Supplementary Online Content

Yu L, Petyuk VA, Tasaki S, et al. Association of cortical β-amyloid protein in the absence of insoluble deposits with Alzheimer disease. *JAMA Neurol*. Published online April 22, 2019. doi:10.1001/jamaneurol.2019.0834

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods.

Clinical diagnosis

Cognitive impairment was determined by a neuropsychologist after reviewing results of cognitive assessments. Dementia diagnosis was provided by a clinician after reviewing both clinical records and cognitive ratings by the neuropsychologist and, in most cases, evaluating the participant. Clinical diagnosis follows the recommendations of the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association[1]. Alzheimer's dementia requires impairment in memory and at least one other cognitive domain. Mild cognitive impairment (MCI) refers to cognitive impairment that did not meet the criteria for dementia[2]. After a participant died, a summary diagnosis opinion was provided by a neurologist after reviewing all available clinical information, without review of neuropathologic data[3].

Sample preparation for selected reaction monitoring proteomics

Briefly, ~20 mg of tissue from each brain was homogenized, and 400 ug protein aliquots were processed for alkylation with iodoacetamide and digestion with trypsin. Tryptic peptide digests were cleaned using solid phase extraction after readjusting concentration to 1 ug/uL. 30 uL aliquots were mixed with 30 uL synthetic peptide mix containing β-amyloid peptide LVFFAEDVGSNK (New England Peptide, Gardner, MA) with C-terminal lysine containing six ^{13}C and two ^{15}N isotopes. This tryptic peptide matches the middle of the Aβ sequence (eFigure 3) and is shared across all Aβ species. The transitions for this peptide include doubly charge parent ion and y8+, y9+ and y10+ fragments.

Neuropathology Measures

β-amyloid load and PHFtau tangle density were quantified in 8 regions (entorhinal, hippocampus, midfrontal, inferior temporal, angular gyrus, calcarine, anterior cingulate and superior frontal) using immunohistochemistry and image analysis. Specifically, β-amyloid was labeled using 1 of 3 monoclonal antibodies: 4G8 (Covance Labs, Madison, WI; 1:9000 dilution), 6F/3D (Dako North America Inc., Carpinteria, CA; 1:50 dilution), and 10D5 (Elan Pharmaceuticals, San Francisco, CA; 1:600 dilution). Following an automated multistage image analysis, percent area positive for β-amyloid was computed and averaged within and across regions to obtain a summary measure of β-amyloid load[4]. Individuals with positive β-amyloid load were not included in the primary analysis. PHFtau was labeled with an antibody specific for phosphorylated tau (AT8; Thermo Fisher Scientific, Rockford, IL, USA; 1:2000 dilution). Quantification was accomplished via the stereological mapping station. PHFtau tangles per mm^2 were computed and averaged within and across regions to obtain a summary measure of tangle density.

Meningeal and parenchymal vessels in 4 regions (midfrontal, midtemporal, angular and calcarine) were examined for amyloid angiopathy using abovementioned β-amyloid immunostaining. β-amyloid deposition in each region was rated, and the scores were averaged across the regions and then classified into a 4 level semiquantitative scale of none, mild, moderate and severe[5]. Cortical Lewy bodies were identified using monoclonal α-synuclein antibodys (LB509; Zymed Labs, Invitrogen Corp., Carlsbad, CA; 1:150 or 1:100 dilution or pSyn#64; Wako Chemicals Inc., Richmond, VA; 1:20,000 dilution), and rated as present if positive in midfrontal, midtemporal, or inferior parietal cortex[6]. TDP-43 was assessed using a monoclonal TDP-43 antibody (TAR5P-1D3; Ascenion, Munich, Germany; 1:100 dilution) and rated on a 4-level scale to capture the staging of TDP-43 progression[7]. Severe neuronal loss and gliosis of CA1 and/or subiculum were assessed using hematoxylin & eosin (H&E) staining for hippocampal sclerosis[8]. The presence of chronic macroscopic infarcts was recorded during gross examination of both hemispheres and confirmed histologically[9]. The presence of chronic microinfarcts was assessed in minimal 9 regions using H&E stained sections[10]. Vessels in the Circle of Willis were gross examined for artery occlusion (atherosclerosis), and arterioles of the anterior basal ganglia were examined for luminal narrowing using H&E stain (arteriolosclerosis)[11]. Both atherosclerosis and arteriolosclerosis were rated on a 4-level scale, ranging from none, mild, moderate to severe.

***APOE* genotype and polygenic Alzheimer's risk score**

APOE genotype was determined by probing rs429358 and rs7412, the two polymorphisms at exon 4 of the *APOE* gene. Genotyping was performed using DNA from peripheral blood mononuclear cells or brain tissue[12]. Individuals with at least 1 ε4 allele (i.e. ε2ε4, ε3ε4 and ε4ε4) were classified as ε4 carriers.

A polygenic risk score for Alzheimer's dementia was computed based on the GWAS meta-analysis results by the International Genomics of Alzheimer's Project (IGAP). Briefly, 6,411 SNPs with *p* values less than 0.001 were identified and SNPs in linkage disequilibrium were pruned. Since we specifically interrogated *APOE* genotype, SNPs in *APOE*/*TOMM40* region were excluded. This results in a total of 455 independent SNPs. The number of risk allele of each SNP was weighted by the corresponding coefficient reported in the IGAP GWAS meta-analysis, and then averaged across all the SNPs. The computation was done using PRSice-2 software[13].

Demographics

Age was based on self-report date of birth and date of death. Sex, race and ethnicity, and years of education were reported by participants at the baseline interview. Race and ethnicity were included to describe the study participants.

eReferences.

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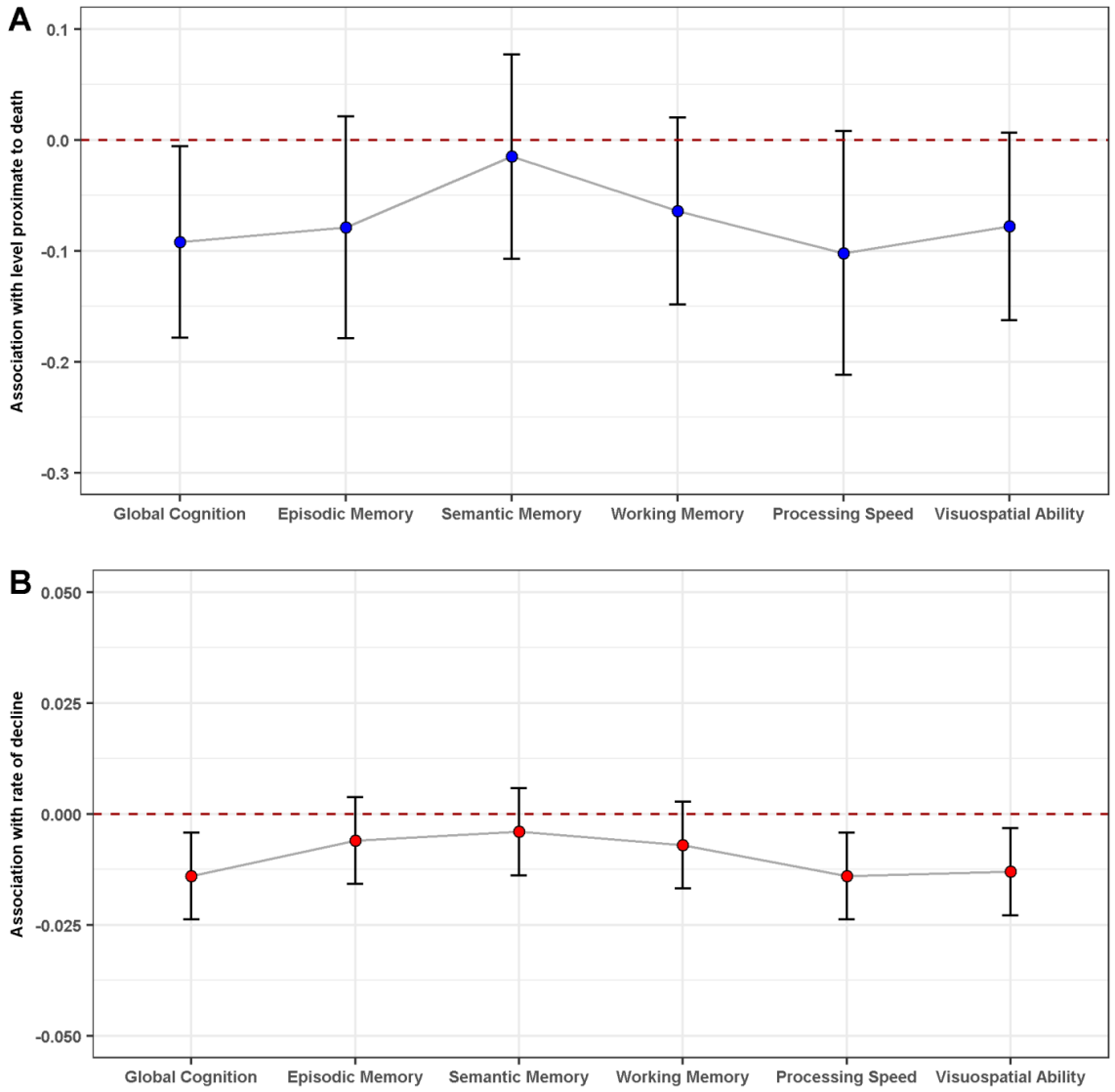
eFigure 1. Pathologic burden of dementia without β-amyloid deposits

37 of 148 individuals without β-amyloid deposits were diagnosed with dementia at death. The Venn diagram illustrates the concomitant occurrence of vascular disease (infarcts, moderate to severe amyloid angiopathy, atherosclerosis or arteriolosclerosis), Cortical Lewy bodies and TDP/HS (TDP inclusion in limbic or neocortex or hippocampal sclerosis).

eFigure 2. –β-amyloid protein associations with cognition



The figure illustrates the associations of β-amyloid protein with both the level of cognition proximate to death (Panel A) and the annual rate of cognitive decline (Panel B). The associations with different cognitive outcomes (x-axis) were examined in separate linear mixed models, adjusted for age at death, sex and years of education. For each model, the point estimate +/- 1.96 standard error of the effect size is shown on the y-axis.

eFigure 3. β-amyloid protein sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

There are two fully tryptic peptides within β-amyloid sequence. The red one is much more detectable than the blue one, thus it is the preferred peptide to quantify total β-amyloid species.