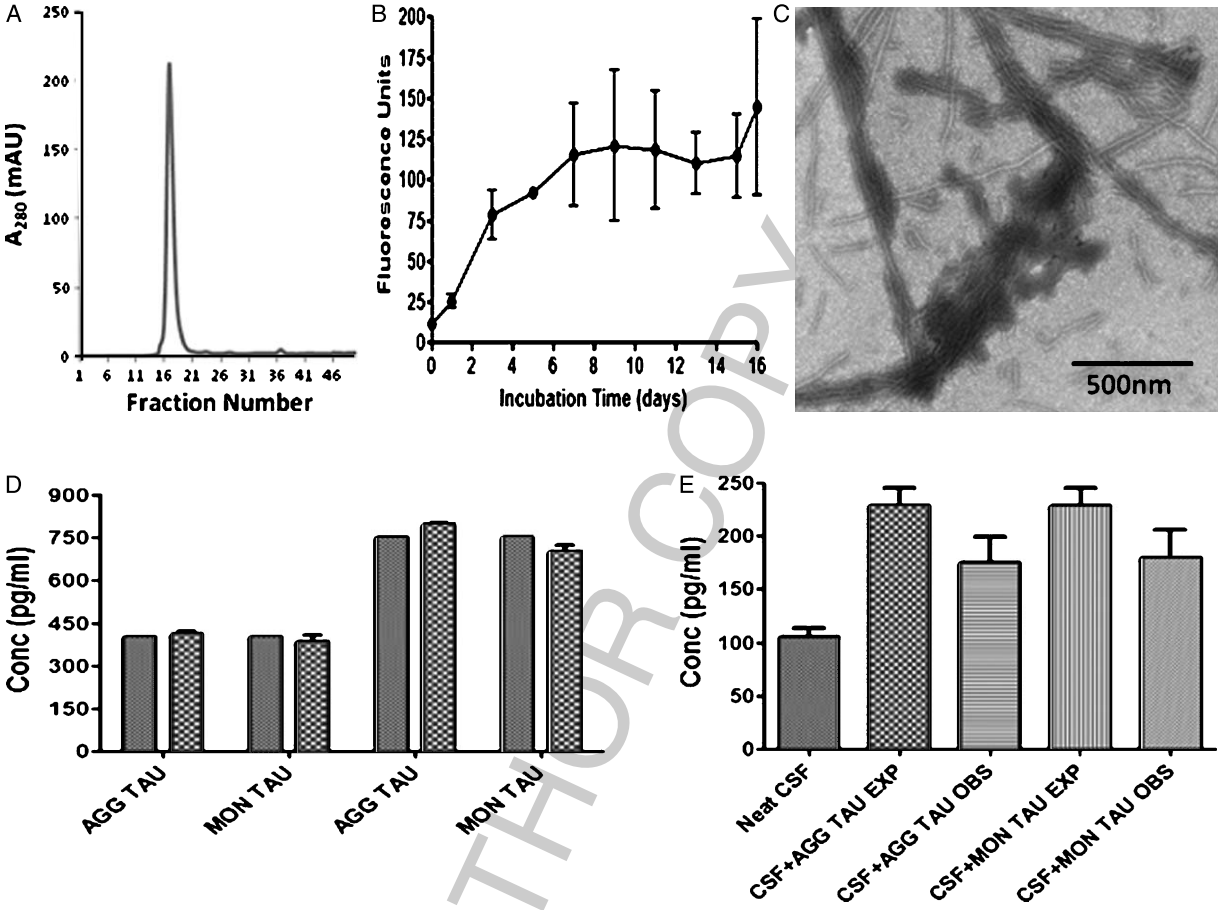


Fig. 2. The INNOTEST® hTau Ag total-tau ELISA detects monomeric and aggregated tau equally well. A) Size-exclusion chromatogram depicting the elution of monomeric recombinant human tau, which was used either directly or after aggregation. B) Aggregation of recombinant tau was induced by incubation with heparin and the reaction progress monitored by thioflavin S binding assay. C) Aliquots were taken at 48 h intervals and examined by electron microscopy. Highly ordered aggregates were detected from day 7 onwards; a representative image, taken on day 9, is shown. D) The Innotest total-tau ELISA detected aggregated tau (AGG TAU) and monomeric tau (MON TAU) equally well. The concentrations determined by ELISA (hatched bars) concurred with the concentrations determined by quantitative amino acid analysis (filled bars). E) A known amount of aggregated recombinant tau (AGG TAU) or monomeric recombinant tau (MON TAU) was spiked into aliquots of the same CSF sample and the mixture analyzed for total tau. Recovery of both aggregated and monomeric species was approximately 60%.

their role in the spread of tau pathology should reveal important information about AD pathogenesis and the use of CSF tau as a biomarker for AD.

ACKNOWLEDGMENTS

We thank Drs. Eckhard and Eva-Maria Mandelkow for supplying the hTau 40 plasmid and for assistance with the expression and purification of recombinant tau.

SO'D is an Irish Institute of Clinical Neuroscience Fellow. This work was supported by a grant from Science Foundation Ireland (08/1N.1/B2033, DMW) and the Mater College for Postgraduate Education and Research, Dublin.

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=1521).

REFERENCES

[1] Blennow K, Hampel H, Weiner M, Zetterberg H (2010) Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol* **6**, 131-144.

[2] Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Meguro K, O'brien J, Pasquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, Scheltens P (2007) Research criteria for the diagnosis of Alzheimer's disease: Revising the NINCDS-ADRDA criteria. *Lancet Neurol* **6**, 734-746.

[3] Grossman M, Farmer J, Leight S, Work M, Moore P, Van Deerlin V, Pratico D, Clark CM, Coslett HB, Chatterjee A, Gee J, Trojanowski JQ, Lee VM (2005) Cerebrospinal fluid

profile in frontotemporal dementia and Alzheimer's disease. *Ann Neurol* **57**, 721-729.

[4] Verwey NA, Kester MI, van der Flier WM, Veerhuis R, Berkhof H, Twaalfhoven H, Blankenstein MA, Scheltens And P, Pijnenburg YA (2010) Additional value of CSF amyloid-beta 40 levels in the differentiation between FTLD and control subjects. *J Alzheimers Dis* **20**, 445-452.

[5] Bian H, Van Swieten JC, Leight S, Massimo L, Wood E, Forman M, Moore P, de Koning I, Clark CM, Rosso S, Trojanowski J, Lee VM, Grossman M (2008) CSF biomarkers in frontotemporal lobar degeneration with known pathology. *Neurology* **70**, 1827-1835.

[6] Noguchi M, Yoshita M, Matsumoto Y, Ono K, Iwasa K, Yamada M (2005) Decreased beta-amyloid peptide42 in cerebrospinal fluid of patients with progressive supranuclear palsy and corticobasal degeneration. *J Neurol Sci* **237**, 61-65.

[7] Aerts MB, Esselink RA, Bloem BR, Verbeek MM (2011) Cerebrospinal fluid tau and phosphorylated tau protein are elevated in corticobasal syndrome. *Mov Disord* **26**, 169-173.

[8] Urakami K, Wada K, Arai H, Sasaki H, Kanai M, Shoji M, Ishizu H, Kashihara K, Yamamoto M, Tsuchiya-Ikemoto K, Morimatsu M, Takashima H, Nakagawa M, Kurokawa K, Maruyama H, Kaseda Y, Nakamura S, Hasegawa K, Oono H, Hikasa C, Ikeda K, Yamagata K, Wakutani Y, Takeshima T, Nakashima K (2001) Diagnostic significance of tau protein in cerebrospinal fluid from patients with corticobasal degeneration or progressive supranuclear palsy. *J Neurol Sci* **183**, 95-98.

[9] Borroni B, Gardoni F, Parnetti L, Magno L, Malinverno M, Saggese E, Calabresi P, Spillantini M, Padovani A, Di Luca M (2009) Pattern of Tau forms in CSF is altered in progressive supranuclear palsy. *Neurobiol Aging* **30**, 34-40.

[10] Jack CJ, Knopman D, Jagust W, Shaw L, Aisen P, Weiner M, Petersen R, Trojanowski J (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* **9**, 119-128.

[11] Stenh C, Englund H, Lord A, Johansson AS, Almeida CG, Gellerfors P, Greengard P, Gouras GK, Lannfelt L, Nilsson LN (2005) Amyloid-beta oligomers are inefficiently measured by enzyme-linked immunosorbent assay. *Ann Neurol* **58**, 147-150.

[12] Tokuda T, Qureshi MM, Ardah MT, Varghese S, Shehab SA, Kasai T, Ishigami N, Tamaoka A, Nakagawa M, El-Agnaf OM (2010) Detection of elevated levels of α-synuclein oligomers in CSF from patients with Parkinson disease. *Neurology* **75**, 1766-1772.

[13] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan E (1984) Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**, 939-944.

[14] Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: A clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* **55**, 181-184.

[15] Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, Freedman M, Kertesz A, Robert PH, Albert M, Boone K, Miller BL, Cummings J, Benson DF (1998) Frontotemporal lobar degeneration: A consensus on clinical diagnostic criteria. *Neurology* **51**, 1546-1554.

[16] Litvan I, Agid Y, Calne D, Campbell G, Dubois B, Duvoisin RC, Goetz CG, Golbe LI, Grafman J, Growdon JH, Hallett M, Jankovic J, Quinn NP, Tolosa E, Zee DS (1996) Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): Report of the NINDS-SPSP international workshop. *Neurology* **47**, 1-9.

[17] Boeve BF, Lang AE, Litvan I (2003) Corticobasal degeneration and its relationship to progressive supranuclear palsy and frontotemporal dementia. *Ann Neurol* **54**(Suppl 5), S15-S19.

[18] McKeith I, Dickson D, Lowe J, Emre M, O'Brien J, Feldman H, Cummings J, Duda J, Lippa C, Perry E, Aarsland D, Arai H, Ballard C, Boeve B, Burn D, Costa D, Del Ser T, Dubois B, Galasko D, Gauthier S, Goetz C, Gomez-Tortosa E, Halliday G, Hansen L, Hardy J, Iwatsubo T, Kalaria R, Kaufer D, Kenny R, Korczyn A, Kosaka K, Lee V, Lees A, Litvan I, Londos E, Lopez O, Minoshima S, Mizuno Y, Molina J, Mukaetova-Ladinska E, Pasquier F, Perry R, Schulz J, Trojanowski J, Yamada M, DLB, Co (2005) Diagnosis and management of dementia with Lewy bodies: Third report of the DLB Consortium. *Neurology* **65**, 1863-1872.

[19] Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, Wood NW, Colosimo C, Dürr A, Fowler CJ, Kaufmann H, Klockgether T, Lees A, Poewe W, Quinn N, Revesz T, Robertson D, Sandroni P, Seppi K, Vidailhet M (2008) Second consensus statement on the diagnosis of multiple system atrophy. *Neurology* **71**, 670-676.

[20] Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O (2012) Cerebrospinal fluid levels of β-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch Gen Psychiatry* **69**, 98-106.

[21] Barghorn S, Biernat J, Mandelkow E (2005) Purification of recombinant tau protein and preparation of Alzheimer-paired helical filaments *in vitro*. *Methods Mol Biol* **299**, 35-51.

[22] Holtzman DM (2011) CSF biomarkers for Alzheimer's disease: Current utility and potential future use. *Neurobiol Aging* **32**(Suppl 1), S4-S9.

[23] Parnetti L, Chiasserini D, Bellomo G, Giannandrea D, De Carlo C, Qureshi MM, Ardah MT, Varghese S, Bonanni L, Borroni B, Tambasco N, Eusebi P, Rossi A, Onofrj M, Padovani A, Calabresi P, El-Agnaf O (2011) Cerebrospinal fluid Tau/α-synuclein ratio in Parkinson's disease and degenerative dementias. *Mov Disord* **26**, 1428-1435.

[24] Harper JD, Lansbury PT (1997) Models of amyloid seeding in Alzheimer's disease and scrapie: Mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu Rev Biochem* **66**, 385-407.

[25] van Harten AC, Kester MI, Visser PJ, Blankenstein MA, Pijnenburg YA, van der Flier WM, Scheltens P (2011) Tau and p-tau as CSF biomarkers in dementia: A meta-analysis. *Clin Chem Lab Med* **49**, 353-366.

[26] Saman S, Kim W, Raya M, Visnick Y, Miro S, Jackson B, McKee AC, Alvarez VE, Lee NC, Hall GF (2012) Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J Biol Chem* **287**, 3842-3849.

[27] Chai X, Dage JL, Citron M (2012) Constitutive secretion of tau protein by an unconventional mechanism. *Neurobiol Dis* **48**, 356-366.

Journal of Alzheimer's Disease 33 (2013) 1–3
IOS Press

Supplementary Data

The ELISA-Measured Increase in Cerebrospinal Fluid Tau that Discriminates Alzheimer's Disease from other Neurodegenerative Disorders is not Attributable to Differential Recognition of Tau Assembly Forms

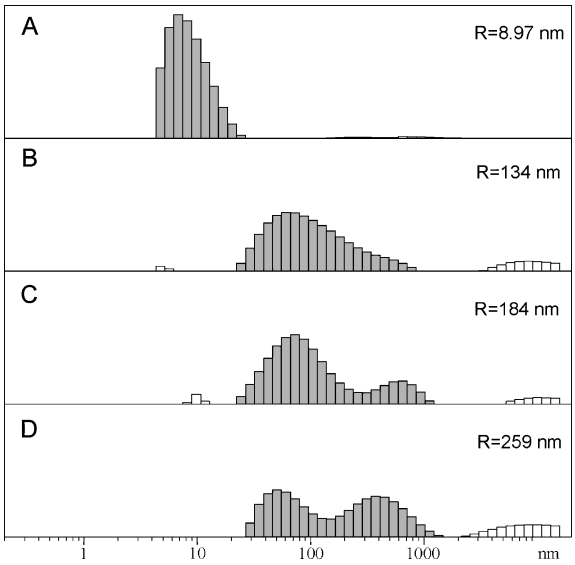
Seán T. O'Dowd[a,b], Mustafa T. Ardah[c], Per Johansson[d,e], Aleksey Lomakin[f], George B. Benedek[f], Kinley A. Roberts[b], Gemma Cummins[b], Omar M. El Agnaf[c], Johan Svensson[e,g], Henrik Zetterberg[h], Timothy Lynch[b] and Dominic M. Walsh[a,*]

[a]*Laboratory for Neurodegenerative Research, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Republic of Ireland*
[b]*Dublin Neurological Institute at the Mater Misericordiae University Hospital, Dublin, Republic of Ireland*
[c]*Faculty of Medicine and Health Sciences, Department of Biochemistry, United Arab Emirates University, Al Ain, United Arab Emirates*
[d]*Department of Neuropsychiatry, Skaraborg Hospital, Falköping, Sweden*
[e]*Department of Endocrinology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*
[f]*Department of Physics and Center for Material Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*
[g]*Department of Endocrinology, Skaraborg Hospital, Skövde, Sweden*
[h]*Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg and Mölndal, Sweden*

Accepted 13 September 2012

*Correspondence to: Dominic M. Walsh, Laboratory for Neurodegenerative Research, Center for Neurologic Diseases, Harvard Institutes of Medicine (Room 921b), 77 Avenue Louis Pasteur, Boston, MA 02115, USA. Tel.: +1 617 5255059; Fax: +1 617 5255252; E-mail: dwalsh3@partners.org.

ISSN 1387-2877/13/$27.50



Supplementary Figure 1. Aggregated tau does not readily disassemble. Dynamic Light Scattering (DLS) was done using an in-house built system with a He-Ne laser operating at 633 nm (Coherent, Santa Clara, CA) as a light source. Light scattered at 90° was collected using image transfer optics and detected by an avalanche photodiode built into a 256-channel correlator (Precision Detectors, Bellingham, MA). The size distribution of scattering particles was reconstructed from the scattered light correlation function using PrecisionDeconvolve deconvolution software (Precision Detectors). Samples were placed in washed tubes, sealed and monitoring begun within 2–5 min. The data shown are normalized distributions of the scattering intensity from particles of different sizes. Average hydrodynamic radii (R) were calculated from the distributions shown in gray. A 2.05 mg/ml solution of monomeric tau contained scattering species with an average $R_H \sim 9$ nm, a value consistent with an intrinsically disordered protein of ~50 kDa (A). In contrast, a 34 μM (1.55 mg/ml) solution of aggregated tau similar to that shown in Fig. 2 was found to contain only high molecular weight species (B). This sample was then diluted 1 : 20 into filtered PBS, used for DLS (C) and readings taken intermittently over 24 h. The size of the scattering species did not change over this time and the sample was then diluted a second time to produce a concentration of 85 nM (3.9 μg/ml) and again used for DLS (D) and monitored for a further 24 h. Even after this second dilution and prolonged incubation there was no evidence of tau monomers. These results demonstrate that when diluted to concentrations substantially below the critical concentration for aggregation, tau aggregates do not readily disassemble.

Supplementary Table 1
Demographic characteristics, Mini-Mental State Exam (MMSE) scores, and CSF tau results. The values for age, MMSE, t-tau, p-tau, and $A\beta_{1\text{-}42}$ are displayed as means ± SE. PSP-RS, progressive supranuclear palsy-Richardson's syndrome; PSP-P, progressive supranuclear palsy-parkinsonism; PNFA, progressive non-fluent aphasia; bvFTD, behavioral variant frontotemporal dementia; MSA-P, multiple system atrophy-parkinsonism

Case	Gender	Age	Diagnosis	Syndrome	MMSE	T-tau (pg/ml)	P-tau (pg/ml)	$A\beta_{1\text{-}42}$ (pg/ml)
1	M	60	PSP	PSP-RS	29	144.1	42.9	310
2	M	75	PSP	PSP-RS	23	243.8	61	394
3	F	61	PSP	PSP-RS	26	288.2	41.5	131
4	M	66	PSP	PSP-P	29	106.4	36.9	39
5	M	67	PSP	PSP-RS	26	332.2	44.6	463
6	M	65	PSP	PSP-RS	28	181.6	46.9	205
7	M	73	PSP	PSP-RS	26	189.2	46.9	198
8	F	79	PSP	PSP-RS	20	191.7	47.4	621
9	F	63	CBD		23	232.2	49.2	392
10	F	62	CBD		23	192.6	39.5	552
11	F	65	CBD		28	822.3	108.7	N/A
12	F	67	CBD		29	169.3	39.7	274
13	F	56	CBD		16	518.5	109.5	251
14	M	72	FTD	PNFA	17	1556	230.2	177
15	F	42	FTD	FTDP-17	30	139.7	35.3	251
16	F	43	FTD	FTDP-17	24	88.2	26.8	89
17	M	45	FTD	bvFTD	29	162.1	42.7	501
18	F	76	MSA	MSA-P	28	520.8	92.4	170
19	F	66	MSA	MSA-P	30	107	22.5	227
20	M	45	IPD		30	63.8	28.4	388
21	M	50	IPD		29	57.5	22.3	200
22	M	72	DLB		18	212.8	51	667
23	F	74	DLB		7	176	41.8	121
24	M	73	DLB		–	135.5	36.2	87
25	M	59	AD		26	345.1	66.6	165
26	F	54	AD		24	910.2	117.6	188
27	M	73	AD		–	748.5	109.5	186
28	M	80	AD		28	214.1	74.7	138
29	F	73	AD		25	419.4	89.2	114
30	M	69	AD		23	506.3	91.5	179
31	F	78	AD		20	328.7	64.3	151
32	M	77	AD		18	399.2	77.8	318
33	M	68	AD		19	559.5	99.9	213
34	M	74	AD		17	500.1	92.8	142
35	F	69	AD		20	639.3	112	76
36	M	72	AD		28	379	81.1	223
37	F	72	AD		30	270.5	63.7	272
38	F	78	CON		–	223	56.6	575
39	F	80	CON		26	329.2	82.4	212
40	F	65	CON		25	272.7	74.8	996
41	M	66	CON		28	179.5	57.6	467
42	F	66	CON		29	244.6	75.1	713
43	M	76	CON		29	109.3	40.6	372
44	M	71	CON		30	65.8	29.1	237
45	F	79	CON		29	191.2	61.5	607
46	F	78	CON		26	238.4	57.8	561
47	M	76	CON		29	262.4	69.1	622
48	F	78	CON		25	191.2	53.8	646
49	M	70	CON		29	115.7	41.4	600
50	F	78	CON		27	149.7	56.6	690
51	M	74	CON		28	323.8	72.1	740
52	M	72	CON		28	267.7	78.6	283
53	F	72	CON		28	261.4	70.4	283