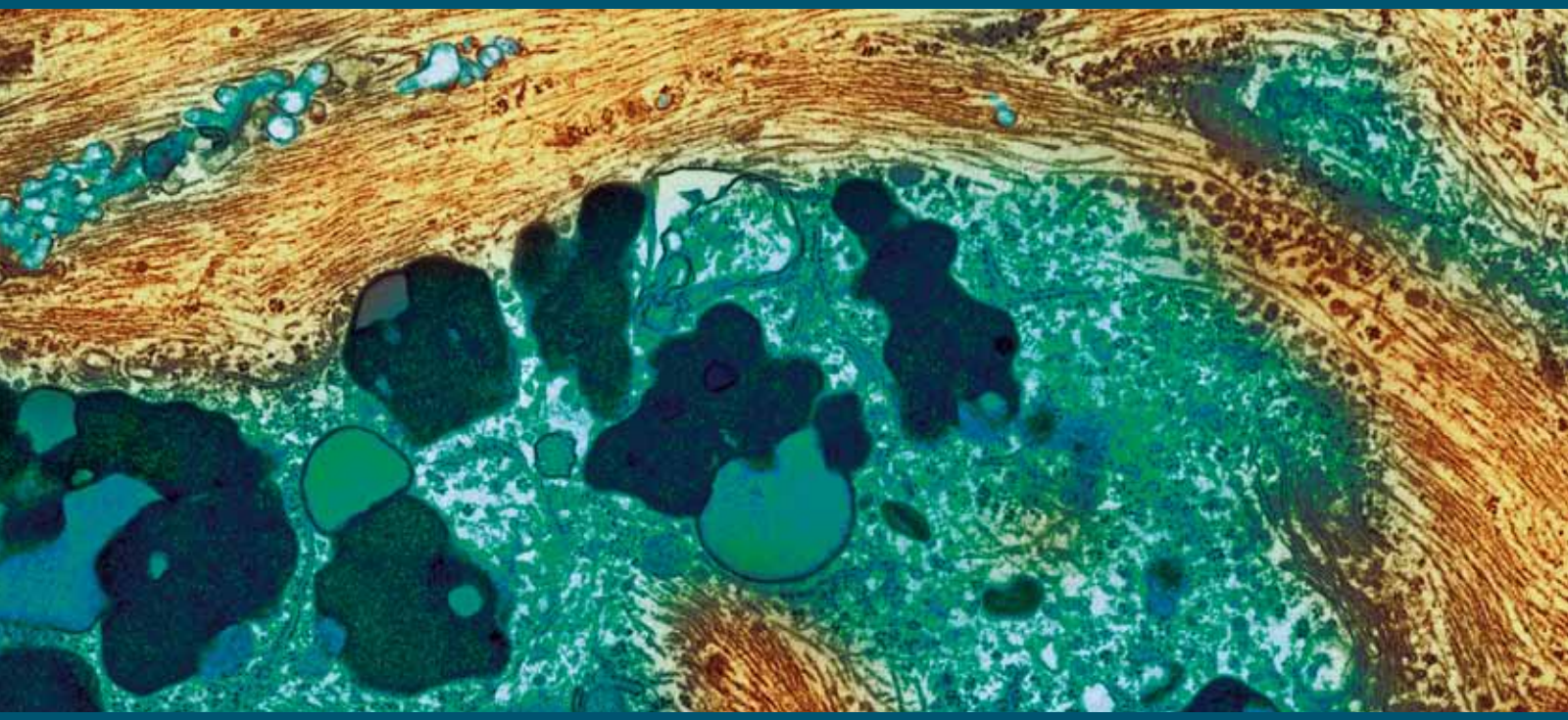
International Journal of Alzheimer's Disease

Biomarkers for Dementia

Guest Editors: Katsuya Urakami, Hiroyuki Arai, Holly Soares, and Giovanni B. Frisoni



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Editorial Board

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Review Article

Diagnostic Utility of CSF Tau and Aβ_{42} in Dementia: A Meta-Analysis

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CSF tau and Aβ_{42} are considered as important markers to diagnose Alzheimer's disease in early stages. Hence, it is important to assess their status in different types of dementia. The main objective of this study was to assess whether these CSF biomarkers can be used to make the differential diagnosis of AD. In the present study, articles published from 1998 till 2009 were taken and meta-analysis was performed to clarify the consistency in trends of biomarkers- CSF tau and Aβ_{42} in AD and other dementias and whether the same can be used as diagnostic biomarkers for its early diagnosis. 11 out of 60 for CSF tau and 07 out of 40 for CSF Aβ_{42}, dementia case-control studies were selected for final analysis. Descriptive statistics shows that median effect size (raw mean difference) of CSF tau was 429 pg/mL (range: 32 to 910 pg/mL) in AD whereas in Dementia due to other causes (DOC) studies it was 69 pg/mL (range: −53 to 518 pg/mL). Similarly the median effect size of CSF Aβ_{42} levels was −442 pg/mL (range: −652 to −41.200 pg/mL) whereas in DOC studies it was −193 pg/mL (range: −356 to −33 pg/mL).

1. Introduction

With the increase in life expectancy, Alzheimer's disease, considered as disease of aging population, has become a major public health problem adding burden to societal costs each year for chronic care and lost productivity in developed and developing countries [1]. Presently, diagnosis of AD is primarily based on the exclusion of other causes of dementia on clinical trials. However, it is not of much help due to overlapping of clinical features of AD with other dementias in early stages. Attempts have been made in the last 4-5 decades to develop and validate specific biological markers which are able to detect the fundamental neuropathological changes occurring in AD in its early stage with high sensitivity (≥80%) and distinguish it from other dementias [2]. Based on these studies, the combination of total tau and amyloid β_{42} (Aβ_{42}) was identified as being among the most promising and informative AD markers to be of use in early diagnosis and as surrogate biomarkers in CSF [2–4]. Increased levels of tau in CSF have been suggested to reflect neuronal and axonal degeneration [5] whereas reduced CSF levels of Aβ_{42} might reflect extracellular accumulation of Aβ_{42} into insoluble senile plaques in the AD brain [6, 7]. However, high CSF concentration of total tau has also been reported in mild cognitive impairment [8], as well as in vascular dementia (VaD) [2, 9–11]. The comorbidity of AD and other dementias can further generate problems. Though studies have reported highly accurate differentiation between AD and normal controls with high sensitivity (50–94%) and specificity (83–100%) [12], CSF-based differentiation of AD with VaD remains a challenge with specificity of 48% only [13]. Also, AD can be associated with other neurodegenerative diseases like Lewy body disease and progressive supranuclear palsy.

In view of the necessity of identifying biomarkers for differentiation of AD from other dementia disorders, the combination of CSF tau and Aβ_{42} has been advocated as diagnostic marker. However, before the diagnostic utility of CSF tau and Aβ_{42} concentration for diagnosis of AD can be established, it is crucial to assess whether the effect sizes of the published studies reported in the last fifteen years show consistent trends in their levels or not in AD cases as compared to controls. The consistency in effect size of tau and Aβ_{42} levels of these studies was explored by advanced

statistical method meta-analysis with three main goals: (i) to test whether the results of studies are homogeneous, (ii) to obtain a global index about the effect magnitude of the studied relation, joined to a confidence interval and its significance, and (iii) to identify possible variables or characteristics moderating the results obtained if there is heterogeneity among studies [14]. In the present study, studies conducted from 1995 till 2009 were taken and meta-analysis was performed to clarify the consistency in trends of biomarkers CSF tau and Aβ_{42} in different types of dementias and whether the same can be used as diagnostic biomarkers for early diagnosis of AD.

2. Materials and Methods

2.1. Search Strategy. Meta-analysis was performed for CSF tau and Aβ_{42} levels, by calculating and combining the effect sizes (raw mean difference), their standard error, and 95% confidence interval after extracting mean, standard deviation, and sample size from 11 CSF studies. 11 studies out of 60 studies for CSF tau levels and 7 studies out of 41 studies were selected for CSF Aβ_{42} levels in dementias from 1996 to 2009 using keywords CSF biomarkers in Alzheimer's disease, tau levels in Alzheimer's disease, and Aβ_{42} levels in Alzheimer's disease.

2.2. Exclusion Criteria. Studies were excluded if they were not in English language, did not provide data for controls, did not mention the diagnostic criteria for AD, and did not report the SD of the mean CSF tau and Aβ_{42} level or their units were not in pg/mL. Also, studies where tau and Aβ_{42} were measured by methods other than sandwich enzyme linked immunosorbant assay were excluded. Only those studies were undertaken in which CSF tau and Aβ_{42} levels were measured in AD and other dementias (vascular dementia, Parkinson's disease-associated dementia, and Lewy body dementia) in controls using Innotest kit from Innogenetics, Belgium. All the studies included had interday and intraday variation less than 10%. Other-dementias group was named dementia due to other causes (DOC). All the values were expressed in pg/mL.

2.3. Statistical Analysis. The raw mean difference (unstandardized) was used for calculating the effect size of each study. The heterogeneity in effect size of both parameters (tau and Aβ_{42}), which may occur from study to study and is one of the serious issues of any meta-analysis, was explored with the help of three statistical tools: (1) test of significance (Q statistics), (2) between-studies variance (T^2), and (3) degree of heterogeneity (I^2). An appropriate model (fixed effect versus random effect model) for getting the pooled effect size was decided after evaluating the heterogeneity in effect size of all the included studies.

The graphical method (Forest plot) has been applied for studying the variability between the effect sizes of individual studies. In Forest plot, each study effect size and respective confidence interval (CI) were plotted on one set of axis along with pooled estimate of effect size, together with its CI.

All the statistical analysis was done using the Meta-analysis report software (Version: Beta3.13) downloaded from the web site on 05.10.10.

3. Results

As CSF tau and Aβ_{42} levels have emerged as two biomarkers for early diagnosis of dementia, in this paper, meta-analysis of published studies for CSF tau and Aβ_{42} level has been carried out to establish the role of combination of two biomarkers in Alzheimer's disease and dementia due to other causes (DOC).

3.1. Meta-Analysis of CSF Tau Level in Dementia. A meta-analysis was performed in 11 published studies of CSF tau in AD and DOC, selected from total of 60 studies. Out of total published studies included in meta-analysis of CSF tau levels in DOC 5 published studies were of vascular dementia (VaD) [7, 18, 22–24], 2 of Parkinson's disease-associated dementia (PDD) [15, 20], 1 of mixed dementia [16], and the rest were non-AD dementia (non-ADD) [17, 19]. Out of 11 studies, only two studies in AD [15, 23] and one study in DOC [23] were having matched controls.

Table 1 shows that details of 11 included studies along with mean, SD, and sample size (N) of CSF tau level for cases and controls. Effect size (raw mean difference), standard error, 95% confidence interval and weight for each included study were calculated. None of studies show effect size zero in both group (Alzheimer's disease and DOC).

Table 2 shows that median sample size (case + control) in 11 Alzheimer's disease studies of CSF tau level was 67 (range: 30–142) whereas it was 49 (range: 26–134) in dementia-due-to-other-causes studies. The median effect size (raw mean difference) of tau level in Alzheimer's disease studies was 429 pg/mL (range: 32 to 910 pg/mL) whereas in dementia-due-to-other-causes studies it was 69 pg/mL (range: −53 to 518 pg/mL). In Alzheimer's disease, CSF tau level is quite high as compared to DOC.

Table 3 shows the estimated pooled effect size of CSF tau level and its 95% confidence interval (CI) of 11 included studies for both groups. Between-studies variance, degree of heterogeneity with 95% CI, Q statistics, degree of freedom, and P value were also shown in the same tables. From Table 3, it can be seen that between-studies variance, degree of heterogeneity, and Q statistics all three approaches clearly indicate that variability in CSF tau estimates across the studies was too high and random effect model for pooling of CSF tau estimates is the only choice. After applying random effect model for pooling of estimates, the pooled estimate of CSF tau in Alzheimer's disease studies was 414.073 pg/mL (CI: 237.170–590.975) while it was quiet low as 83.500 pg/mL (CI: 36.406–130.595) in dementia-due-to-other-causes studies.

Forest plot (Figure 1(a)) shows that out of 11 Alzheimer's disease studies, effect size (raw mean difference) of 6 studies [13, 15, 18, 20, 22, 23] was more than pooled estimate of effect size, and in 5 studies [16, 17, 19, 21, 24], it was below the pooled effect size, whereas out of 11 dementia-due-to-other-causes studies (Figure 1(b)), 5 studies were having

Table 1: Effect size, its 95% CI, and % weight calculations for each selected study of CSF tau in Alzheimer's disease and dementia due to other causes.

S. no.	Author's name and year	Country where the studies were conducted	Tau level (pg/mL)(AD and control)						Effect size (pg/mL)		95% CI	Weight	Tau level (pg/mL) (DOC and control)						Effect size (pg/mL)		95% CI	Weight
			Diseased			Control							Diseased			Control						
			N	M_d*	SD	N	M_c**	SD	RMD+	SE			N	M_d*	SD	N	M_c**	SD	RMD+	SE		
1	Arai et al. (1995) [15]	Japan	19	919	349	19	9	4.5	910.00	80.07	753.06–1066.94	0.005	7	77.2	45.5	19	9	4.5	68.20	17.23	34.43–101.97	0.215
2	Tapiola et al. (1998) [16]	Finland	81	524	351	33	293	140	231.00	45.99	140.86–321.14	0.015	40	403	248	33	293	140	110.00	46.17	19.51–200.49	0.030
3	Galasko et al. (1998) [17]	USA	82	663	481	60	387	167	276.00	57.33	163.64–388.36	0.010	74	456	383	60	387	167	69.00	49.47	−27.96–165.96	0.026
4	Andreasen et al. (1998) [18]	Sweden	43	796	382	18	190	57	606.00	59.78	488.83–723.17	0.009	21	708	382	18	190	57	518.00	84.44	352.51–683.49	0.009
5	Kanai et al. (1998) [19]	Japan	32	489	297.5	41	217	128	272.00	56.26	161.73–382.27	0.010	33	267	146	41	217	128	50.00	32.34	−13.38–113.38	0.061
6	Andreasen et al. (2001) [7]	Sweden	105	759	417	18	264	102	495.00	47.27	402.36–587.64	0.014	23	461	280	18	264	102	197.00	63.14	73.25–320.75	0.016
7	Sjögren et al. (2002) [20]	Sweden	19	919	349	17	342	116	577.00	84.87	410.67–743.33	0.004	15	306	65	17	342	116	−36.00	32.76	−100.21–28.21	0.059
8	Grossman et al. (2005) [21]	US	17	534.6	303.5	13	260.4	93.8	274.20	78.07	121.18–427.22	0.005	73	207.1	270.3	13	260.4	93.8	−53.30	40.95	−133.58–26.98	0.038
9	de Jong et al. (2006) [22]	The Netherlands	61	613	326	30	184	89	429.00	44.79	341.21–516.79	0.016	50	303	307	30	184	89	119.00	46.36	28.14–209.86	0.030
10	Bibl et al. (2007) [23]	Germany	15	700	480	15	200	130	500.00	128.40	248.34–751.66	0.002	15	340	280	15	200	130	140.00	79.71	−16.22–296.22	0.010
11	Ravaglia et al. (2008) [24]	Italy	31	56.3	32.7	36	24.28	5.9	32.02	5.96	20.35–43.69	0.909	13	63.5	40.3	36	24.28	5.9	39.22	11.22	17.23–61.21	0.506

*Mean in diseased group, **Mean in control group and raw mean difference.

Table 2: Descriptive statistics of CSF tau studies in Alzheimer's disease and dementia due to other causes.

CSF tau level (pg/mL)	Alzheimer's disease		Dementia due to other causes	
	Median	Range	Median	Range
Sample size	67	30–142	49	26–134
Effect size	429	32.020–910	69	−53.300–518

Table 3: Pooled estimate of effect size with 95% CI, test of significance and magnitude of heterogeneity of CSF tau studies in Alzheimer's disease and Dementia due to other causes.

Statistics	Alzheimer's disease	Dementia due to other causes
Pooled estimate of CSF tau (pg/mL)	414.073	83.500
95% CI of pooled estimate (pg/mL)	237.170–590.975	36.406–130.595
Between-studies variance (T^2)	85013.958	4343.165
Degree of heterogeneity (I^2)	97.9%	82.4%
95% CI of I^2	97.2%–98.4%	69.7%–89.7%
Q statistics	467.756	56.738
DF	10	10
P value	0.000	0.000

individual effect size more than pooled estimate of effect size [13, 16, 18, 22, 23], while in 6 studies, individual effect size was below the pooled effect size [15, 17, 19–21, 24].

3.2. Meta-Analysis of CSF Amyloid β_{42} Level in Dementia. A meta-analysis was performed in 7 published studies of CSF tau in AD and DOC, selected from total of 41 studies. Out of total published studies included in meta-analysis of CSF Aβ_{42} levels in DOC 3 published studies were of vascular dementia (VaD) [13, 22, 23], 1 of Parkinson's disease-associated dementia (PDD) [20], 1 of semantic dementia [21], and the rest were non-AD dementia (non-ADD) [17, 19].

Table 4 shows that details of 7 included studies along with mean, SD, and sample size (N) of CSF Aβ_{42} level for cases and controls. Effect size (raw mean difference), standard error, 95% confidence interval, and weight for each included study were calculated. None of studies show effect size zero in both group (Alzheimer's disease and dementia due to other causes).

Table 5 shows that median sample size (case + control) in 7 Alzheimer's disease studies of CSF Aβ_{42} level was 73 (range: 30–142) whereas it was 41 (range: 26–134) in dementia-due-to-other causes studies. The median effect size (raw mean difference) of Aβ_{42} level in Alzheimer's disease studies was −442 pg/mL (range: −652 to −41.200 pg/mL) whereas in dementia-due-to-other causes studies it was −193 pg/mL (range: −356 to −33 pg/mL). In Alzheimer's disease, median CSF Aβ_{42} level is quite low as compared to DOC.

Table 6 shows the pooled effect size of CSF Aβ_{42} level and its 95% confidence interval (CI) of 7 included studies. Between-studies variance, degree of heterogeneity with 95% CI, Q statistics, degree of freedom, and P value were also shown in the same tables. From Table 6, it can be seen that between-studies variance, degree of heterogeneity, and Q statistics all three approaches clearly indicate that variability in CSF Aβ_{42} estimates across the studies was too high and random effect model for pooling of CSF Aβ_{42} estimates is the only choice. After applying random effect model for pooling of estimates, the pooled estimate of CSF Aβ_{42} in Alzheimer's disease studies was −363.926 pg/mL (CI: −542.007–−185.845) while it was quiet high as −170.743 pg/mL (CI: −256.912–−84.574) in DOC studies.

Forest plot (Figure 2(a)) shows that out of 7 Alzheimer's disease studies, effect size (raw mean difference) of 2 studies was more than pooled estimate of effect size [19, 21] and, in 05 studies, it was less than the pooled effect size [13, 17, 20, 22, 23]. Of 7 DOC studies (Figure 2(b)), 02 studies were also having individual effect size more than pooled estimate of effect size [19, 21] while in 05 studies individual effect size was below the pooled effect size [13, 17, 20, 22, 23].

Funnel plots showed clear existence of publication bias in both groups.

4. Discussion

Diagnostic markers for AD have been sought for many years for its early diagnosis and differentiating it from other types of dementias. Distinguishing between the two most common forms of dementias, AD and VaD, is one of the most challenging differential diagnoses in geriatrics OPD and is very crucial also because the therapeutic strategies are very different. With the development of cholinergic-based treatments for AD, there is great emphasis on the need for its early and accurate diagnosis to allow initiation of therapy when it will be of most benefit to the patients [17]. CSF biomarkers tau and Aβ_{42} combined together have emerged as such diagnostic markers for early diagnosis of AD. Although combination of CSF tau and Aβ_{42} yields a highly accurate differentiation between AD and controls, CSF-based differentiation of AD from other dementias especially VaD

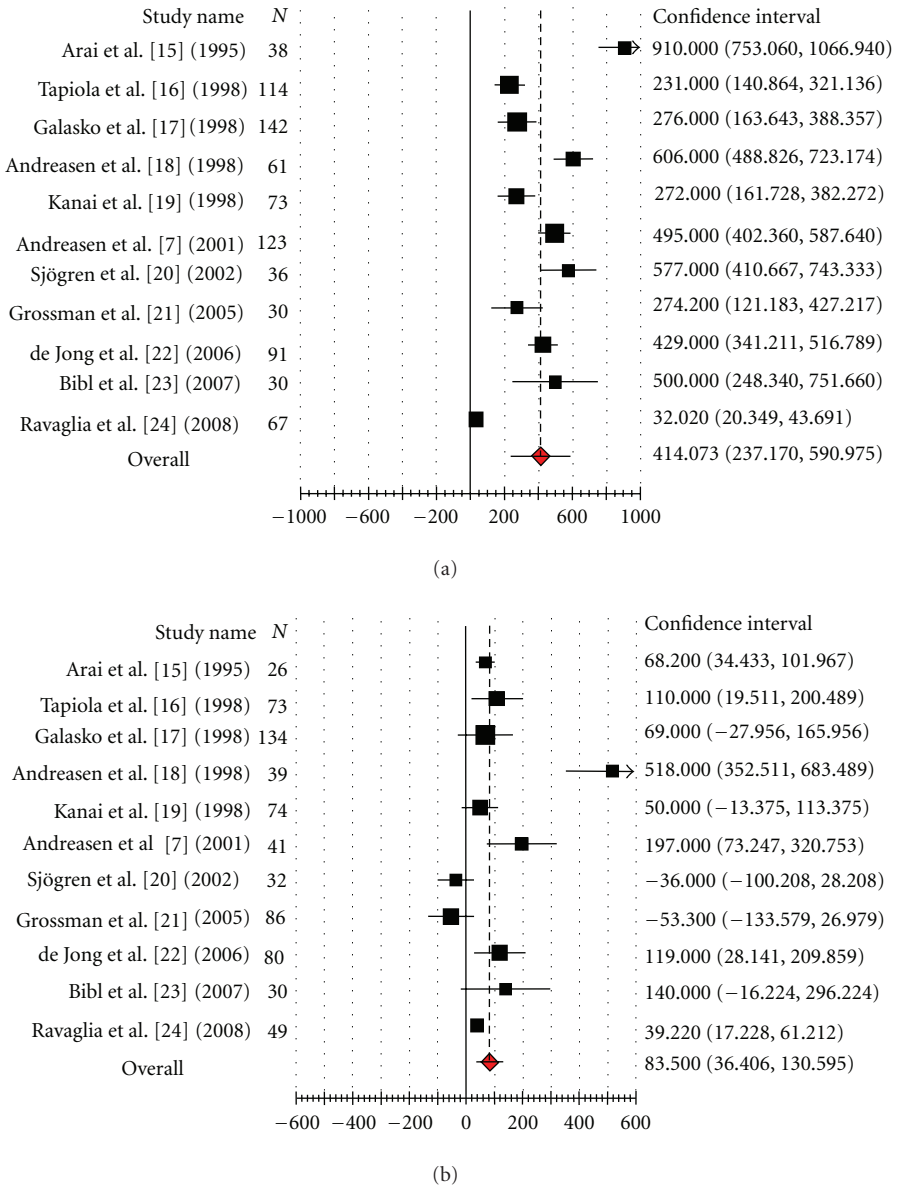

Figure 1: (a) CSF tau level in Alzheimer's disease. (b) CSF tau level in dementia due to other causes.

remains highly challenging as it has low specificity for the same [13].

4.1. Meta-Analysis of CSF Tau Level. A meta-analysis performed in 11 published studies of CSF tau in different types of dementias showed high levels of tau in AD in all the studies whereas 2 studies showed low levels in DOC as compared to controls. A number of studies have found a significant increase in CSF tau levels in AD and DOC as compared with normal ageing [25–27].

Table 2 shows that raw mean difference of CSF tau levels in AD group was much higher than that in DOC group signifying that levels of tau in CSF in AD cases rise much higher than in DOC. This finding supports the studies showing much higher sensitivity and specificity of CSF tau levels in distinguishing the AD patients from control subjects as compared to non-AD dementias [16] and VaD [13].

The assessment of the heterogeneity in meta-analysis is a crucial issue because the presence versus absence of true heterogeneity (between-studies variability) can affect the choice of statistical model. There can be two sources of variability leading to heterogeneity in a set of studies in a meta-analysis. First, variability due to sampling error (within-study variability) is always present in a meta-analysis because every single study uses different samples. The other source of variation is due to heterogeneity among the population effect sizes estimated by the individual studies (between-studies variability). It may be due to variation in the characteristics of the samples, treatment and the design quality of the study [27]. In the present study, three statistical methods were applied to explore the true heterogeneity. They

Table 4: Effect size, its 95% CI, and % weight calculations for each selected study of CSF Aβ_{42} in Alzheimer's disease and dementia due to other causes.

S. no.	Author's name and year	Country where the studies were conducted	Aβ42 level (pg/mL) (AD and control)						Effect size (pg/mL)		95% CI	Weight	Aβ_{42} level (pg/mL) (DOC and control)						Effect size (pg/mL)		95% CI	Weight
			Diseased			Control							Diseased			Control						
			N	M_d*	SD	N	M_c**	SD	RMD+	SE			N	M_d*	SD	N	M_c**	SD	RMD+	SE		
1	Galasko et al. (1998) [17]	USA	82	833	379	60	1485	473	−652.000	74.031	−797.10–−506.90	0.012	74	1129	464	60	1485	473	−356.000	81.475	−515.69–−196.31	0.011
2	Kanai et al. (1998) [19]	Japan	32	109.9	73.2	41	242	180	−132.100	30.947	−192.75–−71.45	0.069	33	209	180	41	242	180	−33.000	42.096	−115.51–−49.51	0.041
3	Andreasen et al. (2001) [7]	Sweden	105	523	180	18	897	242	−374.000	59.684	−490.98–−257.02	0.019	23	704	321	18	897	242	−193.000	87.941	−365.36–−20.64	0.009
4	Sjögren et al. (2002) [20]	Sweden	19	411	99	17	853	161	−442.000	45.173	−530.54–−353.46	0.033	15	680	96	17	853	161	−173.000	46.251	−263.65–−82.35	0.034
5	Grossman et al. (2005) [21]	USA	17	54	15.1	13	95.2	29.7	−41.200	9.015	−58.87–−23.53	0.819	13	44	14.1	13	95.2	29.7	−51.200	9.118	−69.07–−33.33	0.876
6	de Jong et al. (2006) [22]	The Netherlands	61	419	128	30	869	207	−450.000	41.193	−530.74–−369.26	0.039	25	655	220	30	869	207	−214.000	58.003	−327.68–−100.327	0.022
7	Bibl et al. (2007) [23]	Germany	15	310	90	15	810	320	−500.000	85.829	−668.22–−331.78	0.009	15	480	220	15	810	320	−330.000	100.266	−526.52–−133.48	0.007

*Mean in diseased group, **Mean in control group and raw mean difference.

Table 5: Descriptive statistics of CSF Aβ_{42} studies in Alzheimer's disease and dementia due to other causes.

CSF Aβ42 level (pg/mL)	Alzheimer's disease		Dementia due to other causes	
	Median	Range	Median	Range
Sample size	73	30–142	41	26–134
Effect size	−442	−652–−41.200	−193	−356–−33

Table 6: Pooled estimate of effect size with 95% CI, test of significance, and magnitude of heterogeneity of CSF Aβ_{42} studies in Alzheimer's disease and dementia due to other causes.

Statistics	Alzheimer's disease	Dementia due to other causes
Pooled estimate of CSF Aβ_{42} (pg/mL)	−363.926	−170.743
95% CI of pooled estimate (pg/mL)	−542.007–−185.845	−256.912–−84.574
Between-studies variance (T^2)	54859.742	9755.39
Degree of heterogeneity (I^2)	97.8%	83.7%
95% CI of I^2	96.8%–98.5%	69.7%–91.7%
Q statistics	271.051	36.780
DF	6	6
P value	0.000	0.000

strongly recommend the presence of heterogeneity in a set of 11 tau studies (Table 3). The test of significance ($P = 0.000$) suggests the presence of true heterogeneity among the effect size of all 11 studies. The magnitude of the true heterogeneity due to between studies variance ($T^2 = 85013.958$ for AD versus controls, & 4343.165 for DOC versus controls) was much higher and contributed to 97.9% in AD versus control and 82.4% in DOC versus control (degree of heterogeneity I^2) in total heterogeneity. All the studies selected, fulfilling the defined inclusion criteria, report a significant difference in CSF tau levels in AD and DOC participants versus controls. Despite the uniform pattern of CSF tau level changes in AD and DOC as compared to control, there was wide range of effect size among 11 articles under study in AD group (32.02–910.00 pg/mL) as compared to DOC group (−53.30–−518.00 pg/mL). There were large differences in baseline levels across studies; 6 studies' in AD versus control [13, 15, 18, 20, 22, 23] and 05 studies in DOC versus control have effect size more than pooled estimate [13, 16, 18, 22, 23], signify that dementia patients having higher CSF tau levels than control. However, the increase in CSF tau levels in AD was much higher than in DOC as compared to control in most studies. Only Ravaglia et al. 2008 [24] found low effect size (raw mean difference) of tau levels in DOC as compared to AD. A number of confounding factors are responsible for the heterogeneity and wide range of CI of effect size found in the studies undergoing meta-analysis. First, very few studies could be included for meta-analysis as per the inclusion criteria in the present study; second, except one study [17] all of the studies had very low sample size for DOC group. Also most of the studies were not age-, sex-matched, and of 11 AD studies, 7 studies were having more number of cases than controls [13, 16–18, 20–22] while in 4 studies the number of control was either equal or more than the number of cases [15, 19, 23, 24]. Similarly, in DOC group, 6 studies were having more number of cases than controls [13, 16–18, 21, 22]. There are inconsistent findings reported showing discrepancies in the effect of these on CSF tau levels in AD versus controls. Some investigators have reported increase of tau with age [15, 19] and a negative correlation with sex [17] with a tendency for females to have higher tau levels than males whereas others found tau levels unaffected by age, age at onset, and AD duration, severity, and rate of progression and equally increased in early and late disease, in mild and severe disease [17, 19, 21]. Another confounding factor is variation in freezer shelf life introducing variation in storage conditions for CSF.

4.2. Meta-Analysis of Amyloid β_{42} Level. All the published studies undertaken in the present meta-analysis showed low CSF Aβ_{42} levels in AD and DOC as compared to controls. However, one study effect size had wider confidence interval with upper limit more than zero in both AD and DOC [21]. Table 5 shows that median effect size of Aβ_{42} levels in CSF in AD is −442 pg/mL as compared to −193 pg/mL in DOC which indicates that decrease in CSF Aβ_{42} in AD is much higher than in DOC group as compared to controls. It is supported by the studies done in last two decades reporting low CSF Aβ_{42} levels in early stages of AD with high degree of sensitivity and specificity [25] as compared to other dementias-LBD [13] and VaD [13].

Out of 7 studies undertaken, 5 studies in AD versus control group had more number of cases than controls [13, 17, 20–22] while the other 2 studies had either equal number of controls or less than cases [19, 23], whereas in DOC versus control group, only 2 studies had more number of cases than controls [13, 17] and the other 5 studies had either more or equal number of controls than cases [19–23].

Same three statistical techniques as have been applied in CSF tau studies (mentioned above) were used to determine the true heterogeneity (between-studies variability) in effect size of 7 included studies (Table 6). The test of significance ($P = 0.000$) rejects the null hypothesis of similar effect

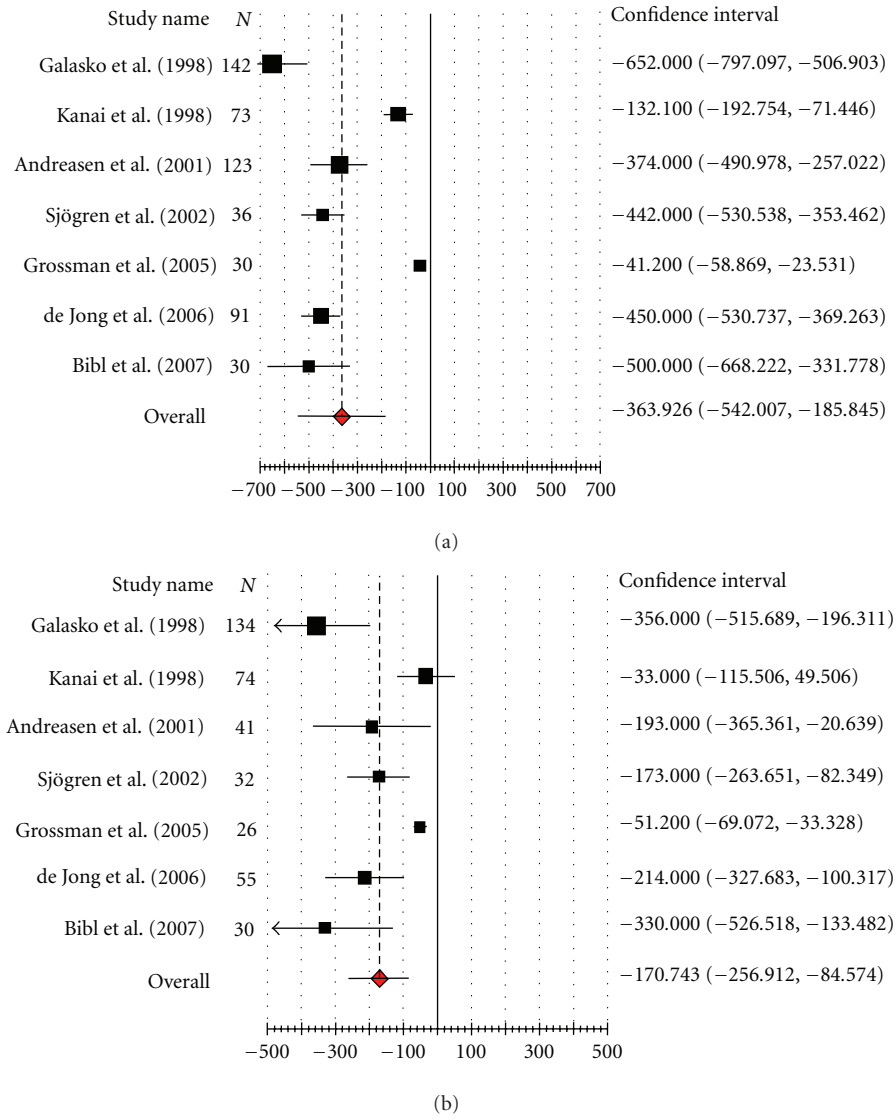

Figure 2: (a) CSF Amyloid β(1–42) level in Alzheimer's disease. (b) CSF amyloid β(1–42) level in dementia due to other causes.

size in all the studies, and the estimated between-studies variance (between studies variance $T^2 = 54859.742$ for AD versus controls and 9755.039 for DOC versus controls) was very much high and between-studies variance contributes the most in total heterogeneity (degree of heterogeneity $I^2 = 97.8$, CI: 96.8%–98.5% in AD and degree of heterogeneity $I^2 = 83.7$, CI: 67.9%–91.7% in DOC). The heterogeneity in effect size of these 07 published studies could be due to different population, small sample size and lack of standardization of assay methods [26]. Though in AD group all 7 studies showed significant reductions in CSF Aβ_{42} levels as compared to control participants, the result of the present study was unequivocal and the range of effect size of 7 studies was quite large (−652 to −41.2 pg/mL). However, the fall in DOC group was much lower than in AD when compared to the control with reduced range of effect size of 7 studies undertaken (−33 to −356 pg/mL). Due to wide range of effect size of these studies, 2 studies effect size was more [19, 21] and that of 5 studies was less than the pooled estimate [17, 20, 22, 23] whereas 1 study [13] was on the vertical line passing through the pooled effect size. Similarly, in DOC group, 2 studies effect size was more [19, 21] and that of 5 studies was less than the pooled estimate [13, 17, 22, 23] whereas 1 study [20] was on the vertical line passing through the pooled effect size.

It has been a challenging task to explain the wide variation in results in the different laboratories due to technical problems in the studies included for studying the diagnostic utility of CSF biomarkers tau and Aβ_{42} in dementia. To overcome this, sensitivity analysis was conducted to explore the sensitivity level of each study. It was observed that there was not much difference in overall effect size on exclusion of one study at a time. Another major problem with this meta-analysis study is the presence of "publication bias." Several lines of evidence show [27] that studies that report relatively high effect sizes are more likely to be published than

studies that report lower effect sizes. In the present study, Funnel plot analysis was done which showed the presence of publication bias for both parameters in AD as well as DOC group supporting the hypothesis that any bias in the literature is likely to be reflected in the meta-analysis study as only statistically significant results are more likely to be published.

4.3. Limitation. During the present meta-analysis, a number of factors was observed which could be causing the limitation in developing hypothesis/coming to conclusion regarding using CSF tau and Aβ_{42} as diagnostic markers in differential diagnosis of AD from dementias due to other causes. One limitation encountered during the present study was simply the amount of effort and expertise meta-analysis takes. Also, lack of complete information in some studies and high level of heterogeneity in individual study effect size for both parameters (tau & Aβ_{42}) added to the limitation. Variation in CSF tau and Aβ_{42} levels in AD, DOC, and controls may be due to the variability introduced during sample collection and storage like using glass or polystyrene tubes[18] than polypropylene tubes, prolonged storage of CSF samples in frozen state or repeated freezing and thawing of CSF, errors introduced due to inadequate study plan like small sample size, less number of controls taken in the study as compared to cases, or case and controls being not age and sex matched [24].

5. Conclusion

Based on our findings of the present meta-analysis, it can be concluded that the combination of high tau and low Aβ_{42} is highly specific for AD and might be useful in screening out the suspected cases of AD, from other types of dementia. However, due to the limited number of studies having large number of sample size with age- and sex-matched samples in control and cases available, the use of these biomarkers has to be verified in further prospective studies.

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Review Article

Potential Peripheral Biomarkers for the Diagnosis of Alzheimer's Disease

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Advances in the discovery of a peripheral biomarker for the diagnosis of Alzheimer's would provide a way to better detect the onset of this debilitating disease in a manner that is both noninvasive and universally available. This paper examines the current approaches that are being used to discover potential biomarker candidates available in the periphery. The search for a peripheral biomarker that could be utilized diagnostically has resulted in an extensive amount of studies that employ several biological approaches, including the assessment of tissues, genomics, proteomics, epigenetics, and metabolomics. Although a definitive biomarker has yet to be confirmed, advances in the understanding of the mechanisms of the disease and major susceptibility factors have been uncovered and reveal promising possibilities for the future discovery of a useful biomarker.

1. Introduction

The incidence of Alzheimer's disease (AD), the most prevalent form of dementia seen in the elderly population, is expected to increase exponentially over the next ten years. With the pervasive nature of such a debilitating disease, extensive research has focused on potential peripheral biomarkers and how they could be utilized to diagnose and monitor the progress of Alzheimer's disease. There are two types of biomarkers: state markers and stage markers. A state marker denotes the severity of the disease in the individual. As the amount of a certain state marker increases, the severity of the disease in the individual increases. Stage markers indicate how far the disease has progressed within the individual. If an individual has a certain degenerative disease, then a state and stage marker will be present [1]. According to the Consensus Report of the Working Group on Molecularand Biochemical Markers of Alzheimer Disease, a biomarker must adhere to certain basic requirements, including the ability to reflect AD pathology and differentiate it from other dementia with an 80% sensitivity, be reliable and reproducible, be easy to perform and analyze, and remain relatively inexpensive [2]. Currently, the most effective methods for ascertaining the diagnosis of AD is limited to imaging technology (e.g., MRI and PET) and the analysis of cerebrospinal fluid (CSF), which requires lumbar puncture [3]. Ideally, both technologies have good specificity and sensitivity but have limitations of expense and risks associated with invasive procedures.

The discovery of a well-established peripheral biomarker that is easily accessible and cost effective is of primary importance when considering the prevalence of this disease. The necessity for a biomarker for AD in blood is so high because of the disadvantages of the status quo. The above consideration suggests the need for a diagnostic biomarker able to detect disease prior to symptomatic onset. In order to achieve this goal, such a diagnostic biomarker needs to be one that could be part of a routine physical exam, as is currently the case for lipid profile. This implies a biomarker that could be obtained in any physician's office or specimen collecting station with minimal invasiveness at reasonable cost and time demand. In order to be adopted for wide use, the biomarker has to have demonstrated consistency in a large number of persons from a variety of populations. Current imaging and CSF biomarker studies satisfy the criteria of having been established in a large number of persons and in a variety of populations. These studies have served to establish the important principle of feasibility of early detection. However, these classes of biomarker do not satisfy the criteria of minimal invasiveness, reasonable

cost, or minimal time demand. These considerations lead to a strong recommendation of the need for an effective biomarker of AD. The criteria of minimal invasiveness, minimal time by the physician, and reasonable cost argue for an easily obtained sample of peripheral tissue that could then be analyzed either in the office or at a central location.

Diagnosis of AD is made mainly from clinical testing, and currently, there is no completely accurate test for diagnosing AD. Obtaining CSF from elderly individuals on repeated occasions is no easy task. Blood is very easy to obtain, and since CSF is absorbed into the blood every day, plasma can supply numerous biomarkers for AD 4. Therefore, finding a peripheral biomarker that uses easily collected samples (e.g., plasma, blood, saliva, and urine) would be doubly advantageous because of its relatively noninvasive procedure and ability to provide an accurate diagnosis. The search to find a peripheral biomarker that could serve as a definitive diagnostic tool that is universally available has created a vast body of studies that spans over several different biological approaches. In this paper, we will examine the current status of potential peripheral biomarkers of Alzheimer's disease, evaluating each from the perspective of being minimally invasive, easily obtainable with a minimal time requirement and reasonable cost. We will further evaluate each biomarker from the perspectives of the ability to detect already diagnosed disease and ability to predict future disease.

2. Evaluable Tissues

The widespread incidence of Alzheimer's has seemingly no pattern of "onset." Because up to 98% of Alzheimer cases are sporadic, it is crucial to identify potential biomarkers from assessable tissues that could diagnose AD on an individual basis [4]. The utility of such an approach is the availability of bodily samples that could provide a noninvasive and rather inexpensive process for diagnostic determination, such as a swab of saliva, a simple blood sample, or a urine test.

The utilization of saliva as a biological marker of AD has been examined and led to the possibility of its utility in diagnosing early onset forms of the disease and differentiating AD type dementia from other forms of neurodegenerative illnesses. Biopsies of the salivary gland can produce significant findings for Alzheimer's, since salivary epithelial cells express amyloid precursor protein and Aβ. Also, it is important to note that changes in the cerebrospinal fluid may perhaps be reflected in the saliva [5]. One study compared a group of individuals with AD to a group of controls matched for age and sex as well as individuals with Parkinson's disease. The findings uncovered that there was a small, but still statistically relevant, increase of Aβ42 in patients with mild AD [5]. It should be noted that there was no noticeable change in the Aβ42 levels of either the controls of Parkinson's patients, which would indicate that the salivary levels of Aβ42 could be used to distinguish AD from other forms of dementia. Studies also suggest there is a connection between the salivary acetylcholinesterase enzyme (AChE) and AD, since it is already established that a decrease in central cholinergic activity is a noteworthy aspect of the disease biochemistry [6]. During early stages, the cholinergic neurons primarily undergo degeneration and result in a notable decrease in acetylcholine. One study revealed that in patients with AD, AChE activity was appreciably lower than in their age-matched counterparts, suggesting that salivary levels of cholinergic activity could be a biomarker [7]. The changes in salivary AChE activity appear to parallel the AD-associated decrease in brain cholinergic activity [7]. In a study done by Sayer et al., subjects receiving treatment with AChE inhibitors were classified based on whether they responded cognitively to the AChE treatment. They found a significant difference in AChE levels between those who did not respond to the treatment and their controls [7]. While these studies demonstrate the possible fronts through which a useful biomarker can be found, there have yet to be conclusive results verifying the diagnostic value of acetylcholinesterase levels and whether peripheral salivary markers truly reflect changes in cerebrospinal fluid.

The use of blood as a tissue to yield potential biomarkers of AD has both its advantages as well as challenges. The most prominent challenge to determining the accuracy of a blood biomarker is in establishing a correlation between brain changes and dectecting those changes in blood [8]. Despite the difficulties presented by a blood-brain barrier, the possibility of a blood protein signature and notable alterations in blood-based proteins may present biomarkers that could be used to predict and monitor disease progression. Along with the ease of accessibility of blood, there is the additional advantage of the multiple tissues present in blood, namely, plasma, serum, and its cellular components (e.g., reds cells, white cells, and platelets).

The use of plasma-based proteins offers some promising risk analysis tools. Plasma is the liquid portion of blood that suspends cells such as erythrocytes, leukocytes, and thrombocytes and proves to be an ideal fluid for biomarker inquiry due to its universal availability. Plasma can be isolated from blood by using an anticoagulant and centrifuging the sample at low speeds. It contains thousands of proteins that reflect the physiological occurrences in the body and affect the brain from the periphery as well as those proteins that are exported from the brain. Several studies have been produced to demonstrate that telomere length in peripheral blood cells are a potential marker for AD, but the relationship between peripheral blood leukocyte telomere length and the proliferation of AD pathogenesis remains unclear. In one study, the telomere length of peripheral blood leukocytes was compared to the telomere length in the cerebellum [9]. Telomere length in the cerebellum was not indicative of inherited telomere length as a determinant of AD susceptibility; rather, acquired shortening of peripheral blood leukocyte telomere lengths could be seen as an indication of chronic stress, supporting an underlying correlation between leukocyte telomere length and risk for developing AD.

Urine samples can be considered as a means of diagnosing AD through noninvasive procedures. Evaluating the proteins in urine for AD could help physicians inform patients of their prognosis. Unfortunately, researchers have attempted to develop an AD biomarker sensitive enough to be used on urine, but no such reliable and reproducible biomarker has been found to date. Early evidence showed

increased concentrations of NTP in the urine of AD patients, generating immense interest among other fellow biomarker researchers. However, attempts to commercialize the test were unsuccessful, because the validity of the test was questioned because of lack of reproducibility. Another protein called the pancreatic exocrine protein, also known as pancreatic thread protein (PTP), contained a fibrillary structure that resembled fibrils located in neuritic plaques in the brains of AD patients [10]. These researchers observed extensive amounts of PTP immunoreactivity in the brains of AD patients. The study found a substantial concentration of NTP in the CSF of AD patients compared to their controls [10]. As researchers tried to reproduce the findings of this study, they found that PTP was 40 times higher in serum than in CSF. Furthermore, PTP immunoreactivity in CSF paralleled with the CSF/serum albumin ratio, which suggests that NTP in CSF is actually PTP from serum [10].

In particular, the AD-associated neuronal thread protein (AD7c-NTP) has been of interest as a biomarker due to its ability to reflect significant irregularities in cellular function [9]. Dementia of the AD type is symptomatic of cell loss that is caused by multiple mechanisms that involve apoptosis and abnormal mitochondrial function. The AD7c-NTP gene codes for a protein associated with causing apoptosis and therefore, the overexpression of the gene could lead to the cell loss seen in the early stages of the disease. Higher levels of AD7c-NTP can be seen in the urine of patients experiencing early AD and can even offer insight onto the severity of the dementia. In clinical uses, AD7c-NTP has been shown to be a very useful biomarker with more than 90% sensitivity for the early detection of AD [11].

To the contrary, there is much speculation about the true utility of the NTP and AD7c-NTP. The nucleotide sequence of AD7c-NTP does not share any resemblance with a pancreatic thread protein, insinuating that these two genes code for completely different proteins. Additionally, when the DNA sequence of AD7c-NTP was compared to chimpanzee and human genomes, various amounts of differences were identified. Coincidentally, these discrepancies were discovered in places where the human and chimpanzee genome were completely identical [10].

3. Genomics

The use of genomic technologies is valuable in identifying potential biomarkers in several neurological diseases, including Alzheimer's, and promises to provide important insight for the future in terms of personalized diagnosis and treatment based on an individuals' predisposition to a particular condition. As the genetic analysis of individuals uncovers heritable risk factors, genomic technologies will lead to a better understanding of the protein products and mechanistic pathways associated with the proliferation of the disease.

One of the ways to finding potentially useful diagnostic biomarkers is through genomics and the human genome, which allows us to better understand disease and the manner in which it proliferates. Mendelian genetic approaches have limited utility in identifying neurodegenerative afflictions like AD, because familial cases account for a rather small amount of those afflicted. It is more important to discover biomarkers that can help explain the more common sporadic cases of the disease. Among the inheritable aspects of Alzheimer's, apolipoprotein $\varepsilon 4$ has been established to have a particularly strong correlation to the development of the disease. While single allele differences are rarely able to confer an accurate indication for risk of disease development, Alzheimer's disease could be an exception. Entire genome single-nucleotide polymorphism studies have been conducted, and they confirm that the ApoE locus is able to indicate, to a certain extent, the genetic susceptibility to developing AD [12, 13]. The usefulness of genome-wide association studies primarily lies in their ability to reveal susceptibility genes of a disease by uncovering DNA variants in a large-scale analysis of the human genome. Based on replication in a large number of studies, the only firmly established genetic susceptibility factor for Alzheimer disease is the $\varepsilon 4$ allele of ApoE [14]. While genome-wide association studies have proven to be useful in detecting variations in DNA that can potentially be linked to the heritability of this disease, they cannot provide the biological basis of the disease.

ApoE is a lipid transport protein that is encoded in a single gene and exists as three different isoforms. Based on the traditional principles of inheritance, the dosage of the ApoE alleles, divided into $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$, is strongly related to the risk for dementia of the Alzheimer's type at an increasing frequency with the increase of $\varepsilon 4$ alleles [15]. Although the Apo E $\varepsilon 4$ allele accounts for only 14% of the general population, approximately 50% of Alzheimer's patients carried the $\varepsilon 4$ allele [16]. Both Carriers and noncarriers of the $\varepsilon 4$ allele develop Alzheimer's, evidence suggests that those without the allele are more likely to show signs of the disease later [17]. As a key component of very low-density lipoproteins that is required for cholesterol transport both centrally and in the periphery, the role of ApoE $\varepsilon 4$ in AD has been related to its effect on the metabolism of cholesterol [18]. Studies of ApoE polymorphisms demonstrate that the $\varepsilon 4$ allele correlates with an elevation in total and low-density cholesterol levels and could, therefore, play a negative role in AD [19]. Conversely, it is suggested that the ApoE $\varepsilon 2$ plays a protective role in the development of AD, lowering risk, and delaying the onset of the disease [20]. The protective ability of $\varepsilon 2$ can be attributed to the opposite effect on cholesterol metabolism it has compared to the $\varepsilon 4$ allele, namely lower levels of total and low-density lipoprotein cholesterol [21].

The different isoforms are also hypothesized to have varying effects on amyloid plaque formation and metabolism. The $\varepsilon 3$ isoform can have an increased binding affinity to Aβ peptides, thereby allowing for the clearance of Aβ and prevention of neurotoxic plaque formation [22]. It is further hypothesized that the neurotoxicity of the Aβ peptides further contributes to neurodegeneration in the $\varepsilon 4$ isoform. While ApoE $\varepsilon 3$ is capable of protecting cells from H_2O_2-induced oxidative stress, the $\varepsilon 4$ isoform is not as successful at this task [23]. There are several mechanistic explanations for the role of ApoE isoforms in relation to Aβ metabolism and

the formation of AD plaques, but a conclusive role requires further studies to be firmly established.

Mechanistically, ApoE4 may be responsible for accelerating the degeneration of neurons, and thereby damaging synaptic stability and causing an earlier onset of dementia. One of the key contributing factors of ApoE on the development of neurological diseases like AD is linked to the manner in which it contributes to neuronal repair, remodeling, and protection. Deleterious insults to neurons could be a result of oxidative stress, ischemia, inflammation, or other stressors associated with aging. ApoE is a contributing factor to the repair of neurons through its lipid transport function. While ApoE $\varepsilon 2$ and $\varepsilon 3$ are effective in this process of neuronal cell maintenance, $\varepsilon 4$ has been observed to be less efficient in this role [24]. This further exacerbates the cognitive decline seen in AD by affecting neuronal connections. Although ApoE isoforms have a definite and striking effect on the clearance of amyloid-β and cytoskeleton stability, there are no unified explanations for the manner in which ApoE4 specifically causes a notably increased risk for AD.

Among the other biomarker candidates that have been uncovered using genomic techniques, growth factor receptor-bound associated binding protein 2 (GAB2) alleles have been shown to have an impact on AD risk for ApoE4 carriers. The GAB2 protein is involved in several important signaling pathways that could be linked to disease proliferation if interference with the expression of GAB2 were to occur, as evidenced by the elevated levels of GAB2 in at-risk neurons and GAB2 proteins found in neurofibrillary tangles [25]. One study examined the possibility of GAB2 as a modifying factor for Alzheimer's risk in ApoE4 carriers and determined a significant correlation between multiple single-nucleotide polymorphisms (SNPs) and an increased risk of disease for $\varepsilon 4$ allele carriers. The study utilized a genome-wide analysis of 502,627 SNPs to characterize and determine susceptibility genes for the onset of AD. Through their surveys of these single-nucleotide polymorphisms, the researchers determined an association of AD with six single-nucleotide polymorphisms found in the GAB2 gene as well as a shared haplotype that encompassed the GAB2 gene. Additionally, it was determined that interference with the expression of normal GAB2 led to an increase in tau phosphorylation, which is typically observed in individuals with AD. Another study further investigated the hypothesis that normally GAB2 protein is associated with reducing tau phosphorylation and the formation of neurofibrillary tangles and that an isoform of the protein could play a part in increasing the susceptibility to phosphorylated tau in at-risk individuals [26]. Further replication of both studies is required, but it does provide important insight into the possibilities to better understand the pathogenesis of this disease that could later contribute to diagnosis and treatment.

Another valuable genomics marker the ApoE gene indicated that a set of single-nucleotide polymorphisms in TOMM40, which is located approximately 15 Kb upstream of ApoE, revealed a linkage disequilibrium in connection with the E4 allele and demonstrated a significant association to increased AD risk [27]. This particular gene is responsible for the formation of an essential mitochondrial membrane protein that plays an active role in protein transport. Since aberrations in mitochondrial structure or causes for oxidative stress to the mitochondria are linked to an increase of AD risk, it would be plausible to propose the TOMM40 as a genetic indicator of risk [28]. The important genetic finding associated with TOMM40 predicts the onset of AD based on the variable length of deoxythymidine homopolymer (poly-T) on the gene. Due to the high linkage disequilibrium between TOMM40 and ApoE that demonstrates an evolutionary relationship between the two genes, it is possible to note that specific variants of TOMM40 are closely associated with each of the ApoE alleles [29]. The very long poly-T variants are categorized as high-risk alleles and are linked to ApoE $\varepsilon 4$ alleles 98% of the time, whereas the ApoE $\varepsilon 3$ variants are subdivided into either very long or very short. While the $\varepsilon 3$ allele was supposedly neutral for AD development, it is more likely the heterogeneity of AD age-of-onset seen in the $\varepsilon 3$ population was a result of a linkage of the allele to both very long or very short poly-T variants [28]. The variable length of TOMM40 is, therefore, a candidate for helping predict the onset of AD and has the potential to be a clinical diagnostic tool. Another significant finding seen through a genome-wide association study was that overlapping or linked single-nucleotide polymorphisms across the TOMM40 and ApoE region showed a significant association with cases of sporadic AD [30]. These finding warrant further investigation to determine the utility of the TOMM40 region as a biomarker of AD.

The results finding both GAB2 and TOMM40 to be possible genetic indicators of AD require further study and confirmation; however, the prospects of genotypic analysis using single-nucleotide polymorphisms at multiple gene loci provides exciting possibilities for the determination of diagnostic risk analysis for this and other diseases.

4. Proteomics and Proteins

Another approach towards uncovering a potential diagnostic biomarker for AD relies on a large-scale analysis of proteins and protein structure that could be used to indicate risk of cognitive decline. Proteomics involves two main steps: (1) separate the proteins using multiplex assays and (2) identify the protein and its origin. The search for novel biomarkers that can be used diagnostically at early stages of AD have looked towards proteomic technologies to determine if there are proteins that can predict disease as well as monitor progression and response to treatments. Many proteomic studies have been coordinated in order to accurately diagnose AD but none have emerged as the definitive method or cluster of identifiable proteins despite early encouraging results [31].

Recent proteomic analysis has found that Alzheimer's patients have a dysfunctional ubiquitin carboxyl-terminal hydrolase system. The main purpose of this system, which contains ubiquitin proteasome, is to destroy misfolded proteins. Ubiquitin proteasome is a protein that safeguards other proteins from unwanted interaction between proteins. In AD patients, disfigured proteins overwhelm the ubiquitin system

and leads to the amassing of many abnormal proteins in the system. Recent studies claim that the ubiquitin proteosome is a target of protein oxidation in AD patients, creating a connection between oxidative stress and Alzheimer's in patients [32].

Through studies of AD patients, researchers were able to find high levels (up to 10x the normal amount) of Glial fibrillary acidic protein (GFAP) in AD patients. Astrocyte cells of the CNS and has many important functions, including cell communication and mitosis. The increased levels of GFAP in AD brains (?) means that the pathway is overcompensating for its lack of influence [32].

Among the more prominently studied biomarkers for AD risk are plasma levels of Aβ42 and Aβ40, but they have not emerged to have a definite value as a predictive tool [33]. The analysis of plasma Aβ40 and 42 levels offers a noninvasive and inexpensive biomarker, since a key pathological characteristic of Alzheimer's is Aβ deposition in senile plaques. Research has indicated that increased levels of tau and phospho-tau and decreased levels of Aβ42 can accurately indicate individuals with AD in CSF [1]. If proven to be a reliable indicator of mild cognitive impairment and AD, plasma levels of Aβ40 and Aβ42 and the ratio of Aβ42/Aβ40 could be a valuable biomarker. Mutations of the amyloid precursor protein (APP), which produces Amyloid β protein,can result in an increase of Aβ42 and Aβ40 in patients prior to the onset of the disease [16]. In all AD patients, regardless of APP mutation, Aβ is found to collect and form deposits in the brain that lead to the creation of senile plaques. Since its role has been extensively studied and is intrinsically linked with AD, there have been therapeutic efforts to interfere with the production of Aβ and disband accumulated amyloid deposits. Due to the different cell types that are capable of producing Aβ, it is hard to establish which cells are most actively contributing to circulating plasma or the pathways of interchange of amyloid between the brain and the periphery [4]. While patients with AD certainly had increased Aβ plasma levels in the brain and skeletal muscles, it is not yet possible to consider Aβ a biomarker, since the pathways of the protein and its dispersion and uptake have yet to be fully understood.

There have been several noteworthy attempts to validate plasma Aβ as a biomarker of AD, but questions as to their reproducibility hinder verification of their ability to accurately diagnose disease. Autopsy confirmed reports illustrate the prevalence of Aβ42 deposits in AD patients as either having it be the only form of amyloid β protein deposited, being the major form, or simply having large levels of both Aβ42 and 40 deposited. One study indicated that while patients who have an elevated Aβ42 levels in the plasma are more at risk for developing AD, after the onset of the disease Aβ plasma levels actually decline to possibly reflect the compartmentalization of Aβ peptides in the brain [34]. Another recent longitudinal study demonstrated that low plasma levels of Aβ40 and 42 had a correlation to a rapid decline in cognition. The hypothesis to support this observation was that an increased deposition of Aβ in the brain would be reflected in a lower Aβ plasma level [35]. While these studies help explain and predict the rapid cognitive decline that is seen in the disease, it does not really elucidate the manner in which plasma levels of Aβ can affect AD risk and development that would indicate its use as a biomarker.

A case-cohort study determined that a combination of a high base-line level of Aβ40 and low base-line concentrations of Aβ42 seemed to correlate with a higher risk of developing dementia [36]. Another study identified no individual correlation between either baseline Aβ40 or Aβ42 with a transition from mild cognitive impairment to AD, but the ratio of Aβ42/Aβ40 did demonstrate a relationship with conversion to AD [33]. The longitudinal study demonstrated that a lower Aβ42/Aβ40 ratio was indicative of a greater decline in patient cognition. Unfortunately, conflicting results and the lack of reproducibility in study findings have made it difficult to determine the exact role of Aβ in the determination of AD diagnosis.

While amyloid β protein has undergone extensive study in its association with AD risk, there are several other proteins in the plasma that have been analyzed as potential biomarkers of the disease. Among the more prominently CSF-based proteins studied in association with AD is tau because of the hyperphosphorylation and aggregation of tau protein that is characteristic of the disease. Tau is a state marker that is located in neuronal axons. Because tau is a state marker, increased concentrations of tau in individuals typically means a higher severity of neuronal degeneration [1]. An increase in tau in AD patients has been discovered in many different studies. However, other dementias, such as vascular dementia, can lead to an increase in tau as well. For these reasons, tau cannot be the sole biomarker for AD, because an increase in tau points to a number of different diseases [1]. There have yet to be any significant studies investigating a blood-based analysis of this particular protein and its potential as a peripheral biomarker, but other plasma proteins have been revealed to be of interest. Studies using the proteomics approach to biomarker identification have yielded plasma proteins that demonstrate a noteworthy alteration in levels in AD patients when compared with controls. One study uncovered that alpha-2-macroglobulin and complement factor H, which are both evident in senile plaques, are present in elevated levels in AD plasma, with the latter only evident in increased levels for AD and not other types of dementia [37].

Phosphorylated tau protein in CSF is a new advancement in the search for AD biomarkers. The concentration of phosphorylated tau directly correlates with the state of tau in the brain. Unlike tau, concentrations of phosphorylated tau does not increase after a stroke or any other diseases, making phosphorylated tau a useful biomarker for AD. Phosphorylated tau protein in CSF has been seen to have high specificity, relative to tau protein for AD. Furthermore, other diseases, such as Parkinson's and depression, have normal concentrations of tau in individuals. Therefore, the specificity of phosporylated protein will prove useful since it will be able to distinguish AD from other types of dementias [1].

In a study focusing on CSF biomarkers and incipient AD, the researchers concluded that combining tau and Aβ42

as a biomarker had an 83% specificity for detection of AD, while combining tau and phosphorylated tau as a biomarker yielded in a slightly higher specificity. Furthermore, T-tau, (what is this? Total tau), P-tau (what is this? Phosph tau), and Aβ42 have been proven to be strong markers for the development of AD in patients with mild cognitive impairment (MCI). If proven by other studies as well, this result could have an enormous impact on the design of clinical trials of patients with MCI. As convincing as these results may seem, more studies are still required to discover which combination of potential biomarkers generates the highest specificity. To increase specificity, a longer follow-up time (preferably more than five years) is required, because some cases in this study could have developed AD after the study had completed. Another useful technique in identifying incipient AD is through neuroimaging methods and cognitive tests. The downfall to these method is that each is correlated with disease severity. Trying to detect incipient AD during the earlier stages would increase the overlap between patients who actually have incipient AD and patients who have other illnesses [38].

Another potential biomarker that is present in elevated levels in AD patients is alpha-1-antitrypsin (A1AT), which can also be found in senile plaques and neurofibrillary tangles. A1AT is a serine protease inhibitor that is responsible for restraining overexpressed proteases during inflammation. Therefore, when it is oxidized to its precursor form and unable to perform this task, there is the characteristic inflammation seen in AD pathology [39]. Another protein associated with the systemic inflammation observed in the AD patients is the elevated presence of alpha-1-antichymotrypsin (A1ACT), which is also a serine protease inhibitor. Increases in A1ACT levels have been shown to have a correlative relationship with the severity of pathology and have also been known to induce hyperphosphorylation of tau in neurons [40].

The search for individual plasma biomarkers has yet to yield a definitive candidate for the diagnosis of AD. However, there is the possibility of using multiple protein markers concurrently to identify the risk for disease. A recent pilot study demonstrated the potential of 18 different signaling proteins found in the plasma that could be used as a diagnostic tool. The study observed the alterations in 18 signaling proteins that could indicate changes in the periphery or central nervous system that are closely linked to Alzheimer's disease [41]. When a study was conducted to reproduce AD diagnosis using the 18 analyte panel, the attempt was unable to produce similar results. The study was able to indicate that a full 89-analyte panel might be useful in diagnosis when used concurrently with other predictive markers such as Aβ [42].

The utility of these and other plasma proteins as diagnostic biomarkers is evident through their accessibility and ability to indicate several pathological processes that are characteristically seen in AD. The differences in levels of plasma proteins between AD patients and controls does aid in the explanation of disease proliferation, but there needs to be further work done in this area to definitively demonstrate the diagnostic efficacy and reproducibility of these findings. Additionally, the requirements of a biomarker to be able to serve as a diagnostic tool as well as to determine disease progression and the effects of treatment make it more realistic to work towards establishing a coordinative plasma biomarker that relies on a combination of different markers to establish AD prognosis and treatment.

5. Epigenetics

Epigenetics is another field of study that relies primarily on the epigenetic regulation of pathology in AD to help elucidate potential biomarker candidates for the disease. The term epigenetics refers to the dynamic regulation in genomic functions that occur independently of DNA sequence and the modification of DNA and chromatin that leads to key characteristic aberrations of the disease. Abnormalities in the amyloid precursor protein, Aβ, and the hyperphosphorylation are implicated in the pathogenesis of AD, and it is plausible that alterations in these genes contribute to the pathways of the disease. By altering the structure of chromatin, and thereby the transcription and expression of the genes, epigenetic processes are capable of altering cellular function. The primary targets of epigenetic regulation are methylation and histone modification of the chromatin; therefore, technologies to determine DNA methylation and histone modification profiles could prove particularly useful in determining genetic variations and genes responsible for the proliferation of AD. Additionally, critical changes are projected to be in epigenetic structures occurring during progression of the disease, leading to significant alterations in the molecular structure of several cells, tissues, and organs.

The transmembrane protein amyloid precursor protein (APP) has been extensively investigated through an epigenetics perspective. There are several studies that support the notion that the abnormalities of epigenetic mechanisms could affect the expression of APP, which plays an essential role in controlling Aβ synthesis and formation of plaques. An earlier study showed that the APP gene was in fact controlled by methylation and determined that variations in methylation-induced APP expression in different parts of the brain and other tissues. The determination that alterations in the methylation of the APP gene directly influence its expression in a region-specific manner suggests that the changes seen in AD could be impacted by epigenetics.

The role of DNA methylation in AD proliferation has also been studied through the analysis of human postmortem brain tissues and the methylation status of various promoters of genes that are closely linked to the pathology of AD. One study of the human cerebral cortex demonstrated an elevation in the methylation of the SORBS3 gene and a decrease in the methylation of S100A2 gene [43]. The former is responsible for encoding a cell adhesion molecule that is seen in neurons and glia, while the latter is a calcium-binding protein. While these alterations in methylation status are normally seen in nondemented aging, the shift was much more evident in AD patients. Another study demonstrated that the promoter regions of the apolipoprotein E (APOE) and (MTHFR) genes were hypermethylated in AD patients in comparison to normal controls [44]. These and other studies

demonstrate the notion that abnormal methylation of genes could certainly have a pronounced effect in AD.

The aberrations in methylation and other epigenetic changes seen in AD demonstrate the need to further investigate this approach to AD pathology in order to elucidate the function of epigenetic regulation in this disease.

6. Metabolomics

One of the more novel approaches to discovering a diagnostic biomarker for Alzheimer's is the study of metabolomics, which utilizes the science behind biochemistry to detect any metabolic disruptions by simultaneously monitoring activity of various metabolites. Any unusual disturbances to activity in the metabolic network could be useful to better understanding the mechanisms of the disease. Although there has yet to be conclusive evidence to illustrate the existence of a metabolomic fingerprint that could serve as a conclusive diagnostic biomarker, this new field is able to make significant progress by creating a comprehensive map of metabolic pathway regulations that are influenced by genes and the environment.

A recent pilot study probed the viability of utilizing this technology to better understand mechanistic pathways and possibly distinguish candidate biomarkers that could undergo further inquiry in the future. The study used postmortem samples of cerebrospinal fluid to attempt to discover any alterations in the metabolic pathways of AD patients and nondemented subjects. There were significant difference changes of tyrosine, norepinephrine tryptophan, purine, and tocopherol pathways in the AD samples when compared to controls [40]. Since the primary aim was to establish the practicability of this field and its potential to elucidate biochemical alterations of interest, there have yet to be any conclusive biomarkers yielded through this approach. Additionally, the study was performed on cerebrospinal fluid, but peripheral metabolomic signatures for AD compared to controls and other disease has not yet been explored. However, this form of exhaustive biochemical analysis could establish unique perspective on the pathways that are modified in disorders like AD that could further ascertain useful diagnostic markers.

7. Conclusion

The neurodegenerative pathology of Alzheimer's disease is the cause for the most prominent form of dementia and affects millions of people worldwide. While there are imaging and CSF-based technologies for the detection of this disease, it is important to inquire into other peripheral biomarkers that could offer a diagnosis that is both noninvasive and inexpensive.

Our review has shown that a wide variety of peripheral biomarkers have been examined. Although all are easily obtained, they vary in their ability to detect already diagnosed disease. We suggest that biomarkers that are less able to detect already diagnosed disease with minimal error are not promising candidates for early detection of disease. In view of the promise of these selected peripheral biomarkers, we suggest that effort be devoted to determining their efficacy in large number of persons from a variety of populations. In addition, it is also essential that peripheral biomarkers that offer promise in terms of their ability to detect already diagnosed disease in large populations need to additionally demonstrate their ability to predict future diagnosis of AD by sufficient number of years to allow effective intervention.

Establishing the utility of a peripheral biomarker may be considered in two phases. In Phase 1, it will be necessary to establish that the biomarker under consideration can detect already diagnosed AD. In Phase II, it will be necessary to demonstrate the ability of the biomarker to detect disease well in advance of the appearance of the current criteria for a diagnosis of AD.

The approaches reviewed offer important insight into the groundwork that has been established towards better comprehending the disease as well as newer fields of investigation that offer promising possibilities. These peripheral biomarkers not only offer the potential to establish diagnostic tools for clinical use, but also lay the foundation for better understanding the mechanisms of the disease that could reveal methods for the treatment and even the prevention of AD.

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Review Article

Biomarkers to Measure Treatment Effects in Alzheimer's Disease: What Should We Look for?

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It is often surprisingly difficult to tell whether a treatment for Alzheimer's disease is effective. Biomarkers might offer the potential of a quantifiable objective measure of treatment effectiveness. This paper suggests several criteria by which biomarkers might be evaluated as outcomes measures. These include biological plausibility, statistical significance, dose dependence, convergence across measures, and replicability. If biomarkers can meet these criteria, then, pending regulatory approval, they may have a role in the evaluation of treatment effectiveness in Alzheimer's disease. If not, their usefulness may be in supplementing, but not supplanting, clinical profiles of treatment effects.

For a compound to be a demonstrably effective treatment for Alzheimer's disease two broad conditions must be met: first, the compound must be effective, and, second, it must be tested in a way which allows that effectiveness to be demonstrated. As formidably difficult as the first challenge is, it also can be surprisingly tricky to show that any treatment for Alzheimer's disease that falls short of cure offers therapeutic potential. Even the tried-and-true Alzheimer's Disease Assessment Scale—Cognitive Subscale (ADAS-Cog) misclassifies important clinical change—typically overestimating decline [1]—so that more than twenty years into the modern era in dementia therapeutics, a recent consensus report has called for a new multidimensional measure for use in dementia drug trials [2].

Into this breech biomarkers seem poised to step. In general, a biomarker for dementia is the term given to "measurable biological characteristics that can either serve as indicators of normal or pathogenic processes in the body, or as tools to track pharmacological responses to therapeutic drugs" [3]. As the accompanying papers in this issue amply demonstrate, there are a host of sometimes ingenious measures that might be employed as biomarkers. Of particular interest is that some such measures might be detectable years before clinical dementia is present; in this way, they serve as targets for therapy, such that with successful treatment the manifestations of Alzheimer's disease are either attenuated or even absent. The challenges to achieving this heroic goal are formidable, requiring an advanced understanding of Alzheimer's disease pathophysiology, and the ability of candidate biomarkers to be measured and tracked over time. In addition to these and other important technical and scientific challenges, however, are some conceptual considerations which need to be considered, and which are the subject of this paper. It will discuss criteria for a biomarker to be used as a measure of treatment effects and some challenges in regard to each criterion. The purpose is not to discourage the very significant advances possible in the implementation of the biomarker agenda, but to lay out some of what needs to be addressed if the full value is to be realized. These criteria for whether a biomarker might be used to measure treatment effects are based on criteria for making inferences about clinical meaningfulness [4] themselves based on the Bradford-Hill criteria for establishing whether an association is causal [5]. Briefly, these criteria are: the biomarkers should, on biological grounds, plausibly be related to Alzheimer's disease; it should be statistically significant; it should show dose-dependent effects; it should show convergence with related measures; it should be replicable. Next, we consider each in a little more detail.

The biomarker should be biologically plausible. This criterion is likely to be the weakest, on three grounds. First,

it will almost always be met for any biomarker, because a theoretical basis for its measurement will likely be the basis on which it is investigated in the first place. Second, as it is inherent that our understanding of biology is always contingent, and that data to the contrary will always trump a good hypothesis, no biologically plausible explanation is likely to withstand data to the contrary. Even so, the role of clinical trials in enhancing our understanding of the biology must be stressed. For example, successive failures of gamma-secretase inhibitors have given credence to the proposition that a new generation of such compounds must be selective for inhibiting a-beta in comparison with Notch signaling endpoints [6]. The ability to confirm this in human studies will help secure this understanding of the underlying biology. Even so, a particular challenge to any argument about the biological plausibility of any single candidate biomarker is this. Dementia is highly age associated. As people grow older, they are more likely to have more than one thing wrong with them, and these cumulative small effects can add up to make dementia more likely [7]; in like manner, we see in older adults, many causes of dementia existing in a single brain [8, 9]. The influence of cooccurring dementia pathologies on disease expression can pose a heavy burden on any prediction which might rest on just one biomarker.

The association between the biomarker and disease expression should be statistically significant. On its face, this seems like a reasonable enough criterion and it might well prove to be true in practice. But what if it turns out that no single biomarker on its own will be sufficiently persuasive? This view is in fact supposed by the Dubois criteria being proposed for the diagnosis of dementia [10]. These new criteria propose several candidate biomarkers be measured, and they suggest increased predictive power will be the case when more biomarkers are present. Note, however, that a recent review suggests that most biomarkers have poor positive predictive value used on their own, and in established clinical disease are inferior to memory testing [11]. How they will work out in combination, especially to track preclinical or "pathophysiological" Alzheimer disease, when clinical symptoms are expected largely to be lacking, will be an important research question. Will it be the case that altering biomarkers of amyloid deposition will alter biomarkers of neurodegeneration to in turn alter clinical disease expression [12]? With regard to established disease, biomarkers that change as disease progresses, such as functional and metabolic markers detected by task-dependent activation on functional MRI and 18F-fluorodeoxyglucose PET, may be candidates for demonstrating statistically significant treatment effects [13].

The biomarker should show a dose-dependent effect. This too seems like an indubitable proposition. Not for nothing is the metaphor of "treating cholesterol" so top of mind at many biomarker discussions. Jason Karlawish of the University of Pennsylvania summed up the situation well in his presentation to the 11th International Congress of Alzheimer's Disease plenary address in Honolulu in July 2010 [14]. There he described the change in the conceptualization of Alzheimer's disease from the leading cause of dementia to a risk state. This new risk state amounts to Alzheimer's disease without dementia, which is proposed to be identified by a series of abnormalities detected by any or some combination of neuroimaging, serum, or cerebrospinal fluid abnormalities [12]. The analogy would be much like the risk of stroke, which is now indicated by an elevation in serum cholesterol. Following the biomarkers model, satisfactory treatment of Alzheimer disease would thereby be demonstrated by altering the level of the biomarker in a favourable direction, although this would be expected to hold only for biomarkers that might be expected to change with treatment—that is, those that were useful for tracking and not just diagnosis. Recent experience with an increase in hippocampal volumes in nondemented older adults who enrolled in an exercise program, compared to continued to decline to those in a sham intervention arm, is perhaps an exciting hint at how a tracking measure might work [15]. Such an effect corresponds well with established work which demonstrated that AD patients with faster cognitive deterioration tend to have greater hippocampal atrophy rates [13, 16]. Likewise, dose-dependence will itself depend on the biomarker and the stage of the patient being treated. For example, in a trial in people with established mild cognitive impairment, amyloid biomarkers might be expected largely to have plateaued whereas an impact on medial temporal atrophy might be postulated for a disease-modifying treatment.

This hope for successful treatment of dementia being reduced to successful treatment of laboratory or imaging tests, has not gone unnoticed by the pharmaceutical and diagnosis industries. These industries have invested hundreds of millions of dollars in the "preliminary validation phase" studies of biomarkers, with the notion that the biomarkers need to be tight against clinical outcomes. But even here there is need for further questioning. If there is one theory that the traditional neuropathological studies have taught us, it is that the correlation between plaques and tangles and disease expression is modest when series include cognitively intact older adults [6, 7, 17, 18]. Whether we should expect more of contemporary biomarkers in living patients is an untested proposition. Recent experience with any amyloid-β_{1-42} biomarker for prodromal AD/mild cognitive impairment illustrates the problem. In detailed simulations based on estimates from the Alzheimer's Disease Neuroimaging Initiative, even though patients with prodromal AD who also had the biomarker (in their cerebrospinal fluid) shared both more impairment at baseline and more cognitive decline, they also showed more variability in outcomes [19]. In other words, the dimensionality of dementia was not reduced by the addition of biomarkers, even though this was an explicit part of the rationale for their use in clinical trials, as was the hope that treatment effectiveness could be demonstrated through biomarker change [20]. Also in pragmatic terms, this means that adding the biomarker did not improve the efficiency of the trial, in that this did not increase power or improve sample sizes.

The criterion of convergence of measures means that if a biomarker were to successfully predict dementia, then it should be reflected in more than one dementia measure. For example, a biomarker that predicted decline in cognition as

measured by the ADAS-Cog might reasonably be expected—at least in the preliminary validation phase—to predict decline in at least some of function, behaviour, quality of life, and caregiver measures. It might also expect to correlate with adverse change in more than one biomarker, on the assumption that dementia is a complex disease state. As with the other criteria, however, there might well need to be nuance in the interpretation. For example, decline in measures will reflect their time frame. If cognitive decline precedes functional impairment, following a preventive strategy, measures might not converge for some time. In addition, if a given biomarker is associated with some disease aspects more than others, convergence of measures might be modest.

As a final criterion, any result about biomarkers must be replicable. As always, this is the highest scientific standard, especially when replicated by entirely independent groups. Given the proprietary nature of much of the biomarkers enterprise, what constitutes independent replication may be a standard short of entirely independent groups. Even so, every effort should be made to achieve this standard. Usually study design consideration—double-blinding being chief amongst them—will constitute the necessary minimum.

This inventory of criteria for using biomarkers to measure treatment effects has focused on how to look. We also must consider what to look for; the recognition of Alzheimer's disease as a complex state has implications in this regard. Chief amongst these is that short of a cure, treatment for Alzheimer's disease will not be the same as having no cognitive impairment, or even age-associated or age-appropriate cognitive function. Rather, it is likely that some features of Alzheimer's disease will be present to only a trivial extent, others will be more modestly attenuated and others still might be unaffected. Whether this diversity of disease modifying effects can be captured in a single biomarker, or a battery of them, is a proposition that remains to be tested.

Disclosure

K. Rockwood is the founder and majority shareholder of DementiaGuide Inc. (stock shareholder). In the last five years, he has attended advisory boards for GlaxoSmithKline, Janssen Alzheimer Immunotherapeutics, and Janssen Ortho Canada (board member/officer). He has given a talk for Shire UK and the ACT-AD coalition, as well as lectures for Alzheimer societies in Australia, Canada, Nova Scotia, and The Netherlands. A. Mitnitski is a part-time employee of DementiaGuide Inc. A. Zeng is an employee of DementiaGuide Inc.

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Research Article

High Throughput ELISAs to Measure a Unique Glycan on Transferrin in Cerebrospinal Fluid: A Possible Extension toward Alzheimer's Disease Biomarker Development

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We have established high-throughput lectin-antibody ELISAs to measure different glycans on transferrin (Tf) in cerebrospinal fluid (CSF) using lectins and an anti-transferrin antibody (TfAb). Lectin blot and precipitation analysis of CSF revealed that PVL (*Psathyrella velutina* lectin) bound an unique N-acetylglucosamine-terminated N-glycans on "CSF-type" Tf whereas SSA (*Sambucus sieboldiana* agglutinin) bound α2,6-N-acetylneuraminic acid-terminated N-glycans on "serum-type" Tf. PVL-TfAb ELISA of 0.5 μL CSF samples detected "CSF-type" Tf but not "serum-type" Tf whereas SSA-TfAb ELISA detected "serum-type" Tf but not "CSF-type" Tf, demonstrating the specificity of the lectin-TfAb ELISAs. In idiopathic normal pressure hydrocephalus (iNPH), a senile dementia associated with ventriculomegaly, amounts of the SSA-reactive Tf were significantly higher than in non-iNPH patients, indicating that Tf glycan analysis by the high-throughput lectin-TfAb ELISAs could become practical diagnostic tools for iNPH. The lectin-antibody ELISAs of CSF proteins might be useful for diagnosis of the other neurological diseases.

1. Introduction

CSF (cerebrospinal fluid), which circulates within the ventricles of the brain and subarachnoid space, reflects the physiological and pathological conditions of the central nervous system [1]. In fact, CSF proteins are used as biomarkers to diagnose neurological diseases such as idiopathic normal pressure hydrocephalus (iNPH) [2–4] and Alzheimer's disease (AD) [5, 6] and may predict the disease onset in a preclinical stage [7]. Since iNPH and AD show similar phenotypes such as dementia and ventriculomegaly, it is difficult to distinguish the two diseases especially in elderly patients. Therefore, simultaneous measurements of a battery of iNPH biomarkers and AD biomarkers could help exact diagnosis of iNPH and AD.

We previously designated Tf-1 and Tf-2 as two isoforms of transferrin in CSF which are separable by SDS-PAGE and showed that the Tf-2/Tf-1 ratio is higher in iNPH patients than in non-iNPH patients [3, 4]. In that study we used immunoblotting method to detect Tf-1 and Tf-2, but the low throughput of immunoblotting makes it impractical for clinical use. Although sandwich ELISA (enzyme-linked immunosorbent assay) or latex photometric immunoassay is high throughput, Tf-1 and Tf-2 cannot be distinguished by these methods because Tf-1 and Tf-2 are different only in their glycan portion, and the antibodies

against the protein portion of Tf cannot distinguish the two isoforms.

To establish a high throughput method to distinguish Tf-1 and Tf-2, we developed ELISAs using a combination of lectins and an anti-Tf antibody (TfAb). Tf-1 has a "CSF-type" biantennary asialo- and agalacto-complex type N-glycans with bisecting β1,4-N-acetylglucosamine (GlcNAc) and core α1,6-Fucose [3, 8], whereas Tf-2 in CSF has a "serum-type" biantennary N-glycans with α2,6-N-acetylneuraminic acid (NeuAc) [3, 9]. Because of their distinct terminal sugars, that is, GlcNAc on Tf-1 and α2,6-NeuAc on Tf-2, we chose PVL (*Psathyrella velutina* lectin) and SSA (*Sambucus sieboldiana* agglutinin) to detect Tf-1 and Tf-2 respectively. Our data showed that the lectin-TfAb ELISAs distinguish the two isoforms to be quantified and that the amounts of SSA-reactive Tf were higher in iNPH patients than in non-iNPH patients. Our newly established lectin-TfAb ELISAs are high throughput methods to measure "glycoforms" of transferrin which might be practical for clinical use.

2. Materials and Methods

2.1. Patients. This study included 28 iNPH patients comprising 14 males and 14 females aged 75.2 ± 6.1 years (mean ± SD) and 18 non-iNPH patients comprising 10 males and 8 females aged 74.9 ± 5.2 years [3]. The iNPH patients were diagnosed using the clinical guidelines for iNPH issued by the Japanese Society of NPH [10]. A bolus infusion test and the tap test were performed routinely. Patients whose gait disturbance improves after the tap test, which removes 30 mL of CSF via a lumbar puncture, were treated with a shunt operation. Those who showed symptomatic improvement 1 month after the shunt operation were defined as iNPH patients while those who did not were defined as non-iNPH patients. In addition, those who did not show improvement after the tap test were classified as non-iNPH patients. The study was approved by the ethics committee of Fukushima Medical University (No. 613), which is guided by local policy, national law, and the World Medical Association Declaration of Helsinki.

2.2. Immunoblotting and Lectin Blotting. CSF samples were dissolved in Laemmli buffer, boiled for 3 min, and loaded onto SDS-polyacrylamide gels (SuperSep Ace; Wako Pure Chemical Industries, Osaka, Japan). After SDS-PAGE, the proteins were transferred to a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, Calif, USA). The membrane was blocked in 3% skim milk, incubated sequentially with an anti-transferrin antibody (Bethyl Laboratories, Montgomery, Tex, USA) and a horseradish peroxidase-labeled anti-goat IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa, USA), and developed using a Super Signal West Dura Extended Duration Substrate (Pierce Biotechnology, Rockford, Ill, USA). For lectin blotting, the transferred membrane was blocked in 1% BSA, incubated with a biotinylated PVL or biotinylated SSA (Seikagaku Corporation, Tokyo, Japan) followed by a horseradish peroxidase-labeled streptavidin (Takara, Shiga, Japan), and developed.

2.3. Lectin Precipitation. CSF was incubated with SSA-agarose (Seikagaku Corporation), and the bound proteins were precipitated by centrifugation. The unbound proteins were further incubated with PVL-agarose (Seikagaku Corporation), and the bound proteins were precipitated.

2.4. Purification of Tf-1. Tf-1 was purified from human CSF as described before [3]. Briefly, CSF was applied to a HiTrap Blue HP column (GE Healthcare, Buckinghamshire, UK). The unbound proteins were applied to a HiTrap Q HP column (GE Healthcare). The bound proteins were eluted with a linear gradient of NaCl from 0 to 300 mM. Tf-1 was eluted at 130 mM NaCl. Tf-1 was further purified by rechromatography with a HiTrap Q HP column. The concentration of the purified Tf-1 was determined by immunoblot analysis with commercially available human Tf (Sigma-Aldrich, St. Louis, Mo, USA) as the standard.

2.5. Lectin-Antibody ELISAs. For PVL-TfAb ELISA, a 96-well C8 Maxisorp Nunc immuno module plate (Nunc, Roskilde, Denmark) was coated with 2.5 μg PVL (Seikagaku corporation) at 4°C overnight and blocked with 0.4% Block-Ace (Dainippon Sumitomo Pharma, Osaka). Purified Tf-1 was used as the standard. The standards and CSF samples were appropriately diluted with PBST (phosphate-buffered saline/0.05% Tween-20), applied to the plate, and incubated at 4°C overnight. After three washes with PBST, the plate was incubated sequentially with anti-Tf antibody (Bethyl laboratories, Montgomery, Tex, USA) and horseradish peroxidase-labeled anti-goat IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa, USA). After three washes with PBST, the wells were incubated with TMB solution (Wako, Osaka, Japan), and 1 N HCl was added to stop the reaction. Absorbances at 450 nm were measured by a plate reader (Bio-Rad Laboratories). CV (Coefficient of variation) of PVL-ELISA was 5.36%.

For SSA-TfAb ELISA, anti-Tf antibodies (Cappel; ICN Pharmaceuticals, Aurora, Ohio, USA) were pretreated with 1.4 mM sodium periodate to abolish SSA epitopes on the antibody and coated on a 96-well plate. Human reference serum (Bethyl laboratories) containing 3 mg/mL serum Tf was used as the standard. The standards and CSF samples were appropriately diluted with TBS (Tris-buffered saline) containing 0.05% Tween 20 and 0.5 mM EDTA and applied to the plate and incubated at 4°C overnight. After three washes with TBST, the plate was incubated sequentially with a biotin-SSA (Seikagaku Corporation) and a horseradish peroxidase-labeled streptavidin (Takara). After three washes with TBST, the plate was incubated with the TMB substrate, and the absorbances at 450 nm were measured. CV of SSA-ELISA was 3.65%.

2.6. Statistical Analysis. Data were analyzed with SPSS version 17 (SPSS, Chicago, Ill, USA). Amounts of SSA-Tf and

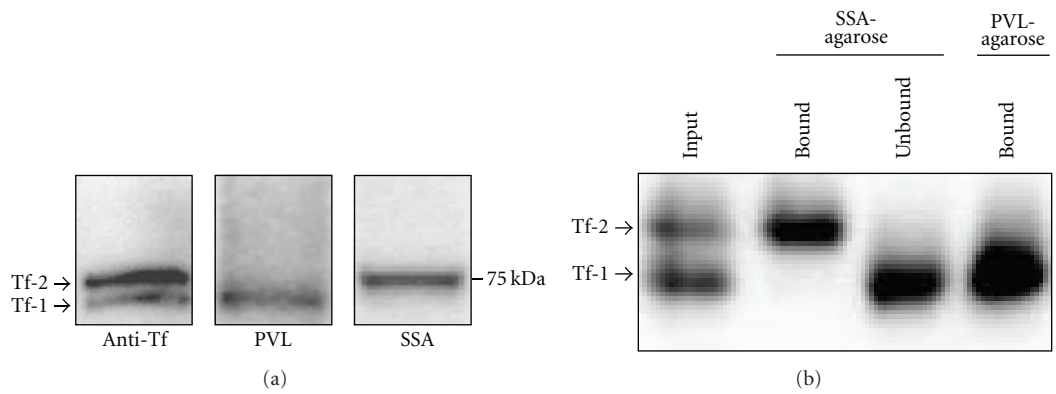

Figure 1: PVL and SSA specifically detect Tf-1 and Tf-2, respectively. (a) CSF was electrophoresed, blotted, and stained with anti-Tf antibody (left panel), PVL (center panel), and SSA (right panel). (b) CSF (input) was sequentially precipitated by SSA-agarose and PVL-agarose. The bound and unbound proteins were electrophoresed and immunoblotted by anti-Tf antibody.

PVL-Tf were analyzed by the Student's *t*-test and Mann-Whitney *U* test, respectively.

3. Results

To establish high throughput lectin-TfAb ELISAs that distinguish "CSF-type" Tf-1 and "serum-type" Tf-2, we first examined whether PVL and SSA specifically detect glycans on Tf-1 and Tf-2, respectively, by lectin-blotting. As we reported previously by immunoblotting, Tf-1 and Tf-2 in CSF were separated on SDS-gel (Figure 1(a) left). When PVL was used as a probe, a band with similar mobility to Tf-1 was detected in CSF (Figure 1(a) center), suggesting that PVL specifically detects the terminal GlcNAc on Tf-1 but not sugars on Tf-2. In contrast, when SSA was used as a probe, a band with similar mobility to Tf-2 was detected in CSF (Figure 1(a) right), suggesting that SSA, detects the terminal α2,6-NeuAc on Tf2 but not sugars on Tf-1. These band signals detected by PVL and SSA (Figure 1(a) center and right) were depleted by TfAb (data not shown), suggesting that the glycan epitopes detected by PVL and SSA reside on the Tf core protein. Moreover, when CSF was precipitated sequentially by SSA- and PVL-agarose, Tf-2 was specifically recognized by SSA, and Tf-1 was recognized by PVL (Figure 1(b)). Taken together, PVL and SSA can recognize Tf-1 and Tf-2, respectively, and distinguish the two Tf glycoisoforms.

Next we investigated whether Tf-1 and Tf-2 were specifically detected by PVL-TfAb ELISA and SSA-TfAb ELISA systems, respectively (Figures 2(a) and 2(b)). The purified Tf-1 was used as a standard to measure Tf-1 in CSF by PVL-TfAb ELISA, whereas Tf in serum was used as a standard to measure Tf-2 by SSA-TfAb ELISA, since serum contains only "serum-type" Tf with very similar glycans to Tf-2 [3]. As shown in Figure 2(c), the purified Tf-1 was successfully detected in a dose-dependent manner (4–64 ng/mL) by PVL-TfAb ELISA, but no significant signals were detected with the serum Tf, indicating that the PVL-TfAb ELISA specifically detects Tf-1. Moreover, SSA-TfAb ELISA detected serum Tf (4–64 ng/mL) but not the purified Tf-1 (Figure 2(d)), suggesting specificity of the SSA-TfAb ELISA for serum Tf and Tf-2. Tfs which were detected by PVL-TfAb ELISA and SSA TfAb ELISA were designated as PVL-Tf and SSA-Tf, respectively.

Finally we measured the concentrations of PVL-Tf and SSA-Tf in CSF from iNPH and non-iNPH patients. As shown in Figure 3, amounts of SSA-Tf were significantly increased in iNPH patients compared to non-iNPH patients while amounts of PVL-Tf were not significantly different suggesting that the SSA-Tf might be an iNPH marker. The SSA-Tf/PVL-Tf ratio were also increased in iNPH patients (not shown), which is consistent with our previous data [3].

4. Discussion

In this study we have developed the high throughput lectin-TfAb ELISAs to measure Tf isoforms that have different terminal sugars. PVL-TfAb ELISA successfully detected Tf-1 but not Tf-2 whereas SSA-TfAb ELISA detected Tf-2 but not Tf-1, demonstrating that the lectin-TfAb ELISAs distinguish the two Tf isoforms to be quantified. Application of the method to iNPH patients revealed that amounts of SSA-Tf were significantly higher in patients with iNPH than in those without, suggesting that the lectin-TfAb ELISAs are promising high throughput methods for diagnosing iNPH. It would be interesting if the SSA-Tf or PVL-Tf is a diagnostic marker for AD or the Tfs can distinguish AD and iNPH.

The lectin-TfAb ELISAs have two differences compared to our previously developed immunoblotting method. First, the lectin-TfAb ELISA is more suitable for clinical use because the ELISA can process more samples. Second, the lectin-TfAb ELISAs detect amounts and structures of glycans on Tf while the immunoblot detects Tf core protein. The immunoblot is a well-established method for discovering biomarkers, but most glycoforms of CSF proteins, in contrast with Tf glycoforms, are not separable by

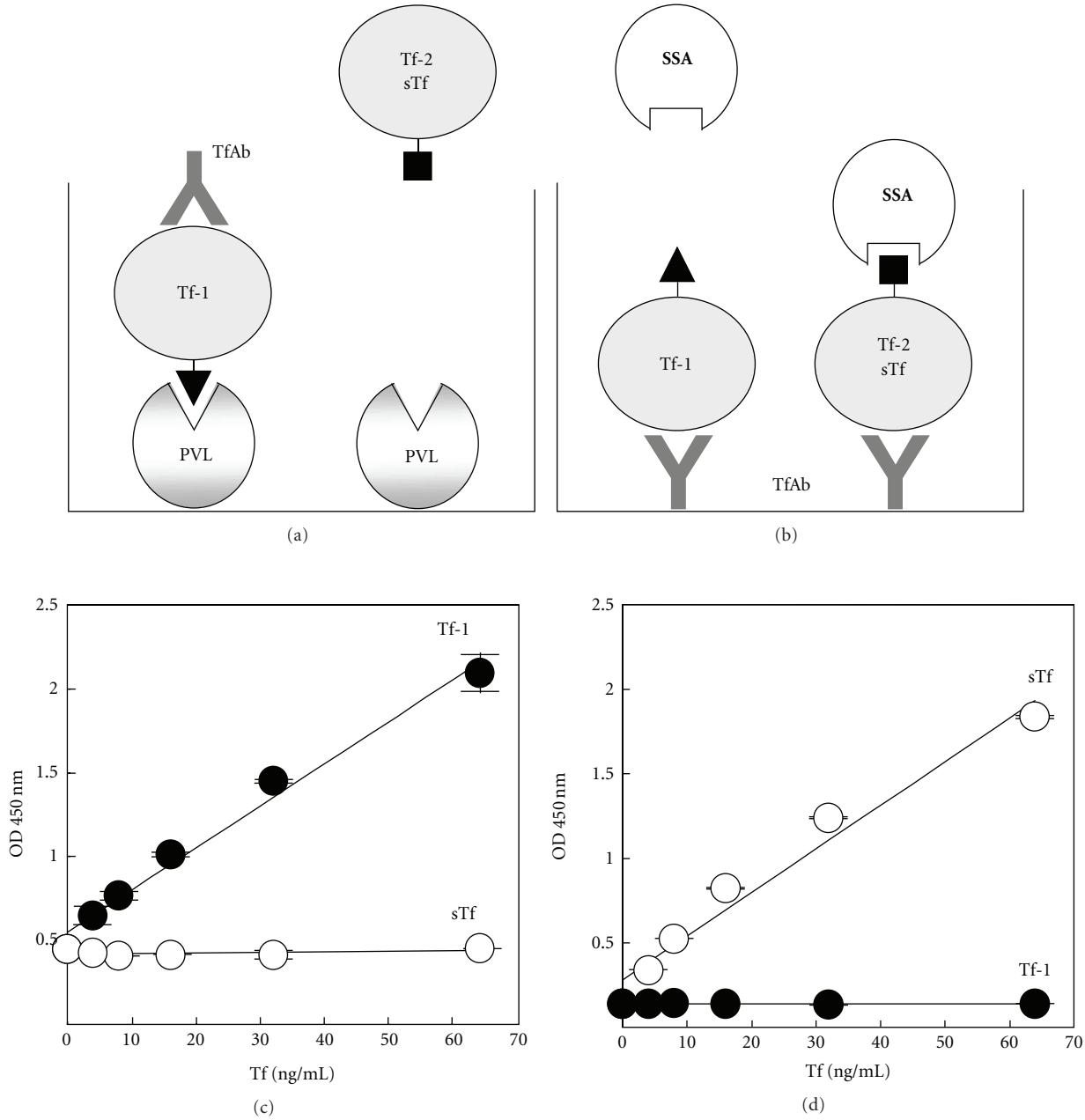

Figure 2: PVL-TfAb ELISA and SSA-TfAb ELISA specifically detect Tf-1 and Tf-2/serum Tf (sTf), respectively. (a) and (b). Schematic representation of lectin-TfAb ELISAs. PVL-TfAb ELISA (a) detects only Tf-1 while SSA-TfAb ELISA (b) detects only Tf-2/sTf. Closed triangles and rectangles represent "CSF-type" and "serum-type" glycans on Tf, respectively. (c) and (d) both the purified Tf-1 and serum Tf were measured in PVL-Tf ELISA (c) and SSA-TfAb ELISA (d). ODs at 450 nm were plotted at each concentration of each Tf. Closed and opened circles show the Tf-1 and serum Tf, respectively.

SDS-PAGE (unpublished observation). In such cases, the lectin-antibody ELISAs are more useful to detect specific glycoforms than immunoblotting. Based on the concept that searches for biomarkers should involve not only "proteomics" but also "glycoproteomics" [11, 12], we are currently trying to develop ELISAs using various combinations of lectins and antibodies and to find new glycobiomarkers for iNPH as well as other neurological diseases such as AD.

5. Conclusion

We have developed the PVL-TfAb ELISA and SSA-TfAb ELISA to measure Tf-1 and Tf-2, respectively. Amounts of the SSA-reactive Tf were significantly higher in CSF of iNPH patients than in non-iNPH patients, suggesting that the lectin-TfAb ELISAs are promising high throughput methods for diagnosing iNPH. The lectin-antibody ELISAs might be useful for a CSF biomarker study of neurological diseases.

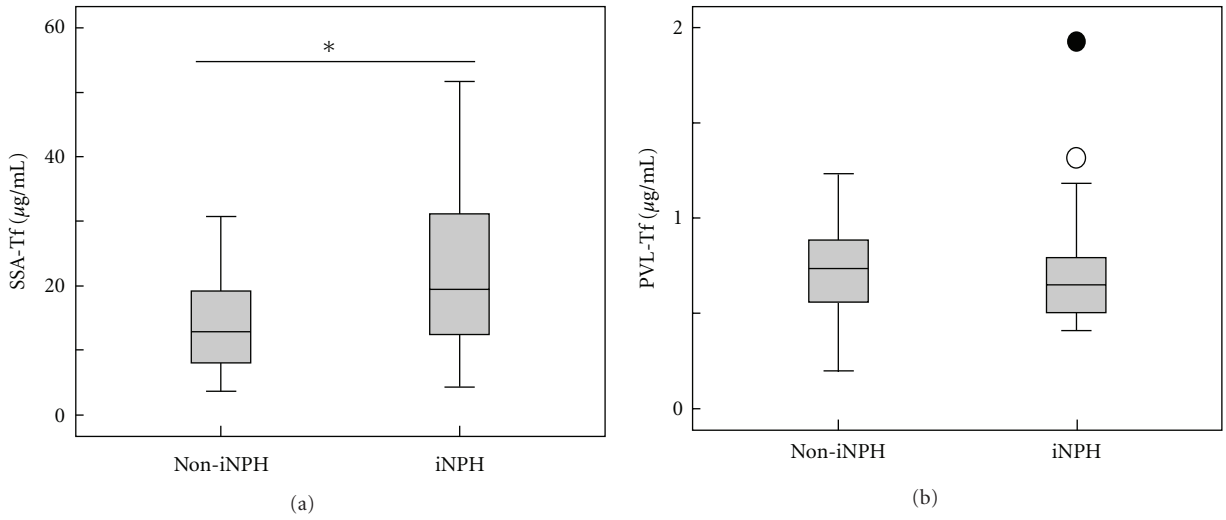

Figure 3: The SSA-Tf is increased in iNPH patients. Concentrations of SSA-Tf (a) and PVL-Tf (b) were measured in CSF of non-iNPH ($n = 18$) and iNPH ($n = 28$) patients, and box plots were shown. An asterisk indicates significantly different ($P < 0.05$). An open and closed circle represent an outlier and an extreme value, respectively.

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Research Article

Genetic Association between Akt1 Polymorphisms and Alzheimer's Disease in a Japanese Population

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A recent paper reported that Aβ oligomer causes neuronal cell death through the phosphatidylinositol-3-OH kinase (PI3K)-Akt-mTOR signaling pathway. Intraneuronal Aβ, a main pathological finding of Alzheimer's disease (AD), is also known as inhibiting activation of Akt. This study aims to investigate whether single nucleotide polymorphisms (SNPs) of the Akt1 gene are associated with AD. SNPs genotyped using TaqMan technology was analyzed using a case-control study design. Our case-control dataset consisted of 180 AD patients and 130 age-matched controls. Although two SNPs showed superficial positive, Hardy-Weinberg equilibrium (HWE) tests, and linkage disequilibrium (LD) analyses suggested that genetic regions of the gene are highly polymorphic. We failed to detect any synergetic association among Akt1 polymorphisms, Apolipoprotein E (APO E), and AD. Further genetic studies are needed to clarify the relationship between the Akt1 and AD.

1. Introduction

The main pathological feature of Alzheimer's disease (AD) is the senile plaque containing aggregated Amyloid β peptide (Aβ). Three genes have been identified as causative genes in familial AD; the amyloid precursor protein (APP), presenilin-1, and presenilin-2 genes. Apolipoprotein E (APO E) is recognized as a genetic risk factor for familial and sporadic AD [1]. In addition, variants of the sortilin-related receptor 1 (SORL1) gene have also been associated with the disease [1, 2]. The evidence from SORL1 suggested that intraneuronal Aβ is significant in early pathogenesis for AD. Recent findings showed that Aβ oligomer is more toxic for neuronal cell [3, 4]. There is widening recognition that the phosphatidylinositol-3-OH kinase (PI3K)-Akt-mTOR signaling pathway is directly affected by Aβ and especially Aβ oligomer modulate cell survival through PI3K-Akt pathway [3, 5–7]. An impaired insulin-mediated signal transduction is one of the pathological features of neurodegenerative diseases [8]. Epidemiological studies note that type II diabetes is risk factor for late-onset AD [9]. Insulin dysfunction might be associated with Aβ and tangles [10, 11]. Intraneuronal Aβ inhibits insulin receptors signaling in neurons by interfering with the association between Akt1 to preclude Akt1 activation [12]. In AD brains, level of PI3K-Akt-mTOR would be decreased [8]. Thus current reports revealed that the dysfunction of PI3K-Akt-mTOR system affect AD pathology [9]. Although genetic variability of PI3K has been reported to affect the risk for AD [13], there are few genetic researches about Akt and AD. In this study, the association between six single nucleotide polymorphisms (SNPs) covering the Akt1 gene and Japanese sporadic AD was investigated.

2. Materials and Methods

DNA was extracted from white blood cells using a standard method. Our sporadic Japanese AD cases ($n = 180$, Male : Female = 79 : 101) were obtained from department of psychiatry, Juntendo university hospital, Tokyo, Japan and department of psychiatry, Juntendo Koshigaya hospital, Saitama Japan. The mean age of the AD group (67.4, S.D. 6.2) was not significantly different from that of the control group

Table 1: Genotypic frequencies of SNPs of the Akt1 gene.

SNP	Location	Genotype	AD	Controls	*P* value	HWE *P* value
		A/A	3	3		
rs113021411	intron 1	A/C	33	45	0.002*	AD: 0.93
		C/C	142	81		Controls: 0.68
		C/C	77	22		
rs2494746	intron 3	C/G	72	85	0.00002*	AD: 0.52
		G/G	29	22		Controls: 0.04*
		C/C	78	22		
rs2494743	intron 3	C/T	71	85	0.00001*	AD: 0.47
		T/T	29	22		Controls: 0.04*
		A/A	27	20		
rs2494738	intron 3	A/G	79	68	0.3	AD: 0.93
		G/G	71	41		Controls: 0.91
		A/A	174	126		
rs3730344	intron 6	A/G	4	3	0.99	AD: 0.99
		G/G	0	0		Controls: 0.99
		A/A	151	114		
rs7140735	intron 13	A/G	27	13	0.17	AD: 0.88
		G/G	0	1		Controls: 0.99

$P^* < 0.05$: Statistically significant (Fisher's exact probabilities test).

Table 2: Linkage disequilibrium (D' value) between SNPs.

	rs1130214	rs2494746	rs2494743	rs2494738	rs3730344	rs7140735
rs1130214 (I1)						
rs2494746 (I3)	**1**					
rs2494743 (I3)	**0.9728**	**0.9933**				
rs2494738 (I3)	0.0554	−0.4329	−0.4273			
rs3730344 (I6)	**−0.9999**	**1**	**1**	0.3476		
rs7140735 (I13)	0.2407	0.2742	0.2838	**−1**	**1**	

(64.4, S.D. 6.7) by the Fisher's exact probabilities test. All the AD cases were diagnosed according to the NINCDS-ADRDA criteria, and none had familial history of AD. The control cases ($n = 130$, Male : Female = 63 : 67) were obtained from healthy volunteers from among staff of our hospital with no history of dementia or other neuropsychiatric diseases. The purpose and significance of this study were explained in detail to each patient and his/her family, and all subjects provided their informed consent. The study protocol was approved by the Ethics committee of the Juntendo University School of Medicine.

Information on the single nucleotide polymorphisms SNPs was obtained from the SNP database (dbSNP) established by the National Center for Biotechnology Information. We selected the SNPs to cover the entire gene, including tagging SNPs. The chosen SNPs were validated, according to the dbSNP and have minor allele frequencies (MAF) greater than 5%. Six SNPs of the Akt1 gene were genotyped using TaqMan technology on an ABI7500 system (Applied Biosystems, Calif, USA). All probes and primers were designed by the Assay-by-Design TM service of Applied Biosystems. A standard PCR reaction was carried out using the TaqMan universal PCR master mix reagent kit in a 10 μL volume. Hardy-Weinberg equilibrium (HWE) tests were carried out for all SNPs for both cases and controls. APO E genotypes for all the samples were determined according to a previous report [14]. Differences in the genotypic frequencies were evaluated using a case-control study design and applying the Fisher's exact probabilities test.

Linkage disequilibrium (LD) between the SNPs as well as a haplotype analysis was performed using SNPAlyse version 5 (DYNACOM, Yokohama, Japan). LD, denoted as D', was calculated from the haplotype frequency using the expectation-maximization algorithm. SNPs were considered to be in LD if D' was greater than 0.75. A case-control haplotype analysis was performed using a permutation method to obtain the empirical significance. The global *P* values represent the overall significance of the observed versus expected frequencies of all the haplotypes considered together using the chi-squared test. The individual haplotypes were tested for association by grouping all others together and applying the chi-squared test with 1 df. *P* values were calculated on the basis of 10,000 replications. All *P* values reported are two tailed, and statistical significance was defined as <0.05. Logistic regression analyses were performed to estimate the relationship among onset of AD, APO E status, and six

Table 3: A case-control haplotype analysis for the 6 Akt1 SNPs.

Haplotype	Overall	AD	Control	Chi-square	*P* value	Permutation *P* value
C-C-C-A-A-A	0.3067	0.3165	0.2969	0.2694	0.6038	0.603
C-C-C-G-A-A	0.2401	0.2818	0.1779	8.8385	$2.95E-03$	0.008*
C-G-T-G-A-A	0.2378	0.2236	0.26	1.0827	0.2981	0.324
A-G-T-A-A-A	0.0658	0.0449	0.0919	5.4284	0.0198	0.044*
A-G-T-G-A-A	0.0549	0.0347	0.0856	7.2736	$7.00E-03$	0.028*

Rare haplotypes with frequencies less than 5% are not shown.
Each nucleotide on the haplotypes represents the SNPs in the following order from left to right: rs1130214 to rs7140735.
$P^* < 0.05$: Statistically significant.

SNPs using SPSS software ver. 17.0 for Windows; (Chicago, Ill., USA). A *P* value of <0.05 was considered statistically significant.

3. Results and Discussion

Our sample set has the power to detect an odds ratio of at least 1.40, assuming a significance level of 0.05, power of 0.70, and an exposure frequency of 0.25 in the controls. Although four SNPs were found to be in HWE, controls of rs2494746 and rs2494743 were not in HWE marginally. Genotypic distribution of the two polymorphisms showed significant difference between our cases and controls (Table 1). Other four SNPs Linkage disequilibrium examination showed strong LD from rs1130214 to rs2494743 and from rs3730344 to rs7140735 (Table 2). The frequency of the C-C-C-G-A-A haplotype was significantly higher in the AD group compared to controls (Table 3). Other two rare haplotypes also showed marginal association.

To date, this is the first study to clarify genetic associations between common SNPs of the Akt1 gene and AD. We found that two SNPs rs2494746 and rs2494743 studied here with Japanese population were not in HWE. Haplotype analysis seemed to be positive superficially. Multiple regression analysis suggested that six SNPs of the Akt1 gene did not associate with the risk for AD and logistic regression analysis for the Akt1 SNPs, APO E and the onset of AD showed no synergetic association (data not shown). SNPs which were not in HWE make us suppose that the regions around such SNPs are highly polymorphic. These SNPs would be triallelic SNPs, or there are large deletions or insertions generally. Since we confirmed that the two SNPs are biallelic, potential large deletion or insertion might exist in the Akt1 gene. Our LD analyses also suggested that the gene consists of two distinct LD blocks. Rs2494738 was thought not to be involved in the two LD blocks. SNPs which are not in HWE with homozygote excess sometimes show positive generally. These SNPs suggest that there are potential polymorphisms including insertion or deletion associated with the disease. Reviewing our raw genotyping data, heterozygotes of control cases of those two SNPs are more frequent than estimated. Thus we guess the SNPs studied here did not affect the disease.

Previous genetic studies for schizophrenic patients are in debate [15–18]. An original report identified Akt1 as a potential schizophrenia susceptibility gene in families of European origins [19]. The additional multi-SNP haplotype analysis showed that specific haplotype is associated with lower Akt1 protein levels [20]. Controversial results have been issued for Japanese schizophrenic patients and Akt1 [15, 16, 21]. The detailed LD analysis from these Japanese studies suggested that there are two apparent LD blocks in the gene [21, 22]. These findings accord with our results. Thus the Akt1 gene is highly polymorphic, and functional SNPs might affect Akt1 levels potentially. We believe that the small size of our dataset may account for the negative results. Our dataset could detect the genetic association between APO E4 and AD. If the SNPs of the Akt1 gene affect the onset of AD, the effects would be smaller than those of APO E4. Previous studies and our findings revealed that the regions of the Akt1 gene are highly polymorphic for Japanese population.

4. Conclusion

Although our pilot study could not show a genetic association between Akt1 and AD, PI3K-Akt-mTOR system has an important role for pathophysiology of AD. Denser SNPing studies would be needed for clarifying the genetic association between Akt1 and AD. Since the relationship between Akt1 and AD remains inconclusive, a meta-analysis would be performed in the future.

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Review Article

Bridging Molecular Genetics and Biomarkers in Lewy Body and Related Disorders

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Recent advances have been made in defining the genetic and molecular basis of dementia with Lewy bodies (DLBs) and related neurodegenerative disorders such as Parkinson's disease (PD) and Parkinson's disease dementia (PDD) which comprise the spectrum of "Lewy body disorders" (LBDs). The genetic alterations and underlying disease mechanisms in the LBD overlap substantially, suggesting common disease mechanisms. As with the other neurodegenerative dementias, early diagnosis in LBD or even identification prior to symptom onset is key to developing effective therapeutic strategies, but this is dependent upon the development of robust, specific, and sensitive biomarkers as diagnostic tools and therapeutic endpoints. Recently identified mutations in the synucleins and other relevant genes in PD and DLB as well as related biomolecular pathways suggest candidate markers from biological fluids and imaging modalities that reflect the underlying disease mechanisms. In this context, several promising biomarkers for the LBD have already been identified and examined, while other intriguing possible candidates have recently emerged. Challenges remain in defining their correlation with pathological processes and their ability to detect DLB and related disorders, and perhaps a combined array of biomarkers may be needed to distinguish various LBDs.

1. Introduction

Over the past decade, dementia with Lewy bodies (DLBs) has arguably become the second most common form of neurodegenerative dementia behind Alzheimer's disease (AD). In addition to progressive decline in cognition, DLB is characterized by fluctuations in cognition with variations in attention and alertness, recurrent formed visual hallucinations, visuospatial dysfunction, and spontaneous parkinsonism. Often, DLB patients also exhibit neuroleptic sensitivity, transient loss of consciousness, falls, and rapid eye movement (REM-) sleep behavior disorder [1]. The clinical separation of DLB from other similar disorders is often difficult resulting in poor diagnostic accuracy, but the relative temporal co-occurrence of parkinsonian features with the typical DLB cognitive and behavioral symptoms such as visuospatial disturbance strongly suggests the diagnosis. Clinical presentation of DLB is also influenced by the amount of AD tau pathology, further complicating the diagnosis [2]. Approximately 20–40% of Parkinson's disease (PD) patients also eventually develop a progressive dementing illness designated as Parkinson's disease dementia (PDD) characterized by a frontal-subcortical clinical presentation [3]. DLB/Diffuse Lewy body disease (DLBD), Lewy body variant of AD (LBV), and PDD comprise an emerging spectrum of clinical phenotypes from relatively pure motor PD to the more predominant cognitive and behavioral disturbance observed in PDD and DLB, yet the reason for the variability remains unknown. Despite the heterogeneity of their clinical phenotypes, a significant neuropathological overlap is observed among these diseases, hence the term "Lewy body disorders" (LBDs) to collectively describe conditions in which Lewy bodies (LBs) and Lewy neurites (LNs) predominate as the hallmark histological lesions. Variation in the distribution of Lewy body pathology is present among LBDs, with more neocortical and limbic system LB in both

DLB and PDD compared to the brains of PD patients without cognitive symptoms and greater neuronal loss in substantia nigra in PDD than DLB [4]. Yet, DLB and overlap disorders such as LBV, more so than PDD, have β-amyloid pathology and basal forebrain cholinergic deficit similar to AD patients [5].

As with other forms of dementia, the pathobiological changes in LBD likely occur decades prior to the onset of clinical symptoms and correspond to widespread irreversible neurodegeneration [6, 7]. It is increasingly clear from the AD therapeutic experience that by the time widespread neuronal injury ensues, symptomatic cholinergic treatments are minimally effective at best, and disease-modifying therapeutic approaches in trials have thus far proven ineffective at altering disease course or in rescuing diseased brain [8, 9]. To demonstrate efficacy, any potential disease-modifying therapy in neurodegenerative dementia must be initiated prior to the expression of the clinical phenotype during the initial molecular pathogenetic events before irreversible neuronal damage has occurred. At present, accurately predicting those individuals at risk for developing neurodegenerative dementia is challenging, and this places greater urgency on developing earlier methods of disease detection. Critically important is not only distinguishing the LBD from AD and other forms of dementia, but also separating DLBs and other LBDs. Although there are many promising candidates for LBD, no biomarkers have yet been validated for clinical diagnostic use, and thus many opportunities exist to develop such tests. Here, we highlight from the perspective of how major genetic discoveries in the LBD and their corresponding biomolecular processes might translate into useful disease markers in biological fluids. Because of many common pathogenetic features among the LBDs, the emerging genetic influences found in PD have readily been translated to DLBs and other LBDs, providing clues to rational approaches for selecting future DLB and LBD biomarker targets for exploration. This paper will highlight several PD and DLB genes and their protein products as candidates for biological disease markers (Table 1).

2. Amyloid and Tau in Lewy Body Disorders

2.1. Amyloid Genetics and Biomarkers in LBD. Aβ, a key component of neuritic plaques in AD brain, is overproduced leading to various degrees of amyloid aggregation and synaptic and neuronal toxicity [10]. As indicated previously, amyloid pathology in the form of neuritic and diffuse plaques can be also found in varying degrees in the brain tissue of patients with DLB, which may interact with LB or synuclein pathology or influence the clinical features of LBDs [4, 11]. The genetic mechanisms of Aβ overproduction in AD are well established; the βAPP gene (chromosome 21), the first identified AD susceptibility gene, encodes a transmembranous protein ranging from 695 to 770 residues, which undergoes a process of regulated intramembranous proteolysis ultimately releasing Aβ peptides, primarily Aβ42 and Aβ40, as well as other fragments. Aβ is generated by the concerted action of β-secretase and γ-secretase complex, while the α-secretase pathway precludes Aβ formation by cleaving βAPP at a site within the Aβ sequence. Genetic analysis of early-onset familial AD cases revealed numerous mutations in the βAPP gene as well as presenilin 1 (PS1; chromosome 14) and presenilin 2 (PS2; chromosome 1) genes, all of which accelerate the processing of βAPP, leading to increased Aβ generation [12]. Specifically, the βAPP KM670/671NL (Swedish) mutation affects the β-secretase site, A692G (Flemish) mutation alters the α-secretase site, and both V717F (Indiana) and V717I (London) mutations affect the γ-secretase processing, leading to elevated Aβ levels. Also important in the Notch developmental signaling pathway which is analogous to βAPP processing, the presenilins are thought to be a component of the γ-secretase enzyme complex, which suggests that missense mutations in the presenilins mechanistically lead to accelerated βAPP processing to Aβ [13].

Therefore, the abnormal proteolytic cleavage of βAPP leads to elevated brain Aβ deposition, and as a result, diminished peripheral levels of Aβ. Reflecting a shift from soluble Aβ to insoluble brain deposits, significant decreases in CSF Aβ42 levels have been demonstrated in AD and more recently in DLB cases [14]. Parnetti et al. found that DLB, compared with PD, PDD, and AD patients, showed the lowest CSF levels of Aβ42 and, when combined with CSF tau, differentiated DLB from PD and PDD, but not from AD [15]. Also, Spies and colleagues showed a greater decrease in Aβ40 in clinical DLB and vascular dementia patients compared with control levels and even with AD. Differentiation of non-AD dementias such as vascular dementia and DLB was improved by comparing the ratio of Aβ42 and Aβ40 [16]. More recently, the detection of amyloid in dementia patients has been greatly enhanced by the use of amyloid-binding agents such as Pittsburgh compound B [17], which also demonstrated amyloid burden in DLB. An Australian study reported more variable cortical PiB binding in DLB patients than in AD [18], whereas a subsequent examination of PiB binding in LBDs including DLB, PDD, and PD, compared with AD and normal patients, showed higher amyloid burden in DLB and AD than in PDD, PD, or NC patients [19]. Amyloid load was highest in LBD patients in the parietal and posterior cingulate regions, corresponding to visuospatial impairments on neuropsychological testing, suggesting that amyloid deposition could partly contribute to the clinical presentation of LBDs.

2.2. Tau Genetics and Biomarkers in LBD. Mutations in the tau gene on chromosome 17 may also present with phenotypic features of PDD or DLB, but they differ pathologically from these disorders in that LBs are generally absent [20]. Tau-bearing neurofibrillary tangles remain one of the pathological hallmarks of AD but are also central to a diverse group of disorders termed "tauopathies" which include progressive supranuclear palsy, corticobasal ganglionic degeneration, frontotemporal dementia (FTD) with parkinsonism linked to chromosome 17, and other disorders [21]. Tau is a microtubule binding protein, which acts to stabilize tubulin polymerization in microtubules critical for axonal

Table 1: Genetics and biomarkers in LBD.

	Biochemical marker	Gene defect	Relevance to LB disorders	Source of biomarker
AD lesions	Aβ	APP: K670M/N671L and so forth. PS1: H163R and so forth PS2: N141I and so forth	Deposited in plaques	CSF, plasma
	Tau	Tauopathy: P301L, N279K, K317M, and so forth	Found in NFT in AD brain, released after neuronal damage	CSF
PD/DLB lesions	α-synuclein (PARK1/4)	A53T, A30P (PD), G209A (DLB), E46K, triplication (PD & DLB)	Mutation $\rightarrow\uparrow$ α-syn aggregation. LB component, toxic oligomers and protofibrils	CSF, skin cells, platelets
	β-synuclein	P123H, V70M (DLB)	Inhibit α-syn aggregation: mutant causes degeneration	CSF
	γ-synuclein	SNP in DLBD	Amyloidogenic: affects neuronal and axonal cytoskeleton	Ventricular CSF
Proteostasis/ oxidative stress	Parkin (PARK 2)	K161N, W453Stop, 202-203delAG, M192L, K211N, and so forth	Ubiquitin E3 ligase, LOF mutation in PD alters mitophagy	ND
	UCHL-1 (PARK 5)	I93M, S18Y (SNP)	Neuronal deubiquitinating hydrolase; impaired synaptic and cognitive function in AD & PD	ND
	PINK 1 (PARK 6)	A168P, A217D, E417G, E240K, and so forth (PD)	Mitochondrial serine/threonine kinase; LOF mutation in PD alters mitophagy	ND
	DJ-1 (PARK7)	M26I, D149A, G78G, R98Q (PD), L166P (PD & DLB)	Redox-dependent chaperone; LOF mutation in PD	CSF, plasma
	LRRK2 (PARK 8)	G2019S, duplication, triplication (PD)	Gain of function mutant in PD?DLB: interacts with α-syn and tau, and with parkin in apoptotic cell death	ND
Cytoskeletal	NF	NEFM (PD)	Disrupted NF $\rightarrow$ abnormal axonal transport; released in cell damage	CSF
Lysosomal dysfunction	GBA	84 dupl G, IVS 2 + 1, N370S, L444P (PD)	Gaucher's disease, abnormal lysosomal function/autophagy in PD	CSF, plasma
Inflammation	IL-1α, IL-1β, IL-6, TNFα	SNP: IL-1$\beta-511$,TNF-$\alpha-308$	α-syn-induced microglial activation $\rightarrow\uparrow$ secretion of neuroinflammatory mediators	CSF

CSF: cerebrospinal fluid; GBA: glucocerebrosidase; Aβ: β-amyloid; NF: neurofilament; ND: not yet determined; PD: Parkinson's disease; DLB: Dementia with Lewy body; UCHL1: ubiquitin carboxy terminal hydrolase L1; PINK 1: PTEN-induced putative kinase 1; LRRK2: leucine-rich repeat kinase 2; LOF: loss of function; SNP: single nucleotide polymorphism.

cytoskeletal integrity and function. In disease, tau protein truncation at Glu 391 or hyperphosphorylation causes microtubule destabilization and aggregation of unbound tau into paired helical filaments (PHFs) leading to characteristic tangle formations [22]. Unlike the tauopathies, no direct pathogenetic tau mutations have been identified in LBDs, but tau pathology appears to be a consistent feature among neurodegenerative dementias including AD and LBDs, and given the pathological overlap, they might share similar pathogenetic pathways (reviewed in Stoothoff and Johnson) [23]. The Ser/Thr kinase and glycogen synthase kinase-3β (GSK3β), in concert with other molecules such as fyn kinase, normally regulate tau function but with aberrant activation accelerate the hyperphosphorylation of tau in neurodegenerative disease. Similarly, the cell cycle family kinase and cyclin-dependent kinase 5 (Cdk5/p35), active during normal brain development and involved in regulatory tau phosphorylation during mitosis, may also contribute to PHF formation.

Consequently, both total tau and hyperphosphorylated forms have been widely investigated and detected in CSF, but not serum, by enzyme-linked immunosorbent assay methods. In the differentiation of dementia types, Arai et al. initially reported elevated total CSF tau levels in AD but not in PD, but subsequently, they showed that total tau was also increased in DLB at similar levels to AD [24]. Yet, others have found differences for both total and phospho-tau (p-tau) in differentiating DLB from AD [25], and levels of total tau and p-tau 181 were significantly increased in autopsy-confirmed DLB patients [26]. In clinically diagnosed dementia cases, CSF p-tau 231 discriminated AD from non-AD dementias as a group, where levels were significantly higher in AD patients compared with DLB, FTD, vascular dementia, other disorders, and control subjects [27]. Separation of DLB from AD, however, was less robust, provided that CSF p-tau 231 levels were also increased in DLB. Clinically diagnosed DLB cases also showed elevated levels of CSF p-tau 181 compared with controls [28], and Hampel et al. reported

that p-tau 181 provided the best discrimination of DLB from AD yielding a sensitivity of 94% and specificity of 64% [29]. In autopsy-confirmed DLB and AD patients, however, sensitivity decreased to 75% and specificity to 61%, with a diagnostic accuracy reported as 73% [30].

3. Synucleins: Genetics to Biomarkers in the Lewy Body Disorders

3.1. Pathogenetics of Synucleins in LBD

3.1.1. Functions of α-Synuclein. LBs are filamentous inclusions consisting primarily of the presynaptic protein α-synuclein (α-syn), which might have several roles in vivo. Studies demonstrate that it is localized to multiple neural tissues, including high expression in neocortex and hippocampus, and that expression increases during acquisition-related synaptic plasticity [31]. Interaction with tubulin suggests α-syn could be a microtubule-associated protein similar to tau [32, 33], and it is highly active in various membrane lipid bilayers such as in presynaptic vesicles acting as a chaperone for soluble NSF attachment protein receptor (SNARE) complex formation [34], in neuronal Golgi apparatus influencing protein trafficking [35] and in the inner membrane of neuronal mitochondrial [36]. The synucleins might act to preserve membrane stability, provide antioxidant function, and assist with membrane turnover, although the actual role of synucleins remains elusive [37, 38]. Because of its association with LB and the tendency to self-aggregate into pathological oligomers and ultimately fibrillar structures [39], α-syn plays a central role in the pathogenesis of LBD, hence the alternate designation "synucleinopathies." The degree of α-syn immunoreactivity in cortical LBs correlates with cognitive severity and disease progression in PDD and DLB [4, 40]. Also, the protein can be recovered from filaments in purified Lewy bodies from PDD and DLB brain [41], and recombinant α-syn tends to form Lewy body-like fibrillar structures in vitro [42].

3.1.2. α-Synuclein Mutations in PD and DLB. In the past decade, tremendous advances have been made in understanding the genetic factors influencing the pathogenesis of Lewy body disorders. Compelling evidence for a genetic basis for PD and DLB followed the discovery of mutations in the α-syn gene (PARK1/4) in patients with autosomal dominant familial Parkinson's disease, and subsequently, mutations were identified in patients with both sporadic and familial DLBs. From a susceptibility marker on chromosome 4q21-23 that segregated with the PD phenotype in Italian and Greek kindreds, A53T [43] and A30P [44] were the first two missense mutations in α-syn associated with familial Parkinson's disease. Clinical analysis of the Italian A53T mutation revealed phenotypic variability over the disease course with several individuals demonstrating moderate to severe dementia [45]. Subsequently, a case of clinically and pathologically well-characterized DLBD in the United States and a Greek proband of DLB with a family history of PD were both determined to have the A53T α-syn mutation [46, 47]. Another mutation, E46K, was discovered in a Spanish family presenting with autosomal dominant DLB [48], and in genetic studies of a large family with the spectrum of Lewy body phenotype ranging from PD to DLB, α-syn gene triplication was described, causing α-syn overproduction similar to the trisomy effect observed in Down syndrome patients [49].

Autosomal dominant point mutations are shown to affect the aggregative properties of α-syn, which has mechanistic implications for the pathogenesis of LBD. Compared to wild-type α-syn, biophysical analyses reveal that α-syn aggregation is folding state dependent, where A53T and A30P mutated proteins cause increased aggregation only from the partially folded intermediate state and not the monomeric state [50]. A53T α-syn transgenic mice have increased oligomerization of the protein in brain regions devoid of inclusions as well as those areas with more abundant lesions and neurodegeneration, and consistent with prior biophysical findings, α-syn toxicity in these mice was dependent on the conformation of intermediate species [51]. In fact, the E46K mutation, as well as the others not only increase the tendency toward aggregation, but also promote formation of annular protofibrillar structures, causes pore formation in various membranes and neuronal damage [52].

3.1.3. β-Synuclein Mutations in DLB. α-Syn is a member of a larger family of synuclein proteins which also includes β-synuclein (β-syn) and γ-synuclein (γ-syn). β-syn has recently been implicated in PD and DLB pathogenesis, but its precise role in disease is still emerging. Despite having strong homology with α-syn, it is not clearly amyloidogenic, but is highly localized to presynaptic sites in neocortex, hippocampus, and thalamus like α-syn [53, 54]. Normal β-syn may act as a biological negative regulator of α-syn. In bigenic α-syn/β-syn-overexpressing mice and in doubly transfected cultured cells, β-syn ameliorated amyloidogenicity, neurodegenerative changes, and motor deficits induced by α-syn overexpression alone [55]. On the other hand, mutated β-syn leads to neuronal damage and disease and augments neurodegeneration, perhaps through a loss of its natural regulator function. Two novel β-syn point mutations, P123H and V70M, were found in highly conserved regions of the β-syn gene in respective familial (P123H) and sporadic (V70M) DLB index cases [56], where abundant LB pathology and α-syn aggregation was present without β-syn aggregation. P123H β-syn overexpression in transgenic mice resulted in axonal damage, gliosis, profound memory, and behavioral deficits [57]. These phenomena may involve α-syn, since bigenic mice overexpressing α-syn with P123H β-syn show greater deficits compared with monogenic mice and compared with P123H β-syn expressed with α-syn knockout, implying that the P123H mutation has a synergistic effect with other synucleinopathies to cause neurodegeneration. P123H as well as V70M β-syn mutations might also injure neurons by disrupting normal lysosomal pathways and corresponding cellular autophagic processes [58].

3.1.4. Association of γ-Synuclein with LBD. Unlike the other synuclein family members, γ-syn or persyn is largely

expressed in the cell bodies and axons of primary sensory neurons, sympathetic neurons, and motor neurons as well as in brain [59]. In cancer biology, γ-syn is associated with abnormally altering cellular mitotic checkpoints in various types of malignancies, making them more aggressively metastatic [60], but as far as neurodegeneration, it is the most recent synuclein member to be linked to LBD neuropathology and the least well understood. Single-nucleotide polymorphisms in all three synucleins have been associated with sporadic DLBD, most prominently γ-syn [61], and in sporadic PD, DLB, and LBV patients, γ-syn antibodies, as well as β-syn and α-syn reveal unique hippocampal axonal pathology [62]. In vivo, γ-syn overexpression in transgenic mice shows age- and dose-dependent neuronal loss throughout the neuraxis, especially in spinal motor neurons, where γ-syn-bearing inclusions, gliosis, and alterations in heat shock protein and neurofilament structure are found [63], perhaps suggesting relevance to motor neuron disease associated with dementia. In vitro evidence further supports a cytoskeletal role for γ-syn in maintaining neurofilament structure; γ-syn overexpression in cultured neurons causes disruption of the neurofilament network by destabilizing the structural integrity of neurofilament-H allowing degradation by calcium-dependent proteases, which has implications for neurodegeneration [64].

3.2. Synucleins as Biomarkers of LBD

3.2.1. Synucleins in the Extracellular Compartment. Synucleins are known as intracellular molecules, but they also appear in extracellular and peripheral fluids from active and passive processes. Evidence suggests that turnover and secretion of these proteins might occur during normal cellular processing, releasing synucleins into extracellular space and hence into peripheral sites. In transfected and untransfected cultured neuroblastoma cells, 15 kDa α-syn is released into surrounding media [65], and furthermore, not only monomeric α-syn but also aggregated forms are secreted in an unconventional exocytic manner into extracellular fluid in response to proteasomal and mitochondrial dysfunction [66]. Remarkably, Desplats et al. recently showed that neuronally secreted α-syn can also be taken in endocytically by other neurons or glia as a means of transmitting pathology [67]. Secreted α-syn interacts with various molecules such enzymes; in cultures, matrix metalloproteinase-3 cleaves native α-syn to smaller proteolytic fragments that enhance its aggregative properties [68]. Whether β-syn and γ-syn also undergo unconventional exocytosis and secretion remains unknown, but given structural and functional similarity to α-syn, the possibility exists. Certainly, synaptic and axonal damage reflecting neurodegeneration may also allow release of synucleins into the extracellular millieu and access to peripheral fluids such as CSF and blood.

3.2.2. α-Synuclein as a PD and DLB Biomarker. Multiple forms of α-syn are released into cerebrospinal fluid (CSF) and other biological fluids. Full-length α-syn has been recovered from lumbar CSF from living normal control, PD and DLB patients [69, 70], and also from postmortem CSF from DLB and other neurodegenerative diseases [71]. Comparative findings regarding differences in CSF α-syn levels among various neurodegenerative diseases, however, are difficult to interpret because of inconsistent observations. In PD, a smaller early study showed that no differences in full-length CSF 19 kDa α-syn have been found in relation to control individuals [69], but a recent effort using a new Luminex assay in a larger sample controlling for extraneous influences showed significantly decreased levels in PD compared to controls with 92% disease sensitivity and 58% specificity [72]. Elevated α-syn levels, however, were found in DLB, AD, and vascular dementia with no differences among them [71]. Perhaps more intriguing, higher-molecular weight aggregated α-syn species in CSF might be associated with PD and DLB. Reduced levels of a 24 kD α-syn-immunoreactive band were found in DLB CSF and correlated directly with declining cognition [73]. Moreover, using a specific enzyme-linked immunosorbent assay (ELISA), soluble aggregated α-syn oligomers in CSF were significantly increased in PD patients compared against control subjects, AD and progressive supranuclear palsy, and specificity ranged from approximately 85 to 87%, while sensitivity was about 53–75% range [74].

Plasma α-syn detected by immunoblotting was decreased in PD compared with age-matched control subjects, and those PD patients with age-at-onset prior to 55 years (early-onset) had significantly lower levels than those with onset after 55 years of age (late-onset) [75]. In addition, soluble oligomeric α-syn detected by specific ELISA was significantly elevated in plasma from PD. This test demonstrated a specificity of approximately 85%, a sensitivity of 53%, and a positive predictive value of 0.818 [76]. Although measurement of plasma α-syn appears interesting as a biomarker, it was reported that skin cells and platelets are also sources for α-syn, and their levels did not correlate with disease presence or severity [77]. Moreover, red blood cells are also a major source of α-syn [78], and thus, plasma could be contaminated by α-syn not originating from brain, which might render interpretation of results difficult. One promising consideration for the future exploration of α-syn as an LBD biomarker will be the development of novel imaging compounds and techniques, similar to amyloid imaging, to specifically target and visualize α-syn distribution in the PD and LBD brain. The availability of such methods will be a significant advance in biomarkers for synucleinopathies.

3.2.3. β-Syn and γ-Syn as Potential Biomarkers in Lewy Body Disorders. Due to their increasing importance in LBD pathogenesis, β-syn and γ-syn, as much as α-syn, might be excellent targets as peripheral markers of disease. As such, levels of these synucleins might be altered in the CSF of patients with PD/PDD and DLB, reflecting the underlying degenerative processes in brain. No studies to date have examined β-syn levels in peripheral fluids in relation to neurodegenerative disease, but a small study reported elevated postmortem ventricular CSF γ-syn levels

in DLB, AD, and vascular dementia patients, with the highest levels seen in DLB patients [71]. More detailed examination of both β-syn and γ-syn as a peripheral disease markers in well-characterized populations of PD, DLB, and other disorders is warranted to determine their specificity and sensitivity in the synucleinopathies.

4. DJ-1 in the Lewy Body Disorders

4.1. Functional Role of DJ-1 in Lewy Body Diseases. Recently, DJ-1 (PARK 7) has emerged as a significant molecular target of interest in LBD principally because of its genetic association with PD and its increasing importance in cellular oxidative neuroprotection. Although its exact role is unknown, multiple functions have been assigned to the DJ-1 protein. Described by Nagakubo et al. as a mitogen-dependent oncogene involved in Ras-related signaling pathways [79], it shares structural homology with the carboxy-terminal domain of *Escherichia coli* HPII catalase and is reported to possess catalase activity which reduces oxidative stress in cultured cells [80]. It also binds to and regulates the PIAS SUMO-1 ligase and is itself posttranslationally modified by sumoylation [81, 82]. Of relevance to Lewy body formation and neurotoxicity, DJ-1 displays redox-dependent chaperone activity conferring proper protein folding and thermal stability, which in fact, also inhibits α-syn aggregation [80]. The overexpression of DJ-1 in rats protects nigral dopaminergic neurons against degeneration involving 6-hydroxydopamine, while mutant DJ-1 in mice causes abnormal dopamine reuptake and susceptibility to 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity [83]. Deletion of DJ-1 homologs in Drosophila renders them sensitive to H_2O_2, paraquat, and rotenone toxicity [84].

4.2. DJ-1 Mutations and Possible Relevance to LBD. No less than 13 gene mutations have been identified in DJ-1 in atypical younger-onset PD patients, but their significance to idiopathic late-onset PD remains uncertain. In autosomal recessive early-onset PD from consanguineous families, a complete DJ-1 deletion in a Dutch family and a point mutation L166P in an Italian case were identified [85]. When expressed in cultured cells, L166P appears to be a loss-of-function mutation which leads to DJ-1 functional instability, degradation by the proteasome system [86, 87], abnormal translocation of DJ-1 to mitochondria, and loss of chaperone activity [80]. The importance of DJ-1 gene alterations in dementia and DLB, however, is uncertain. One report found no impact on dementia risk of the DJ-1 14kb deletion [88], and analysis of an insertion/deletion variant (g.168_185del) in DJ-1 in a larger sample of patients also showed no association with either PD or DLB compared to control patients [89]. Given these early negative findings, the relevance of DJ-1 genetic mutations to DLB and other LBD is not known. At present, no patient harboring a DJ-1 mutation has come to autopsy, so the precise pathology is not known. Although DJ-1 mutant cases may ultimately not be LBDs, it is possible that alterations in DJ-1 may somehow influence the aggregation of α-syn and LB formation [80] or contribute to pathogenesis by other molecular pathways.

DJ-1 is found in brain across a wide range of neurodegenerative diseases including PD, FTD, AD, DLB, and LBVAD, and demonstrates striking association with neuropil threads and neurofibrillary pathology in neocortex and subcortical brain regions in these disorders [90]. Interestingly, this association with tau pathology was seen in DLB and LBV brains, suggesting that as a chaperone molecule, DJ-1 may be involved in tangle formation, and the binding of DJ-1 with these lesions could abolish the normally protective effect of DJ-1, enhancing oxidative neurotoxicity. Wang et al. observed that DJ-1 knockout mice have markedly abnormal hippocampal long-term depression accompanied by a less severe abnormality in long-term potentiation, which was reversed by the D2/3 agonist quinpirole, indicating that DJ-1 has a role in dopamine-dependent signaling in hippocampal plasticity [91]. This implies that DJ-1 may be important in the maintenance of memory and cognition.

4.3. DJ-1 as a Potential Biomarker for Lewy Body Diseases. Given its pathogenetic significance, DJ-1 could be a candidate biological marker for DLB and LB and might serve as a means of monitoring in vivo oxidative damage and protein misfolding. Although intracellular and mitochondrial in localization, DJ-1 is presumed to be secreted perhaps specifically under disease conditions which induce oxidative damage. Using semiquantitative immunoblotting, we previously identified DJ-1 in CSF of sporadic PD patients, where levels were significantly elevated compared with controls. Levels were higher in the earlier stage PD cohort (Hoehn-Yahr stages I-II) than in the more severe patients (Hoehn-Yahr stages III-IV) [92]. Similarly, plasma DJ-1 levels in PD patients were markedly increased compared to controls, but unlike CSF, levels were relatively higher in late stage (III-IV) rather than early stage PD (I-II) [93]. The reason for this difference between plasma and CSF DJ-1 is unknown, but we surmised previously that since CSF DJ-1 originates from a central source produced mainly by reactive glia, early increases in CSF DJ-1 levels probably represent an early protective response to damage, whereas plasma DJ-1, like other plasma disease markers, likely represents peripheral oxidative stress damage. In fact, DJ-1 is secreted into blood in breast cancer, melanoma, familial amyloid neuropathy, and stroke [94–96]. In the largest study to date, Hong et al. developed a more sensitive and quantitative Luminex assay for CSF DJ-1 to complement immunoblotting mass spectrometric and chromatographic analysis methods and found decreasing rather than increasing levels of DJ-1 in PD CSF compared with control patients [72]. The 90% disease sensitivity and 70% disease specificity for PD using this method approaches minimal desired parameters for a clinically useful biomarker for PD. Importantly, the study highlighted the fact that DJ-1 levels are greatly influenced by such variables as the extent of blood contamination and patient age, which could account for some of the variability across studies. Of note, DJ-1 is also subject to oxidative modifications in PD and AD brain tissue, and this might be

measured in peripheral fluids as well, as another monitor of oxidative damage [97]. CSF DJ-1 remains a promising and perhaps clinically useful biomarker for PD, but as far as DLB and other LBD, it is unknown whether CSF levels of DJ-1 are altered. Since plasma DJ-1 is increased in DLB, it is hypothesized that CSF DJ-1 may also be elevated. Further investigation will be necessary to clarify the utility of DJ-1 as a biomarker in DLB and LBD.

5. Glucocerebrosidase as a Novel Biomarker for Lewy Body Disorders

5.1. Glucocerebrosidase Mutations Influence PD and DLB. Many clinicopathologic parallels can be drawn between the lysosomal storage disorders, such as Niemann-Pick, Sandhoff's, Tay-Sachs disease and others, and the age-related neurodegenerative disorders, when considering the aberrant accumulation of pathological substances (e.g., lysosomal sphingomyelin in Niemann-Pick disease versus synucleins in PD and DLB) and the phenotypes of neuronal loss and cognitive deterioration found in both. Common to these diseases are abnormalities in lysosomal and autophagic mechanisms as part of a larger disruption of cellular proteostasis leading to abnormal storage/accumulation of toxic materials and neuronal damage. In the past few years, an altogether unexpected pathogenetic relationship emerged between Gaucher's disease (GD), a prototypic storage disease, and the synucleinopathies. Despite its overall rarity, GD is the most common inherited lysosomal storage disease, especially in the Ashkenazi Jewish population. It is caused by autosomal recessive gene mutations in the glucocerebrosidase (GBA) gene (chromosome 1q21), leading to either partial or complete deficiency of GBA, and hence, toxic lysosomal accumulation of its substrate, glucosylceramide, in multiple cell types including neurons [98]. Recent reports documented an increased incidence of PD in heterozygous relatives of patients with GD [99, 100], but interest in this phenomenon was propelled by the finding that GBA mutations were in fact more common in PD patients of Ashkenazi background compared with AD patients and PD patients in the general population [101–103]. Moreover, more severe GBA mutations such as 84 dupl G and IVS 2 + 1 were associated with a greater degree of PD risk, compared with less severe GBA mutations such as N370S [104]. The relationship between PD and GBA has now been replicated in much larger international studies with the most common mutations being L444P and N370S, and about 28 GBA mutations are presently recognized [105].

Interestingly, in a study of British patients with PD and GBA mutations, all 17 carrier patients demonstrated abundant α-syn neuropathology with Braak stage 5-6 severity and common neocortical LB pathology. Clinically, these patients had earlier age at onset, and hallucinations were present in 45% of patients, while 48% had cognitive impairment or dementia consistent with PDD [106]. Greater severity of GBA mutation also predicted the presence of cognitive impairment in PD patients; 56% of severe GBA mutation carriers had cognitive impairment compared to 25% of mild mutation carriers [107]. These observations suggest a much broader link between GBA mutations and the dementia phenotype of LBD. In fact, examination of GBA gene alterations in DLB patients, with and without concomitant LBV-type AD pathology, showed that the majority of GBA mutations were found in DLB patients rather than in PD, with a mutation rate in DLB ranging from 18 to 23% overall [108, 109]. The proportion of DLB patients with GBA mutations was higher in those with pure neocortical LB pathology compared to those with mixed LB and AD pathology and to those with predominantly brainstem LB. A significant association was also found between GBA mutation status and the presence of LB, indicating that altered GBA might play a role in their formation and in synucleinopathy [108].

5.2. Glucocerebrosidase and Chaperone-Mediated Autophagy in LBD. Important in neurodegeneration, disrupted cellular proteostasis represents a state in which an imbalance exists between effective functioning of the innate cytoprotective machinery and excessive accumulation and aggregation of abnormally misfolded proteins, leading to neurotoxicity. It is increasingly apparent that chaperone-mediated autophagy (CMA) and lysosomal degradation pathways are important in maintaining cellular proteostasis as part of a larger network of cellular actions, with particular relevance for neurodegenerative diseases. Recently, as evidence for CMA dysfunction in synucleinopathies, a significant decrease in autophagy markers was reported in substantia nigra from PD brain [110]. Soluble forms of α-syn, including monomers, oligomers, and even protofibrils, are normally cleared through the CMA/lysosomal degradation by interacting with the chaperone, heat shock cognate-70, and becoming internalized into lysosomes via the Lamp-2a membrane receptor [111, 112]. Studies have indicated that α-syn shares a common pentapeptide structure with other lysosomal substrates, designating it as a target for removal by this pathway [111], and the lysosomal structure is critical to maintaining the internal acidic environment, allowing lysosomal hydrolases to degrade α-syn into peptides released into the cytosol [112]. Mutant GBA could therefore disrupt lysosomal activity leading to abnormal accumulation of nondegraded α-syn, which then aggregates to toxic soluble oligomers and protofibrils. Also, abnormalities in the ubiquitin-proteasome system (UPS) are present in AD and PD, and GBA alterations might secondarily overwhelm the ability of UPS to remove accumulated α-syn, promoting aggregation and neurotoxicity [113]. Pathologically, in GD with parkinsonism, α-syn-positive inclusions were observed in neurons in hippocampal CA2-4 regions, while cortical synuclein pathology was identified in other GD cases [114]. Further, parkin, an E3 ubiquitin ligase also implicated in PD, has been shown to affect the stability of mutant GBA and increase its degradation causing further lysosomal dysfunction [115].

5.3. Glucocerebrosidase as a LBD Biomarker. Because of the importance of mutant GBA function to PD and DLB

pathogenesis, the issue arises as to whether the measurement of GBA activity, or a perhaps other related molecules, might be utilized as a biological marker. The activity of peripherally secreted GBA was measured in plasma and CSF in a 10-month-old female with GD with the aim of monitoring the effect of experimental Cerezyme replacement therapy [116]. Baseline GBA activity was detected in both plasma (2.7×10^{-6} U/μL) and CSF (0.096×10^{-6} U/μL), although CSF activity was several magnitudes lower than plasma. Intravenous Cerezyme, a macrophage-targeted GBA, rapidly raised the plasma activity within 1 hour and CSF activity by 2.3-fold at 3 hours, both returning to baseline after 24 hours. This study suggests the intriguing possibility that GBA activity, especially in CSF and plasma, might be useful in monitoring the efficacy of novel therapies involving CMA and lysosomal function. To extend this observation, Balducci et al. determined that multiple lysosomal hydrolases, including GBA, are significantly decreased in the lumbar CSF of PD patients [117], perhaps supporting a more widespread lysosomal dysfunction in PD not limited to GBA alone. In this regard, other lysosomal enzymes such as mannosidase and β-hexosaminidase might be important additional biomarker targets for neurodegeneration. Moreover, in DLB, AD, and FTD patients, lysosomal enzyme activities in CSF demonstrated a very specific pattern of decrease, in which only DLB showed significant decreases in CSF activity of α-mannosidase, β-mannosidase, GBA, galactosidase, and β-hexosaminidase, whereas in AD and FTD, only CSF α-mannosidase activity was significantly diminished [118]. In DLB, CSF GBA activity showed the greatest magnitude of decrease, reinforcing its importance in the LBD, but also noteworthy is the fact that AD and FTD showed decreased α-mannosidase activity, suggesting that this might be another important factor in lysosomal dysfunction in neurodegeneration. Indeed, these promising candidates need to be investigated further to establish diagnostic accuracy in terms of disease specificity and sensitivity in cohorts of PD, DLB, and other dementing disorders.

6. Miscellanous Candidate Biomarkers

6.1. Inflammatory Cytokines. Polymorphisms in proinflammatory cytokine genes including IL-1α, IL-1β, and TNF-α are associated with increased risk in AD [119]. In PD, several case control genetic analyses have demonstrated that homozygous carriers of the IL-1β−511 and TNF-α−308 promoter region variants have increased disease risk [120, 121], and that earlier age at onset in PD was associated with IL-1β−511 homozygosity at allele 1 [122]. But as yet, no such genetic alterations in cytokines genes have been reported in DLB. Similar to Aβ-induced upregulation of inflammatory cytokines in AD, soluble secreted α-syn in the extracellular space in LBD might also induce the production of a variety of neuroinflammatory mediators into the extracellular fluid. For instance, microglial activation in response to stimulation by secreted α-syn from cultured cells and from overexpression in transgenic mouse models occurs in a dose-dependent manner, causing release TNF-α, IL-1β, and IL-6 [123]. Because secreted CNS cytokines are readily detected in CSF, they have been extensively examined as potential disease biomarkers. IL-1β, IL-2, IL-6, and TNF-α are all upregulated in PD brain, as well as in CSF from PD patients [124–126], and Chen et al. showed that plasma IL-6, but not IL-1β, TNF-α, or other acute phase reactants, predicted risk for future PD in males [127]. In terms of DLB, CSF IL-1β levels, which were relatively low, did not differ compared to AD or normal controls and could not distinguish them apart. Comparable increases in CSF IL-6 levels were found in AD and DLB, but again not significantly different from each other to be of diagnostic value [128]. Indeed, the neuroinflammatory cytokines may be important as a pathogenetic response to CNS injury caused by accumulation of amyloidogenic proteins, but their role as biomarkers for the LBD, especially for DLB, is still unclear.

6.2. Neurofilament Proteins. Disorganization and breakdown in the cytoskeletal network occurs in various LBDs and other neurodegenerative diseases, and as discussed, gamma-synuclein and proteolytic degradation of the cytoskeleton may be involved. As a result, a failure of normal axonal transport results from the accumulation of disrupted neurofilament molecules within the neuropil, causing neuronal demise [129]. Recently, a mutation in the NEFM gene encoding the rod domain 2B of neurofilament M (NF-M) which causes aberrant NF assembly was identified in a single early-onset PD patient [130]. It is recognized that in addition to α-syn, three types of NF protein also comprise the structure of Lewy bodies [131]. Upon cell death or axonal damage, accumulated neurofilament leaks into the extracellular space, subsequently appearing in CSF and perhaps other peripheral fluids. Elevated CSF NF protein was reported in MSA and PSP, but not in PD, and this was suggested to clinically aid in differentiating parkinsonian syndromes [132]. CSF NF protein was also measured in dementia, and although increased levels were observed in DLB, late-onset AD, and FTD, there were no differences among them [28]. Therefore, because cytoskeletal abnormalities are present in many neurodegenerative dementias as well as in PD, NF protein may be more a reflection of nonspecific alterations in neuronal and axonal function, which does not appear to able to clinically separate DLB from other disorders.

6.3. Brain Neurotransmitter Alterations in CSF and by Imaging Modalities. Severe cortical cholinergic deficits originating from deficiencies in the nucleus basalis of Meynert are characteristic of AD brain, but studies have shown that cholinergic deficits are perhaps more severe in DLB brain [5]. This suggests that measurement of cholinergic activity and/or acetylcholine (ACh) might be developed into a potential biomarker for the LBDs. Indeed, early attempts to quantify ACh or its major metabolite, choline, have shown baseline levels to be low and perhaps difficult to measure accurately. In AD, CSF ACh was reported to be significantly lower than control levels [133], while in PD and Huntington's disease patients, despite some cholinergic deficit, lumbar CSF

ACh and choline levels did not differ from normal [134]. No studies have directly examined CSF cholinergic levels in DLB or LBDs, but recently, Shimada and colleagues employed positron emission tomography (PET) mapping of brain ACh activity in DLB and PDD patients and normal controls and demonstrated a marked reduction in cholinergic activity in medial occipital cortex of DLB and PDD, greater than that observed in PD patients without dementia [135]. Some correlation of mapped cholinergic activity with cognitive decline measured by the Mini-Mental State Exam was also found. Although preliminary, this has potential to be a more practical and sensitive cholinergic biomarker for LBD.

Because of similar nigrostriatal loss to PD, a relative dopaminergic deficiency also exists in DLB and LBDs. CSF dopamine (DA) and its metabolites have been investigated previously in PD, and recently, Lunardi et al. showed differences in CSF DA and its metabolites, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC), in PD patients, demonstrating early-stage dopaminergic loss and a correlation with the development of dyskinesia [136]. In DLB, HVA levels were significantly reduced compared with AD, separating the disorders [137]. Similar to cholinergic activity, imaging modalities may also contribute to the assessment of dopaminergic function in the LBDs. In a small study, striatal DA uptake as measured by ^{18}F-fluorodopa PET was decreased in both caudate and putamen in DLB as compared with AD patients and controls [138]. Also, DA transporter loss was determined across multiple studies using ^{123}I-2β-carbometoxy-3β-(4-iodophenyl)-N-(3-fluoropropyl) nortropane ligand with single-photon emission computed tomography (^{123}I-FP CIT SPECT) and demonstrated significant loss of caudate and putaminal DA transport compared with AD and control levels [139–141]. A larger phase III, multicenter study of ^{123}I-FP CIT SPECT in possible and probable DLB patients and non-DLB comparators (mostly AD) demonstrated a mean sensitivity of 77.7% for detecting clinically probable DLB, with a specificity of 90.4% and 85.7% overall diagnostic accuracy [141]. ^{123}I-FP CIT SPECT DA transporter imaging greatly enhanced diagnostic accuracy for DLB over clinical diagnosis alone when coupled with autopsy confirmation, raising sensitivity for DLB from 75% to 88% and specificity from 42% to 100% [139]. Furthermore, DA transporter loss in the caudate may also be inversely associated with depression, apathy, and delusions in DLB patients [142].

6.4. Miscellaneous Imaging Biomarkers in LBD

6.4.1. MIBG Scintigraphy as a DLB Biomarker. Autonomic failure is a common clinical finding in LBD, including PD and DLB, but not in non-LBD dementias, and therefore it has been investigated as an alternative biomarker for the diagnostic separation of DLB from other dementias. Abnormal autonomic function can be determined using cardiac ^{123}I-meta-iodobenzyl guanidine (^{123}I-MIBG) imaging, a technique which assesses cardiac sympathetic nerve function in both cardiac and neurological disorders by measuring the uptake of ^{123}I-MIBG, a norepinephrine analogue [143]. In the last decade, a series of Japanese studies consistently demonstrated delayed heart to mediastinum ratio (H/M) of ^{123}I-MIBG uptake in DLB compared with AD and controls [143–146]. ^{123}I-MIBG scintigraphy was found superior to brain perfusion SPECT imaging [147]. Estorch et al. further showed that in dementia patients followed for four years before "final diagnosis," ^{123}I-MIBG imaging distinguished DLB from other dementias with a sensitivity of 94%, specificity of 96%, and a diagnostic accuracy of 95% [148]. Finally, consistent with autonomic dysfunction in DLB, both early and delayed H/M ^{123}I-MIBG uptake were significantly associated with the presence of orthostatic hypotension in DLB patients and discriminated DLB from AD even in the absence of parkinsonism [149].

6.4.2. Other Structural and Functional Imaging Biomarkers. Various magnetic resonance (MR) imaging modalities have been explored in DLB and PDD, including volumetric imaging, diffusion tensor imaging, and proton magnetic resonance spectroscopy (reviewed in Watson et al.) [150], and although not directly useful as biomarkers at present, they have revealed insights in the pathobiology of LBDs. Using conventional MRI techniques such as voxel-based morphometry and region of interest analysis, some degree of diffusion or focal frontal and parietal atrophy has been observed [151]. Atrophy has been rated at 1.4% per year in DLB brain [152], 1.31% per year in PDD, and 0.31% per year in PD [153]. Not surprising is the fact that unlike AD brain, medial temporal structures are relatively preserved in DLB and PDD, with global hippocampal loss at about 10–20% compared with controls and about 21–25% in AD [154]. Diffusion tensor imaging, an MR technique mapping brain microdiffusion of water in the direction of white matter tracts, has shown decreased fractional anisotropy of water movement in DLB in the precuneus and posterior cingulate areas, perhaps highlighting their role in DLB pathogenesis [155].

Brain perfusion SPECT (^{99m}Tc-HMPAO SPECT) has been evaluated in its ability to diagnostically separate DLB from AD, and in AD, reduced relative cerebral blood flow (rCBF) in the frontal, and medial temporal regions is characteristic, whereas in DLB, occipital hypoperfusion is often observed [156]. Colloby et al. applied statistical parametric mapping to SPECT imaging of DLB patients, more precisely showing large perfusion deficits in the left medial occipital gyrus and the bilateral central, inferior parietal, precuneate, superior frontal and cingulate regions on the brain, which are functionally consistent with frontal-executive and visuospatial deficits in DLB [157]. Across studies, sensitivity ranged from 65 to 85% and specificity from 85–87%, which appears less robust as a potential imaging marker compared with other methods.

6.5. Other PD Genes and Their Protein Products as Possible DLB Markers. Aside from α-syn and DJ-1, numerous other mutations have been associated with familial early-onset PD and possibly LBD (Table 1). Among these gene products

are parkin (PARK 2), UCHL-1 (PARK 5), PINK1 (PTEN-induced putative kinase 1; PARK 6), and LRRK2/dardarin (PARK 8) [158]. Indeed, none of these mutations have yet been associated with prototypic LBD pathology, and it remains to be determined whether they actually represent LBDs or separate diseases with parkinsonian phenotype. Furthermore, no studies have addressed their role as biological markers of disease, but since both synucleins and DJ-1 are detected in CSF and peripheral fluids, it seems plausible that the protein products of other dominant genes in PD could be peripheral biomarker candidates for DLB and other LBD. Parkin, UCHL-1, and PINK1 genes, like DJ-1, all encode proteins important in neuroprotection in terms of maintaining protein homeostasis and preventing stress-related cellular damage, and mutations in these genes cause a loss of these critical functions. Leucine-rich repeat kinase 2 (LRRK2/dardarin), on the contrary, is linked with autosomal-dominant late-onset PD, and mutations result in a toxic gain of function.

LRRK2/dardarin is a kinase consisting of multiple functional domains, and recent evidence suggests that physiologically, its principal function may be to regulate neurite outgrowth. Expression in cultured neurons of several LRRK2 mutations associated with familial PD, such as G2019S, increased kinase activity and significantly reduced neurite outgrowth, whereas expression of a dominant-negative mutation, K1906M, markedly increased neurite length [159]. PD-associated mutations also generated tau-positive axonal inclusions in cultured neurons, suggesting that LRRK2 may be linked to abnormalities in tau. Indeed, expression of mutant G2019S LRRK2 in Drosophila caused activation of the Drosophila GSK-3β homolog and promoted tau hyperphosphorylation leading to microtubule fragmentation and dendritic pathology [160]. Similar tau hyperphosphorylation was also present in transgenic mice expressing G2019S LRRK2, and expression of both wild-type human LRRK2 and G2019S mutant LRRK2 caused abnormal dopaminergic transmission [161]. LRRK2 may also interact with α-syn, another dominantly inherited PD gene, to exert its effect. Lin et al. showed that overexpression of LRRK2 with A53T mutant α-syn in transgenic mice worsened neurodegeneration, while ablation of LRRK2 expression suppressed α-syn aggregation and pathology [162], and α-syn also activates GSK-3β in mice causing tau hyperphosphorylation [163], indicating that LRRK2, α-syn, and tau alterations may all be linked in the same pathway, perhaps with LRRK2 upstream of these events. Although early, evidence has indicated that LRRK2 is also a component of LB in PD and DLB brains [164], and that LRRK2 and α-syn interact in DLB brain and coimmunoprecipitate in cultured cells after oxidative stress challenge [165], suggesting that the LRRK2 may also be important in DLB pathogenesis. Interestingly, genome-wide association studies (GWASs) in a European cohort demonstrated that LRRK2, α-syn, and tau are loci associated with PD risk [166], but examination of tau in a Japanese GWAS cohort failed to identify it as a PD risk locus [167], showing a population difference with regard to this locus. Certainly, population differences might apply to all risk loci examined for PD and LBD, and it is important to determine whether the relationship among LRRK2, α-syn, and tau in PD, DLB, and other LBD is also influenced by population differences. These findings make LRRK2/dardarin an attractive candidate for examination as a potential biomarker, and if identified in CSF or peripheral fluids, they might be used with α-syn and tau as combined biomarkers.

Furthermore, emerging evidence is redefining the roles of PINK1 and parkin in PD pathogenesis. Because energy generation is critical for cellular function, mammalian cells are highly dependent on mitochondria [168]. Depolarization and morphological defects characterize damaged or impaired mitochondria which are targeted for removal through mitophagy, a highly specialized form of autophagy in which parkin and PINK1 play a crucial role (reviewed by Vives-Bauza and Przedborski) [169]. In this process, PINK1 cleavage is inhibited by the loss of mitochondrial membrane potential, causing its lengthening and the recruitment of cytosolic parkin [170, 171]. Voltage-dependent anion channel 1 and other outer mitochondrial membrane proteins are then ubiquitinated in a parkin-dependent manner, and this in turn recruits the binding of adapter proteins such as p62 and histone deacetylase 6 to initiate autophagosome assembly around the damaged mitochondrion and subsequent removal [169]. Of relevance to PD, mutant PINK1 and mutant parkin both cause motor dysfunction, dopaminergic loss, and abnormal mitochondrial morphology in Drosophila [172]. In this paradigm, loss of function PINK1 mutants are rescued by concurrent overexpression with wild-type parkin but not vice versa, indicating that parkin specifically acts downstream of PINK1. Also, parkin mutations have been shown to interfere with ubiquitination and the downstream steps in normal mitophagy [173]. Thus, PD, and possibly related dementias, might be a result, to some extent, of defective mitophagy due to loss of function in PINK1 and parkin such as found in autosomal dominant early-onset PD.

Although LRRK2, parkin, PINK1, and UCHL-1 have not yet been identified in peripheral fluids, PINK1 and parkin may be a promising candidates. Unexpectedly, both PINK1 and parkin, which are normally cytosolic or targeted to mitochondria, were localized extracellularly in AD and multiple sclerosis brain, and colocalized with amyloid plaques, reactive astrocytes, as well as amyloid-affected vessels [174, 175]. This suggests that both PINK1 and parkin are actively released from neurons and glia in response to injury and might be upregulated in CSF and peripheral fluids during neurodegeneration. Interestingly, given a role in mitophagy, they might also be a CSF or peripheral reflection of mitochondrial health and turnover. It remains to be seen whether these gene products can be detected in biological fluids such as CSF as potential biomarkers in PD and LBD.

7. Unbiased Methods in LBD Biomarker Discovery

7.1. Genomics in PD and LBDs. As detailed above, traditional methods for molecular biomarker determination have been derived from targeted analyses of candidate genes/mutations

and corresponding proteins in brain and body fluids such as CSF and blood, with the subsequent exploration of mechanisms in cell culture and animal models. An emerging alternate approach has been to evaluate genomes and proteomes with regard to specific neurodegenerative diseases and their components in an unbiased manner to yield a number of potential pathogenetic, therapeutic, and biomarker targets for further validation. With regard to the genomic analysis of the LBDs, gene expression profiling has proved to be a promising tool. Scherzer et al., for instance, examined transgenic *Drosophila* expressing the human α-syn gene and performed temporal profiling of resultant gene expression [176]. They demonstrated a number of changes, including a downregulation of phospholipase A2 and other lipid genes, downregulation of several mitochondrial respiratory chain molecules, and alteration in membrane transport and energy genes such as voltage-gated calcium channel and lysosomal ATPase, suggesting that mitochondrial integrity might be affected by α-syn overexpression.

In Parkinson's disease brain, RNA from populations of mesencephalic dopaminergic neurons with and without LB were isolated by immunolaser capture microdissection, amplified by polymerase chain reaction and expressed [177]. Interestingly, upregulation of the ubiquitin-specific protease 8 in LB-containing neurons indicated cellular damage and increased levels of ubiquitination in LB, whereas non-LB-bearing neurons showed increased expression of novel cytoprotective genes such as bullous pemphigoid antigen 1, an HSP-70-like gene (STCH) and Kelch-like 1. Although promising, further genomic profiling studies in DLB, PDD, and other LBD are needed to expand the range of novel gene targets for examination and validation.

7.2. Proteomic Profiling in PD and LBDs. As a complement to gene expression profiling and genomic methods, proteomic profiling has also assumed a greater importance in biomarker discovery for neurodegeneration with relevance to the LBD. Advances in methodologies such as 2-dimensional gel electrophoresis (2-D GE), liquid chromatography (LC), high-resolution mass spectrometry (MS), and quantitative proteomics allow analysis of static or condition-dependent protein structure and function associated with PD and LBD in a variety of sample types such as brain or body fluids (reviewed in Shi et al. 2009) [178]. In mice treated with MPTP, a specific mitochondrial toxin, isotope-coded affinity tag assay of brain tissue followed by MS analysis revealed 100 proteins with significantly altered levels including many mitochondrial and metabolic molecules, βAPP and DJ-1 [179].

Basso et al. first examined the proteome of the substantia nigra from Parkinson's disease brain and age-matched controls [180]. Using 2D GE and peptide fingerprinting, of the 44 expressed proteins, 9 proteins differed in PD versus controls, including oxidative and mitochondrial proteins such as peroxiredoxin II, mitochondrial complex III, calcium channel, and others. A subsequent study in PD brain showed decreased frontal cortex levels of mortalin, a novel mitochondrial chaperone protein with roles in energy generation [181]. In addition, LBs isolated by laser-capture microdissection, were analyzed by LC/MS and ultimately demonstrated 156 candidate proteins involved in ubiquitin-proteasome system and synaptic function, from which the heat shock cognate-71, a chaperone involved in neurodegenerative disease, was identified and validated as a candidate target [182]. Abdi and colleagues carried out proteomic evaluation of CSF from AD, PD, and DLB patients and normal control individuals, using chromatography, MS, and isobaric tagging for relative and absolute quantification (iTRAQ), identifying numerous candidate proteins related to PD and DLB, such as lipoproteins ApoC1 and ApoH [183]. Lastly, using surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF) MS analysis of serum from DLB patients compared to AD, a combination of protein peaks provided the ability to separate DLB from non-DLB cases, with a sensitivity of 83.3% and a specificity of 95.8% [184]. Given promising findings, further exploration of the proteomics of the LBDs is warranted, and perhaps consideration should be given to determining whether combining various genomic and proteomic methods will be of value.

8. Conclusions

Over the last decade, tremendous advances have been made in understanding the pathogenetics of PD, PDD, and DLB, which has revealed not only the genetic basis of these disorders, but also related mechanisms common to all the LBD. In parallel, these discoveries have been a catalyst for translating and developing many of the involved proteins into promising biomarkers for disease. A common theme centers on genes that drive a complex network of synergistic and opposing cellular actions underlying pathogenesis. Aggregation of α-syn, the main constituent of intracellular LBs, results in toxic oligomers and protofibrils which not only act intracellularly, but also are actively and passively released into the extracellular environment causing damage to surrounding tissue. Proinflammatory cytokines such as interleukins are also produced which perpetuates the inflammatory cascade. On the contrary, DJ-1, PINK1, parkin, and perhaps others molecules are upregulated to oppose cellular protein misfolding and oxidative stress and maintain mitochondrial function, while autophagy mechanisms attempt to limit the toxic effect of synucleins and other toxins by lysosomal engulfment and digestion. Much of this is reminiscent of a relatively new concept applied to infectious diseases and mechanical tissue injury termed "damage-associated molecular patterning" (DAMP), which is an evolved system to recognize, contain, and repair damage to cells and tissues. It is characterized by the abnormal release of molecules normally confined and operating within healthy cells or from foreign pathogenic agents, that when released into the extracellular space activate receptors and pathways leading to inflammation and multiplying cellular damage (reviewed by Bianchi) [185]. In this regard, events in the pathogenesis of PD, DLB, and related disorders may represent a novel variation of the DAMP response, and in a sense, biological fluid markers are therefore a measurement of DAMP activity as it relates to neurodegeneration.

Despite progress in developing biological markers for PD, PDD, and DLB, clinical diagnosis of this spectrum

of disorders remains challenging. The need for highly sensitive and specific biomarkers that accurately mirror the underlying pathogenetic features of these disorders demands not only that more advanced detection methods be devised and validated in large sample populations, but also that novel biomarker candidates be selected for evaluation based on rational selection from the multiple-associated gene-mechanism associations in the LBD. Gene products including β- and γ-synuclein, GBA, parkin, and PINK1 need to be examined in CSF, blood, and even urine to confirm their presence in biological fluids and threshold of detection. Alterations in the levels of these putative biomarker candidates in CSF and blood can provide further insight into the role these mechanisms may play in disease and also the ability of the potential biomarker to reflect resulting CNS changes. To better understand the relationship of gene mutations, mechanisms, and disease biomarkers in LBD, it would be of great interest to determine whether the levels of these putative biomarkers in CSF and peripheral fluids are altered in patients with known PD, DLB, and LBD mutations. It is likely that combinations of multiple peripheral biomarkers could be needed to monitor the various mechanistic aspects underlying the LBD, but the optimal combination has yet to be determined. Furthermore, both existing imaging modalities as well as novel imaging techniques to detect specific molecular biomarker targets will greatly complement peripheral biomarkers. New specific therapies for the LBD yet to be developed will probably target one or more of the multiple pathways described above, and indeed, this could determine which biomarker or combination of biomarkers would be appropriate as a therapeutic endpoint. Studies are also needed to establish which biomarkers will fulfill the criteria of minimum sensitivity and specificity for the LBD for consistent and reproducible diagnostic use in presymptomatic disease detection and also serve as robust tracking tools and endpoints in monitoring the efficacy of future LBD therapies.

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Research Article

Joint Assessment of Structural, Perfusion, and Diffusion MRI in Alzheimer's Disease and Frontotemporal Dementia

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Most MRI studies of Alzheimer's disease (AD) and frontotemporal dementia (FTD) have assessed structural, perfusion and diffusion abnormalities separately while ignoring the relationships across imaging modalities. This paper aimed to assess brain gray (GM) and white matter (WM) abnormalities jointly to elucidate differences in abnormal MRI patterns between the diseases. Twenty AD, 20 FTD patients, and 21 healthy control subjects were imaged using a 4 Tesla MRI. GM loss and GM hypoperfusion were measured using high-resolution T1 and arterial spin labeling MRI (ASL-MRI). WM degradation was measured with diffusion tensor imaging (DTI). Using a new analytical approach, the study found greater WM degenerations in FTD than AD at mild abnormality levels. Furthermore, the GM loss and WM degeneration exceeded the reduced perfusion in FTD whereas, in AD, structural and functional damages were similar. Joint assessments of multimodal MRI have potential value to provide new imaging markers for improved differential diagnoses between FTD and AD.

1. Introduction

Alzheimer's disease (AD) and frontotemporal dementia (FTD) are two of the most common and devastating disorders that result in dementia in the elderly population. Although the definitive diagnosis of each type of dementia is not possible until autopsy, biomarkers based on magnetic resonance imaging (MRI), providing measurements of brain volume, perfusion, and white matter integrity, have been promising for improved diagnosis and prediction of dementia progression [1]. In AD, which is a progressive dementing disorder associated with cognitive impairments beginning with episodic memory deficits, MRI measurements of brain volume have shown characteristic gray matter (GM) loss primarily in medial temporal lobe regions [2, 3] whereas functional studies using arterial spin labeling MRI or PET/SPECT imaging have shown prominent changes primarily in the parietal lobe (including the posterior cingulate gyrus and lateral temporoparietal areas) [4–7], though regions of structural, and functional alterations can overlap. Furthermore, diffusion tensor imaging (DTI), a unique method to assess the integrity of white matter microstructure, have revealed white matter (WM) alterations in AD, involving the parietal, temporal, and frontal brain regions [8–13]. In FTD, which is associated with impairments of social behaviors, personality, and executive functions, MRI has shown characteristic patterns of structural GM loss [14, 15] and GM dysfunction [16–20] primarily in frontal and anterior temporal lobes. WM volume loss [21, 22] and WM degradation in FTD [23–25] have also been reported in the frontal and temporal regions.

Using biomarkers of neurocognitive measurements to differentiate between AD and FTD are often difficult because of overlapping symptoms. Several studies using imaging markers compared differences in abnormal brain patterns between AD and FTD directly. Structural MRI showed that

AD was associated with greater GM loss than FTD in posterior brain regions [26, 27] whereas FTD was associated with more severe GM loss than was AD in frontal brain regions [26, 28–30]. Similarly, functional imaging such as PET/SPECT and perfusion MRI showed that AD was associated with greater reduced cerebral blood flow or metabolism than FTD in parietal and occipital brain regions [16, 28, 30–32]; whereas FTD was associated with greater frontal dysfunction than AD [16, 30, 32]. In addition to differences in GM, we have previously reported differences in WM between AD and FTD [25]. Specifically, measurements of fractional anisotropy (FA)—a summary measure of DTI indexing WM integrity—indicated greater WM degradation (FA reduction) of frontal brain regions in FTD compared to AD. Furthermore, no brain region in AD was shown to have more WM degradation when compared to FTD. Taken together, these findings suggest that AD and FTD are each associated with disease-specific regional patterns of GM and WM alterations. Recent multimodality strategies [33, 34] of combined radiological markers such as analyzing brain volume and perfusion or WM changes together have shown superior power than that using conventional single modality domain (e.g., brain volume measurement alone) in diagnosis of AD. However, to our knowledge, rarely did MRI studies evaluate GM and WM differences between AD and FTD jointly.

In this study we present a new approach to compare structural, perfusion, and diffusion alterations between AD and FTD using T1-weighted high-resolution structural MRI, arterial spin-labeled perfusion MRI (ASL-MRI), and DTI. The objective was to determine if a joint evaluation of multimodal MRI could provide a biomarker for a differential diagnosis between AD and FTD.

2. Subjects and Methods

2.1. Subjects. Twenty AD patients (mean age and standard deviation: 63.1 ± 6.9 yrs) with a Mini-Mental State Examination (MMSE) [35] score of on average 21.9 ± 5.6, 20 patients with FTD (age: 60.7 ± 9.9 yrs; MMSE: 23.1 ± 5.6) and 21 cognitively normal (CN) subjects (age: 61.9 ± 9.6 yrs; MMSE: 29.6 ± 0.5) were included in this cross-sectional MRI study. A summary of the subject demographics and relevant clinical information are listed in Table 1. The patients with FTD and AD were recruited from the Memory and Aging Center of the University of California, San Francisco. All patients were diagnosed based on information obtained from an extensive clinical history and physical examination. The MR images were used to rule out other major neuropathologies such as tumors, strokes, or inflammation but not to diagnose dementia. The subjects were included in the study if they were between 30–80 years old and without history of brain trauma, brain tumor, stroke, epilepsy, alcoholism, psychiatric illness, or other systemic diseases that affect brain function. FTD was diagnosed according to the consensus criteria established by Neary et al. [36]. All FTD patients were diagnosed with the frontal variant subtype, two of which had combined motor neuron-related symptoms. AD patients were diagnosed according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) [37]. All subjects received a standard battery of neuropsychological tests including assessment of global cognitive impairment using MMSE and global functional impairment using the Clinical Dementia Rating (CDR) scale [38]. Fifty-seven out of all 61subjects had blood drawn for APOE genotyping. Reliable information about the age of onset of symptoms was available from 12 out of 20 AD patients and from all 20 FTD patients. Because it is not unusual for subjects in this age group to have WM signal hyperintensities (WMSH) on MRI, subjects with WMSH were included. An experienced radiologist reviewed all MRI data, and the scores of WMSH were used as covariates in the analysis. The severity of WMSH was classified as mild (deep white matter lesions ≤3 mm, and periventricular hyperintensities <5 mm thickness), moderate (deep white matter lesions between 4–10 mm, or periventricular hyperintensities between 6–10 mm thickness), or severe (deep white matter lesions >10 mm, or periventricular hyperintensities >10 mm thickness), according to the Scheltens' rating scale [39]. All subjects or their legal guardians gave written informed consent before participating in the study, which was approved by the Committees of Human Research at the University of California and the VA Medical Center at San Francisco.

2.2. Data Acquisition. All scans were preformed on a 4 Tesla (Bruker/Siemens) MRI system with a single housing birdcage transmit and 8-channel receive coil. T1-weighted images were obtained using a 3D volumetric magnetization prepared rapid gradient echo (MPRAGE) sequence with TR/TE/TI = 2300/3/950 ms, 7-degree flip angle, 1.0 × 1.0 × 1.0 mm^3 resolution, 157 continuous sagittal slices. In addition, FLAIR (fluid attenuated inversion recovery) images with timing TR/TE/TI = 5000/355/1900 ms were acquired to facilitate the evaluation of WMSH. Perfusion images were acquired using a continuous arterial spin labeling (cASL) sequence [40] with a single-shot echo-planar imaging (EPI) part to map the perfusion signal. cASL-MRI was performed with TR/TE = 5200/9 ms with 2-second long labeling pulses and a one-second postlabeling delay. Sixteen slices with 5 mm slice thickness and 1.2 mm interslice gap, 3.75 × 3.75 mm^2 in-plane resolution were acquired. DTI was acquired based on a dual spin-echo EPI sequence supplemented with two-fold parallel imaging acceleration (GRAPPA) [41] to reduce susceptibility distortions. Other imaging parameters were TR/TE = 6000/77 ms, field of view 256 × 224 cm, 128 × 112 matrix size, yielding 2 × 2 mm^2 in-plane resolution, 40 slices each 3 mm thick. One reference image ($b = 0$) and six diffusion-weighted images ($b = 800$ s/mm^2, along 6 noncollinear directions) were acquired.

2.3. Data Analyses. The assessment of brain volume changes were performed using SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) based on an "optimized" VBM procedure described by Ashburner and Friston [42]. The procedure included several steps. (1) Tissue segmentation: An expectation maximization segmentation (EMS)

Table 1: Demographic and clinical data summary.

	Normal	AD	FTD
Number of subjects	21	20	20
Age (years)	61.9 ± 9.6	63.1 ± 6.9	60.7 ± 9.9
Age range (years)	33~73	51~73	32~74
Sex (M : F)	11 : 10	11 : 9	13 : 7
Years of Education (years)	16.8 ± 2.5	15.7 ± 3.0	16.2 ± 3.2
MMSE	29.6 ± 0.5	21.9 ± 5.6	23.1 ± 5.6
CDR	0	0.8 ± 0.3	1.2 ± 0.6
APOE-ε4 (carriers: non-carriers)	3 : 17[a]	14 : 5[a]	7 : 11[b]
Age of onset (years)	NA	56.2 ± 5.7[c]	55.1 ± 9.9
Symptom duration (years)	NA	3.25 ± 1.6[c]	5.3 ± 4.9
WMSH (severe : moderate : mild)	3 : 2 : 16	1 : 4 : 15	2 : 2 : 16

[a] value for one subject is missing.
[b] value for two subjects is missing.
[c] value for 8 subjects is missing.
WMSH = white matter signal hyperintensities.

algorithm [43] was applied to obtain probabilistic maps of GM, WM, and CSF from the T1-weighted MRI data. (2) Spatial normalization: first, customized GM and WM prior images were created by transforming GM and WM probabilistic maps of all subjects into the standard MNI (Montreal Neurological Institute) space [42]. The segmented GM probabilistic maps in their native spaces were then spatially normalized again to the customized GM prior image using a nonlinear transformation with 16 interactions. (3) Jacobian modulation: the spatially normalized GM images were multiplied by the Jacobian determinants of the transformation (modulation) to obtain volume differences. (4) Smoothing: the modulated GM images were smoothed with an 8 mm full-width-at-half-maximum (FWHM) isotropic Gaussian kernel to reduce variations from misregistrations and to perform voxelwise image statistics.

The assessment of perfusion changes included the following steps. (1) Cerebral blood flow (CBF) image calculation: a perfusion weighted (PWI) image was created by pairwise subtraction of coregistered labeled from unlabeled ASL images. The PWI images were then scaled to obtain a quantitative CBF image based on a single compartment perfusion model [44]. (2) Intermodality coregistration: ALS perfusion and the corresponding T2- and T1-weighted image were coregistered using an affine alignment to establish anatomical correspondence between CBF and segmented GM images and to compute partial volume-corrected CBF in GM. (3) Partial volume correction: partial volume correction of CBF images was performed by rescaling CBF in each voxel proportionately to the GM and WM content, assuming that perfusion of white matter is only 25% of that of GM, as detailed by Du et al. [16]. (4) Spatial normalization: the partial volume-corrected CBF images in GM were spatially normalized to the customized GM prior image that was created from the previous processing of VBM, using the same nonlinear transformation and smoothing parameters.

The assessment of white matter integrity was performed based on DTI and computation of fractional anisotropy (FA) maps, using the dTV.II software [45] and Volume-one software package (URL: http://www.ut-radiology.umin.jp/people/masutani/dTV.htm), supplemented by automatic image denoising and eddy-current corrections. SPM8 software was used for voxelwise analysis of the FA images as outlined in detail elsewhere [25]. In brief, the FA images in the native space underwent the following procedures. (1) Creation of a customized FA template: an averaged FA image was first created from all subjects' FA images that initially transformed to the EPI-derived MNI template in SPM. This averaged FA image was further co-registered to the WM prior image using affine alignment and manual adjustment [46] to archive a customized FA template that accurately correspond to the anatomical information in the WM prior image. (2) Spatial normalization and smoothing: the FA image of each subject in the native space was recursively normalized to the customized FA template using the same nonlinear transformation and smoothing parameters that were applied in the previous VBM procedure.

2.4. Statistics. Paired group differences were evaluated voxel-by-voxel using a general linear model with diagnosis as main contrast and age, sex, and the years of education as covariates. In addition, total intracranial volume (TIV) was used as a covariate in test for volume differences and global mean perfusion was used as a covariate in test for perfusion differences. A threshold of at least 85% GM volume fraction in voxels was applied to restrict GM volume and perfusion analyses to voxels containing predominantly GM. Similarly, a threshold of at least 80% WM volume fraction was applied to restrict FA analysis to voxels containing predominantly WM. The statistical significance for main effects of abnormalities, which was only the prestep for joint analyses, was set to an uncorrected voxel-level P value of .001.

To test if FTD and AD differ with respect to WM and GM abnormalities, we started by determining the number of voxels representing "abnormal" values for each patient and each MRI modality. This was conducted by recording voxelwise differences between an individual's image value (GM volume, GM perfusion, or WM FA) and the respective mean

value of the control group. The difference was then normalized to the standard error of the control mean value to express abnormality as a T-score [47]. High T-scores represent high abnormalities beyond the normal ranges. Next, the number of "abnormal" voxels above a T-score threshold was recorded and normalized to the total number of GM or WM voxels to account for each patient and each modality to quantify the extent of brain abnormality. We termed the number of normalized "abnormal" voxels *load*. Differences in *loads* between AD and FTD patients were evaluated statistically via permutation test with 1000-fold completely random resampling of the diagnostic labels (AD and FTD), using the R project (http://www.r-project.org/). The statistics on the *loads* were evaluated for T-score values ranging from -2 (indicating a mild abnormality level) to -6 (indicating a severe abnormality level). Using the permutation test, we also assessed whether the *load* from a specific MRI modality (e.g., WM FA) relative to another (e.g., GM loss) modality, termed conditional *load*, differed within and across the diagnosis groups. The level of significance for permutation tests was set at $P = .05$.

3. Results

3.1. Demographic Clinical Data. As shown in Table 1, there were no significant differences in age, sex, years of education, and severity of WMSH between each patient group (AD or FTD) and controls. AD and FTD patients had significant lower MMSE ($P < .001$, by ANOVA test) scores compared with controls, as expected. Furthermore, AD patients had a greater proportion of APOE-ε4 carriers than CN ($P = .04$ by χ^2 test) but the FTD patients did not ($P = .35$ by χ^2 test) when compared to CN. AD and FTD patients did not differ significantly with respect to age, sex, years of education, MMSE, CDR, age of onset, symptom duration, and severity of WMSH. To avoid further reductions in sample size due to the missing values, we did not perform the joint analysis including the age of onset or the APOE-ε4 genotyping as covariates across all MRI modalities, although symptom duration was associated with GM volume loss within the FTD group.

3.2. Spatial Distribution of MRI Abnormalities in AD and FTD Compared to CN. Figures 1(a)–1(d) depict the regional distributions of GM loss (in warm color), GM hypoperfusion (in green color), and reduced WM FA (in blue color) in AD and FTD, compared to CN, respectively, as well as the direct comparisons between AD and FTD, based on voxel-wise tests prior to the joint analysis. For better visualization of regional relations, the various distributions are overlaid on each other in Figure 1(d).

Compared to CN, AD patients showed widespread GM loss in bilateral parietal and temporal lobes. The left temporoparietal lobes had the most prominent GM loss. Other regions of GM loss in AD included the posterior cingulate gyrus, thalamus, and bilateral occipital lobes. Compared to CN, FTD patients showed GM loss predominantly in bilateral frontal and temporal lobes. The right frontoinsular gyrus showed the most prominent GM loss. Other regions of GM loss included limbic lobes such as bilateral anterior cingulate gyrus, uncus, subcortical nuclei including the bilateral caudate and the thalamus, and the lateral parietal lobes. Comparing FTD and AD directly, patients with AD showed more GM loss than FTD in bilateral occipital gyrus, left precuneus whereas FTD showed more GM loss in bilateral frontal lobes, including the orbital gyrus, inferior and medial frontal gyrus, and anterior cingulate gyrus.

Regarding perfusion, AD patients showed reductions relative to CN in bilateral temporoparietal lobes, including superior temporal gyrus, precuneus and posterior cingulate gyrus. Hypoperfusion in AD was most pronounced in the left temporal gyrus. Compared to CN, FTD patients showed reduced perfusion in bilateral frontal lobes, including inferior, medial, and superior frontal gyrus, anterior cingulate gyrus, and thalamus. Hypoperfusion in FTD was most pronounced in the right inferior frontal gyrus. Compared to FTD, AD patients showed significant hypoperfusion in the left superior temporal gyrus, claustrum; whereas compared to AD, FTD patients had significant hypoperfusion in right superior, middle, and bilateral medial frontal gyrus.

Regarding WM FA, AD patients had FA reductions relative to CN bilaterally in WM regions in parietal, temporal, and some frontal lobe regions. The periventricular deep WM, posterior corpus callosum and the left posterior cingulum exhibited the most prominent FA reductions. In contrast, FTD patients had widespread FA reductions relative to CN bilaterally in frontal and temporal lobes, and the anterior corpus callosum, and bilateral anterior cingulum were prominently involved. Compared to AD, FTD patients had lower FA values bilaterally in frontal deep WM, anterior corpus callosum and bilateral anterior cingulum, whereas AD patients showed no region with significantly lower FA values when compared to the FTD group.

3.3. Differences between AD and FTD in GM Volume, Perfusion, or WM Damage. We first tested if *loads* differed between AD and FTD. Figure 2(a) displays the *loads* of GM loss (2A-I), GM hypoperfusion (2A-II), and WM FA (2A-III) reduction, respectively, in AD and FTD over a range of T-score levels (deviation from normal). The significance of differences in the *loads* between AD and FTD as a function of T-scores is plotted in Figure 2(b), and separately for each type (GM Vol, GM Perf, WM FA) of *load*. Note, the *loads* and the *P* values are plotted on a logarithmic scale and increasing negative T-scores indicate increasing deviation from normal values. This demonstrates that there is a significantly greater *load* of WM FA reduction in FTD compared to AD at mild abnormality levels (up to T-scores of about -2.3) while the significance gradually vanishes at more severe abnormality levels. There is a trend ($P = .07$ to $.1$) towards more GM loss in FTD compared to AD at moderate to severe abnormality levels (T-scores < -4). However, the *load* of GM hypoperfusion does not differ significantly between AD and FTD across the range of T-scores.

3.4. Joint Assessment of GM Volume, Perfusion, and WM FA Damages in AD or FTD. Figure 3 shows the conditional *loads* (the *load* in one MRI modality relative to another) over

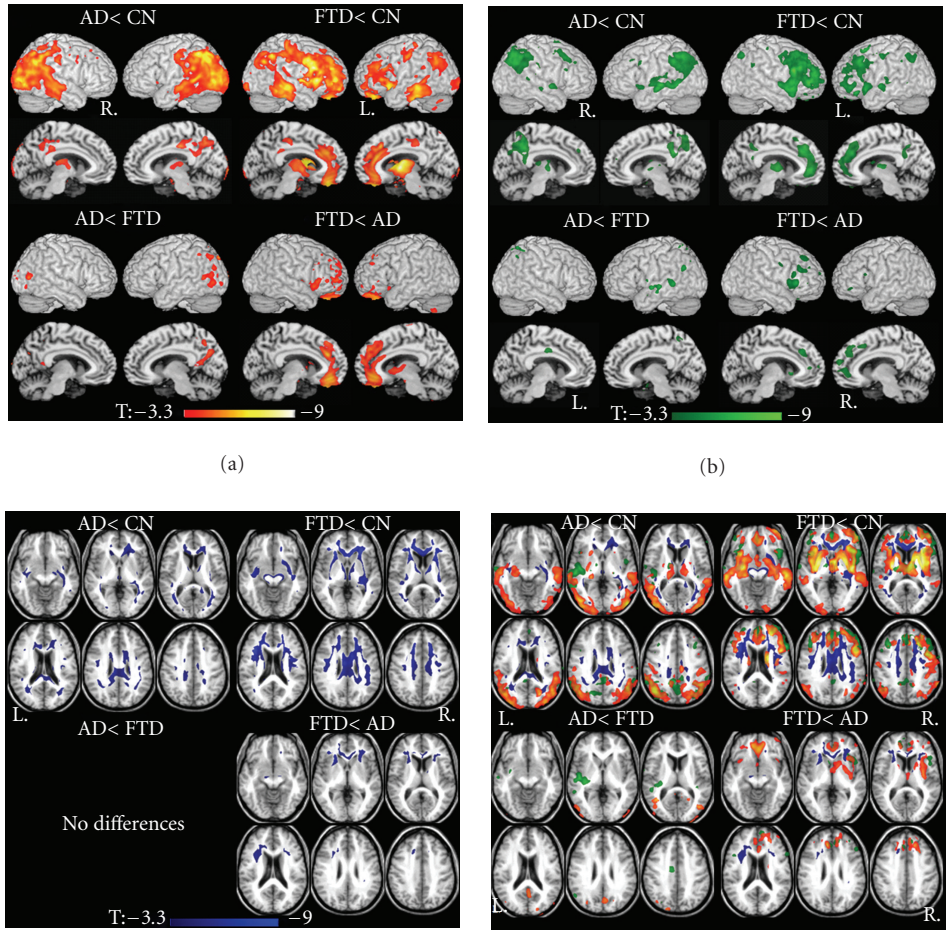

Figure 1: Significance maps of systematic brain abnormalities in FTD and AD patients relative to control subjects (AD < *CN* and FTD < *CN*), and direct comparisons between AD and FTD (AD < *FTD* and FTD < *AD*). (a) GM loss (warm color) and (b) GM hypoperfusion (green color) overlaid on a surface rendered brain template. (c) Reduced WM FA (blue color) in AD or FTD overlaid on an axial brain template. (d) Overlay of the abnormal distributions together. The significant threshold was $P_{\text{uncorrected}} < .001$ for all voxelwise tests. Color scales indicated ranges of significance (T-scores) upon the P threshold.

a range of abnormality levels (T-scores) separately for AD (Figure 3(a)) and for FTD (Figure 3(b)). Note, the plots in Figures 3(a) and 3(b) are on a logarithmic scale. Accordingly, a conditional *load* larger than 1 indicates that the *load* of a particular modality is greater than the *load* of the reference modality, whereas a value smaller than 1 indicates that the *load* of the reference is greater than particular modality. The extent to which differences in one *load* (on particular modality) relative to another (on reference modality) are above chance is depicted in Figures 3(c) and 3(d) for AD and FTD, respectively. Note again, *P*-values in Figures 3(c) and 3(d) are plotted on a logarithmic scale of the base-10 logarithm. The *loads* of GM volume loss relative to GM hypoperfusion as a function of severity (T-scores) are displayed in brown lines, reduced WM FA relative to GM hypoperfusion in purple lines, and GM volume loss relative to WM FA in red lines.

Figures 3(a) and 3(c) show that in AD, the *loads* of the different modalities are not significantly different compared to each other across the level of abnormalities (T-scores) levels. In FTD, by contrast (Figures 3(b) and 3(d)), the *load* of GM volume loss as well as the *load* of reduced WM FA are each significantly greater relative to the *load* of GM hypoperfusion at lower levels of abnormality (up to T-scores = −4). However, as abnormality increases (T-scores < −4), the significance of the difference in the *load* of GM volume loss or WM FA reductions relative to GM hypoperfusion gradually disappears. There are no significant differences between the *load* of GM loss and the *load* of WM FA reductions across all ranges of abnormalities levels in FTD patients.

3.5. Differences between AD and FTD in Joint Modalities. Finally, we also tested if AD and FTD differed with regard to their conditional *loads* from a particular modality relative to that from reference modality but found no significant difference between the groups ($P > .1$) across all ranges of the abnormalities (T-score) levels.

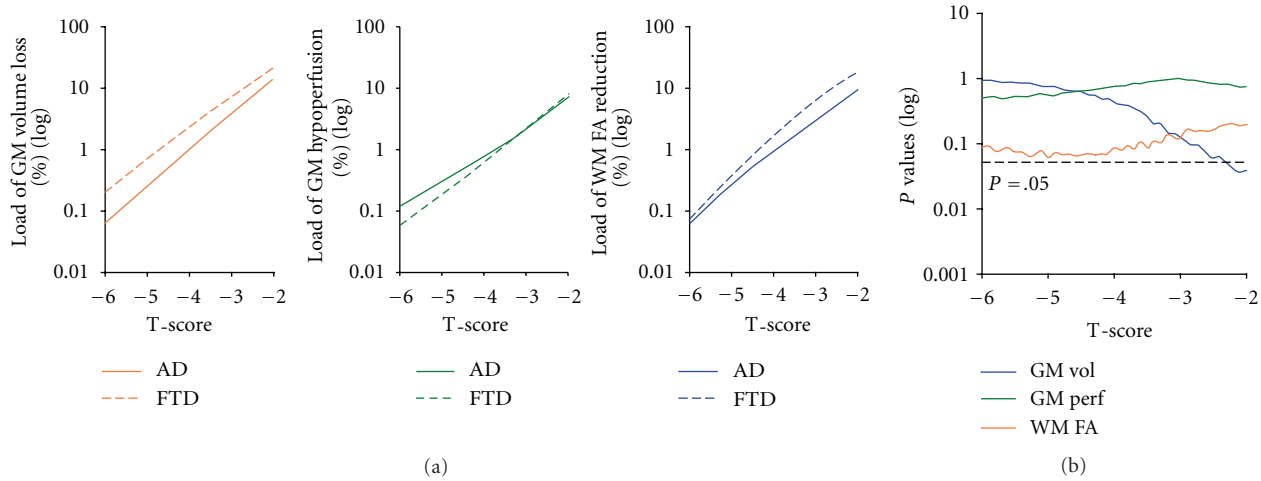

FIGURE 2: Differences in *loads* of GM loss (orange lines), GM hypoperfusion (green lines), and reduced WM FA (blue lines) between FTD and AD across a range of T-scores and significant levels. (a) Mean *loads* of GM loss (I), GM hypoperfusion (II), and reduced WM FA (III) along a range of abnormality levels (T-scores) in AD (solid lines) and FTD (dash lines) patients. (b) Variations in significance of the *load* differences between FTD and AD as a function of T-scores. Note, the vertical axis of all plots in (a, b) uses a base-10 logarithmic scale. The horizontal dotted line indicates $P = .05$ significance.

4. Discussion

We have two main findings. First, FTD patients exhibited more WM damage than AD patients at mild abnormality levels (i.e., small T-scores). The difference vanished gradually for more severe abnormality levels (i.e., larger T-scores). Second, FTD patients had greater GM loss and WM damage relative to GM hypoperfusion, although the differences of damage between modalities gradually vanished with increasing levels of abnormality. In contrast to FTD, AD patients had equal damage in all three modalities, irrespective of the level of abnormality. Taken together, the results suggest that FTD and AD differ in amount of WM and GM structural and functional damages, in addition to their characteristic regional patterns of brain alterations than healthy controls.

Our first main finding of greater WM damage in FTD than AD at mild levels of abnormality suggests that WM may be more sensitive to the pathology of FTD than to the pathology of AD at their early disease stages. The hallmark of FTD is tauopathies or ubiquitin immunoreactive inclusions by the presences of neuronal and glial inclusions [48, 49] in gray and white matter. WM pathologies in FTD have been reported with astrocytic gliosis and oligodendroglial apoptosis, which may ultimately result in axonal degeneration [50–53]. A recent study [54] also reported that oligodendroglial pathology can be predominant in FTD despite severe GM damage. On the other hand, degeneration of frontostriatal networks, which is a characteristic feature of FTD [26], suggests that WM denegation in anterior brain could also be a primary FTD pathology. In contrast to FTD, the hallmark of AD is the deposition of amyloid plaques and neurofibrillary tangles that are associated with loss of neurons and synapses [55, 56]. WM pathology in AD has been suggested to occur secondarily to GM pathology and may include reduction of myelin, axons, and oligodendrocytes [57, 58]. A vascular origin of WM pathology in AD has also been suggested [59]. However, WM pathologies in AD are usually considered mild and potentially reversible [60]. Interestingly, our data showed that differences in WM damage between AD and FTD disappeared at higher abnormality levels. It is possible to suggest that AD and FTD undergo similar WM pathologies at a severe brain damage level. One explanation is that severe WM abnormalities are an outcome of irreversible vascular damage, such as appearance of WMSH, which affects AD and FTD similarly [61]. Another possible explanation is that WM changes resulting from degeneration of corticocortical connections may inevitably occur in both AD and FTD.

In addition, we found a trend of more GM loss in FTD than AD at moderate to severe levels of abnormality. This observation is consistent with histopathological studies showing substantial loss of spindle neurons in the cortex in FTD but not in AD [62]. The finding is also in agreement with several MRI studies [29, 30, 63, 64] which compared FTD and AD directly, showing regionally greater GM loss in FTD when compared to AD but no greater GM loss in AD when compared to FTD. Similarly, several longitudinal studies reported greater rates of GM atrophy in FTD when compared to AD [65, 66]. However, some MRI studies [26, 67] reported greater GM loss in AD compared to FTD, though the extent of the GM losses varied regionally. The discrepancy may result from notorious difficulties to adequately match impairment severity in FTD and AD given substantial differences in symptomatology, although our current study attempted to match the severities (such as the measures of CDR, MMSE) of AD and FTD groups as closely as possible.

The second major finding that GM loss and WM damage exceed perfusion damage in FTD may be explained by loss of brain tissue other than neurons contributing to GM atrophy in FTD. Information about differential loss of various types of brain tissue may be particularly relevant in earlier

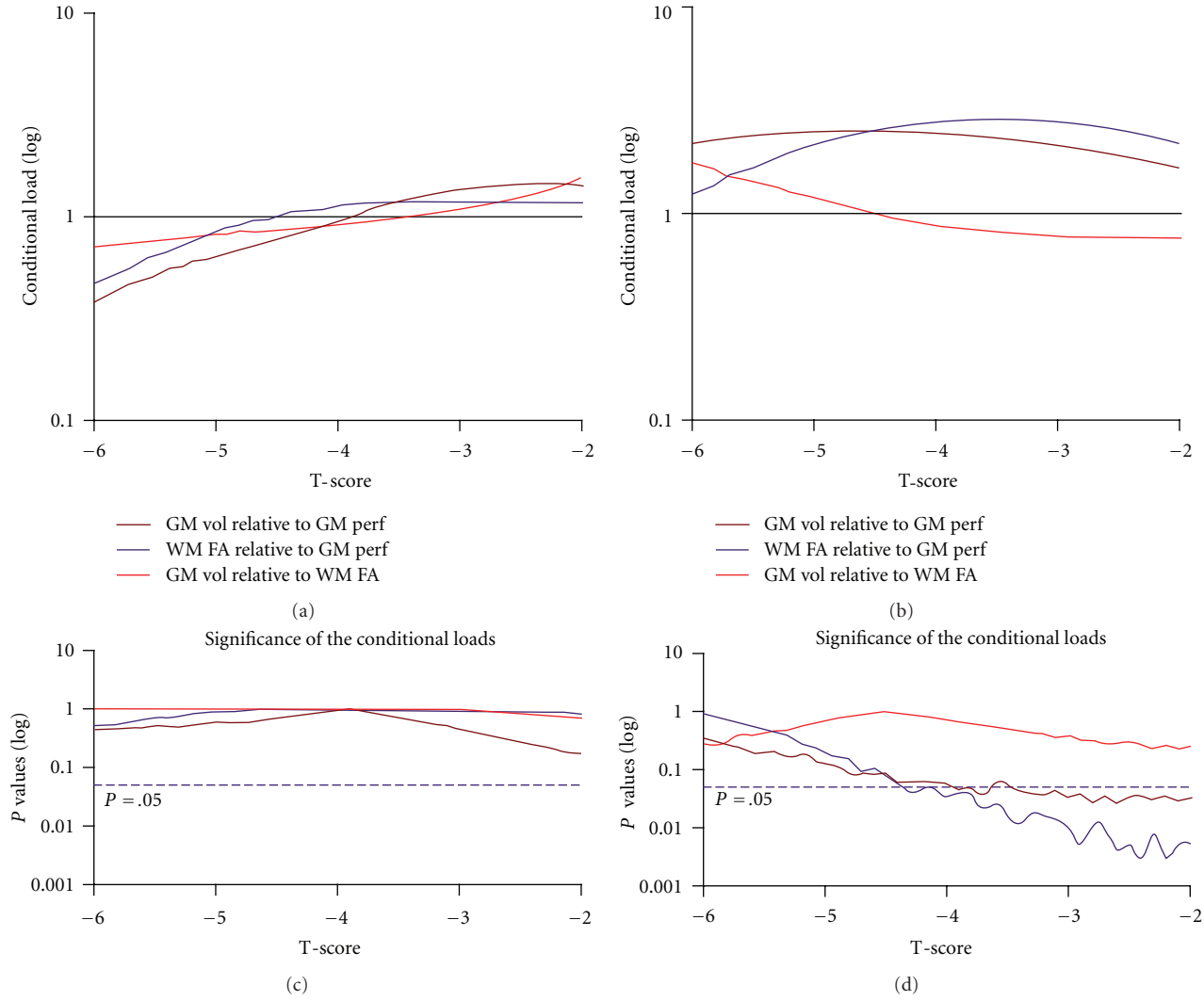

Figure 3: Conditional *load* of one modality relative to another as a function of the level of abnormality (T-score) for AD (a) and FTD (b). Note, vertical axes in (a) and (b) are plotted on a logarithmic scale (base-10) where a value larger than one indicates the *load* from a particular modality was higher than the *load* from reference modality. Conditional *load* lower than 1 indicated opposite relations between modalities. The extent to which differences in the conditional *loads* among modalities are above chance ($P < .05$) across the level of abnormality is indicated in plot (c) for AD and in plot (d) for FTD. Note, P values in (c) and (d) are also plotted on a logarithmic scale. The horizontal dash lines indicate $P = .05$ significance.

and potentially reversible stages of the disease. Our findings are supported by several histopathological studies demonstrating that the earliest cellular changes in FTD occur in astrocytes without neuron loss and that neuronal damage becomes more prominent in later stages of the disease [50, 68]. Therefore, at mild abnormality levels of FTD, the surviving neurons may still function normally as reflected by the normal levels of perfusion. Our findings are consistent with other studies carried out in our laboratory [69] and on different cohorts of FTD and AD patients which have implied dissociation between GM atrophy and perfusion. Specifically, these studies indicated that FTD was associated with greater GM atrophy in absence of significant reduction of perfusion. These results contrast the findings in AD, where the abnormalities across the three modalities were all similar.

Our voxelwise analysis demonstrates the regional patterns of GM loss, hypoperfusion, and reduced WM FA in this sample of AD and FTD patients are consistent with the disease-specific patterns that have been reported in previous MRI studies of brain structure [14, 70], perfusion [5, 6], and DTI [25, 71–73]. Furthermore, the similarity between the regional patterns of GM and WM alterations which appear in the same lobe together for each disease, implies that WM degradation mirrors that of GM damages and is consistent with previous reports [74]. Taken together, these results can demonstrate the well-documented pathological bases of AD [56] and FTD [68].

The new joint analysis approach allows for the investigation of different abnormalities across multiple MRI modalities such as structural, perfusion and diffusion MRI between groups. The approach can be used to test whether groups differ with respect to a single MRI measure as well as multiple measures. The approach was augmented by nonparametric statistical tests via permutations and carried out across

a range of T-scores to reduce measurement bias toward the various brain conditions. The concept can be expanded in principle to conduct a voxelwise joint analysis of multiple MRI measures to determine regional variations in GM loss and hypoperfusion. Other statistical methods for joint analyses of multiple image modalities such as joint independent component analysis (jICA) [75] may provide alternative solutions. The findings with multimodal MRI could potentially be useful to improve the design of AD and FTD clinical trials involving MRI. First, correlations across the MRI measures, potentially boosting sensitivity and specificity, could lead to reduced sample sizes. Second, the finding revealed that FTD presents more white matter involvement relative to AD, thus providing a new biological feature of FTD, and could be used to relax the need to match disease severity in studies recruiting AD and FTD patients.

Limitations of the current study include a small sample size that was reliant on clinical diagnoses which were not sufficiently confirmed by autopsies. Therefore, confidence in the generalization of the results is limited, and potential misdiagnosis of patients may have resulted in spurious findings. Furthermore, we cannot completely rule out that other factors than disease etiology, such as genetic profiles, duration of symptoms, and cardiovascular conditions, which contributed to MRI differences between the patients and thus contaminated the findings. Second, diffusion encoding was limited to the minimum of 6 directions at the time this protocol was initiated, although it is known that many more encoding directions improve the characterization of diffusion such as fewer ambiguities in regions of crossing fibers and better spatial invariance of the noise pattern. Therefore, fiber crossings and the DTI noise pattern may potentially mimic regional differences in FA between these groups. Third, we ignored relationships between brain regions in our joint analysis and therefore under-utilized information from the multimodal MRI data. A more powerful statistical framework [76] that takes spatial relations between multivariate measures into account may provide more power. Finally, the data was artificially scaled to provide a uniform resolution for all MRI modalities, which may have induced a spatial bias as well as altered selectively the sensitivity of each modality. Other approaches that do not require a uniform resolution but can operate on variable spatial scales, such as information theoretic formalisms [77], may lead to differences in results.

In conclusion, our findings suggest that FTD and AD differ regarding their impacts on WM and GM structural and functional abnormalities, in addition to differences between their characteristic regional patterns of brain alterations. Furthermore, the joint assessment of multimodal MRI measures employed in this study has potential value to improve the differential diagnosis between FTD and AD.

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Research Article

Morphological Factor Estimation via High-Dimensional Reduction: Prediction of MCI Conversion to Probable AD

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We propose a novel morphological factor estimate from structural MRI for disease state evaluation. We tested this methodology in the context of Alzheimer's disease (AD) with 349 subjects. The method consisted in (a) creating a reference MRI feature eigenspace using intensity and local volume change data from 149 healthy, young subjects; (b) projecting MRI data from 75 probable AD, 76 controls (CTRL), and 49 Mild Cognitive Impairment (MCI) in that space; (c) extracting high-dimensional discriminant functions; (d) calculating a single morphological factor based on various models. We used this methodology in leave-one-out experiments to (1) confirm the superiority of an inverse-squared model over other approaches; (2) obtain accuracy estimates for the discrimination of probable AD from CTRL (90%) and the prediction of conversion of MCI subjects to probable AD (79.4%).

1. Introduction

A growing body of literature relates the use of machine learning methods to build classification functions from features of interest extracted from medical imaging data (e.g., magnetic resonance images (MRI), positron emission tomography). We focus specifically on applications within the context of aid to clinical diagnostic in Alzheimer's disease (AD) and/or the prediction of future clinical status for individuals with Mild Cognitive Impairment (MCI), a putative precursor to AD [1–6]. These techniques have in common the reduction of large, high-dimensional image vectors into smaller features spaces and the identification of a low-dimensional discriminating function. Authors have reported attempts to further simplify the discriminating function by calculating a single, quantitative scalar measure, for example, the structural abnormality index score [7], the structural-functional biomarker score [8], and the disease evaluation factor [9]. While the first two rely on support vector machine analysis of a feature space composed of grey matter concentration patterns, the latter relies on linear kernel approaches to data reduction and classification of MRI appearance, defined as the combination of T1-weighted intensity and local volume shape characteristics for all voxels within a volume of interest. Using the same features of interest as described in [9], we propose a different morphological factor formulation, extensible to other modalities and to other sources of data. We derive the formulation and estimate its efficiency within the context of aid to diagnostic in probable AD by verifying the hypothesis that it accurately describes current and future clinical status.

2. Methods

2.1. Subjects. A total of 349 subjects were included in this study, with ethics approval obtained from each institution represented.

The first cohort, or *reference group*, consisted in 149 young, neurologically healthy individuals obtained with permission from the ICBM database [10], whose scans were used to create a reference feature space of image data.

The second cohort, or *AD test group*, consisted in 150 subjects: 75 patients with a diagnosis of probable AD (*AD group*) and 75 age-matched controls (*CTRL group*) without neurological or neuropsychological deficit. The probable AD subjects were individuals with mild to moderate probable

AD [11] recruited among outpatients seen at the IRCCS Fatebenefratelli (Brescia, Italy) between November 2002 and January 2005. CTRL subjects were taken from an ongoing study of the structural features of normal aging at the same center [12]. All subjects were followed a minimum of 3 years after inclusion; this longitudinal clinical evaluation constitutes our reference diagnostic.

The third cohort, or *MCI test group*, consisted in 49 MCI subjects taken from a prospective project on the natural history of MCI, carried out at the IRCCS Fatebenefratelli. The project was aimed to study the natural history of nondemented persons with apparently primary cognitive deficits, that is, deficits not due to psychic (anxiety, depression, etc.) or physical (hypothyroidism, vit. B12 and folate deficiency, uncontrolled heart disease, uncontrolled diabetes, etc.) conditions. Patients were rated with a series of standardized diagnostic and severity instruments, including the Mini-Mental State Examination (MMSE; [13]). In addition, patients underwent diagnostic MRI and laboratory testing to rule out other causes of cognitive impairment. These inclusion and exclusion criteria for MCI were based on previous seminal studies [14–16]. Amnestic or nonamnestic, single or multiple domain MCIs were included in the study.

All MCI patients underwent a yearly follow-up visit, consisting of complete clinical and neuropsychological examination, from 1 to 4 years after enrolment. In those individuals that converted to dementia, status was ascertained according to clinical diagnostic criteria for AD [11], subcortical vascular dementia [17], dementia with Lewy bodies [18], and frontotemporal dementia [19]. Within the larger prospective cohort of 100 MCI patients enrolled from April 2002 to December 2006, we have selected patients retrospectively for this study based on their (a) having been followed clinically a minimum of 48 months after their baseline MR scan; (b) having remained either *stable* (MCI-S group; $N = 29$) or *progressed* to probable AD (MCI-P group; $N = 20$; mean progression 1.5 yrs; SD 0.7 yrs). The 48-month longitudinal clinical evaluation constitutes our reference diagnostic.

Data for the last subject (*validation subject*) was obtained with permission from the pilot, multicentric European ADNI project [20] (E-ADNI). It consisted in a healthy volunteer that acted as human quality control phantoms and that was scanned three times at IRCCS Fatebenefratelli (scan; repeat scan, same session; rescan) on the same day.

Ethics Committees approved the study, and informed consent was obtained from all participants.

2.2. Data. The ICBM subjects from the reference group were scanned in Montreal, Canada on a Philips Gyroscan 1.5T scanner (Best, Netherlands) using a T1-weighted fast gradient echo sequence (sagittal acquisition, TR = 18 ms, TE = 10 ms, 1 mm × 1 mm × 1 mm voxels, flip angle 30°). MRI data for probable AD, CTRL, MCI, and E-ADNI subjects were acquired at the IRCCS Fatebenefratelli on a single Philips Gyroscan 1.0T scanner (Best, Netherlands) using a T1-weighted fast field echo sequence (sagittal acquisition, TR = 25 ms, TE = 6.9 ms, 1 mm × 1 mm × 1, 3 mm voxels).

2.3. Data Processing. We provide an overview of the automated image processing methodology, which follows essentially the steps outlined in Duchesne et al. with some modifications [3]. Images from all subjects were processed in an identical fashion using a publicly available toolkit (MINC: http://www.bic.mni.mcgill.ca/ServicesSoftware/HomePage). Processing included intensity inhomogeneity correction [21], nonlocal means denoising [22], intensity scaling, global and linear registration [23], extraction of a predetermined volume of interest centered on the medial temporal lobes, nonlinear registration within the volume of interest towards a common reference target [24], and computation of log-determinants of the Jacobian of the deformation field [25].

2.4. Data Reduction and Feature Selection. The first data reduction step was to construct a feature space based on the $N = 149$ subjects from the ICBM *reference group*. To this end, we used Principal Components Analysis (PCA) of two high-dimensional image vectors within a volume of interest centered on the medial temporal lobe to generate a low-dimensional feature space for classification: (1) the T1-weighted MRI intensity within the volume of interest, transformed into z-score; and (2) log-determinants within the volume of interest. With PCA we moved from a massive amount of data ($2 \times 149 \times 4E10^5$ voxels) to a lower subspace model of maximum N-1 dimensionality, further restricted by using only the first k eigenvectors λ that contribute up to a given threshold r in the description of the total variance of the system:

$$r_k = \frac{\lambda_k}{\sum_{j=1}^{p} \lambda_j}. \quad (1)$$

Once the reference eigenspace was formed, the *reference group* data was no longer used.

We then proceeded by projecting rasterized vectors of intensity and local volume changes for subjects in the *AD* and *MCI test groups* into the reference space. The distribution of eigencoordinates along any principal component for a given population was assessed via quantile plots and Shapiro-Wilke statistics for normal distribution. Following the projection, we used a system of supervised linear classifiers to identify the hyperplane that best separated the groups under study (e.g., CTRL versus probable AD; MCI-S versus MCI-P). To this end, the data was first normalized to guard against variables with larger variance that might otherwise dominate the classification. We employed forward stepwise regression analysis via Wilk's λ method to select the set of discriminating variables $\{\lambda_F\}$, with $F \ll N - 1$, forming the discriminating hyperplane.

2.5. Comparative Morphological Factor Construction. The morphological factor is based on the concept of distance along the restricted set of eigenvectors $\{\lambda_F\}$. In the image-based feature space, this distance d can be calculated in a number of different fashions.

2.5.1. Manhattan Distance. We propose initially the signed difference between subject eigencoordinates along the eigenvector λ_F and the mean of the CTRL distribution for that

eigenvector, denoted $\overline{m}_{\text{CTRL}}$; as this distance increases the likelihood of belonging to the CTRL group decreases:

$$d_i^{\lambda_F} = x_i^{\lambda_F} - \overline{m}_{\text{CTRL}}^{\lambda_F}. \tag{2}$$

2.5.2. Euclidean Distance. We propose the Euclidean distance between position p_i of each subject s_i and both CTRL and probable AD means along the restricted set of eigenvectors $\{\lambda_F\}$ in all F directions, with $F \ll N - 1$. As the distance to one center decreases, the distance to the second should increase. In (3) we demonstrate the distance to the mean of the probable AD group:

$$d_{s_i \to \text{CM}_{\text{AD}}} = \sqrt{\sum_F \left(p_i^f - \overline{m}_{\text{AD}}^f\right)^2}. \tag{3}$$

2.5.3. Weighted Distance. It is possible to weigh each eigenvector by an associated measure of significance, for example, Wilk's λ from the stepwise regression analysis [9] or a factor derived from univariate t-tests. While the Wilk's λ is trivially obtained from the regression analysis, an univariate weight such as the Koikkalainen factor formulation [26] entails performing a t-test comparing the group eigencoordinate distributions (e.g., CTRL versus probable AD; MCI-S versus MCI-P) for each eigenvector of the restricted set, resulting in the P-value $p(\lambda_F)$ for that distribution; from these P-values the significance weight S_F is calculated,

$$S_F = \frac{\ln \min[p(\lambda_F), 0.05] - \ln 0.05}{\ln 0.000001 - \ln 0.05}. \tag{4}$$

The significance increases as the differences between the CTRL and AD groups grow and reaches zero when there is no statistically significant difference (at the $P = .05$ level) between both distributions.

The resulting weighted distance D_i combines the aforementioned distances (Manhattan, Euclidean) with a weight S_F (either Wilk's λ or Koikkalainen factor) over all eigenvectors F from the restricted set $\{\lambda_F\}$ as follows:

$$D_i = \frac{\sum_i^{\lambda_F} S_F d_i^{\lambda_F}}{\sum_{\lambda_F} S_F}. \tag{5}$$

2.5.4. Gravitational Model. As the final formulation, we extend the principle of image-based distance to the context of an attraction field that follows Netwon's Law of Universal Gravitation, whereby any two elements of mass m within the feature space will exert upon one another an attractive force that will vary proportionally to the inverse of the square of the distance between them. In our context the force exerted by one group (e.g., CTRL) decreases as the distance between a subject and the center of mass of the CTRL group grows, while the force exerted by the second group (e.g., probable AD) increases as distance decreases between the same subject and the second group's center of mass. In a multiple group scenario, the calculated combined force serves as a quantitative measure of the likelihood of belonging to one of the groups.

In such a classical formulation the force between any subject s_i with mass m_i, to the centers of mass of, for example, the CTRL group (CM_{CTRL}) and the AD group (CM_{AD}), is expressed as:

$$F_{s_i \to \text{CTRL,AD}} = G m_i \left(\frac{\text{CM}_{\text{CTRL}}}{d^2_{s_i \to \text{CM}_{\text{CTRL}}}} - \frac{\text{CM}_{\text{AD}}}{d^2_{s_i \to \text{CM}_{\text{AD}}}} \right) \tag{6}$$

with

$$\text{CM} = \frac{1}{M} \sum_i m_i p_i, \tag{7}$$

being the formulation for the centers of mass calculations, where M is the total mass for all subjects in the group, m_i their individual masses, and p_i their individual positions in feature space as derived in the previous section. The distance metric that can be used can be anyone of the aforementioned distances; for the purposes of the current study, the Euclidean distance as formulated in (3) was employed.

We chose to retain the concept of "mass" even though it has no real bearing within the present context of an image-based feature space. It could be replaced with different information regarding individuals in the groups, for example, Braak histopathological staging [27]. Alternatively, one can vary the specificity and sensitivity of the attraction field by increasing the "mass" of subjects in one of the groups (e.g., CTRL or probable AD). For these purposes however we set the mass of each subject to unity, and, further, for equal considerations of simplicity, we set the gravitational constant G also to unity. As is, the result is an inverse-squared law relationship.

Statistics and measurements were computed using the MATLAB Statistics Toolbox (The MathWorks, Natick, MA).

2.6. Experiments. Once the reference space was created, all of the experiments that we conducted were performed in a leave-one-out fashion whereby one subject from the study groups was temporarily removed, allowing for an independent estimate of the low-dimensional discriminant function and the calculation of the eigendistribution means and centers of mass. Only then was the left-out subject entered in the system and its morphological factor computed. The final results consist in the comparison of the independently acquired morphological factors for each subject.

We ran three distinct experiments: (a) determination of the relative accuracies of each distance formulation (Manhattan, Euclidean, Weighted Distance (Wilk's λ), Weighted Distance (Koikkalainen), Gravitational model) for the discrimination of CTRL versus probable AD; (b) determination of the accuracy of the best distance formulation for the discrimination of MCI-S versus MCI-P; (c) determination of the resolution of the best distance formulation based on the CTRL versus probable AD discriminant function using the E-ADNI scan-rescan dataset.

3. Results

3.1. Demographics. There were no statistically significant differences for age between the 75 probable AD and 75 NC

Table 1: Demographic information.

	CTRL	AD	MCI-S	MCI-P
N	75	75	29	20
Age (mean, SD)	73.3 (4.6)	73.3 (8.4)	63.5 (14.2)	74.2 (6.3)
Sex	23 M; 52 F	15 M; 60 F	9 M; 20 F	10 M; 10 F
Baseline MMSE (mean, SD)			27.7 (1.5)	26.4 (1.6)

Table 2: Model results.

	Gravity	Weighted distance		Euclidean	Manhattan
		Koikkalainen	Wilk's λ		
Accuracy	0.90	0.86	0.85	0.73	0.78

Table 3: Morphological factor results.

	CTRL	AD	MCI-S	MCI-P
N	75	75	29	20
Mean	0.61	−0.01	0.45	0.24
Std dev	0.32	0.23	0.26	0.27
Std Err mean	0.04	0.03	0.05	0.06
Upper 95% mean	0.68	0.04	0.55	0.37
Lower 95% mean	0.53	−0.06	0.35	0.12

individuals ($P > .05$) in the AD test group. There was a statistical difference for age between the MCI-S and MCI-P groups ($P = .001$) and for baseline MMSE ($P = .01$) (see Table 1).

3.2. Data Processing and Feature Selection. We set the variance ratio r (see Equation (1)) to 0.997, resulting in a reference PCA model composed of 256 intensity and local volume change eigenvectors. We proceeded with forward stepwise regression analysis using Wilk's λ method (P-to-enter = .005) to select the discriminating variables forming the hyperplane separating each group (e.g., CTRL versus probable AD; MCI-S versus MCI-P). This was performed in a leave-one-out fashion to eliminate overlearning of the dataset. The median number of eigenvectors λ_F retained in the discriminating function for either CTRL versus probable AD or MCI-S versus MCI-P was four.

3.3. Morphological Factor Calculation. Table 2 displays the different accuracies obtained for the five different formulations for the morphological factor at the task of discriminating CTRL versus probable AD (leave-one-out). The Gravitational model's accuracy was 90%, superior to the Weighted Distance models.

Using the Gravitational model, we report the results for the morphological factor for the CTRL versus probable AD experiment and the MCI-S versus MCI-P experiment in Table 3. The distributions of morphological factors for all groups, alongside quantile plots to assess normality (CTRL and probable AD groups) are shown in Figures 1 and 2.

The receiver operating characteristic (ROC) curve for the task of discriminating CTRL from probable AD shows the trade-offs possible in sensitivity and specificity (Figure 1(c)). The area under the ROC curve was 0.9444. At the 90% accuracy point (135/150), specificity was 87.5% and sensitivity 92.9%.

With the Gravitational model we computed the ROC curve for the discrimination of MCI-S from MCI-P (Figure 2(c)). The Area under the ROC curve was 0.7940. At 72.3% accuracy, specificity was 62% and sensitivity 75%.

Finally, we computed the morphological factor for the E-ADNI human phantom volunteer, using the CTRL and probable AD cohorts as a training group for the determination of the discriminating function. Using the Gravitational model, the average factor value was −0.4 or 4 standard deviations away from the mean of the CTRL distribution, with an average difference in scan-rescan factor of 4%. Notably, the morphological index obtained via a weighted distance method (Koikkalainen factor) had an average difference in scan-rescan factor of less than 1%.

4. Discussion

The gravitational or inverse-squared law model constitutes a novel development in the strategies towards obtaining a single quantitative factor from data reduction and machine learning of very high-dimensional MRI input data towards

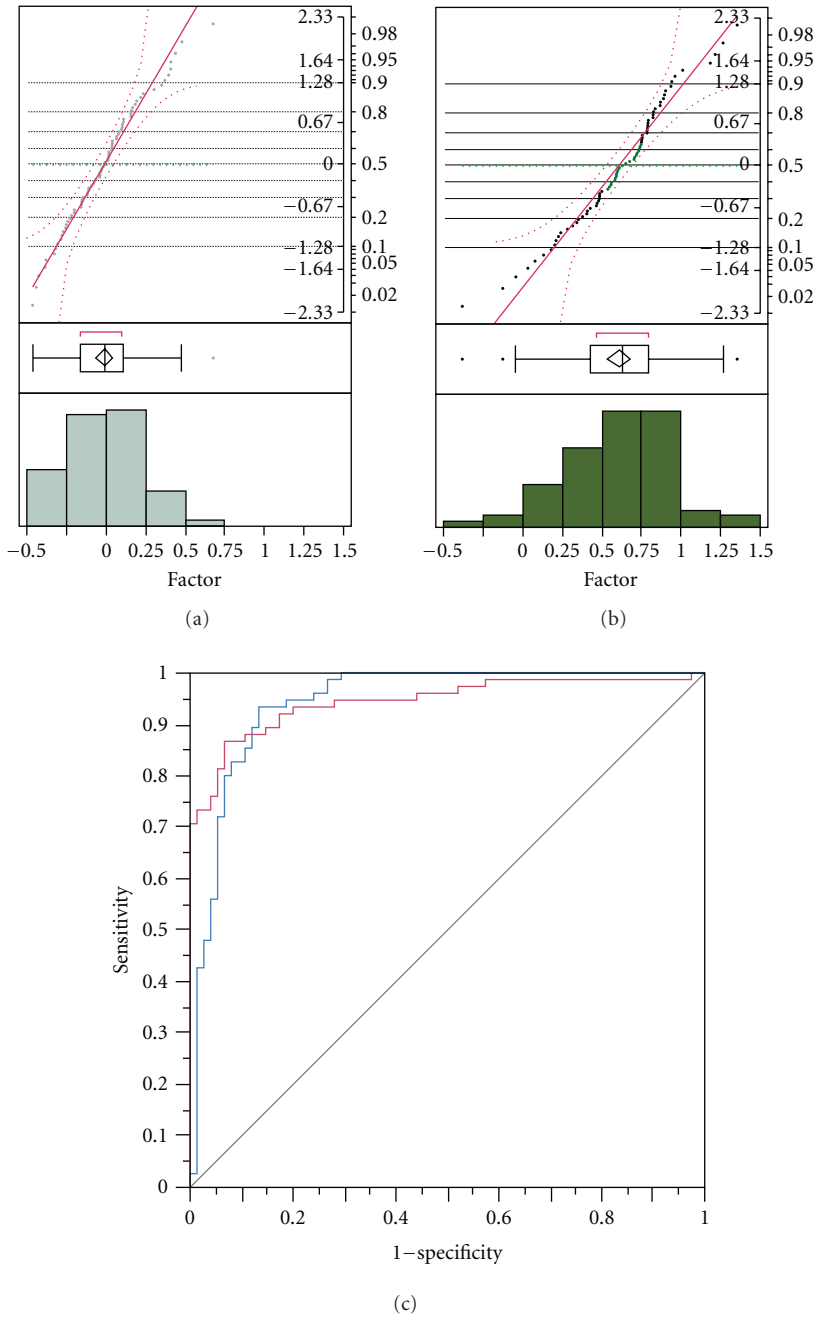

Figure 1: (a, b) Distributions of morphological factors for the CTRL (a) and probable AD groups (b) alongside quantile plots based on the Gravitational model (see Section 2.5.4). (c) Receiver operating characteristic curve (ROC) for the morphological factor displaying the trade-offs between sensitivity and specificity at the task of discriminating CTRL versus probable AD. The area under the ROC curve was 0.9444. At the 90% accuracy point (135/150), specificity was 87.5% and sensitivity 92.9%.

discrimination of individual subjects. Its inherent flexibility makes multigroup comparisons trivial, alongside the introduction of other sources of data. Its performance compares favorably to other results in the MRI literature within the context of discriminating CTRL versus probable AD [2]. As a single dimensional scalar, the morphological factor metric achieves strong accuracy (90%), especially when compared to other multidimensional discrimination functions (e.g., 92% as reported in [3]). It has also a strong result when put within the clinical context of discriminating CTRL versus probable AD, where inclusion evaluations are reportedly 78% accurate (albeit against final histopathological diagnostic). While lower, accuracy figures for the prediction of progression to probable AD in the MCI cohort (on average, 1.5 years before clinical diagnostic) are also strong and compare favorably to published results on MRI data [4, 6]. A study comparing these approaches (e.g., within a monocentric setting, such as the Open Access Series of

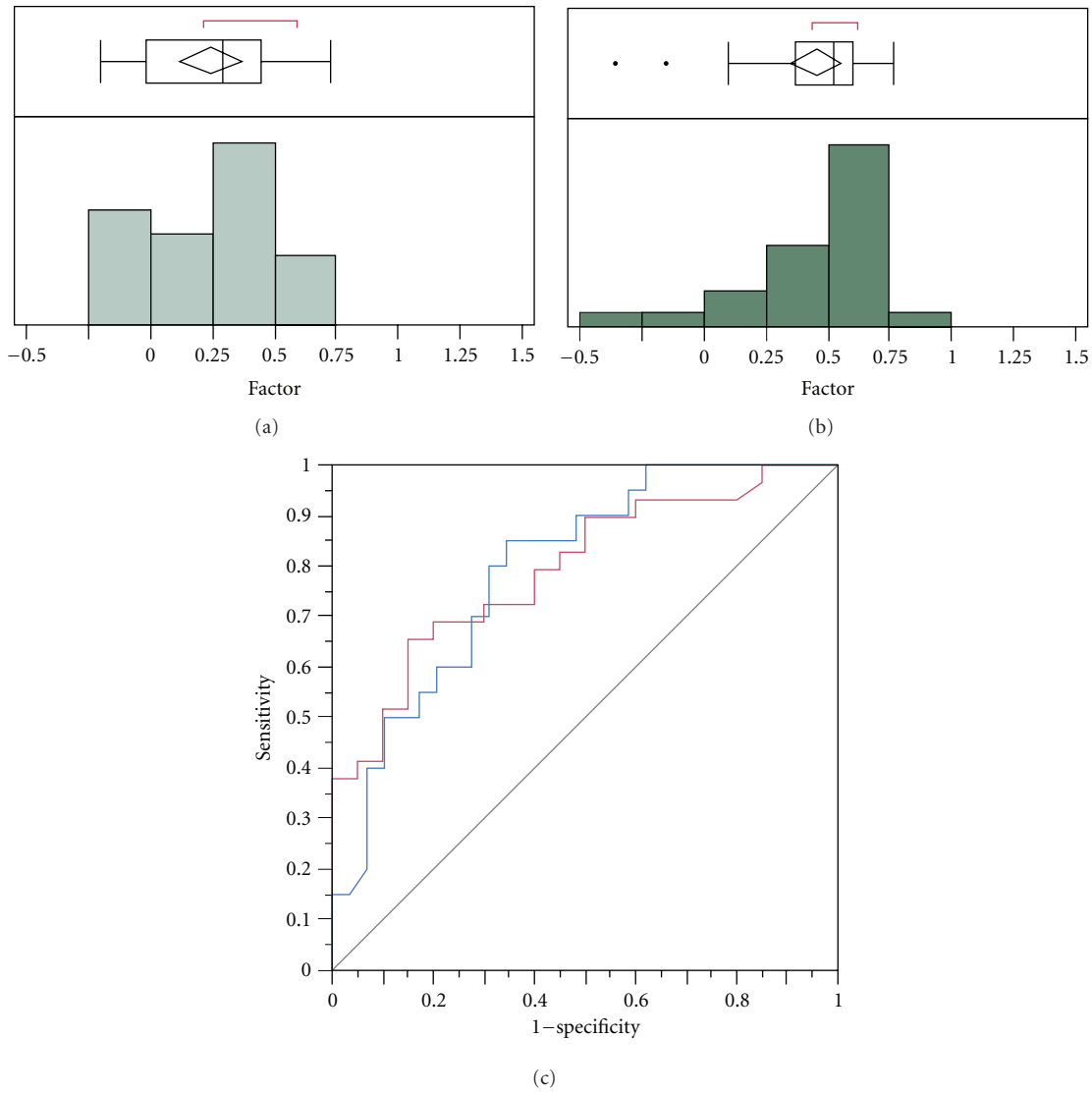

Figure 2: (a, b) Similar distributions of morphological factors for the MCI-S (a) and MCI-P groups (b). (c) ROC for the discrimination of MCI-S and MCI-P. The area under the ROC curve was 0.7940. At 72.3% accuracy, specificity was 62%, and sensitivity 75%.

Imaging Studies [28] or multicentric setting such as the Alzheimer's Disease Neuroimaging Initiative [29]) would be worthwhile.

The paper uses the leave-one-out approach to feature selection (stepwise regression analysis), which allows a correct generalization of the morphological factor as it is not tested on the same data.

Clinical interpretation of changes in image features associated with changes in the morphological factor should provide insight into the development of AD and would need to be compared to existing results from voxel-based morphometry studies, structural studies (e.g., hippocampal and entorhinal atrophy), and histopathological confirmation studies. Overall, we speculate that the specific patterns of intensity and local volume change differences result from different levels of advanced extracellular plaque formation, neurofibrillary tangles accumulation and other pathological processes between CTRL and probable AD, and between stable and progressing MCI. With regards to the features employed in this method, the differences in local volume changes should mirror the changes noticed in other reports, such as visual assessment [30], while differences in grey level might reflect the intensity of neuronal loss induced by the neuropathological changes [31], which precede volume loss as visualized on MRI. Such an evaluation however is beyond the scope of this paper.

The difference in factor averages between probable AD and CTRL was 15%. At this level, the minimum trial size required to detect this difference is 59 individuals for both samples ($\alpha = 0.05$; $\beta = 0.50$) and reaches 75 individuals if we include scan-rescan variability.

4.1. Limitations. There are a number of limitations in this study. One pertains to the fact that the MRI images for the

probable AD subjects were acquired at the time of diagnosis; therefore, some of the patients have had AD for a number of years. In turn, this implies that extensive neurodegeneration has taken place at this point and should artificially facilitate the discrimination with CTRL. However, the fact that the latter were age matched and the fact that the results in the MCI cohort remain significant alleviate part of this concern. It would be useful to assess if the morphological factor correlates with different indices of disease severity, cognitive deficits, or other biomarkers. Neuropathological confirmation is also required to replace the clinical evaluation as a gold standard. Finally, the patterns of abnormalities that can be found by the method are restricted to a space that is built from healthy, young controls. It is not the optimal space to describe normal aging and/or AD-related variability. However, it does tend to maximize the distance between both groups, as we noticed from building a few reference spaces in an N-fold validation of the CTRL/probable AD groups that achieved lower accuracies.

We estimate that the proposed formulation of the morphological factor is relevant within the context of aid to diagnostic and prediction of future clinical status in probable AD.

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Review Article

The Default Mode Network in Healthy Aging and Alzheimer's Disease

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In the past decade, a "default mode network" (DMN) has been highlighted in neuroimaging studies as a set of brain regions showing increased activity in task-free state compared to cognitively demanding task, and synchronized activity at rest. Changes within this network have been described in healthy aging as well as in Alzheimer's disease (AD) and populations at risk for AD, that is, amnestic Mild Cognitive Impairment (aMCI) patients and APOE-ε4 carriers. This is of particular interest in the context of early diagnosis and more generally for our understanding of the physiopathological mechanisms of AD. This paper gives an overview of the anatomical and physiological characteristics of this network as well as its relationships with cognition, before focusing on changes in the DMN over normal aging and Alzheimer's disease. While perturbations of the DMN have been consistently reported, especially within the posterior cingulate, further studies are needed to understand their clinical implication.

1. A Brief Historical and Methodological Introduction on Default Mode Network

Before the emergence of functional magnetic resonance imaging (fMRI), the most classical way to explore brain functional activity associated with different cognitive states consisted in using metabolism or perfusion Positron Emission Tomography (PET). The concept of brain resting-state network arose from observations made when comparing cerebral perfusion during cognitive processing to that measured during passive baseline conditions such as at rest, that is, when subjects lie in the dark and are instructed to think about nothing in particular. Activity decreases in a set of brain areas were then consistently reported during tasks compared to the resting-state, leading to the concept of "deactivations" [1, 2]. In other words, some brain regions appear to be more engaged during rest than during constrained cognitive activity. These observations were then reinforced by works showing greater deactivations with increasing attention-demanding processes [3–7]. In these conditions, deactivations intensity depends on attention load so that a cognitive task requiring low attention levels will induce weak deactivations, while greater deactivations will be associated with tasks requiring high attention levels.

Resting-state brain activity is mainly assessed using ^{18}FDG-, H_2O^{15}-PET or fMRI. fMRI is a noninvasive method that utilizes changes in blood oxygen level-dependent (BOLD) signal to identify areas of increased or decreased neuronal activity [8, 9]. In addition, resting-state activity can be investigated either contrasting cognitive and baseline conditions (see above), or assessing the temporal characteristic of brain activity measured at rest with fMRI. Using this technique, resting-state activity was shown to be characterized by low-frequency synchronized oscillations in large-scale functional brain networks. Resting-state activity is also sometimes assessed while subjects are asleep or sedated (see for instance [10–13]). However, findings reported under those specific conditions have to be considered with caution since the resting-brain activity depends on consciousness levels. In case of light sleep or sedation [14] which are characterized by reduced levels of awareness and arousal, at-rest brain activity is modified so that low frequency fluctuations are attenuated compared to the more classical resting-state conditions described above.

There are two main methods that can be used to analyse data obtained from an fMRI resting-state acquisition. In the first category, Independent Component Analysis (ICA) is the most commonly used. It is an exploratory method

which allows the detection of independent brain networks (components) from a same dataset without *a priori*. This is a data-driven method where maps of coactivated brain regions are computed according to temporal correlations in their activity. The second category mainly refers to Region of Interest (seed) based methods, where a brain region has to be selected according to *a priori* hypotheses. The averaged time course of the BOLD signal in this region is then extracted and correlated with the signal time-course in each voxel of the grey matter. This method is more generally used to explore the cortical functional connectivity at rest or during cognitive tasks, allowing to reveal how components of large-scale distributed neural systems are coupled together in performing specific tasks [15]. Although ICA, functional connectivity and deactivation methods probably give slightly different findings, all are used to study resting-state brain activity without theoretical distinction. Consequently, results will be presented disregarding of the method in what follows. However, results obtained when comparing brain activity at rest versus during a cognitive task will be referred to as "deactivations" while DMN "activity" or "connectivity" will refer to analyses conducted from resting-state scans.

Using ICA and seed-based methods, multiple spatially distributed large-scale functional brain networks have been described and termed as resting-state networks. They mainly include the primary sensory, motor, language, attention and default-mode networks (DMN; see [16] for a recent review about all these networks). Regions included in these networks show a synchronized activity in absence of any specific cognitive activity, that is, at rest, while they are known to be engaged during sensory-, motor-, language- or attention-related tasks, respectively. As for the DMN, it includes brain areas associated with multiple high-order functions described below. This network is now considered as an intrinsic property of the brain, as its activity is widely shared among living beings (for works on monkeys see [17, 18]; for works on rats see [19]) and it seems to emerge in early childhood [20, 21]. As the present paper focuses on the effects of AD onto brain resting-state activity, it will refer to the DMN as it includes the regions known to be the most sensitive to the neurodegenerative processes.

2. Physiology, Anatomy, and Cognitive Role of the DMN

As mentioned above, resting-state networks in general, and the DMN in particular, are defined as sets of anatomically distant brain regions showing temporal correlations in their spontaneous fluctuations, that is, functional connectivity. The DMN mainly includes the posterior cingulate cortex (PCC)/precuneus, dorsal and ventral medial prefrontal, lateral (mainly inferior) parietal cortices, and medial temporal lobes (Figure 1(a)). It is thought to involve multiple subsystems that converge on "hubs" or nodes, such as the PCC, ventral medial prefrontal, and inferior parietal cortices. These hubs are strongly inter-connected and connected to the other regions of the DMN as well [22, 23] (Figure 1(b)). Interestingly, the functional connectivity observed between remote brain regions using resting-state fMRI is consistent with their anatomical connectivity as assessed using Diffusion Tensor Imaging. This suggests that the strength of functional connectivity within the DMN areas at least partly depends on white matter tracts, that is, on the strength of structural connectivity [24–29].

Despite the growing amount of knowledge regarding the DMN physiology and anatomy, the cognitive function of this network is still poorly understood. Interestingly, the different brain regions of the DMN are known to be involved in different high-level cognitive functions. Thus, PCC activity is reported during tasks that imply autobiographical episodic memory and self-referential processes [30–35], the medial prefrontal cortex is associated with social cognitive processes [36], the medial temporal lobe is mainly engaged in episodic memory [37, 38], and the inferior parietal cortex, more particularly the angular gyrus, is implicated in semantic processing and attention [39, 40]. Two main hypotheses have been proposed regarding the cognitive role of the DMN. First, it may subtend an introspection activity, implying numerous abilities such as (i) time-travelling in the past, that is, recollection of autobiographical events [13, 34, 41] and in the future, that is, the "prospective brain" [42, 43] and the self-projection based on mental simulations [13, 34, 41], (ii) theory of mind and social cognition, for which human beings may have a genetic predisposition (see [44, 45] for meta-analyses), and (iii) mind wandering and task-unrelated thoughts [46]. First indication of an association between DMN and introspection came from studies using postscan interview to determine the nature of subjects' thoughts during the resting-state scanning [41, 47–49]. Findings all converge to the presence of inner experiences, from autobiographical memories recalling to inner speech or mental images. Reviews and meta-analyses then reinforced this "introspection hypothesis" by underlining the obvious overlap between neural networks of autobiographical memory, theory of mind, future envisioning, and the DMN [13, 45]. According to a second hypothesis, termed as the "sentinel" hypothesis, the DMN is thought to support a broad low-level focus of attention allowing to monitor the external environment for unexpected events [1, 13, 50]. Several experimental works exploring diffuse attention processes support this hypothesis. For instance, DMN activity is linked to high levels of performance on target-detection tasks where targets appear randomly at multiple possible locations. Conversely, performance is not associated with DMN activity when attention is focussed to a specific location [51]. To date, none of both hypotheses has been fully validated, leading to an open field for future investigations.

3. The DMN in Healthy Aging

Several studies have assessed the effects of normal aging on DMN activity, and they consistently reported a significant effect of age. More specifically, they showed significant reduction with age in the activity measured at rest, or weaker deactivation, within superior and middle frontal

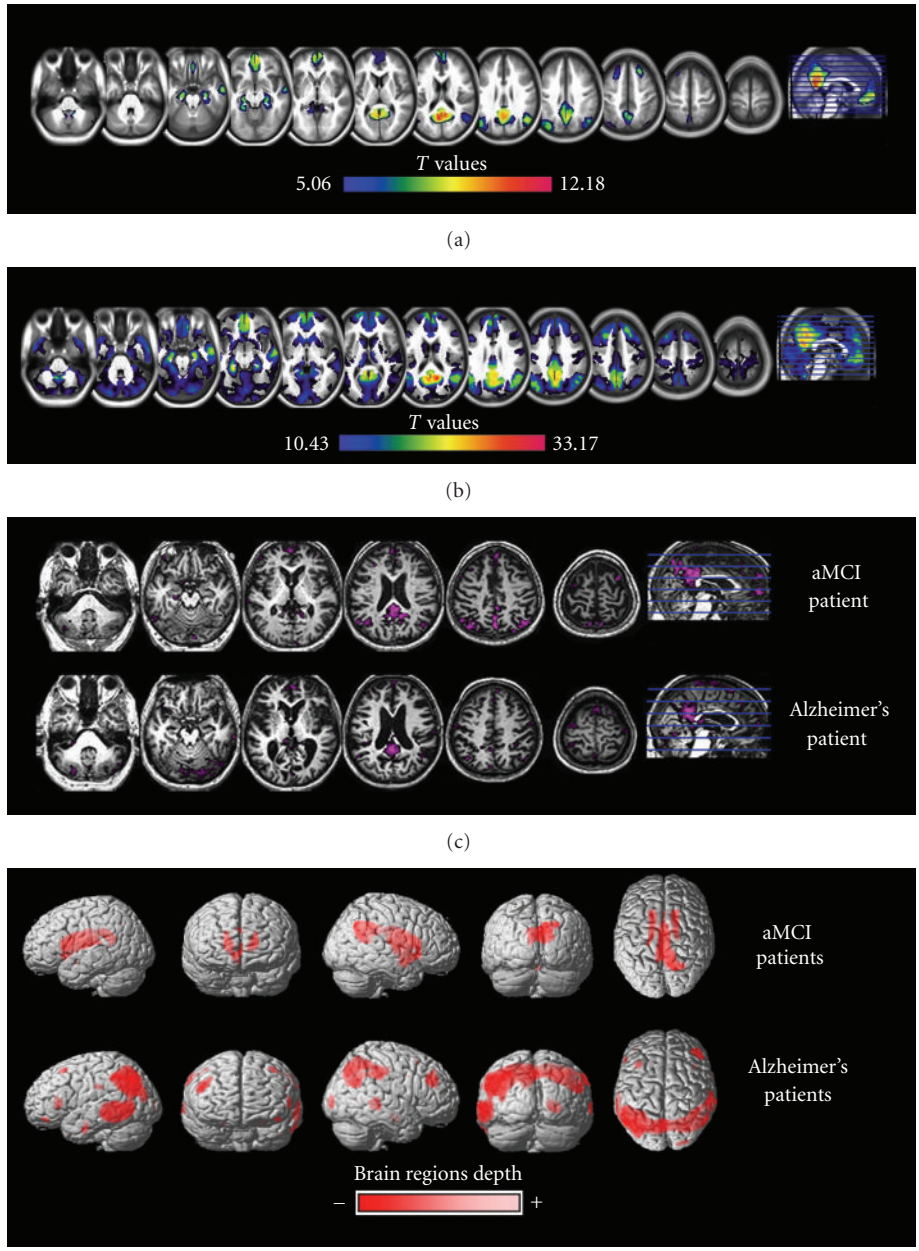

Figure 1: Resting-state fMRI cerebral activity in 71 healthy subjects aged from 19 to 80 years (a) Using an Independent Component Analysis, we identified the Default Mode Network (DMN) encompassing here the posterior cingulate/precuneus, anterior cingulate, orbitofrontal, ventromedial prefrontal, inferior temporal cortices, hippocampi, and angular gyri. (b) Using the posterior cingulate cortex (PCC) as a seed in a functional connectivity analysis, we identified a larger network extending to frontal, occipital, and middle temporal regions, as well as cerebellum, thalami, and motor cortices. Using this same method on (c) amnestic Mild Cognitive Impairment (aMCI) and Alzheimer's disease (AD) patients, a disruption of the connectivity between PCC and anterior then posterior brain areas was observed. (d) ^{18}FDG-PET resting-state measures in two groups of aMCI and AD patients compared to healthy aged controls. While in the former group, hypometabolism was restricted to PCC and subcortical structures, it mainly extended to temporoparietal regions in the latter group.

[52], PCC/precuneus [7, 52–59], middle temporal, superior parietal [7, 52, 60] and medial areas such as medial prefrontal [7, 54, 55, 58, 60, 61], anterior cingulate [53, 54, 61], and hippocampal regions [7, 60]. These disturbances may reflect a reduction in the ability to suspend DMN activity when high-order cognitive processes are required, that is, a difficulty in switching from a "default mode" to a task-related mode of brain function [54, 55]. Older subjects were also found to show greater activity at rest, or greater deactivations, mainly in anterior brain areas, for example, anterior cingulate [16, 54, 56, 58, 62], medial prefrontal, and superior frontal cortices [53]. This increased activity at rest in frontal DMN

regions of elderly adults has been interpreted as a reflect of compensatory processes, that is, an attempt to compensate for the decrease of resting-state activity in posterior DMN areas ([56] see below). Thus, the Posterior Anterior Shift in Aging (PASA) model has been proposed to account for the fact that, while deactivations in occipitotemporal areas of the DMN are reduced, bilateral frontal areas deactivations increase in healthy elderly compared to young subjects [56]. However, this model does not fit with all findings, especially those reporting weak deactivations or activations (instead of deactivations) in older subjects' anterior DMN areas [7, 52–55, 58, 60, 61]. In these studies, older subjects failed in generating reinforced frontal deactivations to compensate for posterior DMN disturbances, which is not in agreement with the PASA model. To conclude, further investigations are needed to better understand healthy aging effects on DMN activity and especially to explore possible functional compensation processes in frontal areas.

There have been an increasing number of studies exploring DMN disturbances in different pathological states including schizophrenia, autism, hyperactivity disorder, epilepsy, multiple sclerosis, and Alzheimer's disease (AD) (see [63] for a review of results in all these pathologies). Studies on AD are the most numerous, which is probably due to the fact that the DMN includes two key areas in AD, that is, the posterior cingulate cortex and hippocampal formation. Indeed, the hippocampus is the region of earliest and most marked atrophy (see [64]; [65] for a meta-analysis on hippocampal atrophy), and the PCC is consistently found to be hypometabolic early in the course of the disease (see [64] for instance; [66] for a recent study; Figure 1(d)). As a consequence, the DMN has been the focus of interest in studies not only on AD patients but also in at-risk populations such as patients with amnestic Mild Cognitive Impairment (aMCI) and asymptomatic APOE-ε4 allele carriers as well.

4. The DMN in APOE-ε4 Carriers

Works aiming at studying subjects with increased risk of developing AD are still rare. However, their results are of high relevance to better understand the pathological processes early in the course of the disease. Except Koch et al. [67] who found no significant differences in the DMN activity between APOE-ε4 carriers and noncarriers, ε4 carriers were found to be characterized by significant changes in brain activity at rest. These disturbances mainly correspond to diffuse decreases in deactivations [68–70] and PCC functional connectivity disruption with the precuneus [68] but also increased functional connectivity between the whole DMN and medial, as well as middle, temporal regions [69]. Furthermore, differences were found in the effect of APOE-ε4 on DMN activity according to the age of the subjects ([71, 72]; see [73] for a review). While young ε4-carriers were characterized by higher DMN activity in retrosplenial, medial temporal, and medial-prefrontal cortices compared to young noncarriers, elderly ε4-carriers showed reduced activity compared to old noncarriers in anterior and posterior cingulate, and cerebellum. As mentioned above regarding age-related effects on DMN activity, increased BOLD signal in young ε4-carriers might also be interpreted as a putative compensatory mechanism to maintain normal cognitive performances. These findings also suggest that the ε4 allele modulates neuronal activity decades before the appearance of the clinical manifestation of the disease [71, 72]. Conversely, attenuated BOLD signal in older ε4-carriers might be attributed to effects of early pathology and especially to interactions between beta-amyloid deposition or clearance and BOLD signal [72]. In addition, disruption of white matter tracts has recently been shown in APOE-ε4 carriers, notably in the cingulum [73] which interconnects DMN areas such as the PCC and the hippocampus. Consequently, white mater disturbances might underlie APOE-ε4-induced DMN activity decreases. Altogether, amyloid plaques and/or white matter disruption could be responsible for DMN functional disturbances characterizing older APOE-ε4 carriers. Finally, according to Trachtenberg et al. [74], future works using fMRI should take into account several considerations which are of importance when investigating the effects of APOE-ε4 on brain activity, such as family history and age, as well as the inclusion of a wider range of APOE genotypes. For instance, there might be a dose-dependent effect so that ε4 homozygotes may be characterized by greater effects on brain functional activity compared to heterozygote subjects. As only two studies with divergent findings have assessed this question to date [68, 75], further investigations are needed to establish clear assertions concerning a dose effect of APOE-ε4.

5. The DMN in Alzheimer's Disease Patients

As for AD, DMN activity changes are in line with those found using FDG-PET measure of resting-state brain metabolism, highlighting the major involvement of the PCC/precuneus region (see [64, 76–78] for PET studies; see [6, 53, 79–82] for fMRI studies; Figures 1(c) and 1(d)). For instance, the functional connectivity between the PCC and the hippocampus seems to be impaired in AD (Figure 1(c)), probably as a consequence of early hippocampal structural alterations. This so-called disconnection hypothesis has received strong support from previous works combining structural MRI and PET. Thus, hippocampal atrophy seems to induce PCC functional perturbation, as well as episodic memory impairment, through disruption of the cingulum bundle [64, 77, 78]. Resting-state fMRI studies showing alterations of the temporal synchrony of PCC and hippocampus activity in patients with aMCI compared to healthy controls [83] are consistent with this hypothesis. Decreases in functional connectivity or deactivation disturbances [6] have also been reported within the PCC of aMCI patients and interpreted as the effect of local atrophy [84]. A recent study rather suggests that disconnection precedes gray matter atrophy in the PCC [66]. According to these authors, PCC atrophy would reflect a long-term effect of brain disconnection and lead to the conversion from MCI to AD (see below). Some studies also reported perturbation of resting-state

activity within the hippocampus in AD compared to controls [79, 82, 85], but also in patients with aMCI suggesting that it is an early process [86]. According to Xu et al. [87], this region is characterized by a perturbation of low frequency fluctuations synchronisation. The magnitude of these asynchronies depends on pathological stages so that the mean index of hippocampal asynchrony is higher in aMCI than in controls and still higher in patients with probable AD compared to aMCI (see also [88] for similar results). Alteration of DMN activity in AD is not restricted to the PCC and hippocampal region as connectivity disruption between these structures and other brain areas have also been reported [66, 85, 89–92]. According to Gili et al. [66] and Zhang et al. [91], these disruptions seem to spread within the cortex as the disease progresses, that is, respectively, from aMCI to AD and from mild to severe AD. Consistently, Rombouts et al. [6] showed that aMCI deactivations were less marked in the precuneus and medial frontal regions, while in AD patients deactivations were restricted to medial frontal areas. Interestingly, this study [6] also indicated that the precuneus BOLD signal in both groups of patients was delayed during an episodic memory task compared to healthy aged controls. As proposed in normal aging (see above), these findings are thought to reflect a difficulty to switch from a resting-state to a task-related mode of brain function, which would mainly be due to a failure of DMN brain regions to show rapid and efficient synchronisation in their activity.

Increases in DMN activity or connectivity have also been reported in patients with aMCI or AD as compared to healthy aged controls. Thus, aMCI patients were found to be characterized by (i) increases in DMN activity located within the PCC/precuneus [93], (pre)frontal [86, 93], lateral parietal, and middle temporal cortices [84] and (ii) increases in functional connectivity between right parietal cortex and left insula [94]. Increases in AD patients were found to concern (i) DMN activity within the PCC/precuneus [93], frontal [89, 91], occipital [95], parietal, and (pre)frontal [91] cortices and (ii) DMN connectivity between left hippocampus and prefrontal dorsolateral cortex [89] or between PCC and left frontoparietal cortices [92]. Altogether, these results point to the existence of potential compensatory processes emerging in the early stages of the disease and located in several DMN areas. It is worth mentioning that cognitive reserve was found to modulate the effect of the pathology on brain function in general and on DMN activity/connectivity in particular. Cerebral or cognitive reserve relates to the capacity of the brain to cope with neuropathology so as to minimize clinical manifestations [96]. Cognitive reserve for instance was found to differentially affect deactivations in healthy elders versus in aMCI and AD patients [93]. Thus, higher cognitive reserve in healthy elderly was found to be associated with lower deactivations within the DMN and lower task-related activity, both thought to reflect increased neural efficiency. By contrast, aMCI and AD patients with high cognitive reserve showed higher activity in task-related brain areas and increased deactivations within the DMN (PCC/precuneus, anterior cingulate) compared to those with low cognitive reserve. This greater reallocation of processing resources from the DMN to brain areas directly engaged in the experimental task could reflect increased reorganization of functional compensatory resources in patients with high cognitive reserve. To sum up, higher cognitive reserve abilities allow a more-with-less mode of brain functioning in normal aging, while it compensates for pathological processes as they appear. As illustrated here for the DMN, the crucial role of cognitive reserve in age- and pathology-related brain reorganization has been widely demonstrated.

One of the main goals of studies assessing the effects of AD on the DMN is to unravel biomarkers that may be useful for the early diagnosis of the disease. The disruption of hippocampus or PCC connectivity could be a good candidate as it intensifies as the disease progresses [90, 92]. Lower deactivations within the whole DMN and especially within (medial) parietal areas [80, 97] were also found to be associated with conversion from aMCI to AD (see [98] for a review). In addition, Koch et al. [67] suggested that the use of multivariate analyses combining measures of the activity of specific DMN areas to measures of the interconnectivity between these regions improved the diagnosis accuracy. Interestingly, using this approach, the disease pattern observed in patients with AD could be identified in a high proportion of aMCI patients, suggesting that such a combination of resting-state measures may be relevant to identify AD at a predementia stage.

All these studies provided accumulating support for a preferential alteration of the DMN hubs in AD, though. However, the reason for the predominant vulnerability of these regions remains unclear. According to Buckner et al. [13, 23], cortical hubs may be preferentially affected in AD because of their continuous high baseline activity and/or associated metabolism which may induce increased vulnerability (notably to beta-amyloid deposition). This hypothesis is supported by studies showing a relationship between amyloid deposition and impaired DMN function in older people without dementia (see [99] for instance). Further multimodal investigations in at-risk subjects and AD patients are needed to better understand this intriguing overlap between the DMN and the distribution of beta-amyloid deposition in the brain.

6. Synthesis and Perspectives for the Future

In conclusion, at-rest brain activity is one of the most important investigation fields of the past decade in neuroimaging. Major advances have been made in the characterisation of the physiological and anatomical properties of resting state networks, and especially those of the DMN. Healthy aging and AD were shown to have significant and distinct effects on deactivations and DMN activity or connectivity. Changes in PCC resting-state activity or functional connectivity within the DMN for instance may be an accurate and early marker of AD. As mentioned in this paper, previous studies have consistently shown the involvement of this region in the course of the disease, even at presymptomatic or predemented stages, that is, in APOE-ε4 carriers and aMCI populations. Given its accessibility and noninvasive nature

compared to metabolism PET measures, fMRI resting-state measurement of PCC activity or connectivity is potentially useful from a clinical point of view. However, further explorations are needed to disentangle the complex and heterogeneous findings, part of which being probably due to the multiple and still suboptimal methods used to explore the DMN. Moreover, several fMRI indices, such as BOLD signal amplitude and temporal derivative (see [6] for instance), may prove to provide complementary information over and above measures of synchronicity. The heterogeneity of findings also certainly reflects the complexity of the disease, as illustrated for instance by the presence of activity increases within the DMN in AD. Those increases may reflect compensatory processes, which may themselves depend on individual cognitive and brain reserve capacity. Finally, further studies are needed to assess the relative accuracy of resting-state fMRI-derived PCC measures as compared to metabolism, hippocampal atrophy, and specific episodic memory measures. It is very likely that, if DMN (PCC) activity proves to be clinically useful, it would have to be considered together with other variables such as education level, and MRI characteristics. An accurate early diagnosis will certainly be achieved only considering overall multiple-source information.

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Research Article

Volumetric Differences in Mapped Hippocampal Regions Correlate with Increase of High Alpha Rhythm in Alzheimer's Disease

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Objective. The increase of high alpha relative to low alpha power has been recently demonstrated as a reliable EEG marker of hippocampal atrophy conversion of patients with mild cognitive impairment (MCI) in Alzheimer's disease (AD). In the present study we test the reliability of this EEG index in subjects with AD. *Methods*. Correlation between EEG markers and volumetric differences in mapped hippocampal regions was estimated in AD patients. *Results*. Results show that the increase of alpha3/alpha2 power ratio is correlated with atrophy of mapped hippocampal regions in Alzheimer's disease. *Conclusions*. The findings confirm the possible diagnostic role of EEG markers.

1. Introduction

Recent studies have demonstrated that hippocampus is not a unitary structure [1]. The hippocampus, including strictly speaking subfields CA1–CA4, and the hippocampal formation, including also dentate gyrus, fimbria, subiculum, and parasubiculum, is a highly sophisticated structure. Stimuli coming from the entorhinal cortex are processed by the dentate gyrus, subfields CA4 and CA3, before being projected outside the medial temporal lobe via CA1 or subicular efferent projections. Moreover, in addition to the unsurprising right–left specialization for verbal and visuospatial material [2], some degree of anterior-to-posterior specialization has been shown by fMRI studies [3].

As a consequence, it is conceivable that local structural changes take place in the hippocampus of patients with Alzheimer's disease (AD) and that different hippocampal subregions are affected in AD. Local changes in hippocampal subregions could be detected through a radial atrophy mapping method able to assess group, based on high resolution MRI at 3 Tesla differences ([1] Frisoni et al. 2008).

A large body of literature has previously demonstrated that in subjects with cognitive decline are present specific modifications of brain rhythms detected by electroencefalography (EEG; [4–11]). In particular, an increase of high alpha as compared to low alpha band has been demonstrated to correlate specifically with hippocampal atrophy ([10] Moretti et al. 2009a) in subjects with mild cognitive impairment (MCI). Moreover, a recent study has shown that the increase of alpha3/alpha2 power ratio is a reliable index to predict MCI patients who will convert in AD [12]. So, the reliability of this EEG marker needs further confirmation in patients with AD, and, if it is possible, with a greater topographic specificity.

In this study we tested the hypothesis that the increase of alpha3/alpha2 ratio is related with volumetric differences in mapped hippocampal regions in AD patients. Up to date, the correlation between mapped hippocampal regions and EEG activity has never been carried out in Alzheimer's disease.

2. Materials and Methods

2.1. Subjects. For the present study, 13 subjects with Alzheimer's disease (AD) were recruited from the memory Clinic of the Scientific Institute for Research and Care

(*IRCCS*) of Alzheimer's and psychiatric diseases "Fatebenefratelli" in Brescia, Italy. All experimental protocols had been approved by the local Ethics Committee. Informed consent was obtained from all participants or their caregivers, according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). The diagnosis of AD was made according to NINCDS-ADRDA criteria [13] and the Diagnostic and Statistical Manual of Mental Disorders IV [14].

2.1.1. EEG Recordings. All recordings were obtained in the morning with subjects resting comfortably. In order to keep the level of vigilance constant, an operator controlled the subject and the EEG traces on-line, alerting the subject any time there were signs of behavioural and/or EEG drowsiness.

The EEG activity was recorded continuously from 19 sites by using electrodes set in an elastic cap (Electro-Cap International, Inc.) and positioned according to the 10–20 International system (Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2). The ground electrode was placed in front of Fz. The left and right mastoids served as linked-mastoid reference for all electrodes. The recordings were used off-line to rereference the scalp recordings to the common average. Data were recorded with a bandpass filter of 0.3–70 Hz and digitized at a sampling rate of 250 Hz (BrainAmp, BrainProducts, Germany). Electrodes-skin impedance was set below 5 kΩ. Horizontal and vertical eye movements were detected by recording the electrooculogram (EOG). The recording lasted 5 minutes, with subjects with closed eyes. Longer recordings would have reduced the variability of the data, but they would also have increased the possibility of slowing of EEG oscillations due to reduced vigilance and arousal. EEG data were then analyzed and fragmented off-line in consecutive epochs of 2 seconds, with a frequency resolution of 0.5 Hz. The average number of epochs analyzed was 140 ranging from 130 to 150. The EEG epochs with ocular, muscular, and other types of artifact were preliminary identified by a computerized automatic procedure [15]. A threshold of $\pm 50\,\mu$V was set to detect muscular artifact. Two expert electroencephalographists manually double-checked and confirmed the automatic selections.

The EEG epochs with ocular, muscular, and other types of artifacts were discarded. The estimation of frequency band range was based on the individually detected spectral peak of the main EEG frequencies. The frequency peak, within a well defined band of interest, is the expression of the synchronization of a functional neuronal population [16, 17]. In this view, the frequency range so obtained could be reasonably less affected by slow or fast artefacts such as, respectively, ocular or muscular activity [15] as well as manifestations of miniature saccades [18].

2.1.2. Analysis of Individual Frequency Bands. A digital FFT-based power spectrum analysis (Welch technique, Hanning windowing function, no phase shift) computed—ranging from 2 to 45 Hz—the power density of EEG rhythms with a 0.5 Hz frequency resolution. Two anchor frequencies were selected according to literature guidelines [19], that is, the theta/alpha transition frequency (TF) and the individual alpha frequency (IAF) peak. The TF marks the transition frequency between the theta and alpha bands and represents an estimate of the frequency at which the theta and alpha spectra intersect. TF was computed as the minimum power in the alpha frequency range, since our EEG recordings were performed at rest. The IAF represents the frequency with the maximum power peak within the extended alpha range (5–14 Hz). Based on TF and IAF, we estimated the frequency band range for each subject, as follows: delta from TF-4 to TF-2, theta from TF-2 to TF, low alpha band (alpha1 and alpha2) from TF to IAF, and high alpha band (or alpha3) from IAF to IAF + 2. The alpha1 and alpha2 bands were computed for each subject as follows: alpha1 from TF to the middle point of the TF-IAF range, and alpha2 from such middle point to the IAF peak [6–9, 20]. Moreover, individual beta and gamma frequencies were computed. Moreover, individual beta and gamma frequencies were computed. Three frequency peaks were detected in the frequency range from the individual alpha 3 frequency band and 45 Hz. These peaks were named beta1 peak (IBF 1), beta2 peak (IBF 2), and gamma peak (IGF). Based on peaks, the frequency ranges were determined. Beta1 ranges from alpha 3 to the lower spectral power value between beta1 and beta2 peak; beta2 frequency ranges from beta1 to the lower spectral power value between beta2 and gamma peak; gamma frequency ranges from beta 2 to 45 Hz, which is the end of the range considered. Moreover, within theta frequency the frequency peak (individual theta frequency, ITF) was also individuated. The mean frequency ranges computed in AD MCI subjects considered as a whole are delta 2.7–4.7 Hz, theta 4.7–6.7 Hz, alpha1 6.7–8.7 Hz, alpha2 8.7–10.7 Hz, alpha3 10.7–12–7 Hz, beta1 12.7–17.2 Hz, beta2 17.2–30.4, gamma 30.4–45. In the frequency bands determined in this way, the relative power spectra for each subject was computed. The relative power density for each frequency band was computed as the ratio between the absolute power and the mean power spectra from 2 to 45 Hz. The relative band power at each band was defined as the mean of the relative band power for each frequency bin within that band. For each subject all electrodes were pooled to obtain an all-scalp region relative power spectrum. Finally, alpha3/alpha2 relative power ratio were computed and analyzed.

2.2. Magnetic Resonance Acquisition. High-resolution gradient echo sagittal 3D MR scans were acquired with a Philips Gyroscan 1.0 T scanner (TR = 20 ms, TE = 5 ms, flip angle 30°, field of view = 220 mm, acquisition matrix = 256 × 256, slice thickness = 1.3 mm, no interslice gap) with a standard head coil. The MRI scanning of AD patients was compared with that of twenty-two healthy normal elderly, chosen as normal controls, in order to verify if correlation between EEG rhythms and hippocampal regions was present in atrophic or nonatrophic areas.

2.3. Image Processing. The 3D images were reoriented along the AC-PC line and voxels below the cerebellum were removed with the MRIcro software (http://www.cabiatl.com/mricro/mricro/mricro.html).

In order to improve extraction of the cerebral cortex in areas adjacent to the cerebellum. The anterior commissure was manually set as the origin of the spatial coordinates for an anatomical normalization algorithm implemented in the Statistical Parametric Mapping (SPM99) software package (http://www.fil.ion.ucl.ac.uk/spm/). A 12-parameter affine transformation was used to normalize each image to a customized template in stereotaxic space, created from the MRI scans of 40 control subjects taken from a well-characterized MRI archive [21, 22]. Warping of one hippocampus to another was based on matching homologous points on a rectilinear surface mesh adapted to the structure boundary. The hippocampi were manually traced on the reoriented and normalized images. A single tracer (A.P.) outlined the hippocampal boundaries on contiguous coronal 1.0 mm thick sections following a standardized and validated protocol [23] using an interactive software program developed at the LONI (Laboratory of NeuroImaging), University of California at Los Angeles (http://www.loni.ucla.edu/Software/index.php). Tracings included the hippocampus proper, dentate gyrus, subiculum (subiculum proper and presubiculum), alveus, and fimbria. Each hippocampus comprised approximately 30–40 consecutive slices, and tracing took about 30 minutes per subject. Test-retest reliability on 20 subjects was good: intraclass correlation coefficients were 0.89 for the left and 0.87 for the right hippocampus.

2.4. Radial Atrophy Mapping. The 3D parametric surface mesh models were created from the manual tracings of hippocampal boundaries [24]. This procedure allows measurements to be made at corresponding surface locations in each subject, which are then compared statistically in 3D [25]. To assess hippocampal morphology, a medial curve was automatically defined as the 3D curve traced out by the centroid of the hippocampal boundary in each image slice. The radial size of each hippocampus at each boundary point was assessed by automatically measuring the radial 3D distance from the surface points to the medial curve defined for individual's hippocampal surface model. Distance field indexing local expansions or contractions in hippocampal surface morphology were statistically compared between groups at equivalent hippocampal surface points in 3D space [26].

2.5. Statistical Analysis. Correlation maps between EEG rhythms and hippocampal surface were computed. The correlation analysis between EEG rhythms and hippocampal volume was performed only in 13 AD subjects; we otherwise compared hippocampal gray matter distribution maps between normal controls and AD patients in order to verify that the AD found correlations between EEG rhythms and hippocampal regions were present in areas where ADS are more atrophic than normal subjects.

Table 1: ANOVA results of demographic variables, that is, age, education, MMSE score, and neurophysiological EEG markers, that is alpha3/alpha2 ratio (see text for details).

	Normal old	AD	*P*-value (ANOVA)
Number of subjects (f/m)	22 (14/6)	13 (8/5)	
Age (years)	73.5 ± 3.4	76.2 ± 2.3	.1
Education (years)	8.8 ± 1.2	4.6 ± 0.9	.4
MMSE	29.2 ± 1.4	21.3 ± 2.5	.01
alpha3/alpha2 ratio	0.9 ± 0.08	1.5 ± 0.2	.02

The correlation maps were generated on 3D models of the hippocampal formation where the dorsal and ventral surfaces can be appreciated. Zones with significant correlations were mapped onto the models based on an atlas where these are shown together with the corresponding MR sections [27, 28] (Figure 1). The correlations and the associated *P*-value maps were plotted onto a colour-coded model of the hippocampal surface. The statistical test for the correlations was computed using linear regression at each surface vertex on the hippocampus [29]. A surface point significance threshold of $P < .05$ was used to visualize the regional specificity of gray matter changes in the cortex. Set level correction for multiple comparisons was carried out by permutation testing at threshold of $P = .05$. Permutation tests are based on measuring the total area of the hippocampus with suprathreshold statistics, after setting the threshold at $P < .05$. To correct for multiple comparisons and assign an overall *P*-value to each *P*-map [30, 31] permutation tests were used to determine how likely the observed level of significant atrophy (proportion of suprathreshold statistics, with the threshold set at $P < .05$) within each *P*-map would occur by chance [29]. The number of permutations N was chosen to be 100,000, to control the standard error SEp of omnibus probability p, which follows a binomial distribution $B(N, p)$ with known standard error [32]. When $N = 8{,}000$, the approximate margin of error (95% confidence interval) for P is around 5% of P [33].

Sociodemographic differences between groups were estimated with analysis of variance (ANOVA) test. Significance was set to $P < .05$.

3. Results

Table 1 shows ANOVA results of demographic variables, that is, age, education, MMSE score, and neurophysiological EEG markers, that is alpha3/alpha2 ratio. Significant results were obtained only for MMSE ($P < .01$) with significant decrease in AD as compared to normal old subjects, and for alpha3/alpa2 ratio ($P < .02$) with significant increase in AD as compared to normal old subjects.

Figure 2 displays the pattern of negative significant associations between alpha 3 rhythm and gray matter volume in the hippocampus; areas of strongest and significant ($r > -.40, P < .05$) correlation mapped to little spots in the left

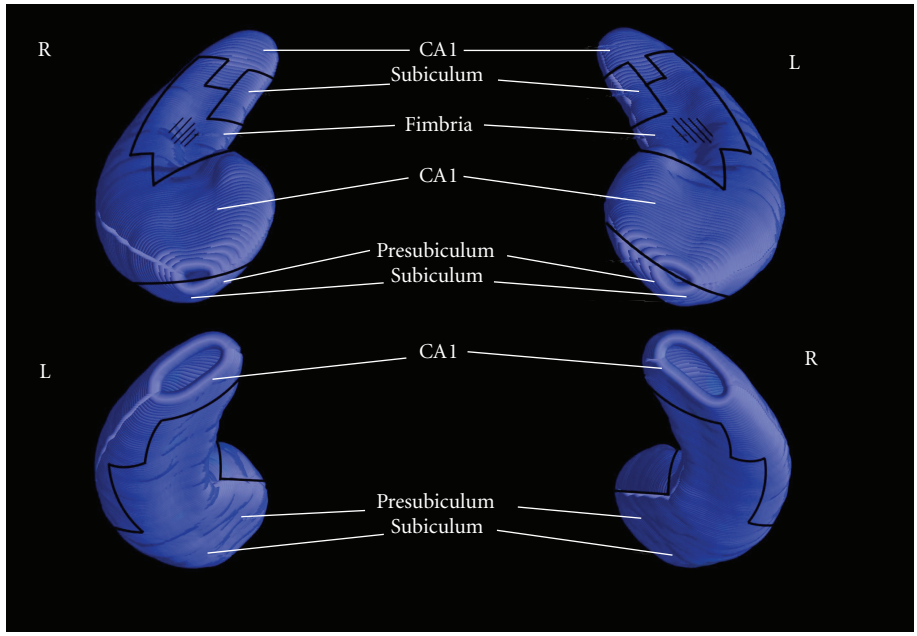

Figure 1: Cytoarchitectonic subregions mapped on blank MR-based models of the hippocampal formation of a healthy subject.

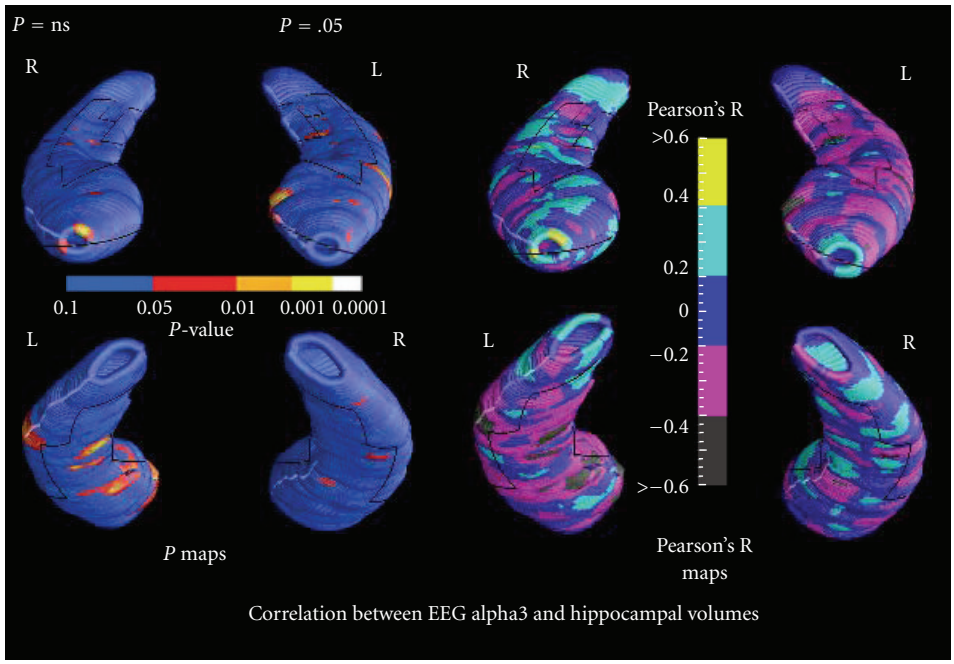

Figure 2: Correlation between alpha3 power rhythm and volumes of hippocampal subregions in AD patients. Permutations overall *P* values are displayed on top of each hippocampus.

dorsal subiculum and CA2-3 sectors of the body and to the CA1 mesial and lateral portion of the head; moreover, large areas of significant correlations were distributed across widespread left ventral subiculum and presubiculum.

Figure 3 shows correlations between alpha3/alpha2 rhythms ratio and hippocampal volumes in AD patients. Again, negative significant associations are found between same areas of AD left hippocampus, although a bit enlarged, and alpha3/2 EEG rhythms. Correlations in right hippocampus resulted not significant at permutation testing ($P > .75$ in both cases).

Although small areas of significant correlation between EEG rhythms and volumes were detected in right hippocampus too, the overall *P*-maps resulted globally not significant (*P* at permutation testing $> .05$ as stated at top of Figures 2 and 3); so we cannot exclude the null hypothesis that for right

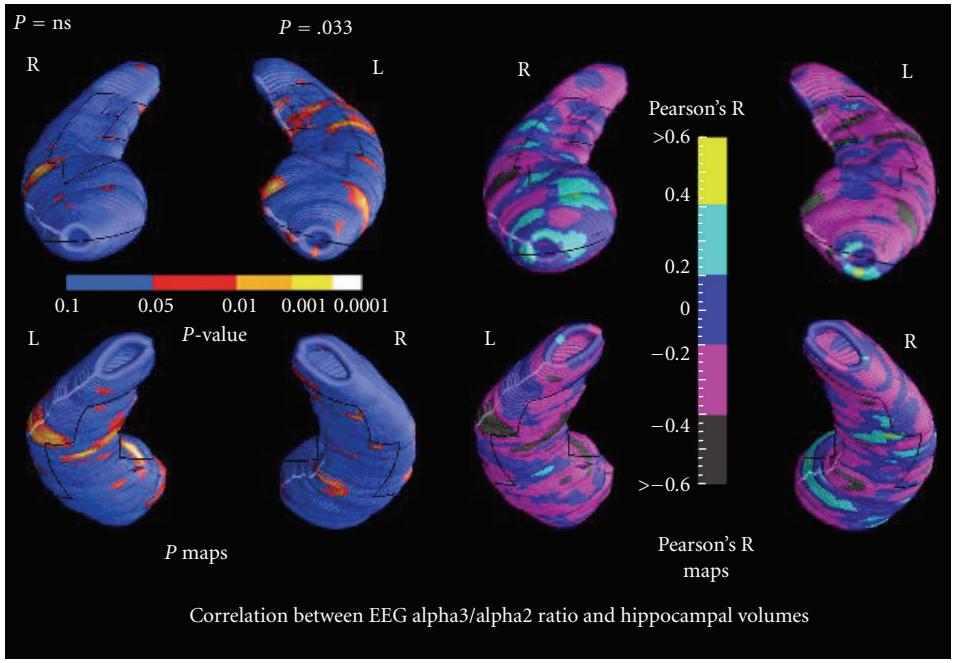

Figure 3: Correlation between alpha3/alpha2 power rhythm ratio and volumes of hippocampal subregions in AD patients. Permutations overall *P* values are displayed on top of each hippocampus.

hippocampus only, all correlations detected occurred by chance. Moreover, for both hippocampi, areas of significant positive correlations are really small and, moreover, localized in a zone, at the very start of the head of the structure, that is usually not taken into account in radial atrophy map analyses. This area could be more often affected by manual tracing bias and systematic bias due to changes in the anatomy of the hippocampus of patients and difficult in well discriminating boundaries between low contrast structures. Some of the bias derive from the resolution of the scanner, in that the voxel size is of the order of 1 mm. Other sources of error derive from the manual tracing method although the reliability for the method has been shown to be high [26].

The hippocampal areas of correlation are all atrophic in AD patients as compared to normal controls.

4. Discussion

The main result of the present study is the finding of a correlation between brain electrical activity collected by scalp EEG and discrete-mapped hippocampal areas in subjects with AD.

The results show that in AD patients the increase of both alpha 3 rhythm spectral power and alpha3/alpha2 power ratio is correlated with the decrease of left hippocampal gray matter volumes.

Our findings confirm the outcomes of two recent meta-analyses. Indeed, a metaanalysis of voxel-based morphometry studies [34], analyzing 429 subjects with amnesic mild cognitive impairment (aMCI), found that the left medial temporal lobe atrophy is the most consistent neurostructural biomarker to predict conversion from aMCI to AD. Moreover a recent metaanalysis focused on the left-right hippocampal pattern asymmetry in AD [35] has considered 28 studies, in which 23 studies included 700 AD patients and 751 controls, 14 studies included 365 MCI patients and 382 controls, and nine studies contain both AD and MCI. The results have demonstrated that the asymmetry in the left and right hippocampal volume, with a less-than-right pattern, exists and may vary with disease progression.

In particular, our results showed that hippocampal areas involved in correlation with EEG markers are presubiculum, dorsal and ventral subiculum, CA2-3 sectors of the body, and CA1 mesial and lateral portion of the head. These findings confirm previous results obtained in a large cohort of patient with mild cognitive impairment (MCI) who convert in AD. Indeed, the increase of alpha 3 band power was found in MCI patient with hippocampal atrophy [7]. Moreover, the increase of alpha3/alpha2 power ratio is the most specific EEG marker of hippocampal atrophy [10, 11], and it is highly predictive of MCI who will convert to AD (12 Moretti et al. in press). Moreover, the prevalence of the modification of EEG rhythms in the left hemisphere in patients with incipient AD was found also in a recent EEG coherence study [8].

The hippocampus is affected early in Alzheimer's disease by neurofibrillary tangle deposition, spreading from the entorhinal cortex to the CA1 subfield, and subicular region, then to the CA2–3 subfields, the CA4 subfield and finally the neocortex [36].

The atrophy of the hippocampal subregions in AD patients seems to affect the polysynaptic pathway of the traditional hippocampal circuitry. This pathway is characterized

by projections by dentate gyrus to CA 2/3 region, through the mossy fiber pathway. The CA3 Schaffer collaterals project to CA1 and, lastly, CA1 projects to the subiculum. This internal circuit of hippocampus is involved in processing details of information through pattern separation and pattern completion processes. In turn, autoassociative networks of the hippocampal subregions, such as that present in CA3 (but also in CA1), have been proposed to be essential for encoding, filtering, and storing memories [37, 38]. This filter or storage function information relies on the integrity of synchronization of the temporal dynamics.

The synchronization of high alpha power has been demonstrated to be involved in top-down cognitive processes [39]. This finding could suggest that there is an the attempt to focus attention on highly selective aspect to prevent interference of irrelevant stimuli (top-down process) in order to maintain a good memory performance [7, 39]. Recently [7], we suggest that MCI subjects could fall in a "hyperattentive state" during the course of disease. Our results confirm this hypothesis, extending those findings to AD patients.

The correlation of the increase of alpha 3 power and alpha3/alpha2 power ratio with atrophic areas could suggest that there is a compensatory synchronization in high alpha rhythm in an effort to balance the degenerative process. Another possible explanation is that the disruption of the order of a stable attractor network prevents the synchronization of large neural assemblies, mirrored by the low alpha synchronization, inducing an increase of high alpha power.

The specific involvement of alpha rhythm and the absence of significant findings in other rhythms, such as theta frequency, could suggest that the hippocampal atrophy in AD is linked to functional changes in a broader network. Indeed, alpha rhythm is not specific of hippocampus like theta frequency. Generation of alpha rhythm arises from thalamus-posterior cortical areas interplay. Of note, the hypometabolism and atrophy of posterior cingulate/retrosplenial and medial temporal cortex pathway, strictly connected with both hippocampus and visual cortex, as well as with low alpha rhythm generation, is well demonstrated in AD [40]. So, the prevalence of high alpha could underlie the disruption of extensive synaptic connection deriving in the formation of smaller cell assemblies. The impairment of the network could explain memory and cognitive symptoms of AD beyond the hippocampal atrophy itself.

5. Conclusions

Our findings confirm the possible diagnostic role of EEG markers when integrated with morphostructural measures in patients with AD.

Conflict of Interests

Authors have no conflict of interests.

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Review Article

Biomarkers of the Dementia

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Recent advances in biomarker studies on dementia are summarized here. CSF Aβ40, Aβ42, total tau, and phosphorylated tau are the most sensitive biomarkers for diagnosis of Alzheimer's disease (AD) and prediction of onset of AD from mild cognitive impairment (MCI). Based on this progress, new diagnostic criteria for AD, MCI, and preclinical AD were proposed by National Institute of Aging (NIA) and Alzheimer's Association in August 2010. In these new criteria, progress in biomarker identification and amyloid imaging studies in the past 10 years have added critical information. Huge contributions of basic and clinical studies have established clinical evidence supporting these markers. Based on this progress, essential therapy for cure of AD is urgently expected.

1. Introduction

The number of patients with dementia have been increasing exponentially with the aging of society in advanced countries and Asian countries. About 24,300,000 people are expected to have dementia worldwide, and there are already more than 2,000,000 people with dementia in Japan. A half of dementia were caused by Alzheimer disease (AD). The development of AD research has clarified that the pathogenesis of AD is initiated by Aβ amyloidosis with secondary tauopathy and provided a strategy for investigating drugs that may improve or cure AD. Mild cognitive impairment (MCI) as a prodromal stage of AD and the pathogenesis of Dementia with Lewy bodies (DLB) and Frontotemporal lobar degeneration (FTLD) as a non-AD type dementia have also been elucidated. Currently, a consortium study by the Alzheimer Disease Neuroimaging initiative (ADNI) is being performed to establish global clinical evidence regarding a neuropsychiatric test battery, CSF biomarkers, neuroimaging including MRI, FDG-PET, and amyloid PET to predict progression from MCI to AD and to promote studies of basic therapy for AD [1]. Several new biomarkers such as Aβ oligomer, α-synuclein, and TDP-43 are now under investigation for further determination of their usefulness to detect AD and other non-AD type dementia.

2. Cerebrospinal Fluid Aβ40, Aβ42, Tau, and Phosphorylated Tau

These biomarkers have been used for a clinical diagnosis of AD, discrimination from the Vascular dementia (VaD) and non-AD type dementia, exclusion of treatable dementia and MCI, prediction of AD onset and evaluation of the clinical trials of an anti-Aβ antibody, Aβ vaccine therapy, and secretase inhibitors [2–4]. Aβ amounts in cerebrospinal fluid (CSF) are controlled by orexin, suggesting the presence of a daily change in the CSF Aβ amounts, that is, Aβ levels are high while awake and low while a sleep. Collection of CSF by lumbar puncture early in morning in a fasting state is recommended [5]. Aβ is produced mainly in the nerve cells of the brain, and it is secreted about 12 hours later into the CSF, then excreted through the blood-brain barrier 24 hours later into blood (Aβ clearance), and finally degraded in the reticuloendothelial system. Aβ levels are regulated in strict equilibrium among the brain, CSF, and blood [6, 7]. In AD brains, Aβ42 forms insoluble amyloids and accumulates as insoluble amyloid fibrils in the brain. The reason Aβ42 levels are decreased in the CSF of AD patients is considered to be caused by deterioration of physiologic Aβ clearance into the CSF in AD brains [2, 3].

CSF total tau levels increase slightly with aging. However, CSF tau levels show a 3-fold greater increase in AD patients than in normal controls [8]. It is thought that the rise in CSF total tau is related to degeneration of axons and neurons and to severe destructive disease of the nervous system. Several diseases show slightly increased tau levels such as VaD, multiple sclerosis, AIDS dementia, head injury, and tauopathy. However, CSF tau levels show significant increases in Creutzfeldt-Jakob disease (CJD) and meningoencephalitis [8].

3. Methods for Measurement of CSF and Plasma Biomarkers

CSF and plasma Aβ40 and Aβ42 amounts can be measured with an Amyloid ELISA Kit (Wako), which is commercially available and used worldwide. The ELISA kit was developed in Japan by Suzuki et al. and shows extremely highly sensitivity and reproducibility [9]. INNOTEST β-AMYLOID1-42(Innogenetics), for Aβ42 is used widely in Europe and America. Several assay kits for total tau and phosphorylated tau are also used for the measurement of CSF tau. Currently, total tau is measured using INNOTEST hTau Ag (Innogenetics). There are 3 ELISA systems for measurement of phosphorylated tau that recognize the special phosphorylation sites at Ser199 (Mitsubishi Chemical Corp.), Thr181 (Innogenetics) and Thr231 (Applied NeuroSolutions Inc.), and phosphorylated tau levels are increased in CSF of AD on assays using these kits. Of these 3 kits, INNOTEST PHOSPHO-TAU (181) (Innogenetics) is commercially available and used widely. Recently, INNO-BIA AlzBio3 by Innogenetics has been able to measure Aβ1-42, total tau, and P-tau181P simultaneously in 75 μL of CSF, which is a very small amount of CSF.

4. Clinical Evidence, Sensitivity, and Specificity

The first large-scale collaborative multicenter study of CSF Aβ40, Aβ42, and total tau as biomarkers of AD was reported by a Japanese study group in 1998 [4]. A total of 236 subjects were followed and evaluated using a combination index (AD index: CSF Aβ40/Aβ42 $\times$ total tau), which showed a diagnostic sensitivity of 71% $\times$ and specificity of 83% in AD. The diagnostic sensitivity rose to 91% on continuous follow-up study. This study continued until 2004. Finally, diagnostic sensitivity was 80% and specificity was 84% in a total of 507 subjects (157 AD patients, 88 control subjects, 108 non-Alzheimer-type dementia patients, 154 nondementia disease patients) [10]. An European and American large scale multicenter study reported that a combination assay of Aβ42 and tau in CSF samples from 100 controls, 84 non-dementia neurological diseases, 150 AD, and 79 non-AD type dementia showed a diagnostic sensitivity of 85% and specificity of 86% [11]. A large-scale multicenter Japanese study of CSF total tau alone in 1,031 subjects (366 AD, 168 non-AD dementia, 316 non-dementia neurological diseases, and 181 normal controls) reported a sensitivity of 59% and specificity of 90% for diagnosis of AD [8]. After these studies, many combination studies reported decreased Aβ42 and increased tau in CSF from AD patients. Practical guidelines for dementia proposed by the American Academy of Neurology in 2001, which were based on a systematic review of all reports between 1994 and 1999, showed that there were 4 Class II or Class III studies on CSF Aβ42 reporting a diagnostic sensitivity of 78~92% and specificity of 81~83%. Regarding CSF tau levels in AD, there were also 4 Class II or Class III studies reporting a diagnostic sensitivity of 80~97% and specificity of 86~95%. Three Class II or III reports were selected for a systematic review of combination study of Aβ42 and total tau in CSF. This review also evaluated the sensitivity as 85% and specificity as 87% [12]. A community-based prospective study showed that the diagnostic sensitivity for AD was 94% (probable AD), 88% in possible AD, and 75% in MCI. The specificity was 100% (mental diseases) and 89% in non-dementia subjects. The specificity was low in DLB (67%) and VaD (48%). Sensitivity was increased in subjects having the ApoE ε4 genotype [13]. A comparative study based on pathological findings reported that the diagnostic sensitivity was 85% and specificity was 84% [14]. Meta-analysis of 17 reports on CSF Aβ42 and 34 reports on CSF total tau (3,133 AD subjects in total with comparison to 1,481 normal controls subjects) was performed in 2003, and showed a final diagnostic sensitivity of 92% and specificity of 89% [15]. Global standardization of the assay system using normal subjects has also been carried out.

In the examination of CSF phosphorylated tau, the first study of p-tau199 was reported as a large-scale multicenter collaborative study by a Japanese group. A total of 570 subjects were analyzed (236 AD, 239 non-AD and non-dementia neurological diseases, and 95 normal controls) and showed a diagnostic sensitivity of 85% and specificity of 85% in a comparison between AD and non-AD disease controls [16]. Assay systems using different epitopes of phosphorylated tau (p-tau231, p-tau181, and p-tau199) have been internationally standardized. When the sensitivity was fixed above 85%, the respective specificities were 83% for p-tau231, 79% for p-tau199, and 60–71% for p-tau181 [17]. Currently, the assay system for p-tau181 tau is widely used to measure phosphorylated tau in CSF. Systematic review of CSF biomarkers for AD in 2001 analyzed 41 studies (2,500 AD, 1,400 controls) of CSF total tau, 15 studies (600 AD, 450 controls) of Aβ42 and 11 studies (800 AD, 370 controls) of p-tau, 5 studies (Mild AD) for diagnosis of early AD, and 9 studies of MCI and showed that the respective specificities and sensitivities were 90% and 81% for CSF total tau, 90% and 86% for CSF A42, 92% and 80% for CSF phosphorylated tau, and 83~100% and 85~95% in the combination assay with CSF Aβ42 and total tau [18]. A summary of major large-scale multicenter studies of CSF biomarkers for the diagnosis of AD is shown in Table 1.

5. CSF Biomarkers for Prediction of the Onset of AD from MCI

During these past several years, studies of CSF biomarkers have investigated the prediction of progression from MCI to

Table 1: Eleven major studies of CSF biomarkers for AD published between 1998 and 2009.

Study	year	subjects	biomarker	sensitivity	specificity	other
Kanai	1998	93 AD, 54 cont, 33 nAD, 56 ND	Aβ40, Aβ42, †Tau	71~91%	83%	Multicenter, prospective
Hulstaert	1999	150 AD, 100 cont, 79 nAD, 84 ND	Aβ42, †Tau	85%	86%	10 European center
Knopman	2001	3 Class II, III studies	Aβ42, †Tau	80~97%	86~95%	System review AAN
Andreasen	2001	163 AD, 23 VaD, 20 MCI, 9 DLB, 8 ND, 18 cont	Aβ42, †Tau	75~94%	89~100%	1 Y-prospective
Itoh	2001	236 AD, 239 nAD/ND, 95 cont,	PTau199	85%	85%	multicenter
Shoji	2002	366 AD, 181 cont, 168 nAD, 316 ND	†Tau	59%	90%	multicenter
Clark	2003	106 dementia, 73 cont	Aβ42, †Tau	85%	84%	2~8 Y follow up autopsy confirmed
Sunderland	2003	17Aβ42 studies, 34 tau studies (3133 AD versus 1481 control)	Aβ42, †Tau	92%	89%	Meta-analysis
Blennow	2003	41Tau studies (2500 AD versus 1400 cont) 15Aβ42 studies (600 AD versus 450 cont) 11p-Tau studies (800 AD versus 370 cont)	Aβ42, †Tau, PTau Aβ42/tTau	86% 90% 92% 85~94%	90% 81% 80% 83~100%	Systematic review Early AD, MCI
Hampel	2004	161 AD/FTD/DLB/VaD, 45 cont	PTau231 pTau181 pTau199	85%	83%, 79%, 60~71%	International harmonization
GTT3	2004	243 AD, 91 cont, 152 nAD, 157 ND	Aβ40, Aβ42, †Tau	80%	84%	Continuous GTT1

AD (Table 2). A study following 52 MCI subjects for 8.4 months found that 29 MCI subjects developed AD and the specificity of CSF Aβ42 assay was 90% [19]. Follow-up study of 273 subjects (55 MCI, 100 AD, 14 DLB, 11 FTD) for 2 years showed that 38% (20/55) of MCI patients already showed alteration of at least 2 CSF biomarkers including Aβ42, total tau, and p-tau181 at the time of the study initiation. Eleven MCI subjects developed dementia 1 year later, while the remaining 33 subjects stable. Eleven showed a further progression of cognitive impairment, still not fulfilling the diagnostic criteria for dementia. Ten of 11 MCI patients who progressed to AD showed alteration of at least 2 CSF biomarkers, and all 11 converters showed high p181tau levels in CSF. Conversely, 29 (88%) of the 33 stable MCI subjects did not show any alteration of CSF biomarkers [20]. The longest prospective study (4–6 years) followed 137 MCI and 39 normal subjects. Of these 57 subjects (42%) developed AD and 21 subjects (15%) developed dementia due to other causes during followup. Fifty-six subjects (41%) did not show any change during an average followup of 5.2 years. Using CSF Aβ42 and total tau assay, onset of AD was predicted with a sensitivity of 95% and specificity of 83%. The hazard ratio was 17.7. The addition of p181tau assay further improved sensitivity and specificity to 95% and 97%, respectively [21]. In comparison studies between CSF biomarkers and amyloid imaging by PIB-PET, CSF Aβ42 levels were decreased and total and p181 tau levels were increased in very mild AD. CSF Aβ42 levels completely related to brain amyloid deposits detected by PIB-PET in patients with or without dementia [22]. The CSF total tau/Aβ42 ratio and the p181 tau/Aβ42 ratio predicted the exacerbation of CDR score [9].

The usefulness of CSF biomarkers for predicting the progression form MCI to AD was strictly validated by the international consortium study, ADNI performed between 2004 and 2009. In US-ADNI, 819 healthy subjects (55~90 y; 229 normal, 398 MCI, 192 mild AD) were selected based on less than 4 points on Hatchiski Ischemic score, more than 6 points on GDS score, and a history of more than 6 years of education. MMSE scores ranged from 24 to 30 points in normal controls and MCI, 20–26 points in AD patients. CDR sore was 0 in normal controls, 0.5 in MCI, and 0.5~1 in AD. On logical memory test II of WMS-R, the score was evaluated as normal ($\geqq$9: normal subjects with more

Table 2: Major studies of CSF biomarker to predict progression from MCI to AD published between 2004 and 2010.

Study	Year	Case	Follow-up	MCI to AD	Marker	Sensitivity	Specificity	Other
Hampel	2004	52 MCI 93 AD 10 cont	8.4 M	29/52 (56%)	Aβ42, †Tau	Aβ42 59% †Tau 83%	Aβ42 100% †Tau 90%	European cohort
Parnetti	2006	55 MCI 100 AD 14 DLB 11 FTD	1 Y	11/55 (20%) 38% of MCI has 2 marker abnormalities	Aβ42, †Tau, pTau181	2 biomarker abnormality in AD converters (91%)	Normal markers in stable MCI (88%)	Mayo Clinic Cohort
Hansson	2006	137 MCI 39 cont	4~7 Y	57 AD (42%) 21 nonAD dementia (15%) 56 stable MCI (41%)	Aβ42, †Tau, pTau181	Aβ42/†Tau 95% Aβ42/†Tau/pTau181 95%	Aβ42/†Tau 83% Aβ42/†Tau/pTau181 87%	Prospective study
Show	2009	100 AD 191 MCI 114 cont	1 Y	37/191 (19%)	Aβ42, †Tau, pTau181	Tau/Aβ42 predicted 89% of AD converters CSF Aβ42 highly correlated with brain pathology		US-ADNI
Mattsson	2009	750 MCI 529 AD 304 cont	>2 Y	271 AD/750 MCI (36%) 59 nonAD dementia/750 MCI	Aβ42, †Tau, pTau181	83%	73%	12 centers Europe/US
Visser	2009	60 SCI 37 naMCI 71 aMCI 89 cont	3 Y	8/22 CSF/AD naMCI (36%) 27/53 CSF/AD aMCI (51%)	Aβ42, †Tau, pTau181	CSF/AD was observed in control 31%, SCI 52%, naMCI 68%, aMCI 79% All AD had CSF/AD CSF/AD is a significant risk in aMCI		DESCRIPA study Europe study

SCI: subjective cognitive impairment; naMCI: nonamnesctic MCI; aMCI: amnestic MCI; CSF/AD: CSF AD profile (decreased Aβ42/increased tau).

than 16 years of education, $\geqq$5: normal subjects with 8~15 years of education, $\geqq$3: normal subjects with 0~7 years of education), and as MCI or AD ($\leqq$8: subjects with more than 16 years of education, $\leqq$4: subjects with 8~15 years of education, $\leqq$2: subjects with 0~7 years of education). The result one year later showed that the mean AD conversion/year was 16.5% (1.4% in those converting from normal to MCI, 16.5% in those converting from MCI to AD, 8 cases reverted from MCI to normal and 2 cases reverted from AD to MCI). About 50% of MCI patients take anticholinesterase or memantine (ChEI: 44% in MCI, 85% in AD; memantine: 11% in MCI, 47% in AD: Combination use of ChEI and memantine: 9% in MCI, 41% in AD). Average deteriorations of ADAS-cog score were 1.1 points/year in MCI and 4.3 points/year in AD. Among all CSF biomarkers, Aβ42 at the initial evaluation was the most reliable marker predicting conversion from MCI to AD and the progression of cognitive impairment. CSF Aβ42 was the most sensitive biomarker for AD in a follow-up study of 100 mild AD, 196 MCI, and 114 normal elderly controls and an associated correlation study using age-matched 52 normal control and 56 AD brains (sensitivity 97%, specificity 77%). The sensitivity and specificity of CSF total tau assay were 70% and 92%, respectively. The sensitivity was 68% and the specificity was 73% for a CSF p-tau181 assay. The sensitivity of the total tau/Aβ42 ratio was 86%, and its specificity was 85%. Aβ42, total tau, and the number of ApoE ε4 alleles were the most sensitive biomarkers in the ADNI cohort. Using CSF total tau/Aβ42 ratio, 33 converters (89%) were predicted before onset among 37 subjects who progressed from MCI to AD within one year [1, 23]. US-ADNI Penn Biomarker core reported that decreased CSF Aβ42 levels and increased total tau levels were prediction markers for AD with the highest evidence level, and that Aβ42 was the earliest marker among total tau, F^{18}FDG-PET, and MRI examination [24]. In addition, there were subgroups of normal subjects who showed rapid deterioration of cognitive function [25]. Hippocampal atrophy detected by MRI was closely related to CSF p-181Tau levels in the ApoE 4 carrier group and CSF total tau in the ApoE 4 noncarrier group. Amyloid accumulation in the precuneus detected by the PIB amyloid PET correlated with hippocampal atrophy [26]. CSF p-tau181 amounts were related with cognitive impairment in the normal group, while CSF Aβ42 amounts were correlated with those in the MCI group. These 2 biomarkers more sensitively detected cognitive impairment than ADAS-cog in the normal and MCI groups. In all groups, subjects with alteration of both Aβ42 and p-tau181 levels showed faster deterioration of cognitive function [27].

A prospective study for more than 2 years by 12 European and American centers reported that 271 patients with MCI developed AD and 59 developed non-AD-type dementia among 750 MCI patients. The sensitivity and specificity of using CSF Aβ42, total tau, and p-tau181 were 83% and 72%, respectively [28]. In the DESCRIPA study, 60 SCI subjects (subjective cognitive impairment), 37 non-amnestic

Table 3: Major studies of plasma Aβ40 and Aβ42.

Study	Year	Follow	Subject	Marker	Results	Journal
Matsubara	1999	—	36 AD 206 cont 6 DS	Lipoprotein free Aβ40, Aβ42	Increased plasma lipoprotein free Aβ42 in AD and Down Syndrome	Ann Neurol
Van Oijen	2006	8.6 Y Rotterdam study	1,756/6,713	Aβ40, Aβ42 Aβ42/Aβ40	396 cases developed dementia during follow up Increased Aβ40 level was a risk for onset of dementia Increased Aβ42/Aβ40 ratio decreased the risk for onset of dementia	Lancet Neurol
Graff-Radford	2007	3.7 Y Mayo Cohort	563 control	Aβ40, Aβ42 Aβ42/Aβ40	53 cases developed MCI and AD Significantly increased risk for onset of MCI and AD (3.1) in lower 25% group with decreased Aβ42/Aβ40 ratio	Arch Neurol
Schupf	2008	4.6 Y	1,125 control	Aβ40, Aβ42 Protofibrillar Aβ42	104 cases developed AD (9.2%) High Aβ42 level increased threefold risk for onset of AD Once developing AD, plasma Aβ42, Aβ42/Aβ40 ratio and protofibrillar Aβ42 were significantly decreased	PNAS
Xu	2008	—	113 AD 205 control	Autoantibody Aβ40, Aβ42	Anti-Aβ42 dimer antibody was absent in AD Aβ40/42 ratio increase with progression of AD	Brain Res
Lambert	2009	4 Y	233 dementia 958 control 8,414 source	Aβ40, Aβ42	prospective 3 city study Aβ42/Aβ40 ratio showed short-term risk of dementia	Neurol
Okereke	2009	10 Y prospective	481 Nurses	Aβ40, Aβ42	Presenile (mean 64 Y) high Aβ42/ Aβ40 ratio correlated with cognitive function 10 years later	Arch Neurol

MCI (naMCI), 71 amnestic MCI (aMCI), and 89 normal controls were followed for 3 years and examined by CSF biomarkers. In the naMCI group with AD-like alteration of CSF biomarkers (AD profile), 8 (36%) of 22 cases developed AD. In the aMC group, 27 (51%) of 53 cases showing with CSF AD profile developed AD. The CSF AD profile was recognized in 31% of control, 52% of SCI, 68% of naMCI and 79% of aMCI. All converters showed the CSF AD profile and the combination of decreased Aβ42 and increased total tau/p-181tau levels in CSF was a significant risk factor in the aMCI group [29] (Table 2).

6. Plasma Aβ40 and Aβ42 as Risk Factors for AD

Since measurement of plasma Aβ42 and Aβ40 by Matsubara, the decreased ratio of Aβ42 and Aβ40 has been reported as a risk factor for AD onset [30]. The Rotterdam study prospectively studied 1,756 subjects randomly selected from 6,713 participants for an average of 8.6 years and reported that 392 subjects developed dementia. In this study, plasma Aβ40 levels at the start related to the risk of the dementia onset. The age- and sex-adjusted upper quartile with a high plasma Aβ40 showed a hazard ratio of 1.07∼1.46 compared with the other 75% of the group. The upper quartile with a high Aβ42/Aβ40 ratio showed a decreased hazard ratio for onset of dementia of 0.74∼0.47 [31]. The Mayo Clinic prospective study consisted of 563 subjects with a mean age of 78 years old followed for 3.7 years. It was reported that 53 of these subjects developed MCI and AD. Significant increase in the risk of MCI and AD onset was recognized in the lower quartile with a low plasma Aβ42/Aβ40 ratio. Relative risk was 3.1 on comparison between the upper quartile and lower quartile. After a adjusting for age and ApoE genotypes, significant deterioration was recognized in subjects with a low plasma Aβ42/Aβ40 ratio [32]. A prospective study of 1,125 cognitively normal elderly subjects for 4.6 year showed that 104 subjects (9.2%) developed AD. High plasma Aβ42 levels at the start of study increased threefold the risk of AD onset. Once AD developed, however, plasma Aβ42, Aβ42/Aβ40 ratio, and protofibrillar Aβ42 were significantly decreased [33]. The presence of anti-Aβ autoantibody was suggested in human plasma, and the Aβ40/Aβ42 ratio was closely related to progression of cognitive impairment in AD patients [34]. By 2009, two additional large-scale studies were reported [35, 36], and the results of the ADNI study are

expected in the near future. These findings are summarized in Table 3.

7. Development of Other Biomarkers

Homocysteine in CSF and plasma were measured in US-ADNI. Significant differences among normal, MCI, and AD groups were observed in plasma, but were not recognized in the CSF. At the same time, there was no significant difference in CSF Isoprostane measured as a marker of oxidation stress [24]. A report of the establishment of ELISA for Aβ oligomer, the main causative molecule of AD, has been attracting attention for measurement of plasma in AD subjects. Plasma Aβ oligomer could be detected in 3 of 10 normal subjects and 19 of 36 AD patients. The level of plasma Aβ oligomer correlated with those of Aβ monomer, and both amounts progressively decreased in familial AD patients [37]. Studies examining CSF α-synuclein and TDP-43 levels as biomarkers for DLB, FTLD-TDP, and ALS were reported from Japan. Levels of CSFα-synuclein were measured by ELISA in 16 DLB and 21 AD patients, but there were no significant differences; however, a correlation with disease duration was recognized in the DLB group [38]. Measurement of CSF DJ-1 and α-synuclein by ELISA in 117 Parkinson's disease (PD) patients, 132 normal control and 50 AD patients suggested that age and contamination of blood caused some artifacts, but showed that both markers were decreased in PD compared with those in normal controls and AD. The sensitivity and specificity for CSF DJ-1 were 90% and 70%, and those for CSF α-synuclein were 92% and 58%, respectively [39]. Assay of CSF TDP-43 was established by Tokuda, and increased levels of TDP-43 were found in early ALS suggesting an early diagnostic marker of ALS. A further detailed study of the usefulness of CSF TDP-43 in ALS and FTDP-TDP is desired in the near future [40].

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Research Article

Feasibility of Predicting MCI/AD Using Neuropsychological Tests and Serum β-Amyloid

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We examined the usefulness of brief neuropsychological tests and serum Aβ as a predictive test for detecting MCI/AD in older adults. Serum Aβ levels were measured from 208 subjects who were cognitively normal at enrollment and blood draw. Twenty-eight of the subjects subsequently developed MCI ($n = 18$) or AD ($n = 10$) over the follow-up period. Baseline measures of global cognition, memory, language fluency, and serum Aβ_{1-42} and the ratio of serum Aβ_{1-42}/Aβ_{1-40} were significant predictors for future MCI/AD using Cox regression with demographic variables, APOE ε4, vascular risk factors, and specific medication as covariates. An optimal sensitivity of 85.2% and specificity of 86.5% for predicting MCI/AD was achieved using ROC analyses. Brief neuropsychological tests and measurements of Aβ_{1-42} obtained via blood warrants further study as a practical and cost effective method for wide-scale screening for identifying older adults who may be at-risk for pathological cognitive decline.

1. Introduction

An exponential rise in Alzheimer's disease (AD) prevalence rates is predicted to parallel the aging of baby boomers creating a potentially unsustainable economic burden to the healthcare system. Delaying the onset or progression of AD, even modestly, by earlier pharmacological intervention could substantially reduce the economic and psychosocial impact of the illness [1, 2]. Unfortunately, many AD patients remain undiagnosed or go undetected until the later stages of disease. Insights into the underlying pathological mechanisms involving beta-amyloid plaque deposition within the brain have led to the development of a host of antiamyloid agents [3] that are in various stages of clinical investigation. There is now a scientific consensus that the pathological events in AD initiate decades before clinical symptoms become apparent, and if disease modification is realized in the coming decades, the need for improved methods of early detection prior to the overt clinical signs will be accentuated.

Traditionally, neuropsychological measures, particularly those that tap cognitive abilities subsumed by the hippocampal formation such as episodic memory, have shown usefulness in identifying cognitively normal elders who subsequently develop AD [4, 5]. Decrements in semantic memory and concept formation have been shown to occur nearly a decade before the development of AD [6]. Performance on visual-spatial and verbal memory measures in midlife have also been shown to predict later memory loss [7]. Neuropsychological measures are noninvasive and generally cost effective. However, individuals with very high premorbid intellectual abilities experiencing incipient cognitive decline may go undetected, and false positives are possible in individuals with a low level of intellectual abilities. Also appropriate interpretation of extensive neuropsychological testing requires a high degree of expertise and training, which limits its use in routine clinical settings.

The advancement of molecular imaging tracers that bind to amyloid, such as Pittsburgh Compound B (PIB) or longer-lived probes (e.g., FDDNP), offers a non-invasive *in vivo* method to detect and quantify brain amyloid deposition [8, 9]. However, this approach for presymptomatic detection is economically impractical for routine use given the current costs and restrictions on "medically necessary" use. Similarly, biomarkers including Aβ_{1-42} and phosphorylated tau (also

implicated in AD pathology) in cerebral spinal fluid (CSF) can predict subsequent cognitive decline [10, 11], but lumbar puncture carries risks and is inconvenient for wide-scale use in cognitively impaired elderly subjects.

Blood-based biomarkers have more practical applicability for routine use and are likely to be more cost effective than both CSF and imaging procedures. Consequently, measurement of $A\beta_{1-40}$ and $A\beta_{1-42}$ in blood is increasingly being explored and shows potential in identifying individuals at the preclinical stage of AD [12–14]. It has been reported that CSF $A\beta$ levels are subject to high diurnal fluctuations with extremely high variability reported over 12 hours [15]. Over days and weeks, $A\beta$ in blood appears more stable than CSF [16–18]. Furthermore, serum contains more $A\beta$ than plasma [16], possibly due to the release of bound $A\beta$ during the clotting process [19]. Hence, serum $A\beta$ appears suitable for use in predicting MCI/AD and optimal sensitivity, and specificity is probably achievable if combined with current diagnostic procedures, such as brief neuropsychological testing.

In this study, we examined the usefulness of brief neuropsychological tests in combination with blood $A\beta_{1-40}$ and $A\beta_{1-42}$ as a predictive test for detecting MCI/AD in at-risk older adults at a pre-symptomatic stage. Such an approach will be more practical for clinical use and be germane in designing large-scale prevention trials.

2. Methods

2.1. Subjects. Participants included a subset of subjects enrolled in the Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT). ADAPT was a randomized, placebo-controlled, multicenter primary prevention trial sponsored by the National Institute on Aging. The Roskamp Institute served as one of six recruitment sites located across the US. Subjects were randomly assigned to one of three groups: celecoxib (200 mg b.i.d.), naproxen sodium (220 mg b.i.d.), or placebo. The primary outcome measure of ADAPT was development of AD. Full details of data collection, measurements, and study procedures are available at http://www.jhucct.com/adapt/manall43.pdf and described elsewhere [20].

The inclusion criteria for ADAPT subjects were age of 70 or older at enrollment, a self-reported family history of AD-like dementia, and normal cognitive performance on a brief battery of neuropsychological tests. Recruitment for ADAPT began in 2002, and the study was completed in 2007. In 2005, the Roskamp Institute initiated a proteomic ancillary study (F. Crawford, PI) involving blood draw from these subjects. The inclusion criteria for this ancillary study stipulated that each subject was an active ADAPT participant and had met all the ADAPT inclusion and exclusion criteria. An approval was obtained from the ADAPT Steering Committee and a centralized IRB. A separate consent was also obtained from each subject who participated in the ancillary study.

Two hundred and fifteen subjects from the Roskamp ADAPT cohort enrolled in the proteomic ancillary study. At the time of blood draw, subjects maintained cognitively normal status as determined by their performance on an annual cognitive assessment battery. These assessments continued for an additional two years following the blood draw. Blood was collected during the semi-annual followup visits, and the cognitive assessments were performed at the baseline visit and at the annual visits. The time from baseline cognitive testing to the diagnosis of MCI/AD was 4.06 years ($\pm$1.3 SD). Timeframe from baseline cognitive testing to blood draw was 2.25 years ($\pm$0.71 SD) and from blood draw to diagnosis was 1.79 years ($\pm$1.2 SD). The cognitive measures completed at baseline and annual followup included the Modified Mini-Mental State Examination (3MS) [21]; the Hopkins Verbal Learning Test-Revised (HVLT-R) [22]; Digit Span (forward and backward) from the Wechsler Adult Intelligence Scale-Revised (WAIS-R) [23]; a Generative Verbal Fluency test (supermarket items); the narratives from the Rivermead Behavioral Memory Test (RBMT) [24]; the Brief Visuospatial Memory Test-Revised (BVMT-R) [25]. The Mini-Mental State Examination (MMSE) [26] was extracted from 3MS. Alternate forms were utilized annually for the HVLT-R, RBMT, and BVMT-R on each subsequent annual visit. Subjects also completed the 30-item Geriatric Depression Scale [27] and a self-rating scale of memory functions [28]. Collateral respondents completed the Dementia Severity Rating Scale (DSRS) [29]. Due to significant intercorrelations between these tests, analyses described below are limited to those baseline cognitive tests that were sensitive to early changes (i.e., verbal learning and memory) associated with MCI/AD [30] or tests that were similar to those previously shown to be associated with $A\beta$ levels [31].

Normative data from the Cache County study was used to develop the standardized cut-off scores utilized in ADAPT [32]. Individuals who scored below the cut scores on annual cognitive assessments underwent further dementia workup including physical and neurological examinations, laboratory studies (i.e., CBC, chemistry count, sedimentation rate, vitamin B_{12} and folic acid levels, thyroid test, and syphilis serological test), and neuroimaging (i.e., MRI or CT), as applicable. A more comprehensive neuropsychological assessment was also administered by a neuropsychologist as part of the dementia work-up. This battery of tests consisted of the expanded Consortium to Establish a Registry for Alzheimer's Disease (CERAD) battery [33]; Logical Memory I and II of the Wechsler Memory Scale-Revised [34]; Benton Visual Retention Test [35] (Benton); a generative fluency test (animals); Control Oral Word Association Test (COWAT; CFL) [36]; The Trail Making Test [37]; Symbol Digit Modalities Test (SMDT) [38]; Shipley Vocabulary [39].

Following completion of all components of the dementia work-up, a consensus team determined cognitive status using published diagnostic criteria. The annual battery was not utilized in diagnostic determination. The diagnosis of AD was made using NINCDS-ADRDA [40] and amnestic mild cognitive impairment (MCI) using Petersen criteria [41]. All MCI patients were considered to be amnestic MCI, as they only had memory impairment, but maintained normal activities of daily living and overall had a well-preserved cognition in other cognitive domains. Ample

evidence indicates that amnestic MCI patients may be in a transitional stage between normal aging and AD with 85% of these subjects converting to AD over a 7-year period [42]. Additional evidence comes from an imaging study which demonstrated that the pattern of brain atrophy in amnestic MCI patients is typical of that observed in AD patients [43]. It is then reasonable to combine these diagnoses in a single category, thus allowing a large enough numbers to supply statistical power. Of the 215 subjects who gave blood for the ancillary study, two developed non-AD dementia, and another subject died with cognitive status unknown. Blood Aβ values were unavailable for 4 subjects. Of the remaining subject pool of 208 used in these analyses, 28 subjects met criteria for either AD ($n = 10$) or MCI ($n = 18$) in the two years following blood draw.

2.2. Sample Collection, Preparation, and Measurements. Blood draws for Aβ measurement and APOE genotyping were conducted by trained phlebotomists. Serum from blood was prepared and processed using standard laboratory procedures [16]. The serum Aβ content was determined, as per manufacturer's instructions, using the ELISA kits for human Aβ_{1-40} and Aβ_{1-42} and the inter-assay CV, and the intraassay CV was reported to be $\leq$10% (Invitrogen, Calif). Additional details are provided elsewhere [16]. DNA was extracted from whole blood for APOE genotyping using Pure Gene Kits (Gentra systems, Calif), and APOE genotyping was performed using previously established methods, as described elsewhere [16]. APOE genotypes were unavailable for 4 individuals, but these were included in the analyses.

2.3. Statistical Analyses. The data set was range checked, and prior to analyses, the dependent and independent variables were examined for missing data, outliers, and violations of the normalcy assumption. Differences among groups on demographic variables, neuropsychological variables, and serum Aβ_{1-40} levels were examined using either the student's t-test or χ^2 analyses, depending on the type of variable measurement. The Mann-Whitney test was employed if parametric assumptions were not met.

Time-updated Cox regression modeling was used to test whether neuropsychological test scores, Aβ, or a combination of both can predict conversion to MCI/AD in individuals who were cognitively normal at baseline. Potential confounding variables shown to impact risk for cognitive decline included age, education, gender, APOE status, serum creatinine, triglycerides, presence of APOE ε4 allele, and history of vascular disease as determined by treatment with statins or antihypertensive medication which were entered as covariates. The latter variables, coded dichotomously, have been previously shown to impact Aβ levels [44]. Because previous analyses revealed a nonsignificant increase of AD risk with naproxen in this cohort [45], we also controlled for this effect.

Logistic regression modeling was employed to construct receiver operator curves (ROC) to examine the predictive performance of neuropsychological measures from the baseline visit and serum Aβ levels in diagnoses of MCI/AD. ROC curve comparisons were based on area under the curve (AUC), SE, and the associated 95% confidence interval (CI). We subsequently calculated sensitivity of the various models using the predicted probability of each subject by logistic regression modeling with specificity of at least eighty percent. Post hoc power calculations using the G-power software for multivariate regression analyses utilized here suggest a power of nearly 100% at the alpha value 0.05 for the current sample size, total number of predictors, and the observed effect size. All analyses were conducted using the SPSS version 16.0 for Macintosh.

3. Results

The mean age and education of the sample was 76.7 (SD = 3.9) and 14.6 (SD = 2.8) years, respectively. The majority of the sample was Caucasian (98.1%), and 51.9% were male. Despite the cohort's self-report of enriched family history, less than one-third of the total sample (31.7%) carried at least one APOE ε4 allele, a frequency similar to the general population [44]. Comparisons on variables between subjects who remained cognitively normal and those who declined over the short follow-up period are reported in Table 1. Although all subjects at enrollment performed within the normal limits based on the established cut-off scores, those that ultimately declined had generally poorer scores on the 3MS, MMSE, and all memory measures. The two groups were also significantly different on serum Aβ_{1-42} levels and Aβ_{1-42}/Aβ_{1-40}ratios prior to diagnoses of MCI/AD. Only 23% of the cognitively normal individuals had serum Aβ_{1-42} in the lowest quartile compared to the nearly 50% of the diagnostic group (44% of MCI subjects and 50% of AD subjects).

Time-dependent Cox regression analyses were performed to examine the relationship between these cognitive tests and Aβ on the prediction of subsequent conversion to MCI/AD. All neuropsychological analyses were adjusted for age, gender, and education, but no adjustment for the study medications was required as these were baseline scores. Cox regression analyses show that the model using neuropsychological tests predicted MCI/AD (-2 log-likelihood = 206.51, $\chi^2 = 52.11$, df = 8, $P < .001$). Significant individual neuropsychological measures were 3MS ($\beta = -0.25 \pm 0.06$, Wald = 17.78, $P < .001$); generative verbal fluency ($\beta = 0.12 \pm 0.04$, Wald = 8.09, $P < .004$); HVLT-R scores ($\beta = 0.24 \pm 0.11$, Wald = 4.58 $P < .032$).

Cox regression analysis showed that Aβ_{1-42} measured in the lowest two quartiles compared to the highest quartile was a significant individual predictor of conversion to MCI/AD in this model (-2 log-likelihood = 197.47, $\chi^2 = 38.41$, df = 15, $P < .001$). The regression analysis utilizing the Aβ_{1-42}/Aβ_{1-40} ratio found similarly significant results (-2 log-likelihood = 204.69, $\chi^2 = 36.10$, df = 14, $P < .001$) with the lowest ratios being most predictive of subsequent conversion to MCI/AD. The final full model, adjusting for confound and the study medications, included HVLT-R, fluency, 3MS, Aβ_{1-42} levels, and Aβ_{1-42} quartiles (-2 log-likelihood = 166.25, $\chi^2 = 74.55$, df = 18, $P < .001$) with

Table 1: Variable comparisons between groups.

Variable	MCI/AD ($n = 28$)	Controls ($n = 190$)
Age	77.8 ± (3.9) years	76.6 ± (3.9) years
Education	14.61 ± (3.2) years	14.63 ± (2.8) years
% Male	67.9%	49.4%
% APOE ε 4 carrier	42.3%	32.4%
	Means ± SD	
3MS	92.93 ± (4.0)*	96.7 ± (3.0)
MMSE	28.29 ± (2.1)*	28.98 ± (1.3)
HVLT-R	8.11 ± (2.1)*	9.85 ± (2.0)
Digit Span:		
Forward Score	8.36 ± (2.3)	8.27 ± (2.0)
Backward Score	6.93 ± (2.1)	6.87 ± (1.9)
Generative Fluency	24.86 ± (5.8)	25.66 ± (6.2)
RBMT	57.14 (25.4)*	75.00 ± (31.2)
BVMT-R	6.46 ± (2.6)*	8.07 ± (2.4)
$A\beta_{1-40}$	138.08 ± (43.72)	146.24 ± (55.37)
	Median (25th, 75th quartile)	
$A\beta_{1-42}$	7.23 (1.97, 17.49)**	12.38 (6.28, 23.20)
$A\beta_{1-42}/A\beta_{1-40}$ ratio	0.05 (0.02, 0.10)**	0.09 (0.05, 0.15)

*t-Test $P < .05$.
**Mann-Whitney U $P < .05$.
Note: 3MS = Modified Mini-Mental State Examination; MMSE = Mini-Mental State Examination; HVLT-R = Hopkin's Verbal Learning Test-Revised, Trial 4; RBMT = Rivermead Behavioral Memory Test; BVMT-R = Benton Visual Memory Test-Revised.

fluency, 3MS, and $A\beta_{1-42}$ in the lowest two quartiles as significant individual predictors of MCI/AD in the model. $A\beta_{1-40}$ was not a significant individual predictor. Similar results were observed when $A\beta_{1-40}$ levels and $A\beta_{1-42}$ quartiles were substituted in this model with $A\beta_{1-42}/A\beta_{1-40}$ ratios (−2 log-likelihood = 168.49, $\chi^2 = 72.90$, df = 17, $P < .001$).

Baseline values for the 3MS, HVLT-R, and generative verbal fluency scores were subtracted from those obtained at the 12-month repeat testing to determine if changes in these measures differ by $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratios. In unadjusted analyses, among subjects who converted to MCI/AD, the greatest decline for HVLT-R was observed among individuals with the lowest quartile of $A\beta_{1-42}$ (−1.17, ± 2.33 SD) and $A\beta_{1-42}/A\beta_{1-40}$ ratios (−0.75, ±2.63 SD) where individuals in the highest quartile of $A\beta_{1-42}$ (1.33, ± 1.86 SD) and $A\beta_{1-42}/A\beta_{1-40}$ ratios improved by nearly one point (0.6 ± 1.82 SD). However, these differences were not statistically significant ($P > .05$). For the 3MS scores, among subjects who converted to MCI/AD, those with $A\beta_{1-42}$ in the lowest quartile declined (−1.83 ± 1.28 SD) as compared to the highest quartile (4.83 ± 1.35 SD), and this difference was statistically significant (F = 3.42, $P = .033$). For MCI/AD subjects with the lowest quartile of the $A\beta_{1-42}/A\beta_{1-40}$ ratios, the 3MS values remained ultimately unchanged (0.16 ± 1.20 SD), while the scores improved among those with the highest quartile of the $A\beta_{1-42}/A\beta_{1-40}$ ratios (4.33 ± 1.20 SD), and these differences were also statistically significant (F = 3.10, $P = .046$). For generative verbal fluency test, a decline was noted in both the lowest quartile (−4.17 ± 1.40 SD) and the highest quartile (−1.17 ± 2.13 SD) of $A\beta_{1-42}$, and these differences were marginally significant (F = 2.63, $P = .073$). For $A\beta_{1-42}/A\beta_{1-40}$ ratios, a similar pattern was observed, but this difference was not statistically significant. Among individuals who remained cognitively normal, while a similar pattern was observed, those with lowest quartile of $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratios had a larger decline than those with the highest quartile for each HVLT-R (−0.28 ± 0.27 SD versus. 0.14 ± 0.33 SD, respectively.) and 3MS (−1.02 ± 0.51 SD versus −0.39 ± 0.44 SD). However, due to the small magnitude of the change in these scores, these differences were not statistically significant. No such change was observed for the generative verbal fluency test (data not shown).

Examination of sensitivity and specificity using ROC analysis revealed the AUC for neuropsychological testing with age, education, and gender as covariates was 0.83 (95% CI [0.75–0.91], $P < .001$). For $A\beta_{1-42}$ (adjusted for presence of APOE ε4 allele, vascular risk factors, and associated medications), the AUC was 0.79 (95% CI [0.70–0.88], $P < .001$). When neuropsychological testing (3MS, HVLT-R, and Generative Verbal Fluency) and $A\beta_{1-42}$ were combined, the AUC was increased to 0.91 (95% CI [0.86–0.95], $P < .001$). For the adjusted (as above) $A\beta_{1-42}/A\beta_{1-40}$ ratios alone, the AUC was 0.79 (95% CI [0.71–0.88], $P < .001$), and when combined with the neuropsychological measures, AUC was 0.91 (95%CI [0.87–0.96], $P < .001$). The various ROC curves are displayed in Figure 1. Optimal sensitivities with specificity of at least 80% predicted probabilities are shown in Table 2. The highest sensitivity and specificity was achieved using a combination of cognitive scores and $A\beta_{1-42}/A\beta_{1-40}$ ratio, but this finding was driven by $A\beta_{1-42}$.

Table 2: Optimal sensitivities with specificities at least 80% for the various models*.

Model	Sensitivity	Specificity	≪R^2	◁Goodness-of-fit test
Neuropsychological tests†	67.9%	80.0%	0.32	9.32
$A\beta_{1-40}$ and $A\beta_{1-42}$	55.6%	80.0%	0.22	12.81
$A\beta_{1-42}/A\beta_{1-40}$ ratio	59.3%	80.0%	0.22	6.28
Neuropsychological tests and $A\beta_{1-40}$ and $A\beta_{1-42}$	85.2%	85.9%	0.47	2.31
Neuropsychological tests and $A\beta_{1-42}/A\beta_{1-40}$ ratio	85.2%	86.5%	0.49	4.48

* Calculations based on predicted probabilities form Logistic Regression.
† Modified Mini-Mental State Examination, Hopkins Verbal Learning Test-Revised Trial 4, supermarket fluency.
≪ Represents Negelkerke R^2.
◁ Hosmer and Lemeshow chi-square test of goodness of fit, a P value of > .05 was noted and indicates that the model adequately fits the data.

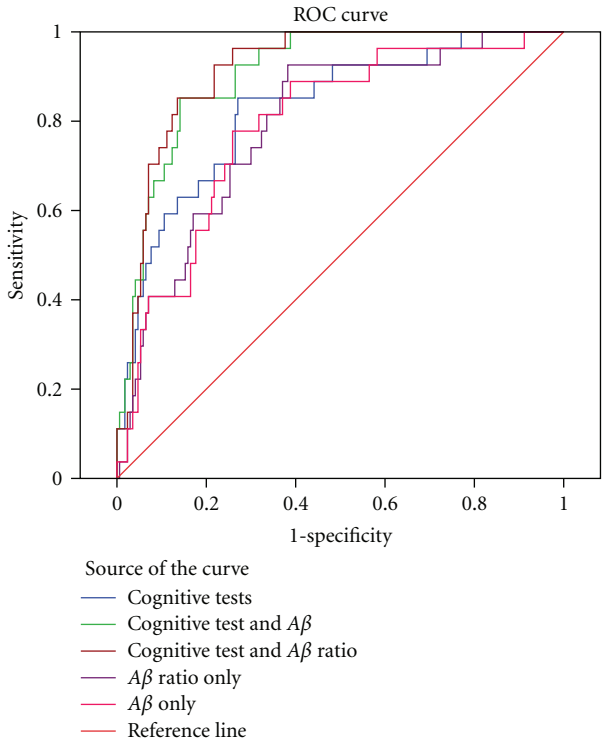

Figure 1

4. Discussion

The pathogenesis of AD is initiated before the clinical symptoms of cognitive impairment and functional decline become apparent in its victims. A simple and pragmatic method for identifying older adults at an increased risk for MCI/AD who may benefit from targeted prevention is therefore of importance in reducing the burden of AD. The combination of brief neuropsychological tests along with blood-based biomarkers of AD represents a reasonable approach with a potential for wide-scale use. Our findings here provide support for this notion and demonstrate that early prediction of risk for developing MCI/AD may be feasible via a combination of brief neuropsychological tests and biomarkers in an at-risk cohort. In this subcohort from ADAPT, measures of global cognitive function (3MS), episodic memory (HVLT-R Trial 4), language fluency, and serum $A\beta_{1-42}/A\beta_{1-40}$ ratio achieved an excellent accuracy of 91%. Furthermore, sensitivity with specificity of at least 80% for the combined measures was superior to neuropsychological measures or to serum Aβ levels alone.

We have recently shown that Aβ levels alone can predict MCI/AD [14], but Aβ levels are influenced by vascular disease and associated medications [44] and require adjustment to observe the full impact of Aβ in predictive modeling. We have also shown that in subjects diagnosed with AD, there is an association between measures of language tests of fluency and object naming and $A\beta_{1-40}$ and that memory performance is associated with serum $A\beta_{1-42}$ [31]. An association between serum $A\beta_{1-40}$ and cognitive measures of memory and language has also been reported in cognitively normal older adults [46]. High baseline $A\beta_{1-42}$ and $A\beta_{1-40}$ with stable $A\beta_{1-42}$ over time is shown to be associated with diminishing cognition [47]. More recently, Yaffe and colleagues demonstrated that low $A\beta_{1-42}/A\beta_{1-40}$ ratios predict cognitive decline over 9 years [48]. In our study, we demonstrate that low $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratios are associated with cognitive decline even within one year. This is extremely valuable from the clinical perspective, as the ability to identify at-risk individuals within a year prior to the onset can significantly improve the quality of care and the recruitment strategy for prevention trials by redirecting those individuals who may not benefit from preventive therapies towards more suitable clinical intervention. This is demonstrated by recent ADAPT findings, which suggest that individuals with low baseline cognitive scores converted soon after the trial initiated and that neither naproxen nor celecoxib intervention was beneficial to these individuals [49]. Collectively, these findings suggest that combining cognitive tests with blood Aβ may be useful for predicting future MCI/AD, which to date has not been explored, particularly as either Aβ or the cognitive tests alone may not have the desired sensitivity or specificity for prediction of future MCI/AD.

This current work presented here provides evidence that the combination of brief neuropsychological tests and blood Aβ has potential utility in predicting MCI/AD at least 2 to 4 years prior to the clinical classification of MCI or diagnosis of AD. In addition, our findings also demonstrate the importance of accounting for factors such as APOE, vascular risk factors, and medications when using Aβ in predicting MCI/AD. Although at present no studies

have reported sensitivity and specificity of CSF $A\beta_{1-42}$ in predicting MCI/AD conversion from normal cognition, a large multicenter study has shown that CSF $A\beta_{1-42}$ predicts transition from MCI to AD [50], while tau alone achieved a high sensitivity (83%) with acceptable specificity (72%). It is interesting to note that our findings using blood and cognitive tests, a far less invasive method, resulted in higher sensitivities and specificities for predicting cognitive decline in at-risk cognitively normal older adults. Despite the limitation that blood sampling was not conducted at the same time point as the cognitive testing, our data provide strong support for further evaluation of this approach, particularly as we have not seen significant fluctuations in $A\beta$ levels over a one-year period (pers. Comm.).

5. Conclusion

Our study provides support that blood-based $A\beta$ levels may have diagnostic utility when combined with neuropsychological measures. This proposed method warrants further investigation to determine its practical applicability in specialized clinic setting by allied health personal and in routine primary care clinics.

Disclosure

The authors report no conflict of interests.

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Research Article

MRI Shows More Severe Hippocampal Atrophy and Shape Deformation in Hippocampal Sclerosis Than in Alzheimer's Disease

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While hippocampal atrophy is a key feature of both hippocampal sclerosis (HS) and Alzheimer's disease (AD), the pathology underlying this finding differs in these two conditions. In AD, atrophy is due primarily to loss of neurons and neuronal volume as a result of neurofibrillary tangle formation. While the etiology of HS is unknown, neuron loss in the hippocampus is severe to complete. We compared hippocampal volume and deformations from premortem MRI in 43 neuropathologically diagnosed cases of HS, AD, and normal controls (NC) selected from a longitudinal study of subcortical ischemic vascular disease (IVD Program Project). HS cases ($n = 11$) showed loss of neurons throughout the rostral-caudal extent of the hippocampus in one or both hemispheres. AD cases ($n = 24$) met NIA-Reagan criteria for high likelihood of AD. Normal control cases ($n = 8$) were cognitively intact and showed no significant AD or hippocampal pathology. The mean hippocampal volumes were significantly lower in HS versus AD groups ($P < .001$). Mean shape deformations in the CA1 and subiculum differed significantly between HS versus AD, HS versus NC, and AD versus NC ($P < .0001$). Additional study is needed to determine whether these differences will be meaningful for clinical diagnosis of individual cases.

1. Introduction

Hippocampal sclerosis (HS) is a highly prevalent pathologic lesion, found in approximately 15% of elderly dementia cases in autopsy series [1]. It is a common cause of memory loss in late life, but is rarely diagnosed before autopsy. HS is characterized by selective neuronal loss with gliosis in the absence of cystic cavitation, involving the CA1 sector of the hippocampus and often extending into the subiculum. A sharp demarcation is often noted histologically between affected and adjacent normal hippocampal subfields. HS can affect one or both hemispheres and can be focal or widespread in its rostral-caudal extent [2]. HS is often accompanied by other types of pathology, but may occur as a relatively isolated finding [1]. The pathogenesis of HS is not well understood, and ischemia/hypoxia, neurodegeneration, or a combination of these nonmutually exclusive processes has been postulated.

A clinical diagnosis of HS is rarely made in late life. Most HS cases are discovered at autopsy and were diagnosed clinically as AD. The typical hallmarks of HS seen in early adulthood (namely, partial complex seizures and T-2 hyperintensity in the hippocampus) are absent in late-onset HS [3–5].

While hippocampal atrophy is the *sine qua non* of AD, it is also observed in HS. Late-life HS is associated with hippocampal atrophy on MRI, both in vivo [3–5] and postmortem [6]. We previously reported MRI hippocampal

volume to be independently associated with HS and AD pathology [3, 4]. Thus, it has been difficult to distinguish HS from AD based on cross-sectional structural MRI.

Recently, automated methods for neuromorphometry have been developed, which allow for the precise statistical modeling of neuroanatomical surfaces [7]. Diffeomorphic mapping applied to the hippocampal surface has been successful in distinguishing AD from normal aging [8] and in predicting the conversion of cognitively normal subjects to very mild dementia [9]. In the present study, we used diffeomorphic mapping to compare patterns of deformation of the hippocampal surface in subjects with HS and AD compared to controls. Using nonbiased stereology counting methods, we previously noted greater neuron loss in HS than AD, especially in CA1 [10]. Therefore we used diffeomorphic mapping to test the hypothesis that HS could be distinguished from AD based on the severity of deformations of the hippocampal surface near the CA1 subfield.

2. Materials and Methods

2.1. Sample Selection. Autopsy cases were obtained from the Ischemic Vascular Dementia Program Project, a prospective, longitudinal study of subjects with subcortical ischemic vascular disease (IVD), Alzheimer's disease (AD), and cognitively normal elderly subjects (NC). The total available autopsy sample consists of 146 cases obtained over the 11 year span of 1997 to 2007. For this study, we considered 100 autopsy cases with bilateral hippocampi and MRI available for review.

Cases were evaluated for neurofibrillary tangle load (Braak & Braak score), neuritic plaque burden (CERAD rating), Lewy bodies (McKeith Lewy body score), and vascular lesions including cystic, lacunar, and microinfarcts. HS was evaluated with the H&E stain. At a minimum, two levels of the hippocampus were reviewed for each hemisphere: the level of the pes and at the level of the lateral geniculate nucleus. More commonly, the entire rostral-caudal extent of the hippocampus from pes to tail was evaluated. The severity of HS was scored as "none, focal, or complete," based on the extent the hippocampal involvement. HS was rated "none" when there was no HS, "focal" when HS was limited to a portion of a CA sector at a single level of the hippocampus, and "complete" when the HS involved the entire pyramidal layer of CA1 and/or subiculum through the rostral-caudal extent of the hippocampus.

Of 100 cases with bilateral hippocampi available for review, 31 had HS. HS cases selected for this study ($n = 11$) had complete hippocampal sclerosis in one or both hemispheres, few or no tangles (Braak and Braak score < III), or neuritic plaques (CERAD = none or sparse). Cases with focal HS ($n = 3$) or hippocampal infarcts ($n = 4$) were excluded as were HS cases with AD pathology ($n = 13$) due to numerous tangles in the hippocampus.

The AD cases ($n = 24$) included in this study had a Braak and Braak stage of V or VI and a CERAD plaque score of moderate or frequent. AD cases with HS or other co-morbidities were excluded.

NC cases ($n = 8$) were cognitively normal (clinical dementia rating scale, CDR = 0) at last clinical evaluation prior to death and had no significant pathology at autopsy, namely, Braak and Braak stage < III, CERAD neuritic plaque score of none or sparse, Lewy body score of 0, and no vascular lesions. All cases meeting these criteria were included. Pathologically normal cases which were not cognitively normal (CDR > 0) were excluded ($n = 7$).

2.2. Imaging. All imaging was performed on a 1.5 Tesla MR system (Siemens Vision System, Germany), using a standard head coil. Structural MRI included volumetric T1-weighted magnetization-prepared rapid acquisition gradient-echo (MPRAGE) image, a multislice proton density, and T2-weighted images based on a dual-echo sequence.

Hippocampal surfaces in each subject were obtained using template-based (UCSF template) high-dimensional brain-warping algorithm (Medtronic Surgical Navigation Technologies, Louisville, CO), which was created from MRI data acquired from five female and five male volunteers, 57 to 94 years of age, mean age 70.5 for all 11.1 years). Details of this method have been described elsewhere [11].

Hippocampal surface zones on the UCSF template surfaces corresponding to underlying subfields were transferred from an existing source (WUSM template) [9, 12] using surface matching techniques [13]. Subfields analyzed correspond to CA1, the subiculum, and a combined subfield comprising combined deformations for CA2, CA3, CA4, and the dentate granule cell layer. Hippocampal surface zones in each subject were obtained by the above brain warping maps. This procedure has been shown to have high intraclass correlation coefficients of the areas of the three surface zones (CA1 −0.97; subiculum −0.97; combined −0.90), comparing manually outlined hippocampal surface zones with the surface zones mapped from the template [12]. Because the transformations from the template to each subject were one to one and onto, all subjects were in-registration with respect to the template. Thus, the different zones on the hippocampal surface could be examined in all subjects using the zones predefined on the surface of the provisory hippocampal template.

Left and right hippocampal volumes in each subject were calculated as the volumes enclosed by the hippocampal surfaces. An average hippocampal surface previously constructed from 86 healthy subjects was used as a reference surface [12], from which normal deformation of each subject's hippocampal surface was calculated at each surface point. For each subject, deformations were averaged within each surface zone to represent surface deformations for CA1, subiculum, and remainder subfields. Using the mean and standard deviation of the nondemented subjects' subfield deformation measures, we computed the z-scores of each subfield zone for each subject in the current study. Negative values of the surface measures represented inward deformation of the surface while positive values represented

Table 1: Characteristics of the three groups.

	HS	AD	NC	*P*values
N	11	24	8	
Age (s.d.)	83.8 (6.7)	80.9 (7.9)	81.3 (6.6)	.54
Sex (F/M)	3/8	7/17	6/2	.07
Education (years) (s.d.)	13 (2.5)	14.4 (3.5)	14.9 (4.5)	.45
Interval last MRI-death (y) (s.d.)	1.8 (0.95)	3.2 (1.9)	3.7 (2.6)	.06
MMSE closest to death (s.d.)	17.1 (8.2)	12.6 (8.5)	29.1 (1.4)	<.001
Duration of illness (years) (s.d.)	7.1 (4.5)	8.6 (3.5)	—	.14
Braak & Braak score (0–6)	1.7 (1.2)	5.6 (0.5)	1.1 (1)	<.001
CERAD score (0–3)	0.7 (0.6)	2.5 (0.7)	0.5 (0.7)	<.001

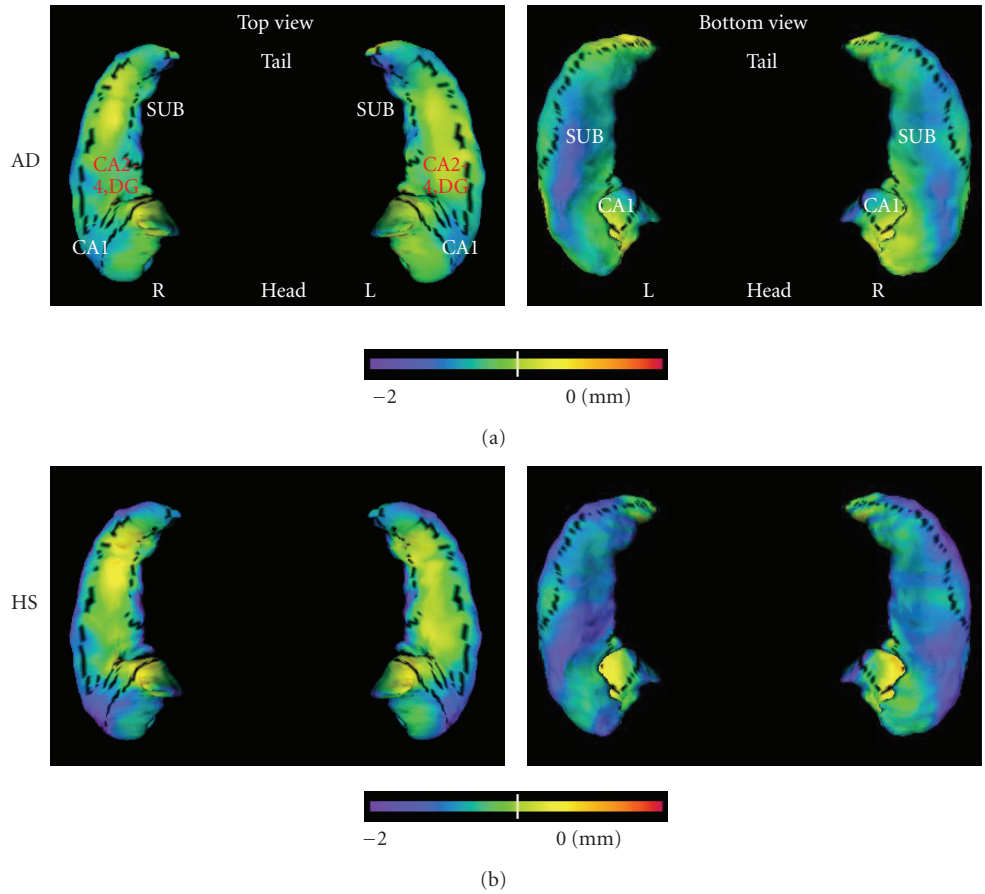

Figure 1: Hippocampal surface deformities in AD (a) and HS (b) compared to NC. Right and left hippocampi are viewed from the top (dorsal surface, left panel) or the bottom (ventral surface, right panel). The flame scale (b) represents the difference between the mean surface of the disease hippocampus and the mean surface of NC hippocampus. Inward deformations are represented by cooler colors (blue to purple), while outward deformations are represented by warmer colors (orange to red). Green to yellow represent near zero deformations. Maximum deformation is 2 mm in either direction. The lateral surface (labeled CA1) is proximal to CA1, where the greatest deformations are found. Labels: SUB = subiculum, CA2-4, DG = combined fields of CA2, CA3, CA4, and the dentate granule cell layer.

outward deformation of the surface. Table 4 summarizes the outcomes of these three groups.

2.3. Statistical Analysis. One-way analysis of variance (ANOVA) was used to compare all of the demographic characteristics in Table 1 except for sex. Fisher's Exact Test was used to analyze the categorical variable sex. Two-way ANOVA using diagnostic group and sex as factors were used to compare hippocampal shape deformations and hippocampal volumes (Tables 4 and 5). Tukey-Kramer follow-up multiple comparison test was used if overall group differences were found. Planned comparisons of HS versus AD were carried out using least square means for hippocampal deformations

Table 2: Dementia severity, clinical diagnoses, and pathological diagnoses for HS cases.

Case	Duration of illness (years)	Final MMSE	Final clinical diagnosis	Pathological diagnosis
1	11	15	AD	HS
2	4	18	AD	HS
3	5	3	AD	HS + IVD
4	1	27	CVD	HS + IVD
5	5	22	DLB	HS + DLB + IVD
6	17	6	FTD	HS + FTD
7	4	28	IVD	HS
8	8	25	possible AD/possible IVD	HS
9	5	14	possible AD/possible IVD	HS + IVD
10	7	12	possible AD/possible IVD	HS +IVD
11	11	18	possible AD/possible IVD	HS + IVD

Table 3: Dementia severity, clinical diagnoses, and pathological diagnoses for AD cases.

Case	Duration of illness (years)	Final MMSE	Final clinical diagnosis	Pathological diagnosis
1	10	20	AD	AD
2	4	6	AD	AD
3	5	10	AD	AD
4	10	17	AD	AD
5	9	21	AD	AD
6	10	0	AD	AD
7	10	4	AD	AD
8	7	15	AD	AD
9	10	3	AD	AD
10	6	8	AD	AD
11	5	20	AD	AD
12	14	3	AD	AD
13	9	2	AD	AD
14	8	18	CVD	AD
15	10	0	FTD	AD
16	0	25	MCI	AD
17	6	20	AD/IVD	AD
18	9	0	AD/IVD	AD
19	7	20	AD/IVD	AD
20	15	18	AD/IVD	AD
21	5	12	AD/IVD	AD
22	14	22	AD/IVD	AD
23	13	14	AD	AD
24	10	24	MCI	AD

and hippocampal volumes. Analyses were two-tailed with the significance level set at $P < .05$ and were carried out with the interactive software SAS 9.1 (SAS Institute, Cary, NC).

3. Results and Discussion

3.1. Case Selection. Because HS in the context of AD presents a confound due to hippocampal neuron loss as a result of both HS and tangle formation, the HS cases included in this analysis were selected to have HS without concomitant AD. Similarly, AD cases with accompanying HS were excluded.

3.2. Demographic Characteristics (Table 1). The 3 comparison groups were comparable in age (81 to 84 years) and education (13 to 15 years). The NC group was predominantly female, which limited our ability to match with AD and HS cases on sex. The interval between last MRI and death was shorter in the HS group. Although the mean minimental status exam (MMSE) score was lower in AD (12.6) compared to HS (17.1) this difference was not statistically significant.

Table 2 details the duration of illness, the final MMSE score, the clinical diagnosis, and the pathological diagnosis for the 11 HS cases. None of these cases was diagnosed clinically with HS. Table 3 lists the dementia severity, clinical diagnosis, and pathological diagnosis for the AD cases, most of which were clinically diagnosed with AD.

3.3. Hippocampal Surface Deformations. Figure 1 illustrates the hippocampal surface deformations for AD hippocampus and HS hippocampus compared to NC hippocampus. To aid the visual identification of the three zones of the hippocampal surface (i.e., CA1, subiculum, and the combined fields of CA2, CA3, CA4, and the dentate granule cell layer) the boundaries that demarcate them are shown black. For the comparison between AD and the NC (Figure 1(a)), areas of hippocampus showing the greatest group differences (as marked by the blue colors) are concentrated in the CA1 and subiculum surface zones. These patterns of deformation resemble our previous findings in nonoverlapping subjects [12]. The comparison between HS and NC hippocampi (Figure 1(b)) shows a deformation pattern with similar extent but increased magnitude (in the negative or inward direction) than that between AD and NC. This observation was further supported by statistical comparison of surface deformation z-scores (Table 4).

Quantitative hippocampal surface deformations expressed as z-scores are presented in Table 4. In comparing AD to NC, there were significant inward deformations of right CA1, left subiculum, right subiculum, and left and right subiculum combined (total) ($P = .001$). As shown in Figure 1, differences were found in the CA1 and subiculum surface zone deformations but not in the CA2+CA3+CA4+DG zone. There was no interaction with sex for any of the comparisons.

Table 4: Hippocampal shape deformations expressed as z-scores.

	NC ($n = 8$)	AD ($n = 24$)	HS ($n = 11$)	$F(2,40)$, P
CA1 Deformation				
Left	−0.31 (0.50)	−0.90 (0.59)	−1.54 (1.08)[1]	$F = 6.69$, $P = .003$
Right	−0.35 (0.44)	−1.07 (0.68)[1]	−1.74 (0.85)[1,2]	$F = 9.37$, $P = .005$
Total	−0.33 (0.42)	−0.99 (0.60)	−1.64 (0.91)[1,2]	$F = 9.02$, $P = .006$
Subicular Deformation				
Left	0.06 (0.19)	−0.39 (0.21)[1]	−0.73 (0.41)[1,2]	$F = 19.14$, $P < .001$
Right	0.09 (0.21)	−0.34 (0.22)[1]	−0.67 (0.28)[1,2]	$F = 23.93$, $P < .001$
Total	0.08 (0.19)	−0.36 (0.20)[1]	−0.70 (0.31)[1,2]	$F = 25.71$, $P < .001$
Combined deformation				
Left	0.24 (0.31)	−0.04 (0.33)	0.007 (0.39)	$F = 2.01$, $P = .14$
Right	0.08 (0.22)	−0.17 (0.29)	−0.15 (0.33)	$F = 2.30$, $P = .11$
Total	0.16 (0.25)	−0.11 (0.27)	−0.07 (0.30)	$F = 2.88$, $P = .07$

[1]Different from NC, Tukey-Kramer ($P < .05$).
[2]Different from AD, Tukey-Kramer ($P < .05$).

Table 5: Left, right, and total hippocampal volumes in NC, AD, and HS.

	Hippocampal volume			
	NC	AD	HS	$F(2,40)$, P
Left	2170.2 (333.8)	1638.1 (305.7)[1]	1294.8 (535.6)[1,2]	$F = 12.27$, $P < .0001$
Right	2235.5 (259.7)	1622.8 (341.5)[1]	1251.9 (352.0)[1,2]	$F = 20.49$, $P < .0001$
Total	4405.7 (541.0)	3260.9 (608.9)[1]	2546.7 (774.5)[1,2]	$F = 19.37$, $P < .0001$

Data are mean (s.d.) in: μm^3.
[1]Different from NC, Tukey-Kramer ($P < .05$).
[2]Different from AD, Tukey-Kramer ($P < .05$).

In comparing HS with NC, there were significant inward deformations of left CA1, right CA1, left subiculum, right subiculum, and left and right subiculum combined (total) ($P = .001$).

In comparing HS with AD, significant inward deformations were found for right CA1, and left and right CA1 combined (total), left subiculum, right subiculum, and left and right subiculum combined (total) ($P = .001$).

The CA1 surface zone deformations of the NC subjects in this study showed an appreciable amount of inward deformation (left −0.31, right −0.35) while the healthy reference group from Wang et al. [12] had an average of 0 (used as reference). This difference could be due to the fact that 20 healthy subjects from a study of schizophrenia were included in the reference group of the previous study. The subjects included in the present study were about 10 years older, and aging may be related to hippocampal volume loss and shape deformities. A similar phenomenon was observed in another study of more elderly subjects with dementia of the Alzheimer type [14].

3.4. Hippocampal Volume (Table 5). There were no statistically significant differences in right versus left hippocampal volume for any group. However, there were statistically significant differences between NC compared to AD, NC compared to HS, and AD compared to HS ($P < .001$). On average, hippocampal volumes for AD were 25.9% less than NC. HS hippocampal volumes were 21.9% less than AD and 42.2% less than NC. There was no interaction with sex for any of the comparisons.

4. Conclusions

In this paper we demonstrate that hippocampal atrophy and the corresponding changes in hippocampal shape distinguish HS from AD on premortem MRI. More atrophy and greater deformation were observed in those portions of the hippocampal surface in proximity to the CA1 and subiculum subfields in HS compared to AD. These results support the possibility that greater severity of hippocampal atrophy and deformation may be useful for the clinical identification of HS. Additional studies will be needed, however, to determine whether severity of atrophy and deformation can be used to distinguish HS from AD on an individual case basis. Better criteria for clinical diagnosis are needed before advances can be made in understanding and preventing HS in late life.

We surmise that the significant inward deformation of CA1 found in this study is probably due to neuronal loss as a result of neurofibrillary degeneration in AD and to unknown pathogenesis in HS. Neuron loss in AD is well documented, and although tangles represent dying or dead neurons, the tangle itself is space-filling, that is, it takes up a volume within the hippocampal subfield, presumably smaller than the healthy neuron. Although significant neuron loss occurs

in AD, there are large numbers of neurons remaining [10]. In contrast, 90% of hippocampal neurons may be lost in HS. Microscopically, it appears that the neuron loss in HS involves primarily CA1 and sometimes subiculum. However, if this condition were restricted to these regions, the volume losses would not be as great as those seen here. In other words, the mechanism leading to the overall volume loss seen in HS affects the entire hippocampus.

A clinical diagnosis of HS is rarely if, ever, made. Indeed, none of the HS cases in this study received a diagnosis of HS. Three cases were clinically diagnosed with possible AD, 2 with possible IVD, 1 with probable DLB, 1 FTD, and 4 were diagnosed with mixed possible AD/possible IVD. A specific biomarker for the clinical detection and diagnosis of HS is greatly needed.

Previous studies have demonstrated a lack of substantial loss of hippocampal volume in healthy elderly control subjects as compared with the younger control subjects [15]. In this study, we excluded cases of AD with HS. Had these cases had been included, the differences between the groups would have been less. Age, years of education, MMSE score, and duration of illness were not significantly different in the two disease groups. The NC group was predominantly female. It is recognized that brain and hippocampal volumes are generally smaller in females compared to males. The predominance of females in the NC group would have affected the AD to NC and HS to NC comparison equally, and might have led to an underestimation of volume differences. However, we did not find any effect of sex on any of these analyses. The shorter interval between MRI and death in the HS group may have contributed to the increased severity of atrophy in the HS group, assuming that atrophy is an equally progressive process in AD and HS. While the group differences in mean severity of hippocampal atrophy and shape deformation are significant, additional study is needed to determine whether these differences will be meaningful for clinical diagnosis of individuals.

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