Effect of Complement *CR1* on Brain Amyloid Burden During Aging and Its Modification by *APOE* Genotype

Madhav Thambisetty, Yang An, Michael Nalls, Jitka Sojkova, Shanker Swaminathan, Yun Zhou, Andrew B. Singleton, Dean F. Wong, Luigi Ferrucci, Andrew J. Saykin, and Susan M. Resnick for the Baltimore Longitudinal Study of Aging and the Alzheimer's Disease Neuroimaging Initiative

Background: The rs3818361 single nucleotide polymorphism in complement component (3b/4b) receptor-1 (*CR1*) is associated with increased risk of Alzheimer's disease (AD). Although this novel variant is associated with a small effect size and is unlikely to be useful as a predictor of AD risk, it might provide insights into AD pathogenesis. We examined the association between rs3818361 and brain amyloid deposition in nondemented older individuals.

Methods: We used ^{11}C-Pittsburgh Compound-B positron emission tomography to quantify brain amyloid burden in 57 nondemented older individuals (mean age 78.5 years) in the neuroimaging substudy of the Baltimore Longitudinal Study of Aging. In a replication study, we analyzed ^{11}C-Pittsburgh Compound-B positron emission tomography data from 22 cognitively normal older individuals (mean age 77.1 years) in the Alzheimer's Disease Neuroimaging Initiative dataset.

Results: Risk allele carriers of rs3818361 have lower brain amyloid burden relative to noncarriers. There is a strikingly greater variability in brain amyloid deposition in the noncarrier group relative to risk carriers, an effect explained partly by *APOE* genotype. In noncarriers of the *CR1* risk allele, *APOE* ε4 individuals showed significantly higher brain amyloid burden relative to *APOE* ε4 noncarriers. We also independently replicate our observation of lower brain amyloid burden in risk allele carriers of rs3818361 in the Alzheimer's Disease Neuroimaging Initiative sample.

Conclusions: Our findings suggest complex mechanisms underlying the interaction of *CR1*, *APOE*, and brain amyloid pathways in AD. Our results are relevant to treatments targeting brain Aβ in nondemented individuals at risk for AD and suggest that clinical outcomes of such treatments might be influenced by complex gene-gene interactions.

Key Words: Alzheimer's disease, amyloid, *APOE*, *CR1*, single nucleotide polymorphism, ^{11}C-PiB PET

Recent large-scale genome-wide association studies (GWAS) have identified novel risk variants for sporadic Alzheimer's disease (AD) (1,2). These findings have since been independently replicated (3,4). Although the identification of novel genetic risk factors for AD is a significant advance, these variants occur commonly in the general population and are associated with small effect sizes. Moreover, they are believed to be merely proxies for true AD risk variants. Their clinical utility as standalone predictors of disease risk is therefore likely to be limited (5). They might, however, be invaluable in the delineation of pathways intrinsic to disease mechanisms or their modifiers in at-risk older individuals. Single nucleotide polymorphisms (SNPs) in the complement component (3b/4b) receptor-1 (*CR1*) were reported to be associated with greater risk of AD (2–4). More recently, the rs6656401^{A} risk allele of *CR1* was also related to greater cognitive decline over time as well as with the extent of neuritic plaque burden at autopsy in older individuals who were nondemented at baseline (6). Together with a large body of evidence supporting a role for the complement system in modulating AD pathogenesis (7), these findings suggest that the AD risk variant of *CR1* might influence pathways related to brain Aβ clearance and/or deposition.

The aim of the present study was to investigate the association between the AD risk variant rs3818361 SNP in *CR1* and brain amyloid burden in nondemented older individuals within the neuroimaging substudy of the Baltimore Longitudinal Study of Aging (BLSA-NI) (8). In light of the findings by Lambert *et al.* (2) in their original GWAS study demonstrating an interaction between this SNP and *APOE* genotype in influencing risk for AD, it was also of interest to examine the effect of *APOE* genotype in modifying associations between *CR1* and brain amyloid during aging.

Methods and Materials

The Baltimore Longitudinal Study of Aging is one of the largest and longest-running longitudinal studies of aging in the United States (8). The community-dwelling unpaid volunteer participants are predominantly white, of upper-middle socioeconomic status, and with an above-average educational level. In general, at the time of entry into the study, participants have no physical and cognitive impairment (i.e., Mini-Mental State

From the Laboratory of Behavioral Neuroscience (MT, YA, JS, SMR); Longitudinal Studies Section (LF), Clinical Research Branch, National Institute on Aging, National Institutes of Health; Department of Radiology (JS, DFW, YZ), Johns Hopkins University School of Medicine, Baltimore; Laboratory of Neurogenetics (MN, ABS), National Institute on Aging, National Institutes of Health, Bethesda, Maryland; and the Department of Radiology and Imaging Sciences (SS, AJS), Center for Neuroimaging, Indiana University School of Medicine, Indianapolis.

Address correspondence to Madhav Thambisetty, M.D., Ph.D., NIA, National Institutes of Health, Room 4B-317, BRC Building, 251 Bayview Boulevard, Baltimore, MD 21224; E-mail: thambisettym@mail.nih.gov.

Received Mar 24, 2012; revised Jul 21, 2012; accepted Aug 5, 2012.

Examination [MMSE] score $\leq$24) and no chronic medical condition with the exception of well-controlled hypertension.

The BLSA-NI began in 1994. The BLSA participants were initially prioritized for admission to the neuroimaging study on the basis of health considerations and the amount of prior cognitive data available for each individual (8). At enrollment, participants were free of central nervous system disease (e.g., epilepsy, stroke, bipolar illness, dementia), severe cardiac disease (e.g., myocardial infarction, coronary artery disease requiring angioplasty or coronary artery bypass surgery), pulmonary disease, or metastatic cancer.

Participants in the current report were 57 (mean age 78.5 $\pm$ 6.3 years) nondemented individuals in the BLSA-NI, who underwent ^{11}C-Pittsburgh Compound-B (^{11}PiB) positron emission tomography (PET) amyloid imaging scans and genome-wide genotyping. They were ascertained from the initial 61 BLSA-NI participants consecutively assessed with ^{11}C-PiB from June 2005 to March 2007 and were representative of the entire BLSA-NI with respect to baseline age, sex, race, and education. We excluded individuals with clinical strokes, brain trauma, and those meeting consensus criteria for AD (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) and mild cognitive impairment as determined by consensus case conference (9,10). This study was approved by the local institutional review board. All participants provided written informed consent before each assessment. Previous studies using ^{11}C-PiB PET data from these BLSA-NI participants have reported on the association of in vivo brain amyloid deposition with cognitive decline during aging (11), brain atrophy (12), and resting state regional cerebral blood flow (13).

The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a multi-center longitudinal study initiated in 2003 by the National Institute on Aging (http://www.adni-info.org; Principal Investigator Michael M. Weiner) (Supplement 1). The principal goal of ADNI is to test whether neuroimaging and other biomarkers, together with clinical assessments can better detect and measure the progression of AD. Data used in the current report were derived from 22 cognitively normal ADNI participants (mean age 77.1 $\pm$ 6.2 years) who underwent ^{11}C-PiB PET imaging and genome-wide genotyping.

Genotyping

Genome-wide genotyping procedures in BLSA and ADNI have been described previously (14–16) (Supplement 1).

^{11}C-PiB Studies

Dynamic ^{11}C-PiB PET studies were performed in the BLSA participants as described previously (13). The PET scanning started immediately after an intravenous bolus injection of 540.2 $\pm$ 33.3 MBq (14.6 $\pm$ .9 mCi) of ^{11}C-PiB with a specific activity of 208.68 $\pm$ 111 GBq/μmol (range: 36.26–540.94 GBq/μmol). The PiB-PET data in ADNI were collected as described previously (17) (Supplement 1).

Magnetic Resonance Imaging-Based Region-of-Interest Definition

In the BLSA PiB-PET study, T1-weighted volumetric magnetic resonance imaging scans were co-registered to the mean of the first 20-min dynamic PET images with the mutual information method in the Statistical Parametric Mapping software (SPM 2; Wellcome Department of Imaging Neuroscience, London, United Kingdom). Besides the cerebellum, which was used as a reference region, 15 regions of interest (caudate, putamen, thalamus, lateral temporal, medial temporal, orbitofrontal, prefrontal, occipital, superior frontal, parietal, anterior cingulate, posterior cingulate, pons, midbrain, and white matter) were manually drawn on the co-registered magnetic resonance images (18).

Quantification of Distribution Volume Ratios in the BLSA PiB-PET Study

Reference tissue model is a compartmental modeling approach that uses a reference tissue, such as cerebellum, time activity curve as input for quantification of ligand-receptor dynamic PET without blood sampling. The distribution volume ratio (DVR) of [^{11}C]PiB binding can be estimated directly by reference tissue models with the reference tissue time activity curve as input (19). The DVR parametric images were estimated by simultaneous fitting of a simplified reference tissue model with linear regression with spatial constraints and the cerebellum as reference tissue (19) (Supplement 1).

Methods for the estimation of global amyloid burden in the ADNI dataset have been described previously (17) (Supplement 1).

Neuropsychological Testing

The BLSA participants completed a battery of 12 neuropsychological tests evaluating six cognitive domains concurrent with the ^{11}C-PiB PET scans (Supplement 1). A similar battery of neuropsychological tests was also administered to the ADNI participants who underwent ^{11}C-PiB PET imaging (20).

Statistical Analyses

Our main aim was to investigate inter-group differences in brain amyloid burden between risk (AG/AA) and nonrisk (GG) carriers of the AD variant rs3818361 SNP in *CR1*. All the analyses were conducted in SAS 9.2 (Cary, North Carolina). During initial exploratory analyses plotting values of PiB DVR across different brain regions, we observed a striking difference in the variability of PiB distribution between the two groups (i.e., AA/AG vs. GG) in most brain regions.

We therefore used generalized least square regression models, which allowed us to investigate the differences in variability of PiB distribution and differences in mean levels of brain amyloid burden between risk (AG/AA) and nonrisk (GG) carriers of the AD variant SNP in *CR1* in one unified model. Mean cortical and regional PiB DVRs were used as dependent variables. We used the group variable (coded 0 for GG and 1 for AG/AA) as the main predictor and included age, sex, and race as covariates to adjust for their effects. We first used two separate residual error variance terms (one for each group) and then used likelihood ratio tests to test whether the residual variances were equal between two groups. One residual error variance (pooled) was used for regions that showed statistically nonsignificant differences in variance, and two residual error variances were used for regions that showed statistically significant differences ($p < .05$) in variance. Once the residual variance terms were determined, the differences in mean levels of brain amyloid burden were then estimated. In the light of previous reports including our own that have shown robust effects of age and *APOE* ε4 status on brain amyloid deposition (11,21–23), we conducted targeted analyses examining whether the effects of age and *APOE* ε4 status on PiB DVRs were different between risk (AG/AA) and nonrisk (GG) groups. In this regression model, the predictors included age, *APOE* ε4 status (ε4-positive or ε4-negative), *CR1*