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Neurobiology of Aging 33 (2012) 2229.e1–2229.e9

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

ADAM10 expression and promoter haplotype in Alzheimer's disease

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Received 2 February 2012; received in revised form 20 March 2012; accepted 29 March 2012

Abstract

Alzheimer's disease is confirmed at autopsy according to the accumulation of brain neuritic plaques and neurofibrillary tangles in the brain. Neuritic plaques contain amyloid-β (Aβ) and lower levels of Aβ correspond to an increase in ADAM10 α-secretase activity. ADAM10 α-secretase activity produces a soluble amyloid precursor protein (APP) alpha (sAPPα) product and negates the pathological production of Aβ. In this investigation, it was hypothesized that genetic variation with the *ADAM10* promoter is associated with ADAM10 expression levels as well as cerebrospinal fluid sAPPα levels. Results from this investigation suggest that the *ADAM10* rs514049-rs653765 C-A promoter haplotype is associated with: (1) higher CSF sAPPα levels in cognitively normal controls compared with Alzheimer's disease (AD) patients, (2) higher postmortem brain hippocampus, but not cerebellum, ADAM10 protein levels in subjects with low plaque scores compared with those with high plaque scores, and (3) higher promoter activity for promoter-only reporter constructs compared with promoter 3' untranslated region (3'UTR) constructs in the human neuroblastoma SHSY5Y cell line, but not in HepG2 or U118 cell lines. Taken together, these findings suggest that ADAM10 expression is modulated according to a promoter haplotype that is influenced in a brain region- and cell type-specific manner.

Keywords: ADAM10; Haplotype; sAPPα; Alzheimer's disease; Reporter assay; Neuritic plaques; Brain; Hippocampus; Cerebrospinal fluid

1. Introduction

Amyloid-β (Aβ), processed from amyloid precursor protein (APP) by β- and γ-secretases, accumulates in the brain as Aβ-containing plaques (Selkoe, 1998); one form, called neuritic plaques, is most closely associated with Alzheimer's disease (AD). Alternatively, α-secretase cleavage within the β-amyloid domain negates the cleavage step that produces pathogenic Aβ (Selkoe, 1998). The membrane-bound disintegrin metalloproteinases (ADAMs) are capable of cleaving APP at the α-secretase site, with ADAM10 as the major α-secretase in the brain (Fahrenholz et al., 2000; Postina, 2008, 2012). An increase in ADAM10 levels in a mouse model corresponds to a decrease in Aβ plaque levels (Postina et al., 2004).

The genomic structure and function of the *ADAM10* promoter has been described (Prinzen et al., 2005). The 5' untranslated region (5'UTR) of *ADAM10* has been reported

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0197-4580/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.03.013

to repress the rate of *ADAM10* translation (Lammich et al., 2010), and retinoic acid and acitretin stimulate promoter activity and increase ADAM10 levels in human cell lines and a mouse model (Endres et al., 2005; Tippmann et al., 2009). Genetic variation within the *ADAM10* promoter has recently been associated with cerebrospinal fluid (CSF) soluble APPα (sAPPα) levels in AD patients compared with cognitively normal control subjects, implicating an important ADAM10 role in AD (Bekris et al., 2011). *ADAM10* mRNA has been reported to be high in AD patients (Gatta et al., 2002), whereas ADAM10 protein levels have been reported to be low in CSF and platelets from AD patients (Colciaghi et al., 2002, 2004; Tang et al., 2006). ADAM10 immunostaining has been reported to be reduced in AD brain (Bernstein et al., 2009). Given the likely influence of ADAM10 expression levels on sAPPα levels in AD, it is important to further characterize the influence of *ADAM10* promoter genetic variation on both transcription and translation (mRNA and protein) of ADAM10 in AD patients compared with cognitively normal control subjects.

Because to our knowledge human brain *ADAM10* mRNA and protein levels have not been previously measured in parallel in the same sample, and because an association between *ADAM10* promoter genetic variation, ADAM10 expression, and neuritic plaque load have not been described, the aim of this investigation was to determine whether *ADAM10* promoter genetic variation is associated with differential ADAM10 brain expression in subjects with a high neuritic plaque score compared with subjects with a low neuritic plaque score and CSF sAPPα levels.

2. Methods

2.1. Population description

Two regions of human postmortem brain (PMB) (cerebellum; CB, hippocampus; HP) from each subject were obtained from the Neuropathology Core of the Alzheimer's Disease Research Center (ADRC) at the University of Washington (UW). Use of human tissue was approved by the University of Washington Institutional Review Board. All tissue was obtained after informed consent, flash frozen at time of autopsy, and stored at −80 °C. Patients with late-onset AD (n = 21) were volunteers in the UW ADRC, where they were diagnosed during life with probable AD per National Institute of Neurological and Communicative Disease—Alzheimer's Disease and Related Disorders (McKhann et al., 1984) and confirmed by postmortem neuropathologic examination to have AD (Braak and Braak, 1991; Mirra et al., 1991) (Table 1). Control individuals (n = 22) were volunteers in the UW ADRC, were never diagnosed with a central nervous system disorder, and postmortem examination showed age-related changes only, including sparse to moderate neuritic plaques (Braak and Braak, 1991; Mirra et al., 1991). Sample size was constrained by limits on postmortem brain interval (PMI), Braak stage, and neuritic plaque score. Sample criteria for inclusion in this investigation were as follows: (1) a low PMI less than or equal to 8 hours to limit mRNA and protein degradation in brain samples and (2) diagnosis in life of AD as well as AD confirmed at autopsy.

Table 1
Population sample description. Cerebrospinal fluid sample used to measure sAPPα levels. Postmortem brain sample used to measure ADAM10 expression. Two-sided Fisher exact *p*-values are shown for the comparison between cognitively normal control subjects and AD patients

	Control subjects	AD patients	*p*-value
Cerebrospinal fluid sample: APPα levels			
n	104	39	
Mean age (range)	67 (52–88)	74 (56–87)	<0.0001
Mean age-at-onset (range)		69 (51–82)	
% Male	50	56	0.574
% *APOE* ε4+	38	72	<0.0001
Postmortem brain sample: ADAM10 levels			
n	22	21	
Mean age (range)	87 (77–95)	81 (60–93)	0.005
Mean age at onset (range)		74 (55–89)	
% Male	50	48	0.833
% *APOE* ε4+	18	67	0.002

CSF sAPPα levels were obtained from 104 healthy cognitively normal control subjects (Table 1). Use of human CSF and DNA was approved by the University of Washington Institutional Review Board. After informed consent, all subjects underwent extensive evaluation that consisted of medical history, family history, physical and neurologic examinations, laboratory tests, and neuropsychological assessment; information was obtained from subjects and from informants for all patients. Mini-Mental State Examination (MMSE) scores of all cognitively normal subjects were between 26 and 30, and Clinical Dementia Rating Scale (CDR) scores were 0 as previously described (Peskind et al., 2006). All AD patients were participants in research clinical cores at respective institutions. Clinical diagnoses of AD were made according to well-established consensus criteria (McKhann et al., 1984; Petersen et al., 1999).

2.2. Cerebrospinal fluid

All CSF samples were obtained as previously described (Peskind et al., 2005, 2006). Samples were separated into aliquots at bedside and frozen immediately on dry ice and stored at −80 °C until assayed. Concentrations of sAPPα in the 10th mL of collected CSF were measured by immunoassays as previously described (Johnson-Wood et al., 1997).