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Article abstract—*Objective:* To evaluate CSF levels of β-amyloid$_{(1-42)}$ (Aβ_{42}) alone and in combination with CSF tau for distinguishing AD from other conditions. *Methods:* At 10 centers in Europe and the United States, 150 CSF samples from AD patients were analyzed and compared with 100 CSF samples from healthy volunteers or patients with disorders not associated with pathologic conditions of the brain (CON), 84 patients with other neurologic disorders (ND), and 79 patients with non-Alzheimer types of dementia (NAD). Sandwich ELISA techniques were used on site for measuring Aβ_{42} and tau. *Results:* Median levels of Aβ_{42} in CSF were significantly lower in AD (487 pg/mL) than in CON (849 pg/mL; $p = 0.001$), ND (643 pg/mL; $p = 0.001$), and NAD (603 pg/mL; $p = 0.001$). Discrimination of AD from CON and ND was significantly improved by the combined assessment of Aβ_{42} and tau. At 85% sensitivity, specificity of the combined test was 86% (95% CI: 81% to 91%) compared with 55% (95% CI: 47% to 62%) for Aβ_{42} alone and 65% (95% CI: 58% to 72%) for tau. The combined test at 85% sensitivity was 58% (95% CI: 47% to 69%) specific for NAD. The *APOE* e4 gene load was negatively correlated with Aβ_{42} levels not only in AD but also in NAD. *Conclusions:* The combined measure of CSF Aβ_{42} and tau meets the requirements for clinical use in discriminating AD from normal aging and specific neurologic disorders.

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With the emergence of specific therapeutic options for AD, there is a definite need for improved diagnostic approaches, including the identification of latent AD in patients with mild cognitive impairment. Levels of β-amyloid$_{(1-42)}$ (Aβ_{42}) and tau in CSF, providing indices related to amyloid deposition and axonal changes or tangle formation, respectively, have been suggested to be of use in early diagnosis and as surrogate markers of AD.[1] An ideal biomarker should have a sensitivity of ≥80% for detecting AD and a specificity of ≥80% for distinguishing other dementias.[1] Ultimately, large studies with precise and reliable assays will define the role of such markers in clinical practice.

The current multicenter study evaluated a novel ELISA for CSF Aβ_{42} determination,[2,3] complementing an ELISA for CSF tau already developed and described.[4-6] The object of this study was to evaluate CSF Aβ_{42} alone and in combination with CSF tau for distinguishing AD from other conditions. The evaluation of the Aβ_{42} assay also included a send-around of a set of quality control (QC) samples.

Subjects and methods. Eight European and two US university centers involved in CSF research participated in this study, which was based on residual CSF archived for research purposes at those centers. The study was conducted in accordance with local clinical research regulations. If required, additional approval from local ethics committees or institutional review boards was obtained by the investigator in advance of the study. CSF samples from healthy elderly volunteers had been obtained after informed consent.

Sample criteria. CSF samples collected by lumbar puncture were to be included if they contained <500 red blood cells per microliter, had been centrifuged at 2,000 g for 10 minutes within 4 hours after lumbar puncture, and had been kept frozen without thawing. CSF samples from

See also pages 1533 and 1687

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Center 1 had undergone an additional freeze-thaw cycle before analysis. Because in-house data indicated artificially low values for CSF $A\beta_{42}$ after storage in polystyrene or glass,[2,3] polypropylene was required as material for CSF storage in all but one center (Center 5). As a control, Center 5 included samples stored in polystyrene. Each study center was to include single CSF samples from patients with AD, other types of dementia, and other neurologic conditions, as well as from age-matched control subjects. Although not all neurologic conditions included are of relevance in the differential diagnosis of AD, a broad range of patient samples was included to provide a general idea about the specificity of the CSF marker changes in a variety of pathologic conditions involving the CNS. Standardized case report forms were used to record demographic data, diagnosis, year of diagnosis, diagnostic criteria used, sampling date and method, and sample storage conditions. Mini-Mental State Examination[7] (MMSE) results at the time of sampling and the number of *APOE* e4 alleles were to be reported if available. Patients with probable AD had to satisfy DSM-IV criteria[8] for dementia of the Alzheimer's type and the criteria for the clinical diagnosis of probable AD as established by a Working Group of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and by the Alzheimer's Disease and Related Disorders Association (ADRDA).[9] Patients received diagnoses of vascular dementia (VAD) if they fulfilled either the State of California Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC) criteria[10] or those of NINCDS with support from the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN).[11] The diagnosis of frontotemporal dementia was made according to the Lund/Manchester criteria.[12] For other conditions, the diagnosis recorded was based on best clinical judgment. Conditions reported for all study patients were grouped into larger categories by a single psychiatrist (K.B.), blinded to the individual study results.

Analyses of CSF samples. Kits for the determination of CSF $A\beta_{42}$ and tau were shipped at 4 °C from Innogenetics NV (Ghent, Belgium) to the participating centers, together with a panel of QC samples (on dry ice) containing seven human CSF samples and two samples with high-performance liquid chromatography—purified $A\beta_{42}$ (Bachem, Heidelberg, Germany). Centers were kept blinded for the testing of the QC samples.

The CSF level of $A\beta_{42}$ and the concentration of total tau, comprising normal tau and paired helical filament-tau, were measured at the centers by sandwich ELISA techniques (Innotest hTAU-Ag and Innotest β-amyloid$_{(1\text{-}42)}$, Innogenetics NV) as described elsewhere.[2-6] The interassay variability of the $A\beta_{42}$ test is <10% (unpublished data, 1998). The assay detection limit (mean of the blank plus 5 SD) is 50 pg/mL, with linearity in the range of 200 to 1500 pg/mL. To prepare standards, a 0.1 mg/mL solution of a pool of different batches of β-amyloid$_{(1\text{-}42)}$ peptides in 0.1% NH_3 were stored at −70 °C and further diluted with a phosphate-buffered solution (pH 7.4) containing Triton X-705 as detergent and casein (0.1%) as stabilizing protein. Patient samples and QC samples were prepared in duplicate, and optical density (OD) was obtained with the ELISA reader available at the center. No training was provided to the study centers before testing. No effort was made to check the performance characteristics of the readers before the study.

OD values of the standard dilution series were fitted according to a sigmoidal dose–response curve implemented in Prism Software version 2.01 (Graphpad Software, San Diego, CA). For samples with OD values below the range of measurement (75 to 1,200 pg/mL for tau and 125 to 2,000 pg/mL for $A\beta_{42}$), the sample concentration was arbitrarily set at 50% of the lowest values of the standard dilution range. Tau levels superior to the range of measurement were similarly set at 1250 pg/mL. No $A\beta_{42}$ levels superior to the measurement range were found in this study.

Statistical methods. In a previous evaluation of CSF levels of $A\beta_{42}$ in 28 AD patients with disease onset after 65 years, mean value and SD were 350 pg/mL and 54 pg/mL (unpublished observations, 1997). Assuming a normal distribution of values with a similar variance in other patient populations as well, a difference in mean CSF $A\beta_{42}$ levels of 54 pg/mL would be detectable with a power of 80% and α level of 0.05 if at least 17 patients per group were analyzed. The analysis plan allowed for pooling of results across centers in the absence of any significant between-center difference. Because the study was based on stored material, possible selection bias was to be considered.

For the primary between-group comparisons (AD versus other subject groups), individual significance levels were calculated by the Bonferroni method, respecting a family level of significance of $\alpha = 0.05$. For the stratified rank sum statistic, p values were calculated by use of the FREQ/Cochran-Mantel-Haenszel procedure in SAS software (SAS Institute, Cary, NC). All other analyses were exploratory. Only p values of ≤ 0.05 were reported (two-sided tests). Where possible, 95% CI were given. Nonparametric statistics were used when normality was rejected (Shapiro-Wilk test, $p < 0.05$), and distributions of laboratory values were described using median and the p25-p75 range. For the generation of classification trees, the algorithm[13] implemented in Systat version 7 (SPSS, Chicago, IL) was used. Receiver operating characteristic (ROC) curve analysis was programmed with Excel software (Microsoft, Redmond, WA). Area under the ROC curve (AUC) and standard error (SE) were calculated by the method of Hanley and McNeil.[14]

Results. *CSF sample characteristics.* In all, 463 CSF samples were included from 10 centers. Each center contributed between 12 and 111 samples. Eight samples were not eligible for statistical analysis because of storage in glass tubes (n = 2), because values for $A\beta_{42}$ or tau were missing (n = 5), or because the sample was a patient's second sample (n = 1). The analysis is based on CSF sampled between 1992 and 1998 from 281 female and 173 male individuals aged 16 to 94 years. One sample collected in 1987 from a 4-year-old boy with neuroborreliosis was also included. A total of 282 individuals were >65 years of age. Ethnic origin was recorded in 436 patients, of whom 428 (98%) were white.

Samples were grouped according to the condition reported for the individual (table 1). The groups consisted of 150 patients with AD (most had probable AD); 4 with mild memory impairment; 100 control subjects (CON) consisting of 42 healthy subjects and 58 neurologic control subjects; 84 patients with neurologic disorders (ND), some of whom had cognitive impairment; 79 patients with non-AD

Table 1 *Demographic data and CSF results by diagnostic group*

Groups and subgroups	Subjects, n (M/F)	Age, y, median (range)	MMSE n	MMSE Median (p25-p-75)	No. cases by number of *APOE* e4 alleles, n (0/1/2 alleles)	Tau, pg/mL, median (p25-p75)	$A\beta_{42}$ (pg/mL), median (p25-p75)	Correctly classified using tau-$A\beta_{42}$ function, n (%) Line	Correctly classified using tau-$A\beta_{42}$ function, n (%) Tree
AD (group AD)*	150 (42/108)	74 (42–90)	127	19 (13–22)	84 (30/44/10)	425 (274–713)	487 (394–622)	127 (85)	122 (81)
AD (Center 2)	58 (14/44)	76 (53–86)	57	20 (16–23)	35 (9/21/5)	464 (375–761)	571 (458–682)	50 (86)	47 (81)
AD (others)	92 (28/64)	72 (42–90)	70	18 (11–21)	49 (21/23/5)	366 (241–677)	428 (335–560)	77 (84)	75 (82)
Mild memory impairment	4 (3/1)	73 (63–79)	4	30 (29–30)	1 (1/0/0)	710 (531–854)	494 (356–631)	NA	NA
All control subjects (group CON)	100 (25/75)	68 (17–87)	61	29 (28–30)	11 (10/0/1)	195 (121–294)	849 (682–1063)	87 (87)	92 (92)
Healthy control subjects (Center 2)	42 (3/39)	74 (60–85)	42	29 (28–29)	NA	219 (134–318)	1001 (797–1214)	39 (93)	41 (98)
Neurologic control subjects (other centers)†	58 (22/36)	57 (17–87)	19	30 (30–30)	11 (10/0/1)	167 (107–290)	769 (608–913)	48 (83)	51 (88)
Non-AD dementia (group NAD)	79 (45/34)	74 (39–94)	64	21 (15–26)	23 (12/5/6)	220 (122–426)	603 (430–744)	46 (58)	38 (48)
Vascular dementia‡	33 (21/12)	73 (39–91)	30	21 (14–26)	17 (7/5/5)	230 (133–451)	597 (425–739)	18 (55)	15 (45)
Frontotemporal dementia	11 (7/4)	75 (46–94)	11	20 (15–23)	5 (5/0/0)	209 (182–468)	640 (599–821)	6 (55)	6 (55)
Normal pressure hydrocephalus	20 (11/9)	74 (51–91)	20	23 (16–29)	NA	154 (86–291)	505 (417–732)	14 (70)	10 (50)
Neurodegeneration with dementia§	15 (6/9)	72 (43–79)	3	18 (10–22)	1 (0/0/1)	249 (113–438)	619 (461–726)	8 (53)	7 (47)
Neurologic disorders (group ND)	84 (38/46)	49 (16–84)	NA		7 (6/1/0)	160 (125–217)	643 (522–786)	72 (86)	73 (87)
Inflammatory neurologic disorders‖	40 (21/19)	41 (16–74)	NA		1 (0/1/0)	145 (105–175)	652 (544–797)	38 (95)	37 (93)
Neurodegeneration without dementia¶	8 (3/5)	65 (55–80)	NA		NA	189 (157–205)	597 (535–711)	7 (88)	7 (88)
Other neurologic disorders**	36 (14/22)	54 (19–84)	NA		6 (6/0/0)	194 (139–269)	642 (501–847)	27 (75)	29 (81)
Vascular events and tumors††	15 (6/9)	56 (35–79)	NA		2 (2/0/0)	329 (249–513)	674 (536–742)	8 (53)	8 (53)
Infections‡‡	21 (15/6)	32 (4–78)	NA		NA	170 (88–363)	574 (378–761)	11 (52)	10 (48)

* Group AD consists of patients with probable AD (n = 146), possible AD (n = 2), or mixed vascular/AD dementia (n = 2).
† Neurologic control subjects include those without dementia with compression mononeuropathy (n = 37), Bell's palsy (n = 5), neurosis (n = 5), tension headache (n = 4), depression without dementia (n = 3), carpal tunnel syndrome (n = 2) and myalgia (n = 2).
‡ Vascular dementia includes Binswanger disease (n = 2) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (n = 1).
§ The subgroup neurodegeneration with dementia includes Parkinson's dementia (n = 7), supranuclear palsy (n = 2), corticobasal degeneration (n = 2), dementia with Lewy bodies (n = 1), subcortical dementia (n = 1), primary progressive aphasia (n = 1), and leukoencephalopathy (n = 1).
‖ The subgroup inflammatory neurologic disorders included MS (n = 20), optic neuritis (n = 5), Guillain-Barré syndrome (n = 9), subacute sclerosing panencephalitis (n = 2), or other inflammatory neurologic disorders (n = 4).
¶ The subgroup degenerative neurologic disorders without dementia includes ALS (n = 3), olivopontocerebellar degeneration (n = 4), and PD (n = 1).
** The subgroup other neurologic disorders includes epilepsia (n = 8), polyneuropathy (n = 9), depression associated with cognitive changes (n = 3), benign intracranial hypertension (n = 2), myopathy (n = 2), oculomotor palsy (n = 1), Parsonage-Turner syndrome (n = 1), and other unspecified neurologic conditions without dementia (n = 10).
†† The group vascular events and tumors includes stroke (n = 8), vascular myelopathy (n = 1), vasculitis (n = 1), subarachnoidal bleeding (n = 1), primary brain tumor (n = 2), brain metastasis (n = 1), and postanoxia vegetative status (n = 1).
‡‡ The group infections includes meningitis (n = 13), HIV infection (n = 2), myelitis (n = 2), encephalitis (n = 1), cerebellitis (n = 1), syphilis (n = 1), and neuroborreliosis (n = 1).

MMSE = Mini-Mental State Examination; NA = no data available.

Table 2 *Comparisons between AD and other groups*

Marker	Technique	Control subjects (n = 100) and AD (n = 150)	Neurologic disorders (n = 84) and AD (n = 150)	Non-AD dementia (n = 79) and AD (n = 150)
$A\beta_{42}$	Stratified rank sum*	$p = 0.001$	$p = 0.001$	$p = 0.001$
	ROC†	AUC 0.847, SE 0.028	AUC 0.697, SE 0.038	AUC 0.629, SE 0.041
		Cutoff: 643 pg/mL	Cutoff: 551 pg/mL	Cutoff: 556 pg/mL
		S 78% (70–84)	S 71% (63–78)	S 65% (57–73)
		Sp 81% (72–88)	Sp 63% (52–73)	Sp 59% (48–70)
Tau	Stratified rank sum	$p = 0.001$	$p = 0.001$	$p = 0.004$
	ROC	AUC 0.824, SE 0.024	AUC 0.889, SE 0.019	AUC 0.740, SE 0.034
		Cutoff: 252 pg/mL	Cutoff: 293 pg/mL	Cutoff: 239 pg/mL
		S 79% (71–85)	S 71% (63–78)	S 81% (74–87)
		Sp 70% (60–78)	Sp 89% (80–95)	Sp 57% (45–68)
$A\beta_{42}$, tau	Linear function	S 85% (79–90)	S 85% (79–90)	S 85% (79–90)
	($A\beta_{42}$ = 240 + 1.18 tau)	Sp 87% (79–93)	Sp 86% (76–92)	Sp 58% (47–69)
	Classification tree	S 81% (74–87)	S 81% (74–87)	S 81% (74–87)
		Sp 92% (85–96)	Sp 87% (78–93)	Sp 48% (37–60)

* Rank sum statistic stratified for center using Cochran-Mantel-Haenszel in Proc FREQ in SAS software (Cary, NC).
† Receiver operating characteristic (ROC) analysis with area under the curve (AUC) and standard error (SE) calculated according to Hanley and McNeil; cutoff given for S + Sp maximized.

S = sensitivity (95% confidence interval); Sp = specificity (95% confidence interval).

dementia (NAD); 15 patients with vascular events or tumors; and 21 patients with infections. Two patients, one with Sneddon's syndrome and cognitive changes, and one with congenital hydrocephalus, were not grouped. Their values for $A\beta_{42}$ and tau fell within the range of control subjects. Analysis of CSF marker results by disease duration was not done because nearly all samples were obtained when the diagnosis was made.

Levels of $A\beta_{42}$ and tau. $A\beta_{42}$ and tau levels among control subjects were analyzed by age, sex, and archiving time, controlled for center. The CSF samples had been archived for a median of 2.5 years. Archiving time did not significantly correlate (stratified Spearman test) with $A\beta_{42}$ or tau levels in control subjects. Normality was rejected ($p = 0.0001$, Shapiro-Wilk test) for the distribution of tau but not for $A\beta_{42}$ in control subjects. Tau and $A\beta_{42}$ levels in control subjects did not differ significantly by age or sex when controlled for center. Therefore, no samples were excluded from any group based on the individual's age or sex, and all results were used for between-group comparisons.

Healthy control subjects, all recruited in Center 2, had higher $A\beta_{42}$ levels ($p = 0.0001$) than did neurologic control subjects, who were included in the other centers. Compared with the other centers, higher values for $A\beta_{42}$ were also found for AD patients in Center 2 ($p = 0.0001$), indicating a general center effect. When Center 2 was removed from the analysis, no center effect was found for $A\beta_{42}$ among the control subjects. The lower number of control subjects per center also rendered this test underpowered. For tau, a slightly significant center effect was found in control subjects ($p = 0.02$) attributable to high tau values observed in Center 3. A center effect tends to reduce the effects between patient groups. However, because the distribution of subject groups per center differed significantly across centers, the between-group effects might exceptionally also be increased after pooling across centers. The analyses by subject groups pooled across centers are therefore to be interpreted with this possible caveat in mind, unless techniques respecting stratification by center are used. For the same reason, data for control subjects and AD patients are also given separately for Center 2 in table 1.

Because one objective of studying these biomarkers was to discriminate groups of AD patients from other relevant groups, contrasts were tested between AD patients and control subjects, ND patients, and NAD patients, respectively (table 2). Statistical techniques including rank sum statistics, ROC curve analysis, discrimination line analysis, and classification tree models were used to explore the between-group differences as well as the discriminatory value of $A\beta_{42}$, tau, and the combination of both markers.

Differences between the AD group and the other groups tested were significant for $A\beta_{42}$ and tau and were associated with varying degrees of between-group discrimination (see table 2). When restricted to a single center (Center 2), the AUC of the ROC analysis (AD versus CON) was even slightly higher with AUC = 0.902 (SE = 0.034) for $A\beta_{42}$ and AUC = 0.883 (SE = 0.026) for tau.

The figure shows the distribution of values for $A\beta_{42}$ and tau for groups AD, CON, ND, and NAD pooled across centers. An exhaustive search algorithm was used to define a cutoff line providing optimal sensitivity and specificity for the discrimination of AD patients from control subjects and ND patients (line plotted in figure). A tree classification algorithm,[13] used earlier in a similar type of study,[15] was also explored (see figure) and resulted in similar levels of discrimination. Discrimination of the AD group from the CON and ND groups was significantly improved by the combined assessment of $A\beta_{42}$ and tau. At 85% sensitivity, specificity of the combined test (linear function) was 86%

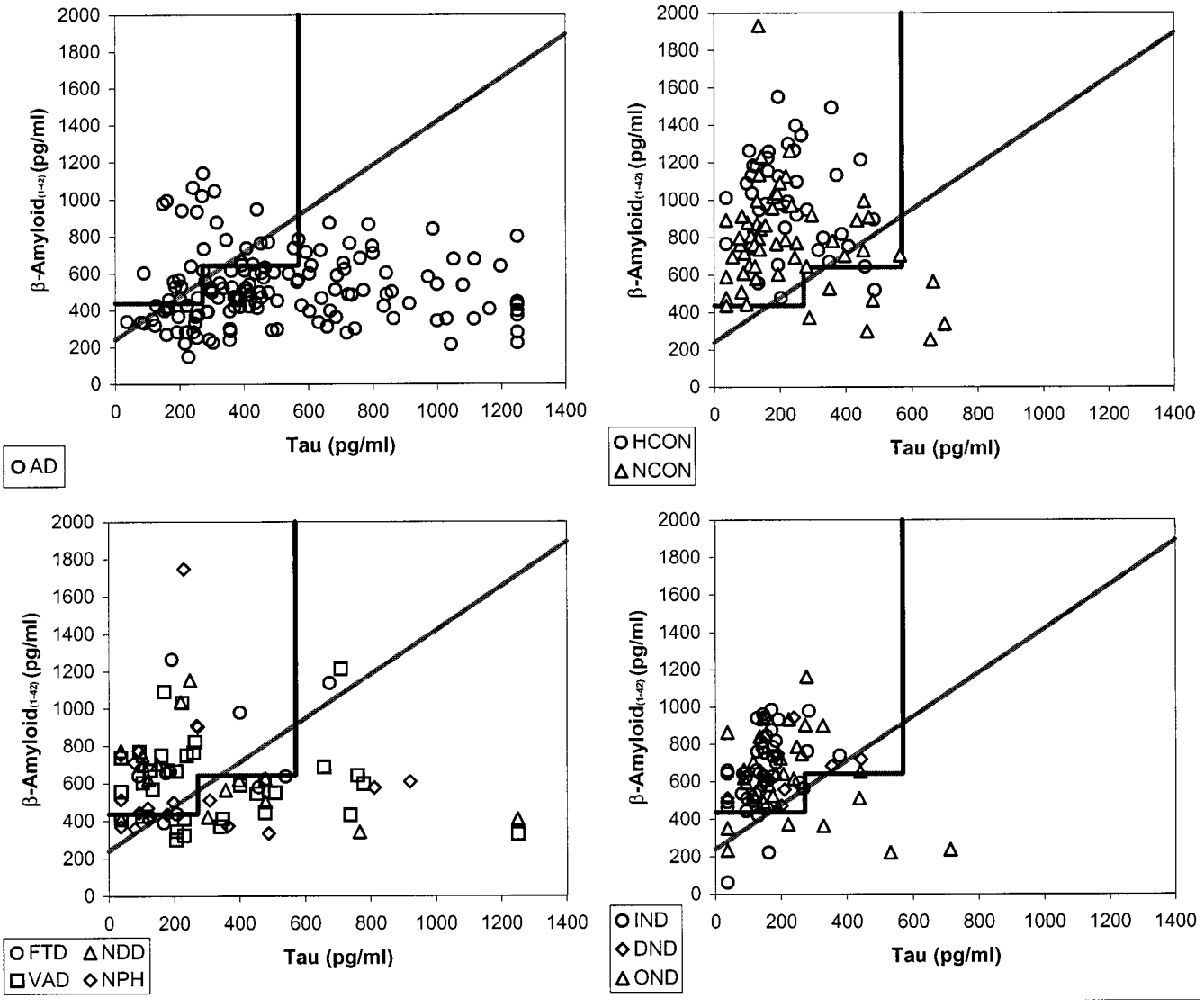

Figure. CSF $A\beta_{42}$ and tau levels (pg/mL) for the AD, disorders not associated with pathologic conditions of the brain (CON), other neurologic disorders (OND), and non-Alzheimer dementia (NAD) diagnostic groups. Different symbols are used for diagnostic subgroups within the various groups. The discrimination line ($A\beta_{42}$ = 240 + 1.18 tau) is shown, together with the function obtained using classification tree analysis. HCON = healthy control subjects; NCON = neurologic control subjects; FTD = frontotemporal dementia; VAD = vascular dementia; NDD = other neurodegenerative forms of dementia; NPH = normal pressure hydrocephalus; IND = inflammatory neurologic disorders; DND = nondementia degenerative neurologic disorders.

(95% CI: 81% to 91%) compared with 55% (95% CI: 47% to 62%) for $A\beta_{42}$ alone and 65% (95% CI: 58% to 72%) for tau. The combined test at 85% sensitivity was 58% (95% CI: 47% to 69%) specific for NAD. The proportion of individuals correctly classified using these classification algorithms is given by group in table 2.

MMSE data at sampling were available for 127 AD patients. The median MMSE was 19, and 23 patients (17%) had MMSE scores >23. Using the linear discriminant function, 16 of these 23 patients (70%, 95% CI: 47% to 87%) were classified as having AD. MMSE values did not correlate significantly with $A\beta_{42}$ or tau levels, except for MMSE with CSF $A\beta_{42}$ in men with AD (n = 36; p = 0.02, Spearman test stratified by center).

A total of 128 subjects were genotyped for *APOE* (see table 1). Subgroup analyses in group AD and group NAD based on number of *APOE* e4 alleles revealed an inverse correlation with $A\beta_{42}$ levels (p = 0.017 and p = 0.026, respectively; Spearman test) but no correlation with tau values. The median $A\beta_{42}$ levels (in pg/mL) in AD patients with 0, 1, and 2 *APOE* e4 alleles were 632 (n = 30), 523 (n = 44), and 401 (n = 10), respectively. In group NAD, corresponding $A\beta_{42}$ values were 667 (n = 12), 597 (n = 5), and 420 (n = 6) pg/mL.

QC sample send-around. A total of seven human CSF samples and two samples containing reference standard were shipped to the centers and analyzed in parallel with the local patient samples. The QC samples covered a range of $A\beta_{42}$ levels from 325 to 1,000 pg/mL. The between-center coefficient of variation based on nine centers varied from 6% to 14% (median 10%) for the various QC samples. Results for one center could not be used because the QC

samples had not been stored appropriately. The median intra-assay coefficient of variation for all samples analyzed for $A\beta_{42}$ in this study, including the QC samples, was 4%.

Discussion. To our knowledge, this is the first report of an international multicenter evaluation of CSF markers for the diagnosis of AD with sample analyses performed locally rather than at a central laboratory. The sample size for the diagnostic groups pooled across centers is also one of the largest published.

Previous studies using simultaneous analysis of CSF $A\beta_{42}$ and tau have demonstrated an association of low $A\beta_{42}$ plus high tau levels with a clinical diagnosis of AD.[15-17] The current study confirms and extends these findings. Levels of CSF $A\beta_{42}$ in AD patients were about half, and tau values were about double, the values in healthy control subjects. The proportion of individuals correctly classified as being AD patients versus control subjects based on a single marker was in the range of values reported from studies in which all assays were performed at a central site.[15,17,18] In this study, the mean of sensitivity and specificity levels of the individual markers analyzed using ROC was at most 74% to 79%. We show that this value is significantly improved to 86% if both markers are considered simultaneously.

Most relevant for the early diagnosis of AD is the discrimination of mild AD from age-associated memory impairment and depressive pseudodementia. It appears that discrimination from normal aging and from other neurologic disorders, some of which are associated with cognitive changes, is possible at the required[1] levels of specificity and sensitivity using the combined assessment of tau and $A\beta_{42}$ in CSF. Moreover, this study demonstrates that this level of discrimination can be achieved in individual laboratories, where the tests were performed without specific training.

The current study design does not allow for calculation of a positive predictive value of these markers. Because the study was based on residual CSF samples frozen for research purposes, the selection of samples may have been biased. The ideal prospective study should include consecutive patients with a differential diagnosis that includes AD. Ideally, these patients should be monitored until the clinical diagnosis can be confirmed postmortem. Considering that the clinical diagnosis of AD is made with a sensitivity of 93% and a specificity of only 55% (mean 74%),[19] sensitivity and specificity levels exceeding those values cannot be expected for CSF markers evaluated in clinically diagnosed patient samples. By their nature, biomarkers should more accurately reflect a pathologic state when compared with clinical phenotype. They may be of value to further improve diagnostic accuracy or guide disease-modifying therapy.

We show that the proposed test combination allows for the detection of 85% of AD patients with only 14% of the control subjects falling within the AD range of values. This figure could be compatible with the frequency of latent AD in these elderly, under the assumption that the biomarkers used are indeed very early indicators of AD. A growing number of studies using CSF tau indeed seem to confirm this hypothesis.[6,20,21] The four patients with mild memory impairment included at Center 3 all had a biomarker profile suggesting latent AD. However, control subjects also had rather high tau values at that center.

Both $A\beta_{42}$ and tau have an intimate link with two different hallmarks of AD pathologic states: plaques and tangles, respectively. Tau is a normal human brain phosphoprotein that binds to microtubules in the neuronal axons, thereby promoting microtubule assembly and stability.[22] Tau levels in the brains of AD patients are increased.[23] Increased levels of CSF tau, probably as a consequence of neuronal/axonal damage, have been reported for AD,[4-6,18] Creutzfeldt-Jakob disease,[24] stroke,[18] and neurotoxicity induced by cytotoxic chemotherapy.[25] In our study, CSF tau was elevated in three of the four samples obtained from patients with primary brain tumor or brain metastases. Most of these conditions, however, are not highly relevant in the context of the differential diagnosis of AD.

Cerebral accumulation in diffuse and senile plaques of β-amyloid, especially $A\beta_{42}$, a proteolytic derivative of the β-amyloid precursor protein, occurs in virtually all patients with AD. β-Amyloid contains 39 to 43 amino acid residues and has a high degree of heterogeneity. In contrast to the total levels of β-amyloid peptide and β-amyloid$_{(1\text{-}40)}$ in CSF, $A\beta_{42}$ concentrations in the CSF are lower in AD patients than in control subjects.[2,3,15-17,26] This decline may reflect an increased recruitment of $A\beta_{42}$ from the CSF and the brain interstitial fluid to deposits in the form of plaques.[17] We did not measure β-amyloid$_{(1\text{-}40)}$ levels in CSF and can only speculate that discrimination could even be further improved using the ratio of the CSF concentrations of both β-amyloid peptides as was described recently.[16] Our data also illustrate that decreased levels of $A\beta_{42}$ in CSF are not specific for AD, because the lowest values were observed in the two patients with subacute sclerosing panencephalitis and in one patient with bacterial meningitis. The mechanisms that could explain these findings remain to be elucidated.

A more important differential diagnostic issue may be that nearly half of the VAD patients clustered in the AD region on the tau by $A\beta_{42}$ plot (figure), whereas the other VAD patients had values in the normal range for both markers. This implies two different types of underlying pathologic conditions in patients with a clinical diagnosis of VAD. Indeed, an inverse correlation of CSF tau with the progression of leukoaraiosis was recently reported,[18] suggesting that patients with "pure" VAD have normal CSF tau levels. The relatively high *APOE* e4 allele frequency in the VAD subgroup (44%, 15/34) and the NAD group overall (37%, 17/46) also raises the question of

the simultaneous presence of AD pathologic states in some of these patients. Neuropathologic studies indicate concomitant AD pathologic states in a large proportion of patients with a clinical diagnosis of VAD.[27,28] Thus, analysis of CSF $A\beta_{42}$ and tau may help identify VAD patients who have concomitant AD pathologic states, i.e., patients with mixed AD/VAD.

APOE e4 gene dosage is correlated with plaque burden in AD.[29,30] In AD and VAD, tau levels do not differ significantly among patients with 0, 1, or 2 *APOE* e4 alleles.[15,18] CSF $A\beta_{42}$ was recently reported to be decreased in AD patients carrying one or two *APOE* e4 alleles.[15] Our subgroup analyses based on number of *APOE* e4 alleles suggest that *APOE* e4 load is a significant determinant of $A\beta_{42}$ but not of tau CSF levels in AD and also in NAD. The additional discriminating power of $A\beta_{42}$ in the diagnosis of AD when *APOE* e4 information is available remains to be established.

The conclusions that emerged from this study could perhaps have been even stronger and the statistical analyses more straightforward if data pooling across centers had not been an issue. Efforts are being made to define the variables that could have caused this center effect, which was not reflected in the analysis of the QC samples. Centers are currently advised to determine their own set of normative data. It is clear that full standardization of the analysis system, including sample collection, handling, storage and preparation, instrumentation, and data interpretation, is required for the establishment of more generally applicable reference ranges for both markers. There is no doubt that between-study variations in results reported for these CSF markers could thus further be reduced. CSF tau levels among control subjects in this study were very similar to those reported in previous studies[5,6,16,18,20,24] using the same ELISA. Standardization of the method for fitting the OD values of the standard dilution series tends to reduce variations in results between studies. For example, mean levels for CSF $A\beta_{42}$ reported[2] using a prototype version of the ELISA become similar to our findings when the OD values of the standard dilution series are fitted using a sigmoidal dose–response curve: 1,218 pg/mL instead of 1,678 pg/mL for control subjects and 585 pg/mL instead of 709 pg/mL for AD patients. It has been demonstrated that the $A\beta_{42}$ levels measured are lower if CSF is collected or stored in polystyrene or glass compared with polypropylene.[2,3] This may be caused by differential levels of tube wall adsorbance of $A\beta_{42}$. In this study, CSF was collected directly in polypropylene only at Centers 2 and 8. This may help explain the somewhat higher levels for $A\beta_{42}$ at Center 2. All centers included CSF that was kept frozen in polypropylene, with the exception of Center 5, where polystyrene was used. Values for $A\beta_{42}$ from Center 5 were not lower than overall values, suggesting that other factors may explain the center effect in this study. For example, artificially reduced levels of $A\beta_{42}$ have been observed after a single additional freeze-thaw cycle.[31] An additional freeze-thaw cycle before analysis of CSF samples in Center 1 could account for the low $A\beta_{42}$ values and incorrect classification of some ND patients (data not shown). Biased selection of AD CSF samples with low tau concentration explains the lower sensitivity for AD observed in Center 9 (data not shown). The high tau values in control subjects reported in Center 3 cannot yet be explained. Once all these variables can be controlled, it seems justified to foresee an even better between-group discrimination.

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High monounsaturated fatty acids intake protects against age-related cognitive decline

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Article abstract—*Objective:* To study the relationships between dietary macronutrient intakes and age-related changes in cognitive functions. *Methods:* We investigated these associations in the prevalence survey (1992 through 1993) of the Italian Longitudinal Study on Aging (ILSA). The population-based sample of 5,632 subjects of the ILSA, age 65 to 84 years, was identified from the electoral rolls of eight Italian municipalities. In this study, standardized test batteries assessing global cognitive functions (Mini-Mental State Examination [MMSE]), selective attention (Digit Cancellation Test [DCT]), and episodic memory (Babcock Story Recall Test), and a semi-quantitative food frequency questionnaire evaluating macronutrient energy intakes, were performed on 278 nondemented elderly subjects from the randomized cohort of Casamassima, Bari (n = 704). *Results:* There was an inverse relationship between monounsaturated fatty acids (MUFAs) energy intake and cognitive decline (MMSE < 24). The effect of education on the odds of having a MMSE score <24 decreased exponentially with the increase of MUFA intakes (over 2,400 kJ; odds ratio, 0.69). Moreover, a significant inverse association was observed between MUFA intakes and DCT score (odds ratio, 0.99). No association was found between nutritional variables and episodic memory. *Conclusions:* In an elderly population of Southern Italy with a typical Mediterranean diet, high MUFA intakes appeared to be protective against age-related cognitive decline. Prospective clinical trials are needed to evaluate the impact of specific dietary macronutrient intakes on the age-related changes of cognitive functions.

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Age-related decline of cognitive functions generally refers to a mild deterioration in memory performance, executive functions, and speed of cognitive processing.[1] These cognitive domains appear to be interrelated because the attentional mechanisms are part of an overall executive system controlling cognition.[2]

The terms age-related cognitive decline (ARCD)[3] and aging-associated cognitive decline[4] have been proposed to indicate an objective decline in cognitive functioning associated with the aging process but within normal limits given the person's age. Whether ARCD is a normal aging process, represents a distinct clinical entity, or has a continuum with dementia is difficult to establish.[5,6]

The causes of ARCD are unknown, but some studies have suggested that it may be prevented.[7] Avoidance of cardiovascular and other chronic diseases, high educational level, and maintenance of vision and hearing have been identified as protective factors from ARCD.[8] On the contrary, hypertension,[9] effects of altered metabolism of steroid hormones, smoking, low-complexity occupation, higher density of persons per bedroom in the home, and low level of physical activity have been identified as risk factors for ARCD.[10]

The role of diet in ARCD has not been extensively investigated. Deficiencies of micronutrients (B_1, B_2, B_6, B_{12}, C vitamins, and folate) have been frequently described in elderly people[11] and significantly associated with cognitive impairment.[12] On the other hand, few data are available on the role of macronutrient intake in ARCD.[13]

The aim of this study was to investigate the relationships between dietary macronutrient intakes and age-related changes in cognitive functions.

Methods. *Subjects.* The subjects of this study are part of a larger study, the Italian Longitudinal Study on Aging (ILSA), promoted by the Italian National Research Council–CNR-Targeted Project on Aging.[14] This was an Italian multicenter, population-based cohort study designed to evaluate age-related diseases as well as physiologic and functional changes of cardiovascular, endocrine, metabolic, and nervous systems in the aging process. The ILSA design included both survey and prospective components.

A sample of 5,632 subjects, age 65 to 84 years, free-living or institutionalized, was randomly selected from the

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electoral rolls of eight municipalities [Genoa, Segrate (Milan), Selvazzano-Rubano (Padua), Impruneta (Florence), Fermo (Ascoli-Piceno), Naples, Casamassima (Bari), and Catania] after stratification for age and sex. Following the equal allocation strategy, in each study center, four age classes (65 to 69, 70 to 74, 75 to 79, and 80 to 84 years) of 88 subjects for each sex were drawn up.

The data of this study have been obtained during the prevalence survey study carried out in Casamassima (Bari, Southern Italy) between March 1992 and June 1993 (prevalence day: March 1, 1992). A total of 404 out of 704 randomized elderly subjects (57.4%) agreed to participate. Of the 300 nonparticipants, 258 subjects refused to participate, 38 died after enrolling, and 4 could not be contacted (moved or never at home). However, 126 subjects (31.2%) were later excluded for not fulfilling all the inclusion criteria. Thus, the study population consisted of 278 free-living elderly subjects, who performed both the neuropsychological evaluation and the dietary assessment.

Selection criteria. The focus of clinical criteria defined by the Working Party of the International Psychogeriatrics Association[4] was mild but objective memory or cognitive decline, in comparison with the standardized performance of age- and education-matched subjects, that could not be attributed to a specific neurologic or psychiatric disorder. Patients who met these criteria could be included in the new diagnostic category of ARCD in the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV).[5] Criteria for entry in this study included no known diagnosis of brain tumor, cerebrovascular malformations, psychosis, epilepsy, multiple sclerosis, stage III syphilis, parkinsonism, stroke (atherotrombotic, hemorrhagic brain infarction, or transient ischemic attack), or dementia. Furthermore, no severe functional limitations and no treatment with any drug that could interfere with the parameters of the study protocol were allowed.

To exclude the diagnosis of dementia, the case finding strategy consisted of a two-phase procedure: all participants were administered an extensive risk factor interview and a screening test battery; those who screened positive underwent a clinical evaluation by a trained neurologist to determine the final diagnosis of dementia. The main screening criteria for cognitive impairment or dementia were Mini-Mental State Examination (MMSE) with a cutoff score of 23/24 or previous diagnosis reported by the proxy respondent. The MMSE has been previously validated in each of the eight study centers against the DSM-III-R[15] clinical diagnosis of dementia; the cutoff point of 23/24 has a sensitivity of 95% and a specificity of 90%.[16] The subjects who scored under 24 on the MMSE were enrolled in this study if the clinical evaluation excluded the diagnosis of dementia. The structured clinical assessment performed by the neurologist consisted of sections B and H of the Cambridge Mental Disorders Examination (CAMDEX),[17] the Pfeffer Functional activities questionnaire,[18] the Hamilton Depression Rating Scale (to exclude depressive pseudodementia),[19] neurologic examination, and review of clinical records. The final diagnosis had to meet DSM-III-R criteria for dementia syndrome, National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria[20] for possible and probable AD, and International Classification of Diseases (ICD-10) criteria[21] for other dementing diseases. To ensure the reliability of diagnoses, before the survey the clinical investigators participated in an interrater agreement study on the application of the above-mentioned clinical diagnostic criteria.[22]

Neuropsychological and functional variables. The MMSE[23] was used to evaluate global cognitive functions (orientation in space and time, concentration and attention span, immediate and delayed verbal memory, constructive praxis, and language). Results of MMSE have been expressed as a score ranging from 0 to 30. Episodic memory was explored with Babcock Story Recall Test (BSRT) (scores ranging from 0 to 16).[24,25] Using a 21-unit story, this test measures immediate and delayed recall and their sum. An event-weighted, hierarchical scoring system was employed, rewarding the degree of organization of the oral recollection given by the subject. Selective attention was assessed by Digit Cancellation Test (DCT) (scores ranging from 0 to 60).[24] As in all other cancellation tests, this test has a basic format with three different matrices made up of 13 strings of 10 digits (0 to 9 in random sequence); each line includes from 0 to 5 targets. Digits have to be crossed out within a time limit (45 seconds/matrix).

Physical functional status was assessed by the Activities of Daily Living (ADL) scale[26] (scores ranging from 6 [all functions preserved] to 18 [all functions lost]). This test evaluates independence in six activities: bathing, dressing, toileting, transferring from bed to chair, continence, and feeding. Ability in home management was assessed by the Instrumental Activities of Daily Living (IADL) scale (scores ranging from 8 [all functions preserved] to 31 [all functions lost]),[27] which considers tasks such as using the telephone, shopping for personal items, preparing meals, doing lightly housework (e.g., washing dishes), or managing money or drugs.

Nutritional and anthropometric variables. Food intake was assessed with a previously validated 77-item semiquantitative food frequency questionnaire.[28] Dietary variables estimated were: energy; total lipids; saturated fatty acids (SFAs); monounsaturated fatty acids (MUFAs); polyunsaturated fatty acids (PUFAs); carbohydrates; proteins; alcohol; total, insoluble, and soluble fibers; and cellulose and noncellulosic polysaccharides (NCP). Subjects indicated how often during the previous year, on average, they had eaten a certain food, choosing from pictures of three different serving sizes or natural units, e.g., a glass of wine. Eight response categories were offered, ranging from never to two or more times per day. Two trained dietitians administered the semi-quantitative food frequency questionnaire in face-to-face meetings in each subject's home. Nutrient intakes were calculated from the questionnaire by multiplying the frequency weight (once per day was equal to one) of each food by the nutrient content of the portion size. The food composition database used to calculate nutrient values was primarily based on The Food Composition Tables of the National Institute of Nutrition.[29]

Measures of height and weight were performed for each participant: vertical stature was measured to the nearest 0.01 m with a stadiometer, weight was measured to the nearest 0.1 kg with an electronic balance, and body mass index (BMI) (kg/m^2) was calculated.

Statistical analysis. Spearman nonparametric correlation was performed to evaluate the relationships between

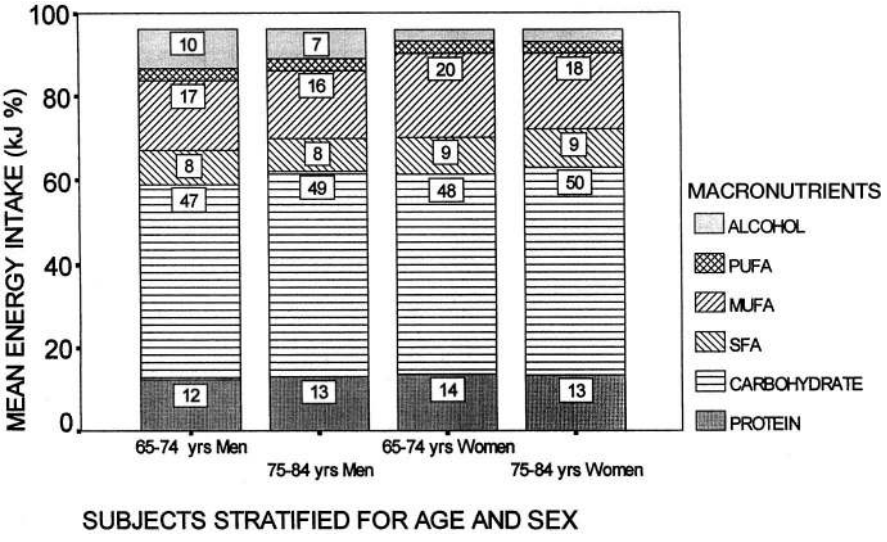

Figure. Distribution of mean energy intakes (%), stratified for age and sex, of the Italian Longitudinal Study on Aging (ILSA-Casamassima), first prevalence survey, 1992–1993. PUFA = polyunsaturated fatty acids; MUFA = monounsaturated fatty acids; SFA = saturated fatty acids.

cognitive and nutritional variables. Subsequently, a multivariate logistic regression model was used to evaluate significant change in odds ratios (OR) of cognitive impairment for macronutrient intakes. These relationships were controlled for covariates that could be effect modifiers or confounders, such as age, sex, education level, and BMI.

For each analysis, the cognitive variable was considered as the dependent variable coded 0 and 1. MMSE results were defined as normal when the score was ≥24 (coded 0),[23,30] or affected when the score was <24 (coded 1). The higher three quartiles of the DCT scores (score ≥ 24) were coded 0 and the lower quartile (score < 24) was coded 1. The higher three quartiles of BSRT total scores (sum of immediate and delayed recall scores ≥1) were coded 0 and the lower quartile (<1) was coded 1. For DCT and BSRT, this stratification was preferred because of lack of age-specific normative data for an Italian elderly population over 79 years old.[24]

The statistical significance threshold was set at 0.05. All calculations were carried out using SPSS 7.5 Windows 95 statistical software (SPSS; Chicago, IL).[31]

Results. No differences in age (74.1 ± 5.5 versus 73.6 ± 5.8, $p < 0.474$, evaluated by separate variance *t*-test) or sex (Pearson $\chi^2 = 2.97$, $p < 0.09$) were observed between participants and nonparticipants (404 participants: 211 [60.6%] men and 193 [54.2%] women; 300 nonparticipants: 137 [39.4%] men and 163 [45.8%] women). A total of 126 out of 404 subjects were excluded for not fulfilling all the inclusion criteria. Of these, we assessed the cognitive status of 102 elderly people. These were characterized by a higher mean age, lower educational level, and lower performance on neuropsychological tests compared to the 278 participants (age 76 ± 6.1 standard deviation [SD] versus 73 ± 5.5 years, $p < 0.01$; education 2.9 ± 2.2 versus 4 ± 2.8 years, $p < 0.01$; MMSE 20.5 ± 5.3 versus 23.6 ± 4.0, $p < 0.01$; DCT 27.0 ± 13.5 versus 33.6 ± 13.1, $p < 0.01$; BSRT 3.9 ± 4.4 versus 6.2 ± 4.9, $p < 0.01$, evaluated by separate variance *t*-test). No significant differences were found between sexes. However, any cognitive assessment of the 300 nonparticipants was not known because the allocation strategy selected a random sample only according to age and sex. Thus, in all, there were 426 subjects (300 + 126) not enrolled in the study. They were characterized by older mean age (74.5 ± 5.7 years versus 73 ± 5.5, $p < 0.01$ evaluated by separate variance *t*-test) and predominance of women (Pearson $\chi^2 = 6.5$, $p < 0.02$) (278 participants: 154 [44.3%] men and 124 [34.8%] women; 426 nonparticipants: 194 [55.7%] men and 232 [65.2%] women) in respect to participants. The participants' mean education level was 4 ± 2.8 years (4.4 ± 2.8 for men and 3.5 ± 2.4 for women).

In our population, analysis of energy intake showed 13% derived from proteins, 48.3% from carbohydrates, 32.9% from lipids (8.4% from SFAs, 17.6% from MUFAs, and 3% from PUFAs), and 5.8% from alcohol. The figure shows the mean energy intakes stratified for sex and age (65 to 74 years and 75 to 84 years) and the differences of mean alcohol energy intake (3% in women versus 9% in men) and MUFAs (19% in women versus 16% in men) between women and men. Daily macronutrient intakes are expressed as mean and SD in table 1. The subjects' mean score on the MMSE, the sum of immediate and delayed recall of BSRT, and the DCT were 23.6 ± 4, 6.2 ± 4.9, and 33.6 ± 13.1, respectively.

The relationships among global cognitive impairment, selective attention, episodic memory, and nutritional variables, performed by Spearman nonparametric correlation, are shown in table 2. The best models were chosen by the results of quartile analysis of each of these variables: the quartiles were determined, and three design variables were created, using the lowest quartile as the reference class. The first and the second quartiles for age (≤71 years old) were coded 0; the third and the fourth (≥72 years old), 1. The first and the second quartiles for education (≤3 years) were coded 1; the third and the fourth quartiles (>3 years), 0. The multivariate logistic analysis was based on the univariate results, choosing the variables and their respective interactions by a hierarchically well-formulated model. In this model, only one interaction was worth pursuing further: education by MUFA. Moreover, this higher-order interaction was highly correlated (close to collinear) with lower-order terms of the logistic model, and it was resolved by centering. Table 3 shows that the effect of education on the odds of cognitive impairment decreased exponentially with the increase of MUFA energy intakes.

Table 1 *Energy, fiber, and nutrient intakes relative to Mini-Mental State Examination (MMSE), Babcock Story Recall Test (BSRT), and Digit Cancellation Test (DCA) scores; ILSA-Casamassima; first prevalence survey, 1992–1993*

		MMSE*		BSRT†		DCT‡	
Intake	Total	<24, n = 107	≥24, n = 171	<1, n = 84	≥1, n = 194	<24, n = 72	≥24, n = 206
Energy (kJ/day)	9524 ± 2857	9522 ± 2946	9525 ± 2809	9493 ± 2805	9487 ± 2850	9448 ± 2744	9521 ± 2900
Proteins (kJ/day)	1230 ± 443	1283 ± 482	1197 ± 415	1234 ± 430	1212 ± 434	1284 ± 445	1203 ± 443
Carbohydrates (kJ/day)	4625 ± 1750	4713 ± 1755	4571 ± 1750	4656 ± 1775	4599 ± 1742	4793 ± 1793	4561 ± 1742
MUFAs (kJ/day)	1584 ± 471	1507 ± 483	1632 ± 458	1549 ± 415	1597 ± 483	1458 ± 449	1625 ± 464
PUFAs (kJ/day)	280 ± 98	266 ± 86	288 ± 105	275 ± 77	281 ± 106	260 ± 87	287 ± 102
SFAs (kJ/day)	781 ± 296	802 ± 303	769 ± 291	747 ± 231	778 ± 301	768 ± 255	772 ± 298
Alcohol (kJ/day)	574 ± 650	491 ± 567	627 ± 693	595 ± 671	573 ± 651	435 ± 529	633 ± 688
Total fibers (g/1,000 kJ)	3.2 ± 1	3.3 ± 0.9	3.2 ± 1	3.3 ± 0.9	3.2 ± 1	3.3 ± 1	3.2 ± 1
Insoluble fibers (g/1,000 kJ)	2 ± 0.8	2 ± 0.7	2 ± 0.8	2 ± 0.7	2.1 ± 0.8	2 ± 0.8	2 ± 0.8
Soluble fibers (g/1,000 kJ)	1 ± 0.3	1 ± 0.3	0.9 ± 0.3	1 ± 0.2	1 ± 0.3	1 ± 0.3	1 ± 0.3
Cellulose (g/1,000 kJ)	0.9 ± 0.4	1 ± 0.4	0.9 ± 0.4	1 ± 0.4	0.9 ± 0.4	0.9 ± 0.4	0.9 ± 0.4
NCP (g/1,000 kJ)	2.2 ± 0.6	2.3 ± 0.6	2.2 ± 0.6	2.3 ± 0.6	2.2 ± 0.6	2.3 ± 0.6	2.2 ± 0.6

Values are mean ± standard deviation.

* Scores ranged from 0–30 (cognitive decline: MMSE < 24).
† Scores ranged from 0–16 (memory impairment: BSRT < 1).
‡ Scores ranged from 0–60 (selective attention impairment: DCT < 24).

ILSA = Italian Longitudinal Study on Aging; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; SFAs = saturated fatty acids; NCP = noncellulose polysaccharides.

Despite the lower education, MUFA energy intake over 2,400 kJ/day was associated with a reduction in OR of cognitive impairment.

The introduction of age as a confounder in the multivariate logistic model containing the interaction term "education by MUFA" was associated with a further increase in OR of cognitive impairment. These results showed that lower education was associated with an exponential reduction in OR of cognitive impairment when MUFA energy intake was over 2,400 kJ/day and this effect was preserved in presence of increasing age. However, as shown in table 3, the growing width of the confidence intervals (CI) indicated that there was considerable uncertainty in these estimates for MUFA energy intake below 1,200 kJ/day.

The role of MUFAs as a protective factor from selective attention impairment was preserved (OR, 0.99; 95% CI, 0.96 to 0.99). However, education and sex had neither modifier nor confounding effect on change in OR for selective attention (odds ratio, 0.99; 95% CI, 0.98 to 0.99). Although age and education were not confounders, they were included in the model because they allowed for a gain in precision (the CI for MUFAs became narrower with than without these two variables). Sex was excluded because it did not show any gain. The relationships between episodic memory and nutritional variables were evaluated, but no association between these variables was found (OR, 0.99; 95% CI, 0.99 to 1.00).

Discussion. In the current study, neuropsychological test scores and macronutrient intakes appeared mutually independent, except for MUFA intakes, which were significantly associated with an improve-

Table 2 *Spearman's correlation coefficients among Mini-Mental State Examination (MMSE), Babcock Story Recall Test (BSRT), and Digit Cancellation Test (DCT) scores and age, sex, education level, dietary intake, and anthropometric variables; ILSA-Casamassima; first prevalence survey, 1992–1993*

Characteristic	MMSE	BSRT	DCT
MUFA energy intake (kJ/day)	0.12*	0.06	0.16†
PUFA energy intake (kJ/day)	0.10	0.004	0.12*
SFA energy intake (kJ/day)	−0.05	0.01	−0.03
Alcohol energy intake (kJ/day)	0.08	−0.0007	0.11
Soluble fibers (g/day)	−0.06	0.007	−0.01
Noncellulosic polysaccharides (g/day)	−0.06	−0.01	−0.005
Protein energy intake (kJ/day)	−0.08	−0.02	−0.11
Cellulose (g/day)	−0.07	−0.05	0.009
Total fibers (g/day)	−0.07	−0.03	−0.02
Carbohydrate energy intake (kJ/day)	−0.03	0.004	−0.05
Insoluble fibers (g/day)	−0.06	−0.03	0.0002
Age (y)	−0.35†	−014*	−0.37†
Sex	−0.06	0.05	−0.02
Education (y)	0.45†	0.44†	0.35†
BMI (kg/m^2)	0.06	−0.02	0.07

* Correlation is significant at the 0.05 level (two-tailed).
† Correlation is significant at the 0.01 level (two-tailed).

ILSA = Italian Longitudinal Study on Aging; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; BMI = body mass index.

Table 3 *Change in odds ratio (OR) of cognitive decline for monounsaturated fatty acid (MUFA) energy intake controlling for education (coded 1 if ≤3 y) and age (coded 1 if ≥72 y); ILSA-Casamassima; first prevalence survey, 1992–1993*

MUFA intake (kJ/d)	Education, y	MMSE score		MUFA, controlling for education		MUFA, controlling for education and age	
		<24	≥24	OR	95% CI	OR	95% CI
≤800	≤3	7	1	33	8.2–133	37.5	9–156
	>3	2	10				
801–1,200	≤3	17	3	14.9	6.2–35.8	16.9	6.7–42.7
	>3	8	24				
1,201–1,600	≤3	12	5	6.7	3.7–12	7.6	3.9–14.6
	>3	12	34				
1,601–2,000	≤3	10	9	3	1.3–6.7	3.4	1.4–8
	>3	16	43				
2,001–2,400	≤3	5	6	1.3	0.4–4.9	1.5	0.4–5.8
	>3	14	23				
2,401–2,800	≤3	1	5	0.6	0.1–4.5	0.69	0.1–4.5
	>3	3	8				

The estimated coefficients for the multivariate model containing centered variables identified in the univariate analysis have been used to estimate the ORs for each category of MUFA, education, and age relative to a referent category (according to a reference cell parameterization).

ILSA = Italian Longitudinal Study on Aging; MMSE = Mini-Mental State Examination; CI = confidence interval.

ment in OR of global cognitive functions and selective attention. Furthermore, the effect of education on the odds of having a cognitive impairment decreases exponentially with MUFA energy intake. These findings appear to be consistent with other studies; they indicate an association between dietary intake and cognitive functions. In a recent longitudinal study of a well-nourished and cognitively unimpaired sample of elderly community residents, a significant association between protein intake and cognitive performance was found.[32] In another study, a significant association between functional variables (i.e., ADL) and alcohol intake was found, likely in relation to a better health status of moderate alcohol consumers.[33] Finally, in a recent study, noninstitutionalized elderly subjects with the best performance on cognitive tests had lower intake of MUFAs, SFAs, and cholesterol, and higher intakes of total food, fruit, carbohydrate, thiamine, folate, and vitamin C.[34]

These apparently conflicting results could be partially due to some questionable features of the study. First, the internal validity of the study must be considered. Ortega et al.[34] used a prospective method making use of a weighed food record for seven consecutive days, but did not provide information about usual dietary intake, despite the variability related to seasonal, monthly, and weekly changes in dietary intake. Dietary records must be repeated in other periods of the year.[35] For our purposes, it was mandatory to use a method to assess the usual diet, i.e., a retrospective method, and to measure the average long-term dietary intake rather than to provide a precise estimate of the current, short-term intake, as the weighed food record does. The previously validated semi-quantitative food-frequency questionnaire is believed to be the best method for dietary assessment in epidemiologic studies because it refers to the whole year and is easy to complete.[36] Secondly, the external validity of the study must be considered. In the study of Ortega et al., the selection of participants was not performed randomly, but within three elderly persons' clubs. These clubs were frequented by healthy elderly people who return to their own homes to sleep.

Some limitations of the current study should be noted. The apparently low reliability of the neuropsychologic tests (BSRT = 69%; DCT = 53%)[24] and of the semi-quantitative food frequency questionnaire (from about 60% for proteins and fibers to 86 to 93% for lipids and alcohol)[28] is approximately similar to other tools exploring the same issues.[37,38] Moreover, strong potential for misclassification—probably the most common cause of artifactual small effects in epidemiologic studies—was observed. In fact, the undefined clinical limits for ARCD and the low reliability of BSRT and DCT end up with a smaller effect estimate (i.e., relationship between attention and MUFA intakes; OR 0.99, 95% CI 0.96 to 0.99) or do not result with a significant effect estimate (i.e., relationship between episodic memory and MUFA intakes; OR 0.99, 95% CI 0.99 to 1.00). The good reliability of the MMSE (88%)[23] in exploring global cognitive functions of aged subjects free from dementing illness warrants a higher accuracy in the subjects' classification of ARCD. The R^2-type coefficients of the logistic analyses were attributable in part to the low reliability of the tools used (1.5% for

the relationship between MUFA intake and global cognitive decline, 2.2% for the relationship between MUFA intake and selective attention impairment).

Owing to the dietary pattern of our population, the typical Mediterranean diet (figure 1), and the rural setting of the study (Casamassima), the mean consumption of olive oil was particularly high at 46 g/day (range, 12.6 to 113.1 g/day). MUFA energy intake was 17.6%, 85% of which was derived from olive oil. The positive effect of dietary habits on cognitive functioning of healthy elderly subjects could be due in part to the antioxidant compounds of olive oil, i.e., tocopherols and polyphenols. In fact, some pathologic conditions, which can be triggered to some extent by an uncontrolled production of free radicals, could probably be prevented or retarded with high intakes of dietary antioxidants[39] (i.e., vitamins A, E, and C, and carotenes) that might yield beneficial effects on frontal/subcortical brain systems, and cognitive functions might be enhanced (increased performance on effortful memory tasks).[12,40,41] However, the antioxidant activity of dietary macronutrients may not exert a protective effect on age-related changes in cognitive functions in every circumstance. In a Japanese population study,[10] high dietary intake of antioxidant compounds was significantly associated with AD and ARCD.

High MUFA intake per se could suggest preservation of cognitive functions in healthy elderly people. This effect could be related to the role of fatty acids in maintaining the structural integrity of neuronal membranes. A study on fatty acid composition of neuronal membranes demonstrated an increase in MUFA content and a decrease in PUFA content with advancing age.[42] It seems that in the aging process there is an increasing demand for unsaturated fatty acids. In fact, in lymphocytic and macrophage-like cells, an increase of Δ^9 desaturase activity, which converts stearic acid in oleic acid and increases the degree of differentiation of cells, has been observed.[43] These findings are consistent with another study in which high PUFA intake is positively associated with cognitive impairment, whereas high fish consumption tended to be inversely associated with cognitive impairment.[44]

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Motor perseverative behavior on a line cancellation task

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Article abstract—*Objectives:* To study the behavioral and neuroanatomic characteristics of perseverative behavior encountered on a target cancellation task in patients with neglect. *Methods:* Motor perseverative behavior during line cancellation task was evaluated retrospectively in 60 patients with left hemispatial neglect from right hemispheric stroke. *Results:* More than 30% of the patients (21 of 60) showed perseveration, manifested as either repetitive cancellation of the same target (18/21) or cancelling extra lines created by the patients themselves (3/21). Neglect severity correlated positively with the frequency of perseverative errors. Perseveration was most prominent in the rightmost portion of the array. Anterior lesions or massive lesions involving anterior and posterior regions were more likely to be associated with motor perseveration than were lesions restricted to posterior areas. *Conclusions:* Although the mechanism of motor perseveration remains to be elucidated, our findings suggest that the combination of aberrant approach behaviors associated with frontal lobe injury and an attentional or intentional bias toward the rightsided targets might explain the behavior.

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Perseveration refers to the continuation or repetition of a behavior, experience, or activity that inappropriately persists after a change in task demands.[1] Perseverative phenomenon can affect motor, sensory,[2,3] or linguistic[1,4-6] domains of behavior. Based on their review of the literature,[7-10] Sandson and Albert introduced a taxonomy of perseverative behavior that included three distinct types.[11,12] Recurrent perseveration

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is the reoccurrence of a previous response to a subsequent stimulus after a temporary cessation of this response. Stuck-in-set perseveration is an inability to switch strategies to new task demands. Lastly, continuous perseveration refers to the compulsive repetition of a movement that has been initiated.

The line cancellation task serves as one of the commonly used tests for evaluation of hemispatial neglect.[13] We noted that patients often repeatedly cancel the same lines but could not find studies that described the characteristics of this perseverative behavior or the lesions associated with this behavior. Therefore, we attempted to analyze the characteristics of perseverative behavior encountered on the line cancellation task in a sample of patients with hemispatial neglect after right hemisphere injuries and to determine whether the lesions of patients with perseveration are different from those without perseveration.

Methods. *Patients.* Patients were 60 consecutive right-handed individuals with left hemispatial neglect on either the line bisection (22/60), line cancellation (5/60), or both tasks (33/60). There were 40 men and 20 women with a mean age of 62.9 ± 12.7 years (range, 24 to 87 years). All experimental subjects had a right hemisphere stroke demonstrated by CT (14/60) or MRI (46/60) performed during hospitalization. Forty-two patients had cerebral infarction and 18 patients had intracerebral hemorrhage. None of the patients had lesions in the left hemisphere except for minor lacunes. All patients were examined within a month after onset, with a mean time of 10.5 ± 8.6 days (range, 1 to 29 days). From an initial set of 72 consecutive patients with right hemispheric injury and neglect behavior, we excluded 12 patients from further analysis. Individuals were excluded owing to initial assessment beyond 30 days after injury (n = 2), more than one episode of stroke (n = 2), isolated thalamic hemorrhage (n = 4), and lack of relevant abnormality detected by neuroimaging despite clinical neglect (n = 4).

Controls. Forty-two individuals without previous history of neurologic or psychiatric illnesses served as control subjects. The group consisted of 19 men and 23 women with a mean age of 59.1 ± 13.2 years (range, 2 to 76 years). All control subjects were right-handed by self report. The control subjects were the spouses or other family members of inpatients hospitalized in the Neurology Department of Samsung Medical Center. All subjects provided written informed consent prior to participation.

Line bisection and line cancellation tasks. The lines for bisection were 242 mm long and 1.5 mm thick. Lines were centered on a white A4 size paper (297 × 210 mm). Subjects were placed in front of a table and instructed to bisect the lines using a pen held in their right hand. Deviations from objective midpoint were measured to the closest millimeter; a positive value denotes rightward deviation. Based on the performance of 42 controls for the 242-mm lines (mean, SD: −0.95, 4.25 mm), left spatial neglect was considered present if the mean of 10 bisections was greater than two standard deviations, which was +7.6 mm.

A modified version of Albert's test was used for line cancellation.[8] The array consisted of 40 black lines (25 mm long, 0.5 or 1.2 mm thick) of various orientations dispersed randomly on a 297 × 210 mm sheet of white paper. Each side of the stimulus sheet contained 18 lines that were divided into 3 columns of 6 lines. The columns were numbered as 1 to 6 from left to right. Four lines in the center column were not included in data analysis. Immediately prior to cancellation, patients were shown a completely cancelled sample sheet. No specific statement was made regarding how many times to cross out each line. Rather, subjects were instructed to achieve the same result as demonstrated on the sample sheet in which each target was crossed out once. All patients performed at least one cancellation task. When line cancellation was administered more than once, only the first trial was used to evaluate perseveration.

Perseveration assessment. Line cancellation performance was analyzed for evidence of motor perseverative behavior. Perseverative responses were defined as targets that had been cancelled with more than one distinct mark crossing the target line. Responses in which the line was crossed twice during the same to-and-fro pen stroke (e.g., without breaking contact with the paper) were not considered as perseverative. Likewise, responses in which a second mark was clearly placed as an extension or clarification of the first cancellation mark were not included as perseverations.

The amount of perseverative behavior is discussed as the perseveration percentage (number of targets with perseverative marks/total number of targets cancelled × 100). The spatial distribution of perseverative responses was assessed by numbering the columns 1 to 6 from left to right. Differences in the distribution of perseverations were analyzed using ANOVA with number of targets marked in a column as the dependent variable and column number as the within-subjects variable.

Lesion analysis. The lesions identified on axial CT or MRI scan were traced on the best fitting template of Damasio and Damasio.[14] Depending on lesion sites, a neurologist who was unaware of patients' clinical information divided the subjects into anterior, posterior, and anterior/posterior groups. The anterior group had lesions in the frontal lobe or basal ganglia and the posterior group had lesions located in temporal, parietal, or occipital lobes. Lesions in the anterior/posterior group were located in both territories. Subjects with lesions restricted to the thalamus were excluded from this study, although basal ganglia lesions with partial involvement of thalamus were included and considered as anterior lesions.

Results. *Perseveration on line cancellation task.* Two types of motor perseveration were identified. One form (type I) was characterized by repetitive crossing out movements (i.e., more than one cancellation mark on the same target) as depicted in figure 1. The second form (type II) was an unanticipated behavior, demonstrated in figure 2, in which subjects drew extra line "targets" of their own and then crossed them out. All 42 control subjects cancelled 100% of target lines. Only four controls showed minimal perseveration of the type I variety, crossing twice a single target from 1 of 40 targets in the array. None of the control subjects made greater than two marks on an individual target item. Based upon control group performance, we therefore considered type I perseveration to be present in the patient group if it occurred on two or more targets and the perseveration percentage (see definition

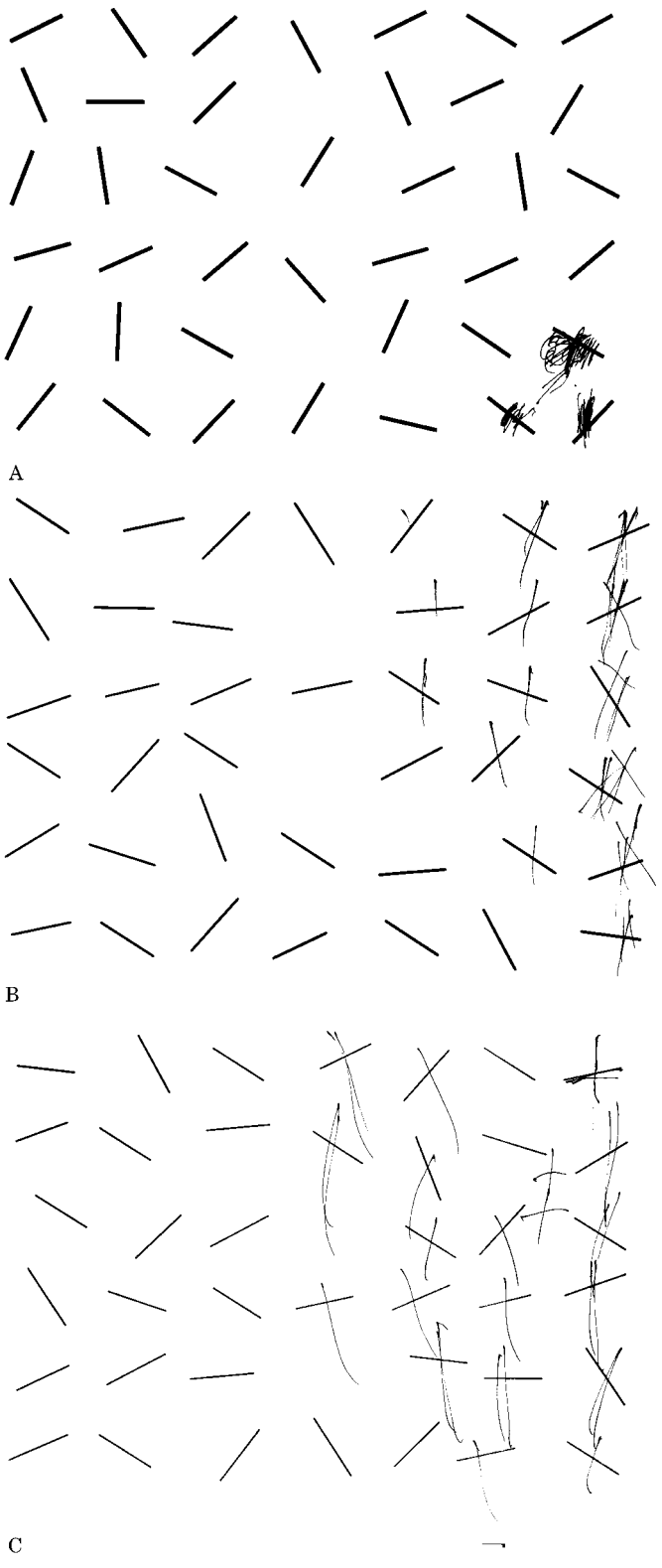

Figure 1. Performance on the line cancellation task showing type I perseveration (multiple cancellation of the same target) in Patient 48 (A) and Patient 35 (B). Note the perseveration of Patient 35 is most severe on the rightmost column. Patient 51 (C) shows mainly type I perseveration combined with a few type II perseverations.

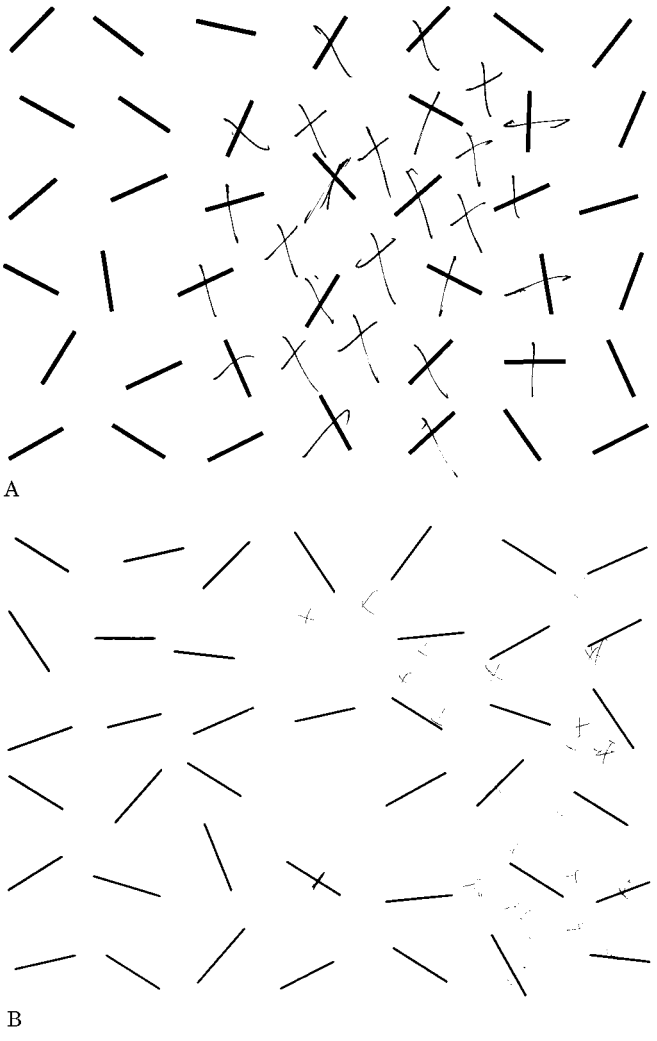

Figure 2. Type II perseveration (crossing the extra lines created by the patient) in Patients 22 (A) and 25 (B).

above) exceeded 10%. No control subject exhibited type II behavior.

Twenty-one of 60 patients showed motor perseveration. Fifteen experimental subjects had type I perseveration, three had type II, and three had both type I and type II (figure 1C). Therefore, only 3 of 18 patients who made type I perseverations also exhibited type II behavior (figure 1B). As shown in table 1, the percentage of perseveration in these 18 patients with type I perseveration varied from 11.1% to 100% (45.8 ± 31.9%).

To learn if there is any regional preponderance in the distribution of type I perseveration, the perseveration percentage was examined as a function of column number. As depicted in figure 3, perseveration was minimal in the columns on the left side, greater in the right columns, and greatest in the rightmost column. These results indicate a propensity for perseverative behavior to occur preferentially on the right side of the stimulus sheet. The strength of this association was tested using ANOVA. There was a significant main effect for column number ($F = 17.3$, df 5,102, $p < 0.0001$). Post hoc analysis using the Tukey honestly significant difference (HSD) test demonstrated that the number of marks in the most rightward column differed significantly from all other columns (largest $p =$

Table 1 *Case summary and perseverative pattern in 21 patients with motor perseveration on the line cancellation task*

Patient number	Sex/age, y	Days postonset	Line bisection error, mm*	Line cancellation "hits," L/R†	Perseveration‡		
					Total (%)	Left 1/2	Right 1/2
2	M/65	15	99.1	0/17	3/17 (17.6)	0	3
4	M/62	20	72.4	0/5	4/5 (80.0)	0	4
17	F/87	15	8.4	16/18	7/34 (20.6)	0	7
18	M/60	14	11.5	18/18	5/36 (13.9)	0	5
20	M/83	2	17.6	0/18	18/18 (100.0)	0	18
22§	M/58	12	5.2	3/10	—	—	—
25§	M/34	28	38.8	0/14	—	—	—
27	M/67	6	9.0	18/18	4/36 (11.1)	0	4
34	M/66	2	99.6	0/2	2/2 (100.0)	0	2
35	M/55	28	94.1	0/14	7/14 (50.0)	0	7
39	M/71	14	46.5	0/12	7/12 (58.3)	0	7
44	M/78	2	15.0	18/18	7/36 (19.4)	2	5
48	M/76	18	84.5	0/3	3/3 (100.0)	0	3
49	M/57	29	12.3	0/12	5/12 (41.7)	0	5
51	M/47	27	23.9	0/15	7/15 (46.7)	0	7
52	M/58	9	96.5	11/18	4/29 (13.8)	3	1
53§	F/70	19	0.5	0/5	—	—	—
54	M/63	3	2.4	14/18	10/32 (31.3)	2	8
55	F/52	7	10.4	17/18	6/35 (17.1)	2	4
59	F/57	29	93.5	0/7	5/7 (71.4)	0	5
60	M/75	3	29.3	0/18	6/18 (33.3)	0	6

* Errors in mm to the right of actual midline.

† Severity of neglect on line cancellation task presented as a ratio of number of cancelled lines on the left side of the page compared to those cancelled on the right side of the page (L/R: the number of cancelled lines out of 18 left-sided target lines/the number of cancelled lines out of 18 right-sided target lines).

‡ Perseveration percentage (total): total number of lines with perseveration/total number of cancelled lines ×100, perseveration percentage (left) and perseveration percentage (right): perseveration percentage in left and right page of stimulus paper respectively.

§ Patients with type II perseveration.

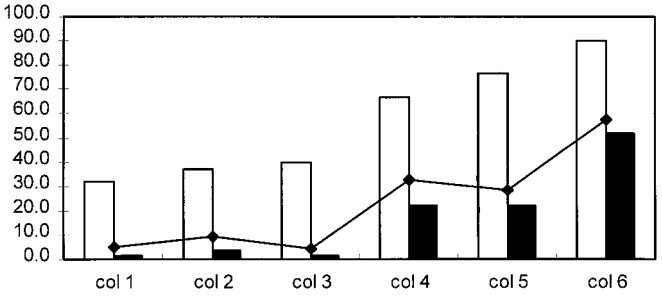

Figure 3. Cancelled targets and perseverative responses as a function of target position. In each column, percentage of cancelled lines (open bars; number of cancelled lines/6 × 100), percentage of lines with perseveration (filled bars; number of lines with perseveration/6 × 100), and perseveration percentage (diamonds; total number of lines with perseveration/total number of cancelled lines × 100) were measured in 18 patients with type I perseveration.

0.004). The next most rightward column differed significantly from all three left-sided columns (largest $p = 0.011$), whereas the least rightward column (third column from the right side of the paper) attained significance only when compared to the most leftward column ($p = 0.02$).

As was shown in table 2, patients with perseveration did not differ from those without perseveration in age, interval between stroke and testing, or degree of line bisection error (t-tests, all p values < 0.05). However, neglect severity on the line cancellation task, as scored by the ratio of the number of cancelled lines on the left side of the page compared to those cancelled on the right side of the page, was greater in patients with perseveration ($t = -3.33$, $p < 0.01$).

Lesion analysis. Of 60 patients examined, 28 had anterior lesions, 20 posterior lesions, and 12 anterior and posterior lesions. Anterior lesions included intracerebral hemorrhage involving the basal ganglia in 14 patients, basal ganglia hemorrhage extending to the thalamic area in two, infarction mainly in the striatum with a surrounding subcortical lesion in nine, infarction restricted to dorsolateral frontal lobe in two, and infarction of the frontal lobe and basal ganglia in one patient. Lesions in the posterior

Table 2 *Comparison of groups of patients with and without perseveration*

Characteristic	Patients with perseveration (n = 21)	Patients without perseveration (n = 39)	*t*-test
Age, y, mean ± SD	63.9 ± 12.4	62.4 ± 13.0	$t = 0.41, p > 0.05$
Time after onset, d, mean ± SD	14.4 ± 9.8	10.0 ± 8.2	$t = 1.61, p > 0.05$
Line bisection, mm, mean ± SD	41.5 ± 38.2	28.2 ± 26.1	$t = 1.58, p > 0.05$
Line cancellation (L/R),* mean ± SD	0.31 ± 0.43	0.67 ± 0.44	$t = -3.33, p < 0.01$
Lesion distribution, n (%)			
Anterior	12 (42.9)	16	
Posterior	3 (15.0)	17	$\chi^2_{(1)} = 4.21, p < 0.05$
Both	6 (50.0)	6	

* Severity of neglect on line cancellation task (see legend of table 1).

group were occipitotemporal infarction in four patients, temporoparietal lesions in eight patients (one hemorrhage, seven infarction), and parietal in eight (two hemorrhage, six infarction). All 12 patients in the anterior/posterior group had total or near total infarctions in the distribution of the middle cerebral artery territory.

Table 3 summarizes the anatomic distribution and etiology of brain injury in neglect patients with perseveration. Overall perseveration occurred in 12 of 28 (42.9%) patients with anterior lesions, 3 of 20 (15.0%) with posterior lesions, and 6 of 12 (50.0%) with anterior/posterior lesion. When only the anterior and posterior groups were compared (chi-square test), perseveration was more frequently associated with anterior than with posterior lesions ($\chi^2_{(1)} = 4.21$, $p < 0.05$). However, these two groups did not differ in mean age (anterior 60.2 ± 11.7, posterior 65.7 ± 15.6, $t = -1.39$, $p > 0.05$), line bisection deviation (anterior 27.5 ± 31.6 mm, posterior 29.3 ± 21.8 mm, $t = -0.22$, $p > 0.05$), line cancellation performance (anterior 0.5 ± 0.5, posterior 0.7 ± 0.4, $t = -0.98$, $p > 0.05$), or interval after onset of injury (anterior 10.7 ± 8.8 days, posterior 10.3 ± 7.2 days, $t = -0.19$, $p > 0.05$).

Of the three patients with type II perseveration, one had a lesion of the dorsolateral frontal lobe and two had basal ganglia hemorrhage that extended to the thalamus.

Discussion. Our results demonstrate that about 30% of patients with left hemispatial neglect from right hemispheric stoke exhibit motor perseverative behavior during the line cancellation task. To date, studies reporting perseverative behavior in association with the neglect syndrome are scant. Although not specifically mentioned, one of the patients (Case 2) in the Tegner and Levander[15] study and two patients (Patients 7 and 12) in the Bisiach et al.[16] study appear to have had repetitive crossing movements on line cancellation performed while subjects were looking through a right-angled mirror. Na et al.[17] also briefly stated that 6 of 10 patients showed similar perseveration during line cancellation while viewing stimuli on closed television circuit. According to our classification, perseveration in these studies appeared to be type I. In these stimulus reversal paradigms, subjects cannot directly view the stimuli or their hands. Thus the tasks place heavy demands on

Table 3 *Anatomic distribution and etiology of brain injury in patients with perseveration*

Patient number	Anatomy and etiology	Category
2	BG hemorrhage	Anterior
4	Total MCA and PCA infarct: F, P, T, O, BG, thalamus	Anterior/posterior
17	Subcortical parietal infarct	Posterior
18	Subcortical frontal/caudate infarct	Anterior
20	Temporoparietal infarct	Posterior
22*	BG hemorrhage extending to thalamus	Anterior
25*	BG hemorrhage extending to thalamus	Anterior
27	Putamen and corona radiata infarction	Anterior
34	Total MCA infarct: F, P, T, insular cortex	Anterior/posterior
35	Total MCA infarct: F, P, T, insular cortex	Anterior/posterior
39	Parietal lobar hemorrhage	Posterior
44	Dorsolateral frontal lobe infarct	Anterior
48	Total MCA infarct: F, P, T, BG, thalamus	Anterior/posterior
49	BG hemorrhage	Anterior
51	Total MCA infarct: F, P, T, insular cortex	Anterior/posterior
52	BG hemorrhage	Anterior
53*	Dorsolateral frontal lobe infarct	Anterior
54	Multifocal subcortical infarct: F, P, BG	Anterior/posterior
55	Striatocapsular infarct	Anterior
59	Total MCA infarct: F, T, P, BG	Anterior/posterior
60	Striatum and insular cortex	Anterior

* Patients with type II perseveration.

BG = basal ganglia; F = frontal; P = parietal; T = temporal; O = occipital; MCA = middle cerebral artery; PCA = posterior cerebral artery.

Figure 4. Patient 54's copy (B) of the Ogden picture (A). The perseveration on Ogden picture is most severe on the right side. Patient 51's copy (D) of the two daisy picture (C) demonstrates left spatial neglect and motor perseveration (multiple petals).

visuomotor coordination, thereby potentially inducing "scribbling" movements rather than true motor perseverations. Unlike the studies with reversal paradigms, the perseveration in our study was observed while the patients performed line cancellation directly. Although there was little overlap between these type I and II perseverations, the group of patients with type II perseveration was small and we need further studies before we can draw conclusions about lesion localization or mechanisms.

We limited our observation of motor perseverative behavior to the line cancellation task but motor perseveration can occur in other tests of neglect. For example, we have noted some of our patients perseverate while copying the two daisy line drawing[18] and Ogden picture[19] (figure 4). Although perseveration was not the focus of their article, some of the patients reported by Marshall and Halligan[18] showed similar motor perseveration, adding extra petals in the two daisy copying task. Further studies might be necessary to explore the relationship between the perseverations observed during cancellation and drawing tasks.

The motor perseveration we observed during line cancellation is similar to what has been termed continuous perseveration.[8-12] The neuropsychologic mechanism that induces continuous perseveration is largely unknown. Liepmann referred to the same

phenomenon as clonic perseveration and regarded the disorder as probably ideational in origin but made no further comments (cited in reference 8). Luria[7] called it "efferent" perseveration when compulsive repetition of the same action occurred whereas switching from one action to another presented no difficulty, and suggested that it is a form of pathologic inertia. Sandson and Albert[12] suggested that continuous perseveration results from disruption of an attentional system, and Heilman classified motor perseveration as one of the four types of intentional motor disorders.[20]

Our results suggest that rightward bias (attentional and intentional bias) associated with right hemispheric damage might have contributed to the motor perseveration. When the group of patients with perseveration was compared with those without perseveration, the perseverating group had more severe neglect. Therefore, the spatial bias associated with neglect behavior might have influenced motor perseveration. Alternatively, motor perseveration might have augmented the spatial bias associated with neglect. Patients with left neglect due to right hemispheric lesions characteristically initiate cancellation from the right side of an array and proceed from right to left.[21,22] Having to make a discrimination between cancelled and uncancelled lines may have required more focused attention that further taxes limited attentional resources.[23] Marking targets increases their salience, and increased stimulus salience may either draw attention or retard disengagement from an attended stimulus.[24,25] A third possibility is that motor perseveration and neglect, although associated, represent defects in separate systems. However, if the perseverative and neglect behaviors are independent, the perseverative errors should occur in the cancelled lines regardless of their location on the page. However, our results revealed that not only were the patients more likely to perseverate on the right half than the left half of the stimulus sheet, but also within the right half of the page, subjects were most likely to perseverate in the rightmost column (figure 1B).

Rightward bias associated with right hemisphere injury has been explained as defective motor exploration toward or in the left hemispace (motor-intentional mechanism),[26,27] hypoattention to targets on the left side,[28] or hyperattention to[29] or a failure to disengage from the targets on the right side[24] (sensory-attentional mechanisms). Whereas some patients in our study perseverated on one line after another, several patients cancelled the lines one by one and then in the middle of or after completion of a search returned to the previously cancelled lines and cancelled them again. This perseverative behavior may also be attributed to some form of a "right capture." According to Kinsbourne, a rivalry exists between the two hemispheres and each hemisphere inhibits activation of the opposite hemisphere.[30] Motor perseverative behaviors in patients with right hemisphere damage may reflect the release of the intact left hemisphere's intentional system from right hemisphere control, resulting both in an ipsilesional orientation bias and impaired inhibitory control over motor actions toward or within right hemispace. Although right capture or unopposed "hyperintention," along with patients' motivation to bisect lines, may have produced motor perseveration, neither mechanism can explain why, when a subject attends to a target line and sees it has been cancelled, he or she cancels the line again. It would seem unlikely that subjects would see the target line and not their own cancellation mark. However, it is possible that there is a defect in working memory such that the patient does not recall the target's configuration.

Previous studies reported various forms of repetitive motor behavior in patients with right hemisphere damage. These include ipsilateral instinctive grasp reaction,[31] hypergraphia,[32] and "response-to-next-patient-stimulation" (respond to stimuli directed to other patients).[33] Motor perseveration on line cancellation may be related to this hyperkinetic behavior associated with right hemisphere injury.

The current findings indicate that perseveration during target cancellation most frequently follows anterior brain (basal ganglia and frontal lobe) lesions or large injuries involving both the anterior and posterior cortex. The results concur with previous studies that perseveration is a particularly common and conspicuous consequence of prefrontal pathology.[7,34,35] Using both verbal and nonverbal tasks to elicit perseveration, Sandson and Albert[12] posited different anatomic correlates for each type of perseveration. Specifically, they associated recurrent perseveration with left temporo-parietal damage, continuous perseveration with right hemisphere damage, and stuck-in-set perseveration with injuries that undermined dopaminergic projections to the frontal and subcortical structures. Intrahemispheric comparisons between patients with and without frontal lobe involvement failed to reveal significant group effects for any measure of perseverative behavior. However, it is not clear from the report whether Sandson and Albert made neuroanatomic distinctions on the basis of neuroimaging or clinical data. Using only patients with radiographically definable brain injury, the current study, when only the anterior and posterior groups were compared, found that patients with anterior lesions more frequently perseverated than did those with posterior lesions. Although we also found that patients with severe neglect were more likely to perseverate, this finding did not confound anatomic correlation as there was no difference in neglect severity between anterior and posterior groups.

That anterior lesions produce more motor perseveration than posterior lesions but not more severe neglect supports the capture hypothesis of motor perseveration. Denny-Brown and Chambers[36] suggest that, whereas the frontal lobes mediate avoidance behaviors, the parietal lobes mediate approach

behaviors. Therefore, patients with frontal lobe damage demonstrate abnormal approach behaviors such as utilization behavior and magnetic apraxia. Diseases of frontal-basal ganglia systems have also been associated with compulsive behaviors. Accordingly, the continuous perseveration observed in the current study may reflect a manifestation of utilization or compulsive approach behavior whose expression is constrained by prevailing attentional and intentional biases that underlie the neglect syndrome.

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Improved discrimination of AD patients using ß-amyloid(1-42) and tau levels in CSF

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