ELSEVIER

Neurobiology of Aging 32 (2011) 2113–2122

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Alzheimer disease pathology in cognitively healthy elderly: A genome-wide study

Patricia L. Kramer[a,b,*], Haiyan Xu[e], Randall L. Woltjer[c], Shawn K. Westaway[a,q], David Clark[c], Deniz Erten-Lyons[a], Jeffrey A. Kaye[a,d], Kathleen A. Welsh-Bohmer[f], Juan C. Troncoso[g], William R. Markesbery[h], Ronald C. Petersen[i], R. Scott Turner[j,k], Walter A. Kukull[l], David A. Bennett[m], Douglas Galasko[n], John C. Morris[o], Jurg Ott[p]

[a] *Department of Neurology, Oregon Health & Science University, Portland, OR 97239, USA*
[b] *Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR 97239, USA*
[c] *Department of Pathology, Oregon Health & Science University, Portland, OR 97239, USA*
[d] *Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR 97239, USA*
[e] *Laboratory of Statistical Genetics, Rockefeller University, New York, NY 10065, USA*
[f] *Department of Psychiatry and Medicine (Neurology), Duke University Medical Center, Durham, NC 27710, USA*
[g] *Department of Pathology and Neurology, Johns Hopkins University, Baltimore, MD 21205, USA*
[h] *Department of Pathology and Neurology, University of Kentucky, Lexington, KY 40536, USA*
[i] *Department of Neurology, Mayo Clinic, Rochester, MN 55905, USA*
[j] *Department of Neurology, University of Michigan, Ann Arbor, MI 48105, USA*
[k] *Georgetown University Medical Center, Washington DC, 20057, USA*
[l] *National Alzheimer Coordinating Center and Department of Epidemiology, Seattle, WA 98105, USA*
[m] *Rush University Medical Center, Chicago, IL 60612, USA*
[n] *Department of Neurosciences, University of California at San Diego, San Diego, CA 92037, USA*
[o] *Department of Neurology, Washington University School of Medicine, St. Louis, MO 63108, USA*
[p] *Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100029, China*
[q] *Department of Cardiology, Oregon Health & Science University, Portland, OR 97239, USA*

Received 23 November 2009; received in revised form 11 January 2010; accepted 14 January 2010
Available online 7 May 2010

Abstract

Many elderly individuals remain dementia-free throughout their life. However, some of these individuals exhibit Alzheimer disease neuropathology on autopsy, evidenced by neurofibrillary tangles (NFTs) in AD-specific brain regions. We conducted a genome-wide association study to identify genetic mechanisms that distinguish non-demented elderly with a heavy NFT burden from those with a low NFT burden. The study included 299 non-demented subjects with autopsy (185 subjects with low and 114 with high NFT levels). Both a genotype test, using logistic regression, and an allele test provided consistent evidence that variants in the *RELN* gene are associated with neuropathology in the context of cognitive health. Immunohistochemical data for reelin expression in AD-related brain regions added support for these findings. Reelin signaling pathways modulate phosphorylation of tau, the major component of NFTs, either directly or through β-amyloid pathways that influence tau phosphorylation. Our findings suggest that up-regulation of reelin may be a compensatory response to tau-related or beta-amyloid stress associated with AD even prior to the onset of dementia.

Keywords: Genome-wide association study; Alzheimer disease; Non-demented elderly with AD neuropathology; Non-demented elderly without AD neuropathology; Reelinneurofibrillary tangles

* Corresponding author at: Department of Neurology, CR131, Oregon Health & Science University, Portland, OR 97239, USA. Tel.: +1 503 494 7196; fax: +1 503 494 7499.
E-mail address: kramer@ohsu.edu (P.L. Kramer).

0197-4580/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2010.01.010

1. Introduction

Prevalence rates for Alzheimer disease (AD) climb steadily from ∼1% in 65-year-olds to as high as 40% by the age of 85 (Hebert et al., 2003). Despite these dire figures, some elderly individuals remain dementia-free throughout their life and, of these, a proportion exhibit substantial AD neuropathology, in the form of amyloid plaques and neurofibrillary tangles (NFTs), at autopsy (Erten-Lyons et al., 2009; Bennett et al., 2005). Thus, individuals differ in their capacity to maintain normative cognitive function even within the context of neuropathological structural changes associated with AD.

There are a number of ways in which genetic mechanisms might promote cognitive health. On one hand, genetic variants may prevent development of the neuropathological substrate that underlies susceptibility to cognitive dysfunction. Alternatively, in the presence of neuropathology, genetic variants may mitigate the effects of AD-related lesions that otherwise result in cognitive decline. The first case provides greater guarantee of cognitive health. Thus, it is important to identify genetic variants associated with development of AD neuropathology within the context of cognitive resilience.

We conducted a genome-wide SNP association study (GWAS) to identify genetic mechanisms involved in healthy brain aging. This study was based on a sample of non-demented, deceased subjects with autopsy who were members of longitudinal healthy aging cohorts at 10 NIA-funded Alzheimer Disease Centers (ADC). These subjects comprised one group with little or no evidence of NFT formation, and one with substantial or severe levels of NFT formation in critical brain regions. We report results that implicate variants in the glycoprotein reelin (*RELN*) as key elements in the molecular basis of AD-related neuropathological processes. We provide additional support for these statistical findings with immunohistochemical data for reelin expression in AD-related brain regions in a subset of our study subjects. To our knowledge, this is the first GWAS that specifically addresses genetic mechanisms of AD neuropathology in non-demented elderly with post-mortem examinations.

2. Methods

2.1. Subjects

Subjects were recruited from aging research cohorts collected over the last two decades at 10 NIA-funded ADCs across the country. Eligibility criteria included the following: (1) ≥65 years old at enrollment; (2) deceased, with autopsy; (3) clinical diagnosis of "no dementia" at enrollment and death; (4) ≥1 clinical evaluation within the year before death; (5) DNA available; (6) Caucasian ancestry. A total of 412 subjects met initial criteria. All subjects had been previously genotyped for apolipoproteinE (*APOE*) status and these data were provided by the respective ADC Data Cores. The study was approved by the IRB at OHSU.

2.2. Neuropathologic diagnosis

One of the hallmarks of AD is the occurrence of NFTs in limbic and neocortical regions of the brain. The Braak score (Braak and Braak, 1991) is a measure of the location and frequency of NFTs and is a key to establishing the pathologic diagnosis of AD (NIA, 1997). Braak scores (BS) range from 0 (no NFTs) to 6 (NFTs in primary motor and/or sensory neocortex), and were obtained for all subjects from the ADC Clinical Data Cores.

We classified subjects on the basis of NFT burden to obtain subsets of individuals for analysis. Autopsy reports were reviewed for consistency with the Braak score provided in the data files from each ADC by a three-member team including a neuropathologist (RW), a neurologist (JK) and a geneticist (PK). Subjects were classified into three groups: LO Braak (≤2), MED Braak and HI Braak (≥4):

(1) If the Braak score and information in the autopsy report were consistent with low NFT levels, the subject was classified as LO Braak ($n = 182$).
(2) If the Braak score and information in the autopsy were consistent with high NFT levels, the subject was classified as HI Braak ($n = 105$).
(3) If the Braak score and autopsy information were at odds with respect to classification of LO or HI NFT burden, preference was given to the autopsy ($n = 12$).
(4) If the Braak score and autopsy information agreed with a Braak score of 3, the subjects was classified as MED Braak ($n = 73$); furthermore, if the Braak score was 2, 3 or 4, but the autopsy report lacked sufficient detail to confirm these scores, the subject was also classified as MED Braak ($n = 17$). In order to maximize phenotypic homogeneity between groups, and thus increase power, we excluded this group from the initial analysis.

The final sample consisted of 299 subjects (185 LO Braak, 114 HI Braak).

2.3. Clinical diagnosis

All study subjects were deceased and had been evaluated for cognitive decline and dementia within 12 months prior to death. Assessments for determining absence of dementia were consistent with standardized protocols in the DSM-III-R.

To minimize the extent of any cognitive impairment in the two groups, we obtained longitudinal clinical data for all subjects, consisting of Clinical Dementia Rating (CDR) scores (Morris, 1993) and/or Mini-Mental State Exam (MMSE) scores (Folstein et al., 1975), from the time of enrollment until death. The CDR is a dementia staging-tool in which a score of 0 represents no cognitive impairment, .5 may represent some cognitive impairment, and a score ≥1 represents dementia; MMSE scores ≥26 are generally indicative of no dementia.