ELSEVIER

Neurobiology of Aging 32 (2011) 551.e13–551.e22

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Review

ACE variants and risk of intracerebral hemorrhage recurrence in amyloid angiopathy

Sophie Domingues-Montanari[a], Mar Hernandez-Guillamon[a], Israel Fernandez-Cadenas[a], Maite Mendioroz[a], Mercè Boada[a,b], Josep Munuera[c], Alex Rovira[c], Olga Maisterra[a], Mireia Parés[a], Maria Gutierrez[a], José Alvarez-Sabin[a], Pilar Chacón[d], Pilar Delgado[a], Joan Montaner[a,*] for the Stroke Project Cerebrovascular Diseases Study Group, Spanish Society of Neurology

[a] *Neurovascular Research Laboratory and Neurovascular Unit, Neurology and Medicine Departments, Universitat Autònoma de Barcelona, Institute of Research, Vall d'Hebron Hospital, Barcelona, Spain*

[b] *Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain*

[c] *Magnetic Resonance Unit, Department of Radiology, Vall d'Hebron Hospital, Barcelona, Spain*

[d] *Lipid Unit, Clinic Biochemistry Service, Vall d'Hebron Hospital, Barcelona, Spain*

Abstract

Cerebral amyloid angiopathy (CAA) is a well-established cause of lobar intracerebral hemorrhage (ICH). The aim of the authors was to investigate the influence of clinical characteristics and genetic variants in the *ACE*, *LRP*, *MMP9*, *Tafi*, *VEGFA*, *CYP11B2*, *A2M* and *APOE* on ICH recurrence in a cohort of CAA-related ICH patients. Sixty patients were enrolled and new symptomatic ICHs in the 36 mo following the index event were recorded. Leukoaraiosis degree, microbleeds count and variants in the *APOE* and *ACE* were associated with ICH recurrence. The rs4311 variant of the *ACE* was an independent risk factor ($p = 0.001$), resisting Bonferroni correction. Moreover, carriers of ε2 of the *APOE* and TT of the rs4311 of the *ACE* reached 100% recurrence before 18 mo ($p < 0.001$). Finally, ACE protein level was measured in serum of controls and depended on the rs4311 genotypes, TT carriers presenting higher level than CC carriers ($p = 0.012$). These results suggest that variants in the *ACE* are associated with CAA-related ICH recurrence, possibly by modulating ACE protein level.

Keywords: *ACE*; *APOE*; Cerebral amyloid angiopathy; Genetics; Hypertension; Intracerebral hemorrhage; Leukoaraoisis; Microbleeds; Protein level; Stroke

1. Introduction

Spontaneous intracerebral hemorrhages (ICH) account for 10–15% of all strokes and expose patients to high mortality rates and poor prognosis (Qureshi et al., 2001). Lobar ICH patients present important white matter damage, associated with higher incidence of cognitive impairment and higher recurrence of ICH with rates up to 23% per year (Greenberg et al., 2004; Hanger et al., 2007). One of the main causes of lobar ICH, particularly in elder patients, is cerebral amyloid angiopathy (CAA), a disorder characterized by the deposition of the β-amyloid protein within the leptomeningeal and cortical arteries (Yamada, 2000). Unfortunately, definitive diagnosis of CAA can only be performed by brain necropsy, although several clinical features allow the estimation of a diagnosis as *probable* or *possible CAA* according to the Boston criteria (Knudsen et al., 2001). CAA is also a common pathological finding in Alzheimer disease (AD) patients with a frequency up to 98% in some autopsy series (Jellinger, 2002).

The mechanisms of vessel rupture due to β-amyloid deposition have not been elucidated, but it might be due to the mechanical stress inflicted on the vessel walls that are already damaged by β-amyloid. Moreover, proteolytic sys-

* Corresponding author at: Neurovascular Research Laboratory, Institut de Recerca, Hospital Vall d'Hebron, Pg Vall d'Hebron 119-129, 08035 Barcelona, Spain. Tel.: +34934894029; Fax.: +34934894102.

E-mail address: 31862jmv@comb.cat (J. Montaner).

0197-4580/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2010.01.019

tems might be implicated in this phenomenon. Indeed, the β-amyloid protein is capable of stimulating synthesis of the metalloprotease 9 (MMP-9) in mice endothelial cells (Lee et al., 2003). This protein is able to degrade components of the extracellular matrix (ECM) such as those of the basement membrane of the vessels (Montaner et al., 2003). Another metalloprotease, the angiotensin-converting enzyme (ACE), shows increased activity directly correlated with parenchymal β-amyloid load and accumulated perivascularly in vascular ECM in severe CAA (Miners et al., 2008). Additionally, exposing neurons to β-amyloid seems to increase ACE level and activity, suggesting an upregulation of ACE by β-amyloid (Miners et al., 2009).

Although CAA can be inherited as an autosomal dominant condition, the most common form is sporadic, and the possible genetic determinants of CAA are not yet clearly identified (Woo et al., 2005b; Yamada, 2004). The apolipoprotein E (*APOE*) gene is the only well recognized risk factor for CAA and it was shown that carriers of the ε2 or ε4 alleles of the *APOE* are at increased risk of recurrent ICH (Greenberg et al., 1996; Greenberg et al., 1998; Tzourio et al., 2008; Woo et al., 2005a). Other genetic variants related with the presence of β-amyloid have been described, including the presenilin 1 (Sanchez-Valle et al., 2007), alpha 1-antichymotrypsin (Yamada et al., 1998), alpha2-macroglobulin (*A2M*), butyrylcholinesterase and paraoxonase genes (Yamada, 2002), as well as other genes associated with β-amyloid deposition like the LDL receptor related protein (*LRP*) (Christoforidis et al., 2005) and tumor growth factor β1 (*TGFβ1*) (Hamaguchi et al., 2005) or with β-amyloid degradation such as MMPs (Backstrom et al., 1996; Roher et al., 1994), neprilysin (Clarimon et al., 2003b) and *ACE* (Clarimon et al., 2003a).

Nowadays, no tools are available to diagnose high risk of ICH recurrence with accuracy and the identification of genetic markers for this purpose could have a great impact on patients' prognosis. Therefore, the authors aimed to investigate genetic variants that could affect ICH recurrence in a sporadic CAA cohort, and to analyze the significant results at a functional level.

2. Methods

2.1. Study patients

Consecutive patients aged ≥ 55 years who presented to the Vall d'Hebron Hospital over a 10-year period and were diagnosed as primary lobar ICH were recruited. For this purpose, all patients underwent a set of diagnostic tests, including routine blood biochemistry, blood cell count, coagulation tests, computed tomography (CT) scan and brain magnetic resonance imaging (MRI) with either CT-angiography or MR-angiography when appropriate, as well as conventional angiography in selected patients. From the 407 initially evaluated patients, the authors excluded those with a lobar ICH related to vascular malformation, impaired coagulation or oral anticoagulant intake, traumatic brain injury, tumoral bleedings and the patients who underwent a surgical procedure. The authors also excluded patients who died within the first month after the ICH (n = 159 or 39%), because in those cases, death was related to the index event. Those patients were older than patients who survived (78 ± 8 years, v second, 73 ± 12 years, $p = 0.006$) and a higher number was under anticoagulation treatment (29.5% v. 15.1%, $p = 0.06$). The authors did not detect any very early recurrence, although they may have missed recurrence in dying patients who did not have a second CT scan because neurological deterioration from the first ICH was considered the cause of death. Patients with a baseline MRI study showing multiple lobar and deep microbleeds, suggesting the existence of a mixed pathology (CAA and hypertension) were excluded as well. Baseline clinical and demographic characteristics were collected and patients were classified as having a *probable CAA* or *possible CAA*, according to the Boston criteria at baseline (Clarimon et al., 2003a; Knudsen et al., 2001). Briefly, the presence of multiple and exclusively lobar, cortical, or corticosubcortical hemorrhages, detected by gradient-echo MRI sequences, was defined as *probable CAA*-related ICH, whereas the diagnosis of *possible CAA* was defined by a single lobar hemorrhagic lesion, without any other finding explaining the ICH. Patients were followed-up every 6 mo at the outpatient clinic, as part of their clinical evaluation. Follow-up duration was variable, ranging from 20 to 36 mo. At each visit, patients and relatives were asked about the occurrence of a new symptomatic hemorrhagic event and available clinical records regarding these events were reviewed to extract the exact date and location of the ICH. Time of death was also collected. Control participants were healthy volunteers, older than 65 years, classified free of neurovascular and cardiovascular history, and familiar history of stroke, by direct interview before recruitment. Details on socioeconomic and demographic characteristics were obtained from all subjects by questionnaires, together with information on smoking, dyslipidemia, hypertension, diabetes mellitus and current medication use. Informed written consent was obtained from all subjects, and the local Ethics Committee approved the study. All subjects were of Caucasian ancestry.

2.2. MRI protocol

A baseline MRI was performed in all patients during the first week. All MRI examinations were obtained using a T1.5 whole body scanner. Images obtained included axial T2-weighted turbo spin-echo [(3700/90/2) (TR/TE/excitations)], axial T1-weighted spin-echo [(550/14/2)], turbo fluid-attenuated inversion recovery [(9000/110/2)] and axial T2-weighted susceptibility-based echo-planar gradient-echo sequence [(0.8/29/1)]. All images were evaluated by a neurologist and a neuroradiologist blinded to the clinical information to detect hemorrhages (microhemorrhages and