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Proteomics-derived cerebrospinal fluid markers of autopsy-confirmed Alzheimer's disease

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Abstract

The diagnostic performance of several candidate cerebrospinal fluid (CSF) protein biomarkers in neuropathologically confirmed Alzheimer's disease (AD), non-demented (ND) elderly controls and non-AD dementias (NADD) was assessed. Candidate markers were selected on the basis of initial two-dimensional gel electrophoresis studies or by literature review. Markers selected by the former method included apolipoprotein A-1 (ApoA1), haemopexin (HPX), transthyretin (TTR) and pigment epithelium-derived factor (PEDF), while markers identified from the literature included Aβ1-40, Aβ1-42, total tau, phosphorylated tau, α-1 acid glycoprotein (A1GP), haptoglobin, zinc α-2 glycoprotein (Z2GP) and apolipoprotein E (ApoE). Ventricular CSF concentrations of the markers were measured by enzyme-linked immunosorbent assay (ELISA). The concentrations of Aβ1-42, ApoA1, A1GP, ApoE, HPX and Z2GP differed significantly among AD, ND and NADD subjects. Logistic regression analysis for the diagnostic discrimination of AD from ND found that Aβ1-42, ApoA1 and HPX each had significant and independent associations with diagnosis. The CSF concentrations of these three markers distinguished AD from ND subjects with 84% sensitivity and 72% specificity, with 78% of subjects correctly classified. By comparison, using Aβ1-42 alone gave 79% sensitivity and 61% specificity, with 68% of subjects correctly classified. For the diagnostic discrimination of AD from NADD, only the concentration of Aβ1-42 was significantly related to diagnosis, with a sensitivity of 58%, specificity of 86% and 86% correctly classified. The results indicate that for the discrimination of AD from ND control subjects, measurement of a set of markers including Aβ1-42, ApoA1 and HPX improved diagnostic performance over that obtained by measurement of Aβ1-42 alone. For the discrimination of AD from NADD subjects, measurement of Aβ1-42 alone was superior.

Keywords: *Alzheimers' disease; non-Alzheimer's disease dementias; cerebrospinal fluid; biomarkers; ELISA; Aβ; tau; apolipoprotein A-1; α-1 acid glycoprotein; haptoglobin; haemopexin; transthyretin; pigment epithelium-derived factor; zinc α-2 glycoprotein; apolipoprotein E*

Introduction

Alzheimer's disease (AD) is the most prevalent dementia in the elderly. While existing clinical diagnostic methods have a high sensitivity for AD, about 90%, they have a low specificity, about 55%, as determined by comprehensive studies of data from National Institute on Aging Alzheimer's Disease Research Centers (Mayeux et al. 1998). The low specificity is due to the difficulty of distinguishing AD from a number of other, less common diseases, known as non-AD dementias (NADD). Collectively, NADD comprise at least 25% of all elderly dementias and are often clinically indistinguishable from AD (Kazee et al. 1993, Victoroff et al. 1995, Litvan et al. 1996, Kosunen et al. 1996, Jellinger 1996, Lim et al. 1999).

Analysis of cerebrospinal fluid (CSF) may offer the greatest potential for developing predictive and diagnostic markers. CSF is contiguous with the brain interstitial fluid and thus reflects brain chemistry (Zheng et al. 2003). Therefore, it is likely that neurodegenerative abnormalities in the brain will ultimately

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produce a characteristic biochemical signature that will become evident in the CSF proteome. A critical deficiency with most CSF tests that have been reported for AD, however, is that they have rarely been evaluated against the gold standard for diagnosis, which is neuropathological examination of the brain at autopsy. Diagnostic methods that use an antemortem neurological diagnosis of AD as the gold standard cannot, by definition, be shown to improve on this and therefore the numerous CSF studies that claim 80–90% sensitivity and specificity compared with the clinical diagnosis of AD are misleading in that they actually represent only perhaps 60–70% accuracy with respect to the true neuropathological diagnosis. Postmortem CSF studies are thus essential to sorting out the CSF profile for AD and other neurodegenerative diseases. It is recognized, however, that postmortem CSF changes (Lescuyer et al. 2004, Finehout et al. 2006, Burgess et al. 2006, Dayon et al. 2008) must be distinguished from changes due to disease.

We initially used two-dimensional gel electrophoresis (2DE) to screen CSF pools from neuropathologically diagnosed AD, NADD and non-demented (ND) elderly subjects (Castano et al. 2006). Apolipoprotein A-1 (ApoA1), haemopexin (HPX), transthyretin (TTR) and pigment epithelium-derived factor (PEDF) were found to be differentially abundant. In the present study, we further tested, using enzyme-linked immunosorbent assay (ELISA) methods, the differential presence of these proteins in CSF samples from individual subjects and evaluated their performance as diagnostic markers for neuropathologically confirmed AD compared with NADD and ND subjects. Four other markers, α-1 acid glycoprotein (A1GP), haptoglobin (HPTG), zinc α-2 glycoprotein (Z2GP) and apolipoprotein E (ApoE), were selected for evaluation on the basis of a literature review (Elovaara et al. 1985, Johnson et al. 1992, Mattila et al. 1994, Davidsson et al. 1999, Davidsson et al. 2002, Puchades et al. 2003, Hansson et al. 2004, Jung et al. 2008). Additionally, we measured the CSF concentrations of Aβ1-40, Aβ1-42, total tau protein (t-tau) and phosphorylated tau protein (p-tau) in the same subjects as these have previously been identified as the most useful diagnostic marker proteins (Galasko et al. 1998, Montine et al. 2001, Sjogren et al. 2001, Clark et al. 2003, Maddalena et al. 2003, Engelborghs et al. 2008).

Materials and methods

Study site

The study took place at the Sun Health Research Institute (SHRI), which is part of a non-profit, community-owned and operated healthcare provider located in the Sun Cities retirement communities of northwest metropolitan Phoenix, Arizona. The SHRI is a member of the Arizona Alzheimer's Consortium (AAC) and the Arizona Alzheimer's Disease Core Center (AZADCC). The SHRI Brain Donation Program (SHRI-BDP) serves as the neuropathology core for both the AAC and the AZADCC (Beach et al. 2008). All subjects participating in the SHRI-BDP have signed an informed consent approved by the Sun Health Institutional Review Board.

Study subjects

The study subjects consisted of 47 individuals with AD, 43 ND elderly and 17 with NADD (Table 1). The subjects with NADD consisted of four with progressive supranuclear palsy (PSP) and dementia, three with hippocampal sclerosis dementia (HSD), four with a non-specific sporadic tauopathy and/or argyrophilic grains, two with frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) and progranulin gene mutations, and one each with frontotemporal dementia (FTD) with tau gene mutation, normal pressure hydrocephalus (NPH), corticobasal degeneration (CBD) and dementia lacking distinctive histology (DLDH). The subjects from all groups were elderly, of mixed gender with mean ages ranging from 77.5 to 84.1 years. There were no group differences in terms of postmortem interval (PMI). The *APOE* group allelic frequencies, neuritic plaque densities and Braak stages differed as expected, based on the selection criteria (Table 1).

Subjects were chosen by searching the SHRI-BDP database for CSF availability from individuals who died with less than a 4-h PMI and had clinicopathological diagnoses of ND control (no clinical history of dementia, parkinsonism or other neurological disorder and no neuropathological findings meeting criteria for a defined disorder), AD or NADD. All subjects had received standardized neuropathological examinations as described previously (Beach et al. 2008). Specific clinicopathological consensus diagnostic criteria were used for AD and other

Table 1. Characteristics of study subjects. Means and standard deviations are shown for age, postmortem interval (PMI), CERAD neuritic plaque density and Braak stage.

	Age (years)	Gender	PMI	*APOE* allelic frequency	Neuritic plaque density	Braak stage
ND controls ($n=43$)	84.1 (5.97)	20M/23F	2.62 (0.75)	ε2 = 0.07 ε3 = 0.77 ε4 = 0.16	1.30 (0.91)	2.67 (0.92)
AD ($n=47$)	79.1 (9.91)	19M/28F	2.58 (0.66)	ε2 = 0.04 ε3 = 0.64 ε4 = 0.32	3.0 (0.00)	5.53 (0.50)
NADD ($n=17$)	77.5 (12.46)	12M/5F	2.64 (0.84)	ε2 = 0.06 ε3 = 0.88 ε4 = 0.06	0.71 (0.77)	2.06 (1.03)

ND, non-demented; AD, Alzheimer's disease; NADD, non-Alzheimer's disease dementia; ApoE, Apolipoprotein E.

neurodegenerative disorders, following a recently published algorithm (Dickson 2005). For AD, cases received the diagnosis if they were classified as 'intermediate' or 'high' probabilities according to criteria published under the auspices of the National Institute on Aging and the Reagan Institute (1997). All AD cases also met CERAD criteria for 'definite' or 'probable' AD (Mirra et al. 1991). For subjects with AD and subjects with NADD, an additional criterion for inclusion was that they did not have a second major neuropathological diagnosis. In particular, AD cases were to have no concurrent neuropathological diagnosis of NADD and NADD cases were to have no concurrent diagnosis of AD.

All individuals were clinically characterized by review of medical records, interviews with contacts and/or standardized neurological and neuropsychological assessments at SHRI. The standardized assessments included the Global Deterioration Scale, Mini Mental State Examination, Unified Parkinson's Disease Rating Scale and a neuropsychological test battery, as described (Beach et al. 2008). The *APOE* genotype had been previously determined for all subjects as part of the standard protocol for the Brain Donation Program (Beach et al. 2008). CSF samples were collected in the postmortem period from the lateral cerebral ventricles, centrifuged at 5000 rpm for 10 min to pellet out cells and then immediately aliquotted into 1 ml samples and stored at -70–80°C in polypropylene tubes until use.

CSF marker protein determinations

The concentrations of CSF marker proteins were determined using commercially obtained ELISA kits, according to the manufacturers' instructions (Table 2). For Aβ1-42, total tau (t-tau) and phosphorylated tau (p-tau), kits were obtained from Innogenetics, Ghent, Belgium. Other kits were obtained from Immuno-Biological Laboratories, Minneapolis, MN, USA (Aβ1-40), GenWay Biotech, Inc., San Diego, CA, USA (A1GP), ALerCHEK, Portland, ME, USA (ApoA1), Immunology Consultant Laboratories, Inc., Newberg, OR, USA (HPTG, HPX, TTR), BioProducts MD, Middletown, MD, USA (PEDF), BioVendor, Candler, NC, USA (Z2GP) and MBL International Corp., Woburn, MA, USA (ApoE). Samples from each subject were run in duplicate. Samples that were out of the linear portion of the standard curve were repeated after dilution. Values that remained outside 2 standard deviations from the mean were discarded. The assays for all proteins were done without knowledge of the subjects' diagnoses.

Statistical analysis

Group differences between marker protein concentrations, adjusted for age and gender, were assessed using analysis of covariance (ANCOVA). Specificity and sensitivity were calculated by means of multinomial logistic regression modelling, with diagnosis as the dependent variable. Separate models were constructed, using those marker proteins found to differ significantly ($p < 0.05$) on ANCOVA, to compare AD vs ND subjects and AD vs NADD. The probability level was set at 0.05 for each of these comparisons.

Results

CSF concentrations of marker proteins and ANCOVA analysis

A graphic portrayal of the mean CSF concentrations of the marker proteins in the three diagnostic groups is given in Figure 1. Results of the ANCOVA are given in Table 3. The CSF concentrations of Aβ1-42, A1GP, ApoA1, ApoE, HPX and Z2GP differed significantly ($p < 0.05$) among the three diagnostic groups, with adjustment for age and gender. No statistical differences were observed for t-tau and p-tau among the three compared groups. Linear regression analysis of all marker concentrations vs PMI found no significant relationships. The correlation coefficients were all extremely low (range 0.01–0.17) and the *p*-values were all greater than 0.12 (range 0.12–0.91).

Table 2. Protein marker enzyme-linked immunosorbent assay (ELISA) kits used, with manufacturer and references.

ELISA	Manufacturer	References
Innotest β-Amyloid 1-40	Immuno-Biological Laboratories	(Clark et al. 2003, Engelborghs et al. 2008)
Innotest β-Amyloid 1-42	Innogenetics	(Clark et al. 2003, Engelborghs et al. 2008)
Innotest total tau	Innogenetics	(Clark et al. 2003, Engelborghs et al. 2008)
Innotest phosphorylated-tau$_{(181P)}$	Innogenetics	(Clark et al. 2003, Engelborghs et al. 2008)
α-1 Acid glycoprotein (orosomucoid)	GenWay Biotech	
Apolipoprotein A-1	ALerCHEK	(Kawashiri et al. 2002, Roberts et al. 2006)
Haptoglobin	Immunology Consultants Laboratory	
Hemopexin	Immunology Consultants Laboratory	
Pigment epithelium derived factor	BioProducts MD	(Dittmer et al. 2008, Sreekumar et al. 2008)
Transthyretin (prealbumin)	Immunology Consultants Laboratory	
Zinc α-2 glycoprotein	BioVendor	
Apolipoprotein E	MBL International Corporation	

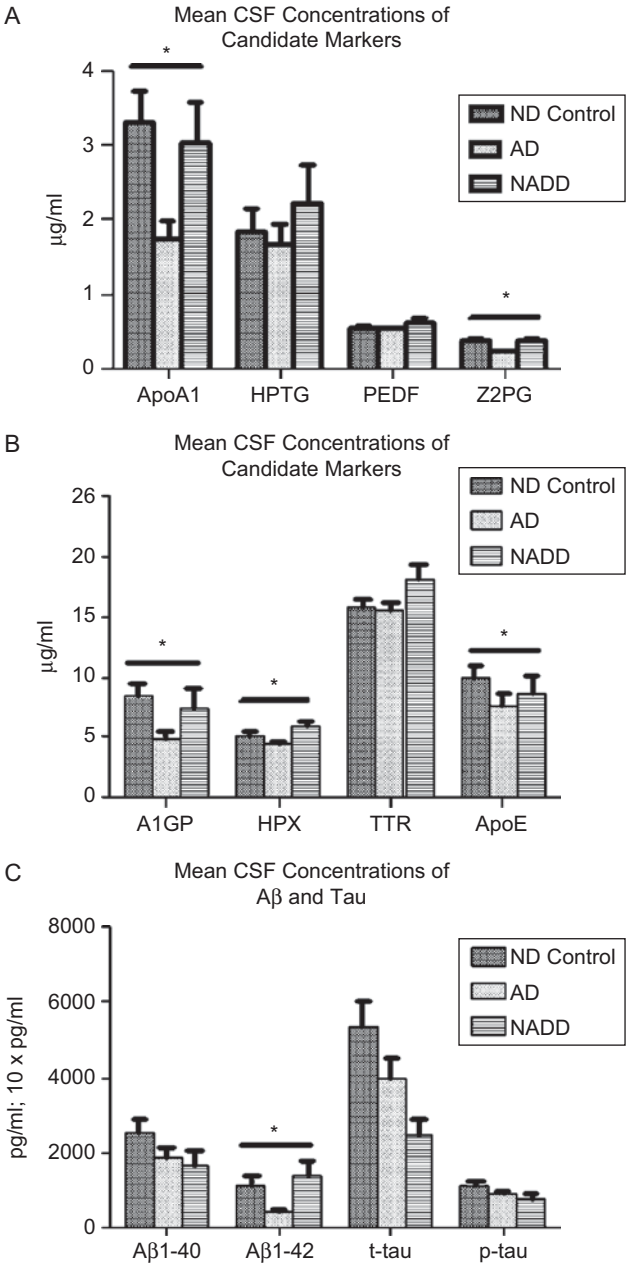

Figure 1. Graphs depicting mean cerebrospinal fluid concentrations of the candidate marker proteins as measured by enzyme-linked immunosorbent assay (ELISA). All markers in images (A) and (B) are expressed as µg ml^{-1}. In image (C) Aβ1-40 and t-tau are expressed in pg ml^{-1}, while Aβ1-42 and p-tau are expressed as pg ml^{-1} × 10. Asterisks denote that the group means differ significantly ($p < 0.05$). See Table 3 for pairwise comparisons. CSF, cerebrospinal fluid; ND, non-demented; AD, Alzheimer's disease; NADD, non-Alzheimer's disease dementia; ApoA1, apolipoprotein A-1; HPTG, haptoglobin; PEDF, pigment epithelium-derived factor; Z2PG, zinc α-2 glycoprotein; A1GP, α-1 acid glycoprotein; HPX, haemopexin; TTR, transthyretin; ApoE, apolipoprotein E; t-tau, total tau; p-tau, phosphorylated tau.

Table 3. Results of ANCOVA comparing cerebrospinal fluid concentrations of the marker proteins in the three diagnostic groups. Results shown are adjusted for age and gender.

ELISA	ANCOVA Significance (p-value)
β-Amyloid 1-40	0.09
β-Amyloid 1-42	0.0001
t-Tau	0.21
p-Tau	0.13
α-1 Acid glycoprotein	0.009
Apolipoprotein A-I	0.001
Haptoglobin	0.82
Hemopexin	0.0065
Pigment epithelium derived factor	0.16
Transthyretin	0.17
Zinc α-2 glycoprotein	0.0018
Apolipoprotein E	0.002

ELISA, enzyme-linked immunosorbent assay.

Results of logistic regression models

The results were analysed to calculate diagnostic performance of the marker panel in terms of its ability to discriminate AD from ND subjects as well as AD subjects from NADD subjects. The CSF markers Aβ1-42, A1GP, ApoA1, ApoE, HPX and Z2GP, which differed significantly ($p < 0.05$) on ANCOVA, were used in a logistic regression model with diagnosis as the dependent variable. Initial modelling determined that, for the discrimination of AD from ND subjects, three markers, Aβ1-42, ApoA1 and HPX, were significantly and independently contributory so the final model included only these three, resulting in 84% sensitivity and 72% specificity, with 78% of subjects correctly classified (Table 4). In comparison, the use of Aβ1-42 alone was less accurate, giving 79% sensitivity and 61% specificity, with 68% of subjects correctly classified (Table 5). For the diagnostic discrimination of AD from NADD, only the concentration of Aβ1-42 was significantly related to diagnosis, with a sensitivity of 58%, specificity of 86% and 86% correctly classified (Table 5).

Discussion

The results of this study support the general strategy taken, in which candidate marker proteins were initially identified with a proteomic technique on pooled postmortem CSF from diagnostic groups, and then subsequently tested with ELISA of individual CSF samples. We used two-dimensional gel electrophoresis (2DE) to screen CSF pools from AD, NADD and ND elderly subjects for candidate diagnostic protein markers (Castano et al. 2006). Four proteins found to be differentially abundant in these studies, ApoA1, HPX, TTR and PEDF, were then studied in CSF using ELISA, along with several

Table 4. Diagnostic performance of the marker panel for discriminating Alzheimer's disease from non-demented control subjects. Accuracy is the percentage of cases correctly classified.

	p-Value	OR	95% CI of OR		Accuracy	Sensitivity	Specificity
Aβ1-42	0.0003	0.95	0.93	0.98	78%	84%	72%
ApoA1	0.001	0.44	0.27	0.72			
HPX	0.008	1.83	1.17	2.87			

OR, odds ratio; CI, confidence interval; ApoA1, Apolipoprotein A-1; HPX, haemopexin.

Table 5. Diagnostic performance of Aβ1-42 alone for discriminating Alzheimer's disease (AD) from non-demented control subjects (ND) and non-Alzheimer's disease dementia subjects (NADD). Accuracy is the percentage of cases correctly classified.

	p-Value	OR	95% CI of OR		Accuracy	Sensitivity	Specificity
AD vs ND	0.0002	0.96	0.94	0.98	68%	79%	61%
AD vs NADD	0.007	0.96	0.93	0.99	86%	58%	86%

OR, odds ratio; CI, confidence interval.

other proteins identified by literature review, including Aβ1-40, Aβ1-42, t-tau, p-tau, HPTG, A1GP, ApoE and Z2GP. Of the initial four markers identified by 2DE, two, ApoA1 and HPX, were found to be significantly different by ANCOVA analysis of ELISA results in the present study and these same two markers were also significant with logistic regression. The 2D gel method differs from the ELISA method in that the former is based on the electrophoretic properties of molecular size and charge, as well as on protein abundance. The ELISA method relies on immunochemical identification of proteins. Also, our initial 2D gel experiments were intended to be a screening method only, as we used pools of CSF from multiple subjects, testing samples of pooled AD CSF against samples of pooled control CSF. It is not possible to obtain diagnostic statistics such as sensitivity and specificity from such experiments. The final results confirm the validity of our strategy in that screening of CSF pools by 2D gel yielded four candidate proteins of which two were ultimately proven statistically significant and diagnostically useful with ELISA of CSF samples from individual subjects.

All of the markers have previously been implicated in AD pathogenesis and/or similar or related pathological processes and/or have been found to be altered in AD (Elovaara et al. 1985, Johnson et al. 1992, Mattila et al. 1994, Davidsson et al. 1999, Davidsson et al. 2002, Hansson et al. 2004, Castano et al. 2006, Jung et al. 2008). In particular, ApoA1 has been intensively studied with respect to a possible pathogenic role in AD, suggesting that its differential presence in the CSF may have mechanistic implications.

ApoA1 is a major component of the high-density lipoproteins (HDL). Decreased ApoA1 serum and CSF concentrations have previously been reported in subjects with AD (Liu et al. 2006), as well as decreased risk of dementia for subjects with decreased serum ApoA1 (Saczynski et al. 2007). ApoA1 has been found in association with amyloid deposits of various types, including Aβ deposits in the brains of AD patients, and has been suggested to modulate amyloid toxic effects at several levels, including a capacity to inhibit aggregation physically and prevent β-sheet organization (Wisniewski et al. 1995, Harr et al. 1996, Koldamova et al. 2001, Sakata et al. 2005). There is evidence that ApoA1 may protect the brain from Aβ precursor protein (APP) C-terminal toxicity through a synergistic interaction with α-tocopherol (Maezawa et al. 2004). It is well established that ApoA1, as a principal component of HDL, protects blood vessels against atheromatosis by removing cholesterol and shunting it to the liver (Luoma 1997). The discovery that the ApoA1 Milano variant can reduce atheroma volume has generated interest in using ApoA1 and its mimetic peptides as therapeutic agents (Nissen et al. 2003, Navab et al. 2004). However, there is some evidence against the potential role of ApoA1 in AD as, in an APP transgenic mouse model of amyloidosis, gene deletion of ApoA1 did not alter brain Aβ deposition (Fagan et al. 2004).

HPX, an acute-phase reactant protein, is induced by interleukin (IL)-1 and IL-6. HPX is synthesized in the brain by ventricular ependymal cells, hence it is found in the CSF. Its functions include maintaining metal ion homeostasis due to its ability to bind metal ions (Mauk et al. 2005) and preventing oxidative damage to cells by transporting heme to the liver (Tolosano et al. 1999, Fasano et al. 2002). It protects against free heme-mediated toxicity and participates in heme iron homeostasis within the brain (Morello et al. 2008). There is evidence that in AD there is a leaky blood–brain barrier and capillary microbleeds that allow the escape of erythrocytes into the brain parenchyma (Jellinger 2002, Soffer 2006), with consequent release of toxic levels of iron. HPX has been previously found to be 6.5 times higher in AD plasma when compared with controls (Yu et al. 2003).

Our findings regarding the diagnostic efficacy of CSF Aβ and tau as diagnostic markers for AD, adds to a large existing literature (Galasko et al. 1998, Montine et al. 2001, Sjogren et al. 2001, Nagga et al. 2002, Maddalena et al. 2003, Gloeckner et al. 2008). However, the high sensitivities and specificities, many well over 80%, reported

by studies using the clinical diagnosis of AD as a gold standard are misleading and cannot really be regarded as indicative of the true, neuropathological diagnostic accuracy. There are still only three published studies of CSF markers that have estimated sensitivity and specificity compared with the neuropathological diagnosis. The first of these (Tapiola et al. 2000) reported, for various combinations of Aβ1-42 and t-tau, sensitivities and specificities well under those reported by clinical studies, ranging from 52.5% to 68.8% for sensitivity and 59.3% to 92.3% for specificity, depending on the combination of markers and the comparison (AD vs ND control or AD vs NADD). A subsequent study (Clark et al. 2003) reported high sensitivities and specificities for t-tau (85 and 84%) in the comparison of AD and controls while for AD vs NADD sensitivity and specificity were only 72 and 69%. In this study, Aβ42 concentrations did not add to diagnostic accuracy. Additionally, only four of the 73 normal control subjects were autopsied in this study. The most recent study (Engelborghs et al. 2008), done in association with the ELISA test kit manufacturer Innogenetics, reported high sensitivities and specificities for various combinations of t-tau, p-tau and Aβ1-42 (80–93%) but the study was seriously flawed due to the use of a control group of much younger age (mean 46 vs 75 and 72 for the dementia groups) and because of the exclusion of 34 subjects for 'out-of-range' data. Our results for Aβ1-42, t-tau and p-tau, based on the largest autopsy-confirmed study to date that used age-comparable ND controls, most resemble those of Tapiola et al. 2000, in that we found much lower sensitivities and specificities than those generally reported by studies using clinical diagnosis as the gold standard. Additionally, we did not find that either t-tau or p-tau measurements were useful in the discrimination of AD from either ND controls or NADD subjects. Only Aβ42 concentrations were diagnostically useful, giving sensitivities and specificities of 83% and 49% for the discrimination of AD from ND controls, while for the comparison of AD and NADD the respective values were 25% and 98%.

The use of postmortem CSF as a medium for identifying diagnostic markers must be approached with caution, as recent proteomic studies have shown that many proteins show postmortem concentration changes (Lescuyer et al. 2004, Finehout et al. 2006, Burgess et al. 2006, Dayon et al. 2008). Despite this potential confound, the suggestion that such changes entirely negate the value of postmortem CSF for the investigation of human neurological disease must be firmly rejected. The investigation of postmortem brain and CSF chemistry has a long and productive tradition within the biomedical sciences, and has yielded what might arguably be regarded as the most relevant and useful discoveries within the field of neurodegenerative disease. The cholinergic and dopaminergic neurotransmitter deficits found within the postmortem brains of subjects with AD and Parkinson's disease, respectively, remain the basis of the major current pharmacological therapies for these diseases. The data presented in the current study would appear to represent genuine disease-related changes, as the diagnostic groups did not differ in terms of their mean PMI and none of the marker protein concentrations correlated significantly with PMI. Also, as mentioned above, all of the proteins in our initial proteomics study had before or since been reported to be altered in AD or related to its pathogenesis.

The negative findings with respect to t-tau and p-tau, however, are puzzling, in that previous autopsy studies did find these markers to be of diagnostic value. There are reports of rapid postmortem changes in both p-tau and t-tau (Schwab et al. 1994, Gartner et al. 1998, Ferrer et al. 2007) and it is possible that these could be more rapid and extensive in the ventricular CSF, which we used, compared with the lumbar spinal CSF utilized in the other studies. Intriguingly, the concentrations of t-tau in the ventricular CSF were in all three categories, AD, ND and NADD, several orders of magnitude higher than those reported for the lumbar CSF using the same ELISA Innogenetics kits (Vanderstichele et al. 2006, Engelborghs et al. 2008). We believe that these measurements are reliable, as samples from the three diagnostic groups were randomly distributed within the assay plates and the assays were performed by one operator who was blinded to their identity. Furthermore, those individuals with high t-tau also had high p-tau levels that overall demonstrated a correlation coefficient of $R = 0.80$. The biochemical differences between ventricular and lumbar CSF are probably due to the dynamics of protein production/clearance that may alter the protein levels between the two sites, with brain-derived proteins being elevated (specifically tau) in the ventricular CSF (Reiber 2001).

A recent study by Ray et al. (2007), found that a set of 18 plasma biomarkers classified clinically diagnosed AD and control subjects with close to 90% accuracy. While this study is of interest, it is not directly relevant to our work in the following ways: (1) these authors classified subjects into AD and control subjects by clinical diagnosis only, which would have misclassified a proportion of subjects compared with neuropathological diagnosis; (2) their study used plasma while we used CSF; (3) these authors used a hypothesis-driven approach to selecting their biomarker proteins, in that they screened for 120 known signalling proteins; we used a non-biased comprehensive proteomic approach using 2D gel electrophoresis initially to identify our candidate biomarkers (Castano et al. 2006), as well as using four other biomarkers identified by literature search. We compared these markers to the current gold standards for AD CSF, namely Aβ and tau forms. None of our markers were assessed by Ray et al.

Employing our panel of three protein markers increased the diagnostic accuracy of the distinction between AD and ND controls by 10% over that achieved by measuring Aβ1-42 alone. Additional investigations, using CSF obtained during life on subjects later autopsied and neuropathologically diagnosed, are necessary to determine whether this marker panel increases diagnostic accuracy when performed on CSF obtained from living subjects. Further identification of additional CSF markers of AD, through proteomics or other means, may incrementally further increase sensitivity and specificity.

It is disappointing that the panel did not distinguish AD from NADD with greater efficacy than Aβ1-42 alone, as this is currently a major problem in clinical diagnostics. While the separation of AD from ND subjects is less important than the separation of AD from NADD, it is possible that these markers may ultimately prove useful for the separation of ND elderly with significant AD pathology from those without significant AD pathology (Riemenschneider et al. 2002, Skoog et al. 2003, Parnetti et al. 2006, Li et al. 2007, Bouwman et al. 2007, Fagan et al. 2007, Andersson et al. 2008, Sundelof et al. 2008). This may be even more important than separating different types of dementia, as an effective marker panel for 'preclinical' or incipient AD might prove to identify those at high risk for progressing to AD. These subjects would be ideal for AD prevention trials.

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