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

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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

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Examination [MMSE] score ≤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 ± 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 ± 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 ± 33.3 MBq (14.6 ± .9 mCi) of ^{11}C-PiB with a specific activity of 208.68 ± 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*

Table 1. Characteristics of Participants from BLSA in ^{11}C-PiB PET Study

	N	Age	Sex	Race	*APOE* ε4 Carriers, *n* (%)
Total	57	78.5 (6.3)	25 F (44%)	48 W (84%)	18 (33%)
GG	40	78.8 (6.7)	16 F (40%)	37 W (93%)	13 (35%)
AG(15)/AA(2)	17	77.8 (5.1)	9 F (53%)	11 W (65%)	5 (29%)
Difference (*p* value)		.59	.37	.0154	.68

Data are presented as mean (SD).

AG/AA, risk carriers; GG, nonrisk; BLSA, Baltimore Longitudinal Study of Aging; F, female; PET, positron emission tomography; W, Caucasian; ^{11}C-PiB, ^{11}C-Pittsburgh Compound-B.

group (AA/AG or GG), interaction between age and *CR1* group, and interaction between *APOE* ε4 status and *CR1* group. Sex and race were included in the model as covariates. Significant interactions indicate whether the effects of age or *APOE* ε4 status on brain amyloid burden differed between *CR1*-risk (AG/AA) and nonrisk (GG) groups. To control for potential type 1 error due to multiple comparisons, we report False Discovery Rate adjusted *p* values (p_{adj}) on the basis of the method described by Benjamini and Hochberg (24).

In our replication study in the ADNI ^{11}C-PiB dataset, our main aim was to confirm our findings of differences in brain amyloid burden between *CR1* risk and nonrisk groups among BLSA participants. Our replication analyses used a measure of global brain amyloid burden that has been previously validated by ADNI investigators both as a quantitative phenotype in genetic analyses as well as to derive cutoff measures to establish PiB positivity/negativity (17,25,26). In restricting the replication study to a single validated measure of global amyloid burden, we avoided making multiple comparisons across several brain regions in the much smaller ADNI dataset. In the replication analysis, the null hypothesis tested was that our original observation of lower brain amyloid in *CR1* risk-carriers was a false positive finding. The *p* value reported for the replication analysis is therefore for a one-sided *t* test comparing mean values of global brain amyloid burden between the *CR1* risk and nonrisk groups.

Results

Sample Characteristics

The two groups (risk carriers, AA/AG; and nonrisk carriers, GG) did not differ significantly in age, sex, number of years of education, or *APOE* ε4 status. Their MMSE scores and domain-specific (memory, language, executive function, visuospatial function, and attention) cognitive performance did not differ significantly. There were a significantly higher number of African American participants in the risk (AG/AA) group (Table 1). Frequencies of alleles in the rs3818361 polymorphism were G/G in 40 subjects (70.2%), A/G in 15 subjects (26.3%), and A/A in 2 subjects (3.5%). Thus 29.8% of our participants carried the risk A-allele. The frequency of the minor allele (A) in our sample was .16, and that of the major allele (A) was .84. There were no significant differences in the age distribution of *APOE* ε4 alleles between the *CR1* risk and nonrisk groups (Table 2).

CR1 and Brain Amyloid Burden

We observed widespread and statistically significant decreases in brain amyloid burden among carriers of the risk allele (AA/AG) of rs3818361 relative to noncarriers (GG). These differences were observed in mean cortical DVR [$t(52) = 3.61$, $p_{adj} = .0016$] orbitofrontal [$t(52) = 2.78$, $p_{adj} = .013$], prefrontal [$t(52) = 3.76$, $p_{adj} = .0011$], superior frontal cortex [$t(52) = 4.07$, $p_{adj} = .0011$], anterior [$t(52) = 3.85$, $p_{adj} = .0011$] and posterior cingulate cortex [$t(52) = 3.05$, $p_{adj} = .0072$], and in the parietal [$t(52) = 2.76$, $p_{adj} = .013$], lateral temporal [$t(52) = 2.70$, $p_{adj} = .014$], as well as occipital cortices [$t(52) = 2.61$, $p_{adj} = .016$] (Fig. 1). Significant differences were also observed in the caudate [$t(52) = 4.43$, $p_{adj} = .0008$], putamen [$t(52) = 3.91$, $p_{adj} = .0011$], and thalamus [$t(52) = 3.80$, $p_{adj} = .0011$]. No significant differences were found in the pons [$t(52) = 1.38$, $p_{adj} = .19$], mid brain [$t(52) = .36$, $p_{adj} = .72$], and white matter [$t(52) = 1.11$, $p_{adj} = .29$], regions associated with nonspecific PiB binding (22) and medial temporal cortex [$t(52) = 2.03$, $p_{adj} = .059$]. We did not observe significant interactions between age and *CR1* group in any of the brain regions examined, indicating similar effects of age on brain amyloid for the risk (AG/AA) and nonrisk (GG) groups.

In addition to differences in mean level of amyloid burden in association with *CR1* genotype, we also observed a statistically significant increase in variability in brain amyloid burden in risk noncarriers (GG) of rs3818361 relative to the risk group (AG/AA). These differences were found in mean cortical DVR [$\chi^2(1) = 19.3$, $p_{adj} < .0001$], orbitofrontal [$\chi^2(1) = 16.4$, $p_{adj} = .0001$], prefrontal [$\chi^2(1) = 21.8$, $p_{adj} < .0001$], and superior frontal cortex [$\chi^2(1) = 23.0$, $p_{adj} < .0001$]; anterior [$\chi^2(1) = 18.3$, $p_{adj} < .0001$] and posterior cingulate cortices [$\chi^2(1) = 19.9$, $p_{adj} < .0001$]; and in the parietal [$\chi^2(1) = 10.3$, $p_{adj} = .0023$], lateral temporal [$\chi^2(1) = 7.1$, $p_{adj} = .012$], as well as occipital cortices [$\chi^2(1) = 5.0$, $p_{adj} = .036$]. Significant increases in variability in PiB DVR in the nonrisk group were also observed in the caudate [$\chi^2(1) = 19.1$, $p_{adj} < .0001$], putamen [$\chi^2(1) = 19.1$, $p_{adj} < .0001$], and thalamus [$\chi^2(1) = 4.5$, $p_{adj} = .045$]. No significant differences in variability of PiB DVR were found in medial temporal cortex [$\chi^2(1) = 1.4$, $p_{adj} = .30$], pons [$\chi^2(1) = .2$, $p_{adj} = .65$], mid brain [$\chi^2(1) = 1.0$, $p_{adj} = .37$], and white matter [$\chi^2(1) = .2$, $p_{adj} = .65$].

To confirm that our observations of statistically significant differences in both mean levels and variability of brain amyloid

Table 2. Age Distribution of *APOE* ε4 Alleles Among *CR1* AG/AA and GG Groups

	APOE ε4		
	Noncarriers, yrs (SD)	Carriers, yrs (SD)	Difference (*p* value)
GG ($n = 40$)	79.9 (7.5)	76.5 (4.2)	.13
AG/AA ($n = 17$)	77.4 (6.1)	78.7 (.8)	.64
Difference (*p* value)	.31	.25	Overall $p = .38$

AG/AA, risk carriers; GG, nonrisk.

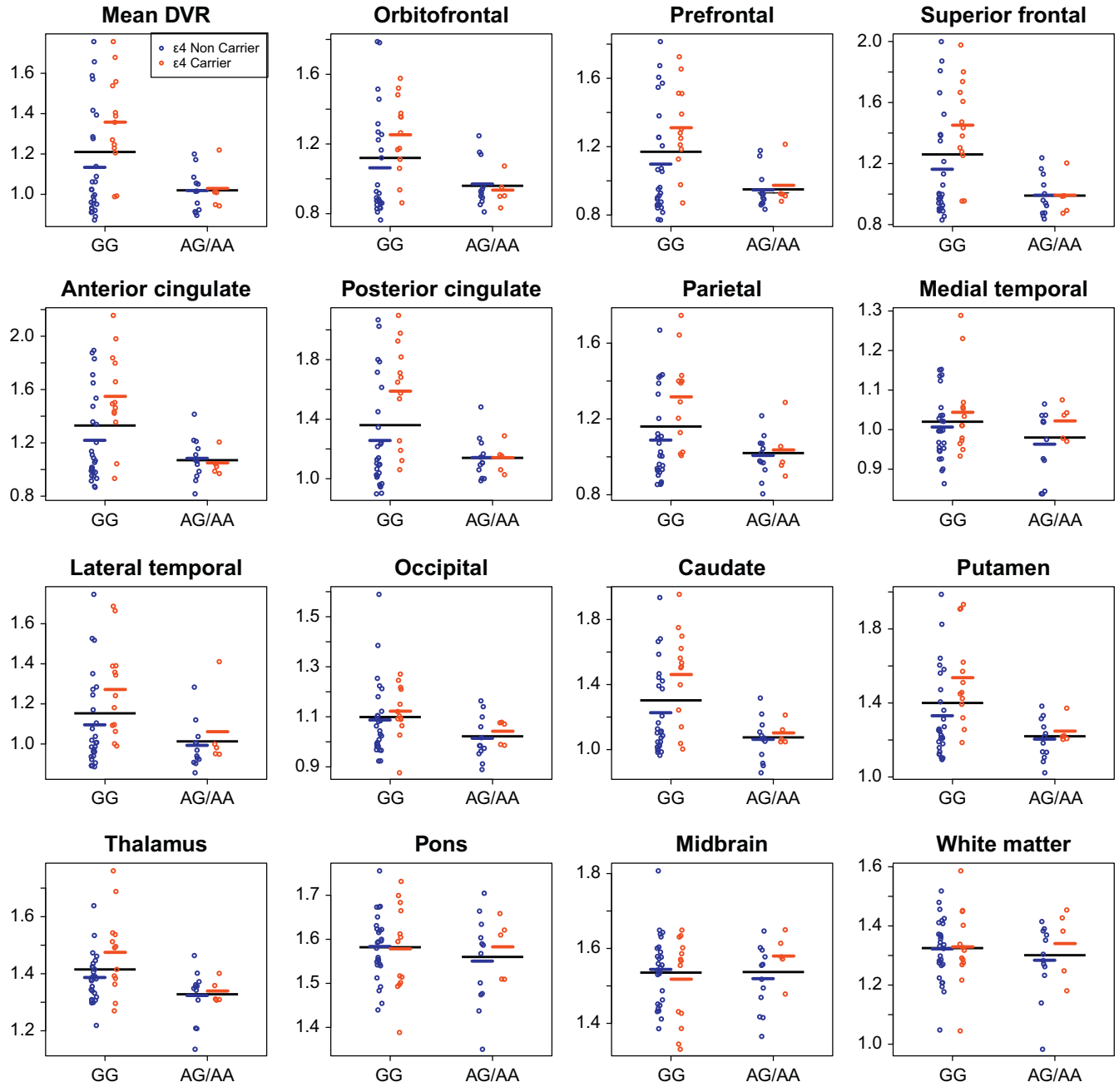

Figure 1. Scatter plots showing the inter-group (AA/AG, risk carriers vs. GG, risk noncarriers) differences in mean cortical and regional Pittsburgh Compound-B distribution volume ratios (DVRs). Individual values are shown in open circles. Red and blue circles denote *APOE* ε4 carriers and noncarriers, respectively. Black lines indicate mean values in risk and nonrisk groups; colored lines indicate mean values for *APOE* ε4 carriers and noncarriers.

burden between the *CR1* risk (AA/AG) and nonrisk (GG) groups were not driven by differences in racial distribution, we repeated the aforementioned analyses after excluding all African-American participants and obtained identical results (Tables S1 and S2 in Supplement 1).

To investigate factors responsible for the increased variability in brain amyloid deposition in noncarriers of the *CR1* risk allele, we investigated whether *APOE* genotype modified the effect of *CR1* on brain amyloid. We found significant interactions between *APOE* genotype and *CR1* group in several brain regions, indicating differential effects of *APOE* genotype on amyloid burden for risk versus nonrisk groups in these regions. Among individuals who did not carry the risk allele of rs3818361 (GG), *APOE* ε4 carriers showed greater brain amyloid burden than *APOE* ε4 noncarriers. In contrast, amyloid burden was similar for *APOE* ε4 carriers and noncarriers in the risk (AG/AA) group. Significant interactions between *CR1* and *APOE* genotype were observed for mean cortical DVR [$t(50) = -2.76$, $p_{adj} = .029$], orbitofrontal [$t(50) = -2.79$, $p_{adj} = .029$], superior frontal [$t(50) = -3.02$, $p_{adj} = .029$], anterior [$t(50) = -3.71$, $p_{adj} = .0080$], and posterior cingulate [$t(50) = -2.71$, $p_{adj} = .029$] cortices. Similar effects were observed in the caudate [$t(50) = -2.57$, $p_{adj} = .035$].

Finally, to replicate our main finding of reduced brain amyloid burden among *CR1* risk allele carriers in an independent sample,

Table 3. Characteristics of Participants from ADNI in ^{11}C-PiB PET Study

	N	Age	Sex	Race	*APOE* ε4 Carriers, *n* (%)
Total	22	77.1 (6.2)	8 F (36.4%)	20 W (90.9%)	6 (27.3%)
GG	18	76.9 (6.3)	5 F (27.8%)	18 W (100%)	5 (27.8%)
AG/AA	4	78.1 (6.3	3 F (75%)	2 W (50%)	1 (25%)
Difference (*p* value)		.75	.12	.026	1.00

Data are presented as mean (SD).
ADNI, Alzheimer's Disease Neuroimaging Initiative; other abbreviations as in Table 1.

we analyzed ^{11}C-PiB PET data available in 22 cognitively normal older individuals in the ADNI sample. The mean age of this sample (77.1 ± 6.2 years) was similar to that of participants in our own ^{11}C-PiB PET study. The *CR1* risk ($n = 4$) and nonrisk groups ($n = 18$) were well-matched in age and sex as well as the number of *APOE* ε4 carriers (Table 3). Similar to our findings, there were no significant differences in MMSE scores and domain-specific (memory, language, executive function, visuospatial function, and attention) cognitive performance between the *CR1* risk and nonrisk groups. Identical to our findings in the BLSA sample, we found that *CR1* risk allele carriers showed a significantly lower global brain amyloid burden than nonrisk allele carriers in the ADNI dataset (1.308 ± .308, and 1.619 ± .328, respectively; $p = .049$).

Discussion

Our main aim in this study was to examine the relationship between the AD risk variant SNP rs3818361 in the *CR1* gene and in vivo brain amyloid burden measured with ^{11}C-PiB PET in nondemented older individuals. In light of recent GWAS studies that showed a greater risk of AD in carriers of the A-allele of this SNP (2,27) as well as an interaction between the *CR1* and *APOE* genes in conferring risk for AD, our primary goal was to examine whether the carriers of the risk allele of *CR1* had significant differences in brain amyloid burden relative to noncarriers and whether the *CR1* × *APOE* interaction might influence brain amyloid deposition in nondemented older individuals. We found widespread and statistically significant decreases in brain amyloid burden in individuals carrying one or two copies of the risk allele (AG/AA) relative to risk noncarriers (GG) in the BLSA sample and also confirmed this finding in an independent sample from the ADNI dataset.

In addition, we observed significantly greater variance in brain amyloid deposition in the nonrisk group (GG), an effect that seems to be influenced in part by *APOE* genotype. Thus, among GG but not AG/AA individuals, *APOE* ε4 carriers exhibited greater amyloid deposition in several brain regions relative to *APOE* ε4 noncarriers (Figure 1).

To the best of our knowledge, this is the first report of an association between genetic variation in the *CR1* gene and brain amyloid deposition quantified by in vivo PET imaging in nondemented older individuals. Our findings run counter to the direction of effect on brain amyloid deposition observed in nondemented carriers of the *APOE* ε4 allele, the most robust genetic risk factor for sporadic AD. Although the *CR1* risk allele was associated with decreased fibrillar amyloid in nondemented individuals in the current study, we and others have demonstrated increased brain amyloid levels in carriers of the *APOE* ε4 allele relative to noncarriers in cognitively normal older individuals (21,22). Our present findings also suggest that the *CR1* risk allele might modify the relationship between *APOE* genotype and brain amyloid deposition. This finding is especially relevant against the background of the index GWAS study by Lambert *et al.* (2), which demonstrated a differential effect of the *CR1* rs3818361 SNP on AD risk between *APOE* ε4 carriers and noncarriers. Our current findings further suggest that the *CR1* × *APOE* interaction also influences an alternative phenotype relevant to early changes in AD pathogenesis by showing that this interaction modulates brain amyloid deposition even in nondemented older individuals.

Our findings merit examination, in light of a recent study by Brouwers *et al.* (28), which showed that four *CR1* SNPs in two haplotype blocks were associated with elevated cerebrospinal fluid $A\beta_{1\text{-}42}$ levels in AD patients—a finding that is similarly counterintuitive in suggesting that *CR1*-associated risk for AD might not be associated with increased brain Aβ accumulation. A recent study, however, did not find an association between other *CR1* SNPs associated with AD risk and cerebrospinal fluid levels of Aβ (29), suggesting that these findings indicate a complex relationship between polymorphic variations in *CR1* and regulation of brain Aβ clearance. Brouwers *et al.* (28) also showed that the common AD risk association with *CR1* might be explained by a low copy number repeat in high linkage disequilibrium with the risk variant that encodes a longer isoform (*CR1*-S) of the *CR1* protein. This longer isoform has an increased number of C3b/C4b cofactor activity sites, which might have a positive effect on Aβ clearance through a C3b-mediated mechanism. However, similar to our present findings, this mechanism suggests that *CR1*-associated risk for AD in older individuals might not be mediated through increased accumulation of Aβ in the brain.

Alternative mechanisms that might mediate the association between *CR1* and brain amyloid levels include its role as an inhibitor of complement activity. However, the net effects of *CR1*-mediated complement modulation on AD pathogenesis are unclear. Such effects might include, for instance, both a deleterious reduction in C3b-mediated clearance of neurotoxic Aβ species from the brain as well as a potentially protective effect through limiting immune-mediated damage of healthy neurons (30).

It is interesting to note that recent studies examining the effect of *APOE* genotype on AD risk associated with genetic variation in *CR1* have been inconsistent. Although a recent meta-analysis showed no evidence for an interaction between *APOE* genotype and *CR1* in mediating risk for AD (31), other reports suggest that the increased risk of AD in carriers of the risk variant of rs3818361 is strongest in *APOE* ε4 carriers (2). Our current results suggest a complex interaction between *CR1* and *APOE* that influences brain amyloid levels in nondemented older individuals.

Our findings showing widespread decreases in brain amyloid burden in nondemented carriers of an AD risk variant gene might also be relevant to recent efforts aimed at lowering Aβ production or enhancing its clearance in asymptomatic individuals at increased genetic risk for AD (32,33). It is worth noting in this context that some previous studies suggest that Aβ deposition in the brain might be a protective adaptive response to neuronal stress and therapeutic strategies against it might exacerbate the disease process (34). Our results showing lower brain amyloid burden in nondemented carriers of the AD risk variant *CR1* suggest that, at least in this group of older individuals, further lowering brain amyloid levels might be of doubtful clinical benefit. Furthermore, by showing a robust interaction between the *CR1* and *APOE* genes, our findings also suggest that clinical outcomes of such therapeutic approaches in presymptomatic individuals might be determined by complex gene-gene interactions.

It must be noted that the participants included in the ^{11}C-PiB PET study described herein are derived from the BLSA-NI and represent a highly educated and healthy sample of nondemented older individuals. The nondemented individuals in our study remained so over intensive follow-up of more than 10 years after their entry into the BLSA-NI. Our findings might thus suggest robust compensatory mechanisms in at-risk participants in this cohort that serve to maintain cognitive health. We must also acknowledge that, although we were able to independently replicate our main finding of lower brain amyloid in *CR1* risk carriers in the ADNI sample, the small number of subjects in the replication analyses did not allow us to test the presence of a *CR1* × *APOE* interaction on brain amyloid in this sample.

Our findings merit consideration in light of a recent study on the effect of the *CR1* rs6656401 SNP on neuritic plaque burden in AD. In their study reporting an association between the rs6656401^{A} SNP in *CR1* and higher neuritic plaque burden in the brain, Chibnik *et al.* (6) studied 553 older individuals who came to autopsy, of whom 220 carried a pathological diagnosis of AD. It is worth noting that the minor allele frequency (MAF) in our current report (.16) is comparable to that in their autopsy sample (.17). However, there are a number of methodological differences between our study and that of Chibnik *et al*. The latter study was based on an autopsy sample of individuals consisting of both pathologically confirmed AD cases as well as non-AD control subjects. It is not clear whether the association of the rs6656401^{A} SNP with neuritic plaque burden in their study remained significant when the analysis was restricted to healthy control subjects and whether there was a statistical interaction between the *CR1* and *APOE* risk alleles. Another methodological distinction between our current report and theirs is our use of in vivo amyloid imaging to quantify brain fibrillar amyloid burden in a variety of brain regions that are not typically examined in postmortem brain tissue with Consortium to Establish a Registry for Alzheimer's Disease criteria (35). It also is notable that the observed MAF in the rs3818361 SNP in our study is comparable to the index GWAS study by Lambert *et al.* (2) in a European population where the MAF for this SNP among more than 8000 control subjects was reported to be .19. Similarly, a recent meta-analysis of studies describing the association of the *CR1* rs3818361 SNP with AD risk included six separate cohorts with a range of the MAF among more than 19,000 control subjects being .17–.23 (36). Nevertheless, replication of our present findings with in vivo amyloid imaging in a larger sample that is more representative of community-dwelling elderly persons and inclusive of individuals with cognitive impairment might be informative.

Conclusions

In summary, our findings suggest a complex effect of the common AD risk variant *CR1* on brain amyloid deposition and its modulation by *APOE* genotype. These findings are relevant to emerging disease-modifying treatments targeting brain Aβ deposition in pre-symptomatic individuals at risk for AD.

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.ucla.edu/wpcontent/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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Supplementary material cited in this article is available online.

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