The Effect of *CR1* on Brain Amyloid Burden during Aging and Its Modification by *APOE* Genotype

Supplemental Information

Subjects and Methods

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research, approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years and 200 people with early AD to be followed for 2 years. For up-to-date information, see www.adni-info.org.

Genotyping Procedures in the Baltimore Longitudinal Study of Aging (BLSA)

The Illumina Infinium HumanHap550 genotyping chip (ver1 and ver3 chips were used; Illumina, Inc., San Diego, CA) product assays 555,000 unique single nucleotide polymorphisms (SNPs) derived primarily from stages I and II of the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/). Experiments were performed as per the manufacturer's instructions using 750 ng of genomic DNA extracted from whole blood. After processing, chips were scanned on Illumina BeadStation scanners. All data were analyzed in BeadStudio (version 3; Illumina). Genotype calls were made using the standard cluster files provided by Illumina. Though many polymorphisms were genotyped, this study focuses on the rs3818361 polymorphism found within the *CR1* gene. Prior to extraction of the rs3818361 genotypes, standard quality control of genotyping data was conducted as previously described (1). In brief, individuals were excluded due to call rate < 95% genome-wide and cryptic relatedness due to proportional sharing (pi_hat) > 0.125 with another participant in the BLSA. Self-reported ethnicity was verified by multidimensional-scaling analysis using HapMap reference populations. SNPs were excluded due to minor allele frequency < 1%, a missingness rate > 5%, Hardy-Weinberg equilibrium *p*-value < 1E-5 to remove poorly clustered SNPs, and non-random missingness by haplotype *p*-value < 1E-5. All quality control of genotype data was undertaken using PLINKv1.05 [PMID: 17701901]. *APOE* genotype analysis was performed on DNA extracted from fresh blood by restriction enzyme isoform genotyping in all participants (2).

Genotyping Procedures in ADNI

The Human610-Quad BeadChip (Illumina, Inc.) was used to analyze samples with all sources of DNA according to the manufacturer's protocol. SNP genotypes were generated from

bead intensity data using Illumina BeadStudio 3.2 software. The *APOE* SNPs (rs429358, rs7412) defining ε2, ε3 and ε4 alleles were not available on the Illumina Human610-Quad BeadChip. These SNPs were genotyped separately and were made available in the ADNI database (3, 4).

^{11}C-PiB PET Studies in ADNI

Approximately 10–15 mCi of [^{11} C]PiB was injected intravenously, followed by a 50-min uptake period (5, 6). PET scans were then acquired from 50 to 70 min in 4–5 min frames. Raw PiB-PET frames were realigned to the first frame and averaged to create a single image. This image was then set to a standard orientation and voxel size, intensity normalized using a cerebellar gray matter region of interest (ROI), and smoothed to a common resolution of 8 mm full-width at half-maximum. The resulting image was subsequently made available for download from the LONI ADNI site (http://adni.loni.ucla.edu/).

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

The mean DVR in 15 ROIs was obtained by applying ROI masks defined on MRI to the DVR parametric images. The mean cortical DVR was calculated by averaging values from orbitofrontal, prefrontal, superior frontal, parietal, lateral temporal, occipital, and anterior and posterior cingulate regions. Parametric images were then spatially normalized using an R_1 template (R_1=K_1/K_1(reference tissue), the target to reference tissue ratio of tracer transport rate constant from vascular space to tissue) (7), and smoothed for the group analysis with a Gaussian filter of 8, 8, and 8 mm in the Talairach x, y, and z planes, respectively.

Estimation of Global Brain Amyloid Burden in the ADNI Dataset

Normalized PiB uptake values from four ROIs (anterior cingulate, frontal cortex, parietal cortex and precuneus) generated by the University of Pittsburgh were obtained for initial PiB-PET scans of all participants from the ADNI database (http://adni.loni.ucla.edu/). The average PiB uptake from these four brain regions has been previously used to define a cut-off value (average PiB uptake = 1.50), to classify participants as either positive for amyloid deposition (average PiB uptake > 1.50) or negative for amyloid deposition (average PiB uptake < 1.50) (6). This measure of global brain amyloid burden has also been validated as a quantitative phenotype in studies of genetic influences on brain amyloid deposition (8).

Neuropsychological Testing in the BLSA

Memory was assessed using the California Verbal Learning Test and Benton Visual Retention Test. Word knowledge and verbal ability were measured using Primary Mental Abilities Vocabulary. Verbal fluency was assessed by Letter (i.e. FAS) and Category fluency tests. Attention and working memory were measured by the Digit Span Test of the Wechsler Adult Intelligence Scale-Revised, and the Trail Making Test. Digits Backward, Trails B, and Verbal Fluency (categories and letters) assessed executive function. The Card Rotations Test assessed visuospatial function.

Supplementary Tables

Results from analyses comparing the estimated variance and mean values of PiB DVRs between *CR1* risk (AG/AA) and non-risk groups (GG) after excluding African American participants.

T**able S1**. Test for equality of variance between *CR1* risk and non-risk groups using log likelihood ratio test in the generalized least square model

	Variance Estimates		**Chi square-test statistics**			**FDR-adjusted *p*-value**
RS3818361_T	**(GG)**	**(AG/AA)**		***df***	***p*-value**	
Mean DVR	0.072	0.011	9.2	1	0.0024	0.0096
Caudate	0.082	0.013	8.6	1	0.0034	0.010
Putamen	0.060	0.011	7.7	1	0.0055	0.011
Thalamus	0.012	0.0071	0.9	1	0.34	0.42
Lateral Temporal Cx	0.058	0.023	2.5	1	0.11	0.18
Medial Temporal Cx	0.0086	0.0069	0.2	1	0.65	0.69
Orbitofrontal Cx	0.077	0.014	8.0	1	0.0047	0.011
Prefrontal Cx	0.089	0.012	10.1	1	0.0015	0.0096
Occipital Cx	0.019	0.0083	2.1	1	0.15	0.22
Superior Frontal Cx	0.12	0.011	12.8	1	0.0003	0.0048
Parietal Cx	0.065	0.021	4.0	1	0.046	0.082
Anterior Cingulate Cx	0.12	0.017	9.4	1	0.0022	0.0096
Posterior Cingulate Cx	0.14	0.024	8.4	1	0.0038	0.010
Pons	0.0063	0.011	1.2	1	0.27	0.36
Midbrain	0.010	0.0083	0.1	1	0.75	0.75
White matter	0.012	0.015	0.2	1	0.65	0.69

Cx, cortex; DVR, distribution volume ratio; FDR, false discovery rate.

Table S2. Age and sex-adjusted means for PiB DVR values in each group (GG; non-risk, AG/AA; risk) and their difference.

	Mean Estimates (SE)		**Diff**	***t*-test**			**FDR-adjusted**
	(GG)	**(AG/AA)**	**GG-A**	**statistics**	***df***	***p*-value**	***p*-value**
Mean DVR	1.19 (0.045)	1.02 (0.031)	0.17 (0.055)	3.03	44	0.0041	0.011
Caudate	1.28 (0.048)	1.06 (0.034)	0.22 (0.058)	3.74	44	0.0005	0.0080
Putamen	1.37 (0.041)	1.21 (0.031)	0.16 (0.051)	3.20	44	0.0026	0.0090
Thalamus	1.41 (0.018)	1.31 (0.031)	0.098 (0.036)	2.74	44	0.0088	0.020
Lateral Temporal Cx	1.14 (0.038)	1.01 (0.068)	0.13 (0.078)	1.74	44	0.089	0.12
Medial Temporal Cx	1.02 (0.015)	0.96 (0.027)	0.064 (0.031)	2.03	44	0.048	0.070
Orbitofrontal Cx	1.11 (0.046)	0.97 (0.035)	0.14 (0.058)	2.32	44	0.025	0.044
Prefrontal Cx	1.15 (0.050)	0.96 (0.033)	0.19 (0.060)	3.16	44	0.0028	0.0090
Occipital Cx	1.09 (0.022)	1.02 (0.039)	0.072 (0.044)	1.61	44	0.11	0.14
Superior Frontal Cx	1.22 (0.057)	1.00 (0.032)	0.22 (0.065)	3.46	44	0.0012	0.0090
Parietal Cx	1.15 (0.043)	1.02 (0.043)	0.13 (0.061)	2.16	44	0.037	0.059
Anterior Cingulate Cx	1.30 (0.058)	1.07 (0.040)	0.23 (0.070)	3.26	44	0.0021	0.0090
Posterior Cingulate Cx	1.35 (0.063)	1.15 (0.047)	0.20 (0.079)	2.55	44	0.014	0.028
Pons	1.58 (0.015)	1.55 (0.025)	0.033 (0.030)	1.12	44	0.27	0.29
Midbrain	1.54 (0.017)	1.54 (0.030)	0.002 (0.034)	0.07	44	0.94	0.94
White matter	1.33 (0.019)	1.28 (0.034)	0.048 (0.039)	1.21	44	0.23	0.26

Cx, cortex; DVR, distribution volume ratios; FDR, false discovery rate.

Supplemental References

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