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Caspase-6 activity predicts lower episodic memory ability in aged individuals

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ABSTRACT

Caspase-6 (Casp6), a cysteinyl protease that induces axonal degeneration, is activated early in Alzheimer Disease (AD) brains. To determine whether Casp6 activation is responsible for early cognitive impairment, we investigated the abundance of Casp6 activity, paired helical filament–1 (PHF-1) phosphorylated Tau and amyloid beta peptide (Aβ) pathology by immunohistochemistry in the hippocampal formation of aged non–cognitively impaired (NCI) individuals. Casp6 activity was restricted to the entorhinal cortex (ERC) and CA1 regions of the hippocampus. Pathology scores were then correlated with cognitive scores obtained within 1 year of death. Regression analyses revealed that ERC and CA1 Casp6 activity were the main contributor to lower episodic memory performance, whereas ERC PHF-1 pathology predicted lower semantic and working memory performance. Aβ did not correlate with any of the cognitive tests. Because Casp6 activity and PHF-1 pathology are intimately associated with AD pathology and memory decline is an early event in AD, we conclude that Casp6 activity and PHF-1 immunoreactivity in ERC identifies aged individuals at risk for developing AD.

1. Introduction

Caspase-6 (Casp6) activity is present in neurofibrillary tangles (NFT), neuropil threads (NPT), and neuritic plaques (NP) of sporadic Alzheimer's disease (AD) brains and in familial AD caused by amyloid precursor protein (APP), presenilin I, or presenilin II mutations (Albrecht et al., 2009, Albrecht et al., 2007). Previously, we have shown that Casp6 activity occurs at all stages of AD and is equally abundant and distributed in mild, moderate, severe, and very severe forms of AD (Albrecht et al., 2007). Surprisingly, Casp6 activity was detected in some non–cognitively impaired (NCI) brains, and the levels of Casp6 activity correlated inversely with cognitive scores measured within a year of death. However, although Casp6 activity was quite abundant in the hippocampal formation and in the cortices of AD brains, high levels of Casp6 activity in NCI brains were mostly limited to the entorhinal cortex (ERC), the first area to be affected pathologically by AD according to Braak staging (Braak and Braak, 1995, Lace et al., 2009).

Casp6, is involved in inflammation and apoptosis, does not induce cell death when activated in AD neurons or in mammalian cell lines (Gray et al., 2010, Guo et al., 2004, Klaiman et al., 2009), but induces axonal degeneration in primary cortical human neurons and commissural and sympathetic mouse neurons (Klaiman et al., 2008, Nikolaev et al., 2009, Sivananthan et al., 2010). Casp6 cleaves several cytoskeletal (alpha-tubulin, Tau) or cytoskeletal-regulating proteins including post-synaptic actin regulating proteins, Drebrin, Spinophilin, actinin-1, and actinin-4 (Klaiman et al., 2008). In addition, Casp6 activation increases the production of the amyloid beta peptide (Aβ) (LeBlanc, 1995); however, the effect is not through direct cleavage of APP as originally thought (Gervais et al., 1999, Pellegrini et al., 1999, Weidemann et al., 1999) but rather occurs by caspase-dependent cleavage of an inhibitor of the beta secretase (Tesco et al., 2007, Tesco et al., 2003). Furthermore, Casp6 cleaves valosin-containing protein and impairs its ability to target misfolded and ubiquitinated proteins to the proteasome for degradation (Halawani et al., 2010). Together, these studies indicate that Casp6 activity affects a number of parallel degenerative pathways that are likely to impair neuronal function.

To determine the impact of Casp6 activity on the cognitive performance of NCI individuals compared to accepted AD pathological markers, we analyzed the extent of active Casp6, amyloid beta peptide (Aβ), and PHF-1 immunopositive pathology in different

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areas of the hippocampal formation of 17 NCI individuals and correlated these pathological scores with cognitive scores obtained within a year of death.

2. Methods

2.1. Collection of brain tissues, fixation, and preparation of slides

Brain tissue was obtained from subjects who participated in the Religious Orders Study (ROS). Details of the clinical and pathologic evaluation have been previously reported (Bennett et al., 2012). The ROS includes more than 1150 older nuns, priests, and brothers who have agreed to yearly clinical evaluations and brain donation at time of death. The clinical evaluations include a medical history, and neurologic and cognitive assessments performed on a yearly basis. The cognitive evaluation includes 21 tests: the Mini Mental State Examination (MMSE), and 20 other tests, 19 of which can be summarized as follows: visuospatial ability (VSA); perceptual speed (PS); and episodic, semantic, and working memory by converting the individual tests within those domains to Z scores and averaging. All 19 Z-scores can be averaged to yield a global cognitive score. Participants were diagnostically classified by a clinician following the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association (NINCDS-ADRDA) criteria. An AD diagnosis was assigned to persons with a history of cognitive decline and evidence of impairment in memory and other cognitive domains. Mild cognitive impairment (MCI) referred to individuals who displayed cognitive impairment upon neuropsychological evaluations but were not clinically diagnosed with dementia. Non–cognitive impairment (NCI) referred to persons without dementia or MCI. Standard neuropathologic assessment, including Braak Stage determination, was done at autopsy by a neuropathologist shielded from all clinical data.

2.2. Memory tasks and scores

The memory scores represent an average z scores based on the mean and standard deviation of 19 cognitive performance tests at the baseline clinical evaluation. Essentially, a score of 0 refers to the average score of the cohort at baseline, and =1 and −1 are 1 standard deviation above and below the mean. Details of the development and derivation of the scores can be found in prior papers (Wilson et al., 2002). The episodic memory scores were based on a composite score of the following seven instruments: logical memory, word list recall (immediate), word list recall (delayed), word list recognition from the Consortium to Establish a Registry for AD (CERAD), and immediate and delayed recall of story A from the logical memory subset of the Wechsler Memory Scale–revised, and immediate and delayed recall of the East Boston Story. Visuospatial abilities were composite scores of 2 instruments: the 15 items version of Judgment of Line orientation and a 16-item version of standard progressive matrices. Perceptual speed is a composite score of the oral version of the symbol digit modalities test and number comparison. Semantic memory scores are a composite score of 4 instruments: verbal fluency from CERAD, subsets of items from the Boston naming test, extended range vocabulary test, and the national adult reading test. Working memory scores are a composite score of 4 instruments: digit span subtests forward and backward of the Wechsler memory scale revised, digit ordering, and alpha span. The MMSE is the widely used 30-item standardized screening measure of dementia severity.

2.3. Immunostaining of tissue sections with anti-active Casp6, TauΔCasp6, PHF-1, and Aβ

Formalin-fixed, paraffin-embedded 4-μm thick hippocampal formation tissue sections were deparaffinized, rehydrated, and treated with antigen retrieval buffer (10 mmol/L Tris Base, 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.05% Tween 20, pH 9) for 20 minutes at 97 °C in the Pascal Dako Cytomation apparatus and immunostained using the Dako Autostainer Plus automated slide processor and the EnVision Flex system (Dako, ON). Tissue sections were treated with peroxidase for 5 minutes, blocked with Serum-Free Protein Block (Dako, Burlington, ON) for 30 minutes, and subjected to primary antibodies diluted in EnVision Flex Antibody Diluent for 30 minutes. Active Casp6 was detected with p20Casp6 10630 antiserum (1/5000) and TauΔCasp6 10635 antiserum (1/25,000) (Guo et al., 2004), Aβ was detected with F25276 antiserum (1/2000) (LeBlanc, 1995). The PHF-1 antibody (1/5000) was generously provided by Dr Peter Davies (Department of Neuroscience,

Table 1
Demographics and clinical characteristics of the study group

Case patient	Sex	Age (y)	Braak	GCS	MMSE	Memory			VSA*	PS**	Education (y)	APOE allele
						Episodic	Working	Semantic				
1	F	79.12	3	1.22364	30	1.60395	0.95262	0.90475	1.0366	1.27859	18	3/3
2	F	84.75	3	0.71149	30	1.48841	−0.15070	0.96480	0.29079	−0.23121	16	3/3
3	M	72.43	2	0.69258	30	0.45572	0.40186	0.86009	1.18376	1.17663	23	3/3
4	M	74.29	0	0.59127	27	0.60954	0.74473	0.63723	0.73764	0.23726	19	3/3
5	M	83.73	4	0.52824	29	1.29833	−0.28511	0.71240	0.89198	−1.26619	16	3/3
6	F	85.48	4	0.40283	29	0.95316	0.31588	−0.22709	0.71407	−0.65848	20	3/3
7	F	89.41	3	0.33139	30	1.07033	0.09619	0.28166	−0.46610	−1.00030	19	3/4
8	F	77.48	3	−0.09110	29	−0.13994	−0.50318	−0.23723	0.44705	0.30807	18	3/3
9	F	92.67	1	−0.14633	27	0.44284	−1.19912	0.55572	−1.37839	−0.25670	18	3/3
10	M	92.83	3	−0.18231	26	−0.06912	−0.66609	0.34252	−0.14436	−0.69320	20	3/3
11	M	90.29	5	−0.23766	27	−0.17313	−0.18859	0.29502	−0.26917	−1.47179	16	3/3
12	M	78.54	4	−0.40004	30	−0.22092	−0.68774	0.28922	−0.58024	−1.51319	25	3/4
13	F	91.05	4	−0.61728	28	−0.07310	−1.25225	0.10650	−1.35791	−1.58537	20	3/3
14	M	82.97	3	−0.62227	28	−0.74148	−1.12674	−0.13982	0.30176	−0.86870	16	3/3
15	M	84.16	4	−0.68013	25	−0.60590	−0.83912	−0.63451	−0.92203	−0.51771	15	3/3
16	M	83.54	4	−1.13457	27	−1.38893	−1.36202	−1.23970	0.60258	−1.44238	18	3/4
17	M	83.40	3	−1.20115	22	−1.00056	−1.31209	−1.57093	−0.89131	−1.20052	15	2/3
Average	10 M	83.89	3.12	−0.04891	27.88	0.206121	−0.41538	0.11180	0.011157	−0.570895	18.35	
SD	7F	6.12	1.20	0.69	2.18	0.89	0.74	0.73	0.88	0.89	2.74	

Key: APO E, apolipoprotein E; F, female; GCS, global cognitive score; M, male; MMSE, Mini-Mental State Examination; PS, perceptual speed; SD, standard deviation; VSA, visuospatial ability.

* $p = .0173$.

** $p = .0062$.