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Specificity of AD7C-NTP as a Biochemical Marker for Alzheimer's Disease

■ The 41-kD protein AD7C-NTP is present in neurons. It is selectively upregulated in the Alzheimer's disease (AD) brain and is associated with the pathology of the disease. In situ hybridization and immunostaining studies have localized AD7C-NTP gene expression in neurons. Overexpression of AD7C-NTP in transfected neuronal cells promotes neuritic sprouting and cell death. Using an enzyme-linked sandwich immunoassay constructed with antibodies to the recombinant protein, AD7C-NTP levels have been measured in cerebrospinal fluid samples from cases of AD as well as age-matched controls and a variety of neurological disease controls, including cases of stroke, Pick's disease, amyotrophic lateral sclerosis, diffuse Lewy body disease, and certain psychiatric disorders of the elderly. The mean AD7C-NTP level in the possible/ probable AD group (4.3 ±3.2 ng/mL) was significantly higher ($p < 0.0001$) than the age-matched non-AD-demented control group (1.1 ±0.9 ng/mL). However, there was no significant difference between AD7C-NTP levels in the non-AD-dementia control group and age-matched normal controls (1.1 ±0.9ng/mL vs. 1.2 ±0.9ng/mL). Levels of AD7C-NTP greater than 2.0 ng/mL were found in 83% of possible/probable AD, and in only 6% of the non-AD-demented control group. The data clearly confirms specificity of AD7C-NTP as a biochemical marker for Alzheimer's disease. ■

Keywords: AD7C, neuronal thread protein, Alzheimer's disease, bio-markers, diagnosis

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a chronic deteriorating course of impaired intellectual function and memory loss. The definitive diagnosis of AD is made by pathologic examination of postmortem brain tissue (Smal, Robins, and Barry, 1997). Clinical diagnosis, however, is often missed or delayed (Roses, 1995). A reliable biochemical marker can be a significant help in the accurate and early diagnosis of AD. There are some biochemical markers available, but overall their clinicopathologic correlations are far too low to make them clinically useful in most cases. Apolipoprotein E4 (ApoE4) allele is merely a risk-factor consideration (Arai, Terajima, and Higuchi, 1995). The amyloid β-protein (Aβ)level in the cerebrospinal fluid (CSF) has been reported to decrease in AD, and the tau protein level in the CSF has been reported by several groups to increase in AD patients (Jensen, Basun, and Lannfelt, 1995; Pirttila, Kim, and Mehta, 1994). However, a significant overlap between AD and non-AD levels limits the usefulness of both amyloid β-protein and tau protein. An Alzheimer's test based on the combination of amyloid β-protein and tau protein has been proposed (high tau, low Aβ for AD; low tau, high Aβ for normal). However, this combination marker still suffers from excessive overlap of AD and non-AD patients, as well as nondeterminant regions where tau and amyloid β-protein levels are both low or both high (Motter, Vigo-Pelfrey, and Kholodenko, 1995). Other potential biochemical markers, such as P-97, are still in a preliminary stage (Kennard, Feldman, and Yamada, 1996). Although Alzheimer's Disease Associated Protein (ADAP) has been observed to be an excellent AD marker in the brain (Ghanbari, Miller, and Haigler, 1990), its utility as a CSF marker has not yet been demonstrated. Genetic markers such as presenilin 1 and 2 mutations are trait markers in a subgroup of early-onset familial AD, and are irrelevant to the vast majority of cases of Alzheimer's disease (Tanzi et al., 1996).

The protein AD7C-NTP (41 kD) is present in neurons. It is selectively upregulated in the Alzheimer's disease (AD) brain, and is associated with the pathology of the disease. In situ hybridization and immunostaining studies have localized AD7C-NTP gene expression in neurons (de la Monte, Carlson, and Brown, 1996; de la Monte, Volicer, and Hauser, 1992). Overexpression of AD7C-NTP in transfected neuronal cells promotes neuritic sprouting and cell death (de la Monte, Ghanbari, and Frey, 1997). An early biochemical marker of AD, AD7C-NTP is detected in the brain prior to Aβ and tau proteins (de la Monte, Carlson, and Brown, 1996). The overexpression of AD7C-NTP has been demonstrated in the CSF (de la Monte, Ghanbari, and Frey 1997). This overexpression is not due to nonspecific injury or aging (de la Monte,

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Ghanbari, and Frey, 1997). Moreover, AD7C-NTP levels in the CSF were positively correlated with Blessed dementia scale score (de la Monte, Ghanbari, and Frey, 1997).

This study was undertaken to examine the specificity of AD7C-NTP as a biochemical marker of AD. The AD7C-NTP levels in CSF were compared in possible/probable AD, early AD, normal controls, non-AD dementia controls, and other neurological disease groups.

Materials and Methods

The AD7C-NTP Assay

Specimens were analyzed for AD7C-NTP under standardized conditions in the Nymox Reference Laboratory (CLIA certified) in Rockville, MD. The AD7C test configuration is a sandwich enzyme immunoassay with monoclonal antibody (N_3I_4) as the capture and polyclonal antibody (ADRI) for the detection (de la Monte, Ghanbari, and Frey, 1997; Ghanbari and Ghanbari, 1998). Both N_3I_4 and ADRI were generated with recombinant AD7C-NTP (de la Monte, Carlson, and Brown, 1996) and affinity purified in protein-A or protein-A/G columns (Slatko et al., 1998). Antibody binding specificity to recombinant AD7C-NTP and not to nonrelevant proteins such as human albumin or gamma globulin was demonstrated in direct-binding radioimmunoassays (Slatko et al., 1998). The AD7C-NTP ELSIA was highly sensitive (30 pg), reproducible (Coefficient of variation [CV] < 10%), and linear up to 100 ng/mL (r2 > 0.9) with a recovery rate of more than 90%, based on additions of AD7C-NTP recombinant protein. Duplicate CSF samples (100 µL) were analyzed without knowledge of clinical diagnosis, and the values obtained were in the linear range of the standard curve. The clinical records were reviewed without knowledge of the assay results.

Cerebrospinal Fluid Samples

The CSF samples for the possible/probable Alzheimer's group, as well as the majority of the non-AD-dementia group, came from individual physicians throughout the USA (48 individuals and 2 groups). They were taken from patients who were being investigated for possible AD by physicians who were independently evaluating the AD7C test. The clinical diagnoses for these cases were obtained from the patients' physicians by retrospectively surveying the patients for at least six months after the AD7C-NTP analysis. Sources for the dementia-group samples are described in Table 1. There are six postmortem CSF samples from patients with other neurological diseases (Table 1). The diagnostic criteria for the cases used in this study were not controlled, and may be different from physician to physician; but the study reflects a realistic situation in the field. The clinical diagnoses of cases in the non-AD groups (Table 1) were according to the physicians participating in the survey. The AD and non-AD groups were age-matched.

Table 1 Patient Profiles and AD7C-NTP Levels in the Cerebrospinal Fluid of non-AD Control Patients

Diagnosis	*Age (Years)*	*AD7C-NTP (ng/mL)*	*Source/ Type*
Cerebrovascular accident	76	1.2	(a)
Cerebrovascular accident	72	0.9	(a)
Amyotrophic lateral sclerosis	72	1.4	(a)
Multiple system atrophy	71	0.0	(a)
Pick's disease	74	3.1	(a)
Pick's disease	72	0.0	(a)
Depression	62	1.9	(b)
Depression	71	1.3	(b)
Depression	56	1.1	(b)
Memory loss	67	0.0	(b)
Anxiety disorder	55	0.9	(b)
Normal forgetfulness	60	1.2	(b)
Vascular dementia	75	0.0	(b)
Lewy body disorder	73	0.8	(b)
Hepatic encephalopathy	68	0.0	(b)
Normal forgetfulness	71	1.8	(b)
Neuroleptic malignant syndrome	58	0.0	(b)
Lewy body disorder	73	1.9	(c)
Lewy body disorder	75	1.8	(c)
Lewy body disorder	71	1.8	(c)
Lewy body disorder	68	2.3	(c)
Lewy body disorder	78	0.4	(c)
Creutzfeldt-Jakob disease	52	1.8	(d)

(a) Loyola University (Chicago), postmortem
(b) Individual physicians, antemortem
(c) Massachusetts General Hospital (Boston), antemortem
(d) Autopsy-confirmed diagnosis

Data Analysis

The data were analyzed using descriptive statistics to determine group mean (± SD) and CSF AD7C-NTP levels. Intergroup differences in mean age and CSF AD7C-NTP levels were analyzed by analysis of variance and post hoc Duncan and Fisher's Least-squares difference (LSD) tests. The data analysis was performed using the Number Cruncher Statistical System software, version 6.5 (JL Hintze, Kaysville, Utah).

Results

Patient profiles and AD7C-NTP levels in the CSF of patients serving as non-AD dementia controls and other neurological disease controls are listed in Table 1. Neu-

Table 2 Population Profiles and AD7C-NTP Levels in Cerebrospinal Fluid

Group	*Number of Cases*	*Age (Years*)*	*AD7C-NTP (ng/mL*)*
Early Alzheimer's disease**	89	66.5 ± 8.6	4.6 ± 3.4***
Normal control**	18	61.8 ± 13.3	1.2 ± 0.9
Possible or probable Alzheimer's disease	35	68.5 ± 10.5	4.3 ± 3.2***
Dementia control	17	68.3 ± 7.4	1.1 ± 0.9
Other neurological disease, postmortem	6	72.8 ± 1.8	1.1 ± 1.1

* Mean ± SD, cases over 50 years old

** Values from de la Monte et al. (1997)

*** Statistically significant differences ($p < 0.0001$) relative to normal, demented, and other neurological disease control groups

rological disease controls included Lewy-body disorder (six cases), Pick's disease (two cases), cerebrovascular accident (two cases), vascular dementia (one case), multiple system atrophy (one case), hepatic encephalopathy (one case), drug toxicity (one case), and Creutzfeldt-Jakob Disease (one case). The remainder of the dementia controls consisted of psychiatric non-AD cases which at one time were investigated for possible AD.

The AD7C-NTP concentrations in the CSF samples of patients with possible/probable AD and patients serving as dementia control as well as other neurological controls are summarized in Table 2. The previously reported data for early-AD cases and normal cases (de la Monte, Ghanbari, and Frey, 1997) are also listed in Table 2 for comparison. The AD7C-NTP concentration in the possible/probable AD group (4.3 ± 3.2 ng/mL) was significantly elevated ($p < 0.0001$) compared to the non-AD-dementia control group (1.1 ± 0.9ng/mL). AD7C-NTP mean concentration in non-AD-dementia controls was not significantly different from normal controls (1.10 ± 0.9ng/mL vs. 1.2 ± 0.9ng/mL). Similarly, the mean AD7C-NTP concentration in possible/probable AD cases was comparable to that found in cases of early AD (4.3 ± 3.2ng/mL vs. 4.6 ± 3.4ng/mL). There was no significant difference between the mean ages in AD (68.5 ± 10.5 years) and the non-AD groups (68.3 ± 7.4 years).

The distribution of the individual values for AD7C-NTP concentrations in possible/probable AD cases as well as those in the non-AD-dementia cases are plotted in Figure 1. With a cutoff line of 2.0 ng/mL, 83% of possible/probable AD values were above the line (sensitivity 83%), and 94% of values for dementia cases were below the line (specificity 94%). There were 7 out of 24 cases in the non-AD-dementia and other neurological groups with an AD7C-NTP concentration of 0.0 ng/mL, compared to 0 out of 35 cases of the possible/probable AD group.

Discussion

The data clearly indicate that AD7C-NTP concentrations in the CSF of early-AD patients as well as possible/probable AD patients are significantly elevated when compared with age-matched non-AD groups. More importantly, there is no statistically significant difference between AD7C-NTP concentrations in the CSF of patients serving as the non-AD-dementia control, other neurological disease control, and age-matched normal control groups. The dementia group includes a wide variety of cases commonly presenting to physicians. This study represented a typical and realistic situation in the field, and has involved the participation of over 50 physicians. The physicians were independently evaluating the AD7C test during a trial period. The patient information and case diagnoses were unknown to the technologists who received and analyzed the samples.

The specificity of the marker is demonstrated by the specificity of the assay for normal controls as compared to non-AD-dementia controls (89% vs. 94%). The specificity of about 90% is consistent with the fact that AD7C-NTP is an early marker of AD (de la Monte, Ghanbari, and Frey, 1997, de la Monte, Carlson, and Brown, 1996) and there is always a possibility of some "presymptomatic" AD being present in the non-AD control groups. For example, in the demented and other neurological disease groups, the only patients whose AD7C-NTP concentrations were above 2.0 ng/mL were one individual with Pick's disease (3.1 ng/mL) and one with Lewy-body disorder (2.3 ng/mL). The possibility of these cases also having early changes consistent with Alzheimer's disease cannot be dismissed. It is interesting to note that 6 of the 23 patients in the non-AD group (Table 2), but none of the 35 patients with AD, had an AD7C concentration of 0.0 ng/mL.

It should be noted that the cases reported here represent examples of more difficult cases of possible/probable AD candidates for clinical diagnosis, which prompted CSF samples being referred to the Reference Laboratory. For example, the postmortem-confirmed case of Creutzfeldt-Jakob disease (Table 1) was originally diagnosed as AD after an extensive diagnostic workup and laboratory tests.

We attribute the specificity of the assay to highly specific antibodies such as N_3I_4 and N_3C_{11}. These antibodies were produced against the recombinant form of NTP or AD7C-NTP, and were selected for their specificity from among more than 100 monoclonal antibodies. Hence one should differentiate the results reported here versus earlier reports on the elevation

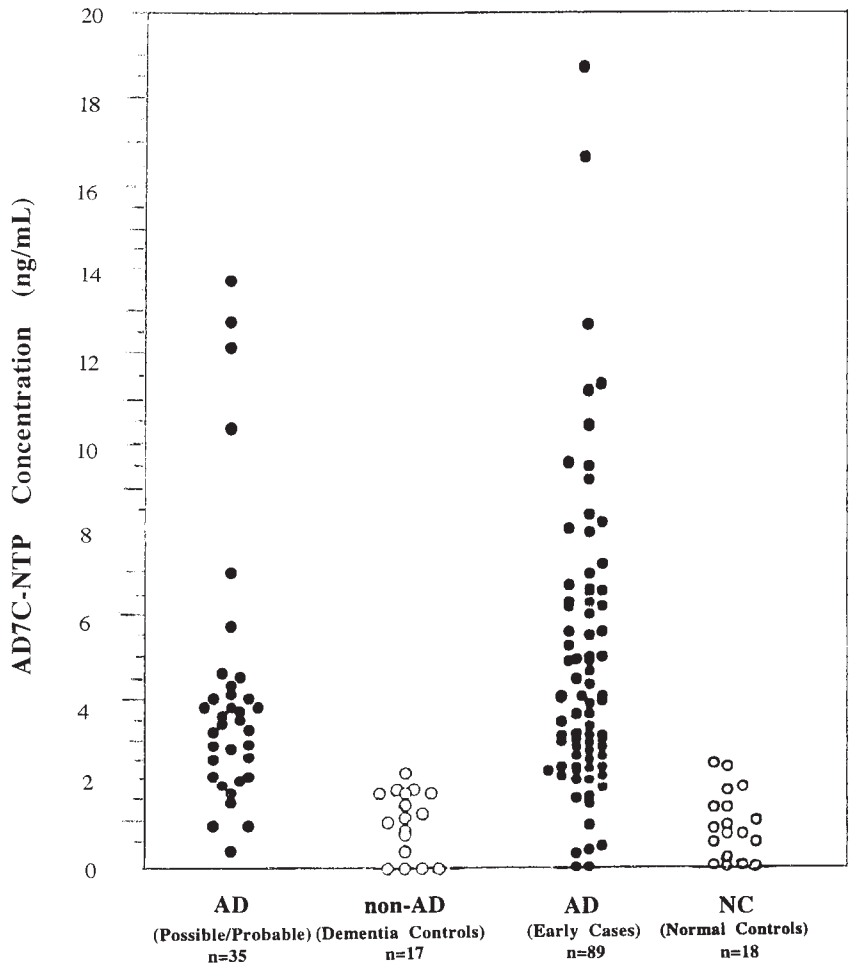

Figure 1 Distribution of AD7C-NTP levels in antemortem cerebrospinal fluid samples (refer to Tables 1 and 2 for population profiles and diagnoses of cases in the scattergram). There were 83% of patients with possible/probable AD and 89% of patients with early-AD with AD7C concentrations above 2.0 ng/mL (sensitivity); 94% of patients serving as non-AD dementia controls and 89% individuals studied as normal controls had AD7C concentrations under 2.0 ng/mL (specificity). All groups were age-matched. There were 6 out of 23 patients in the non-AD demented and other neurological disease groups that had an AD7C-NTP concentration of 0.0 ng/mL, compared to 0 out of 35 individuals in the possible/probable AD group.

of NTP in AD that was generated using cross-reacting antibodies (de la Monte and Wands, 1992).

Conclusion

Concentrations of AD7C-NTP in the CSF of patients with possible/probable AD are significantly higher than those found in individuals with non-AD dementia and other neurological diseases. Moreover, the AD7C-NTP concentrations in patients with non-AD dementia and those serving as other neurological disease controls and normal controls are nearly identical. The specificity and sensitivity of CSF AD7C-NTP (94% and 83%, respectively) in this study (possible/probable AD vs. non-AD-dementia controls) are similar to those previously published for early AD vs. normal controls (89% and 89%, respectively). The data clearly confirm the specificity of AD7C-NTP as a biochemical marker of Alzheimer's disease.

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