"Genome-wide interaction study of brain beta-amyloid burden and cognitive impairment in Alzheimer's disease"

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Supplementary Methods, Supplementary Table1, and Supplementary Figure1.

Supplementary Methods:

1. ADNI study:

The Alzheimer's Disease Neuroimaging Initiative (ADNI)[1] is a multi-site study that was launched in 2003 and collects serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological data from healthy elderly, mild cognitive impairment (MCI) and early Alzheimer's disease (AD) patients. Participants were aged 48 to 91 years old at baseline. Details regarding data collection can be found at http://www.adni-info.org/. All participants provided written informed consent, and each site's institutional review board approved study protocols.

ADNI was conducted in three phases (i.e. phases 1, GO, and 2). Given the similarities between the protocols of ADNI phases GO and 2, we merged data from these studies together. Participants originally enrolled in ADNI-GO/2 underwent baseline [^{18}F]Florbetapir-PET imaging, in addition to clinical and neuropsychological assessment, blood and cerebrospinal fluid (CSF) sampling and brain MRI, and were genotyped using the Illumina HumanOmniExpress BeadChip. Baseline data from this sample were included in the genome-wide SNP-by-[^{18}F]Florbetapir-PET Aβ deposition interaction study in relation to cognitive impairment. Cognitive dysfunction was evaluated using the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog), which consists of 11 tasks in cognitive domains mainly consisting of memory, language, and praxis[2], and verbal memory performance was assessed using Rey Auditory Verbal Learning Test (RAVLT)[3]. A subset of participants originally enrolled in ADNI-1 were also followed up

to phases GO/2 and underwent [^{18}F]Florbetapir-PET scan at the time of their enrollment in ADNI-GO/2. These individuals were genotyped using the Illumina Human610-Quad BeadChip, and had been examined by the ADNI neuropsychological battery at multiple time points prior to their PET scan. Given the differences mentioned above, data from these individuals were analyzed separately as a replication sample.

1.1. [^{18}F]Florbetapir-PET preprocessing:

Details of brain Aβ [^{18}F]Florbetapir-PET imaging and preprocessing in ADNI have been described elsewhere[4]. Briefly, structural T1-weighted MRI scans were parcellated into individual cortical and subcortical regions of interest (ROI) using FreeSurfer[5] v4.5. Overall gray matter [^{18}F]Florbetapir standardized uptake value ratios (SUVR) were calculated as the average uptake of four cortical ROIs (frontal, anterior/posterior cingulate, lateral parietal and lateral temporal regions) relative to uptake in the reference region (whole cerebellum white and gray matter).

1.2. Genetic preprocessing and genome-wide interaction study:

Genetic quality control, multidimensional scaling, and imputation were performed for both ADNI-GO/2 and ADNI-1 samples separately. Genetic quality control was conducted using PLINK[6] v1.07 according to a standard protocol as outlined by Anderson et al[7]. SNPs with call rate <95%, departure from Hardy-Weinberg equilibrium with P-value $<1\times10^{-6}$, and minor allele frequency <0.01 were excluded from further analyses. Participants were excluded from the study if they met any of the following criteria: (*i*) discrepancy between stated sex and sexual chromosome markers; (*ii*) genotyping rate <90%; (*iii*) related individuals with pi-hat >0.125 in identity-by-descent analysis; (i*v*)

ethnic outliers as measured by pairwise identity-by-state distances >4 standard deviation; (*v*) low or high heterozygosity rate. To limit possible confounding effects of population heterogeneity, all analyses were confined to the participants with European ancestry (CEU/TSI) as identified by multidimensional scaling (based on the ENIGMA2 protocol http://enigma.ini.usc.edu/wp-content/uploads/2012/07/ENIGMA2_1KGP_cookbook_v3.pdf and using HapMap3 reference data). After aligning the genetic data to the human assembly GRCh37/hg19 using UCSC's liftOver tool[8], haplotypes were prephased using SHAPEIT[9] v2.r790, and imputation was performed using IMPUTE2[10] v2.3.1, with the 1000 Genomes Phase1 integrated haplotypes as the reference panel. All SNPs with an IMPUTE2 info score of less than 0.5 were excluded from further analyses.

To reduce the likelihood of false-positive results in the context of modest sample size, the genome-wide interaction analysis was carried out on imputed ADNI-GO/2 SNPs with minor allele frequency >0.1, using GWASTools[11] implemented in R v3.1.1 (http://www.r-project.org/), while controlling for the effects of age, sex, and years of education in general linear models. Model-robust estimates of standard errors ("sandwich" standard errors) were used to correct for potential inflation of the false positive rate due to model mis-specification in genome-wide gene-environment interaction studies[12, 13].

1.3. CSF $A\beta_{1-42}$ level measurement:

Details of CSF collection were described elsewhere[14]. The $A\beta_{1-42}$ level was measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with

Innogenetics (INNO-BIA AlzBio3, Ghent, Belgium; for research use–only reagents) immunoassay kit–based reagents. The capture monoclonal antibody was 4D7A3 and the detection antibody was 3D6. CSF analyses were performed in 4 batches, and results were rescaled (transformed) based on "2013 BASELINE ADNI results" (http://adni.bitbucket.org/docs/UPENNBIOMK7/ADNI_Methods_Template_Shaw%20Trojanowski%20for%202014%20ADNI%20II%206%209%202014.pdf). As per the ADNI Biomarker Core's Recommendation for cross sectional studies of ADNI-GO and ADNI-2 at baseline, all baseline results from the four batches were combined.

1.4. Structural MRI preprocessing and voxel-based cortical thickness analysis:

All high-resolution 3D T1-weighted images were acquired on 3.0T MR scanners. Preprocessed nonaccelerated T1-w images that had undergone several correction procedures including gradwarp (correction of image geometry distortion) and N3m correction (a customized method for bias field correction)[15] were downloaded from the ADNI database and processed using Advanced Normalization Tools (ANTs)[16] v2.1.0. Briefly, the antsCorticalThickness.sh[17, 18] script was used to estimate cerebral cortical thickness (including hippocampus and amygdala) of each participant, and the resulting cortical thickness maps were nonlinearly registered to a template derived from the ADNI cohort using antsIntroduction.sh script[19]. Registered cortical thickness images were spatially smoothed using an isotropic Gaussian kernel (σ=2mm). Smoothing with surrounding zero signals was compensated by dividing the smoothed cortical thickness maps by the smoothed cerebral cortex mask[20]. Smoothed images with sub-optimal registration were excluded from further analysis.

The resulting individual cortical thickness images were then used for voxel-based analysis. Non-parametric statistical analysis with 10,000 permutations was performed on the images using Randomise[21] in FSL (www.fmrib.ox.ac.uk/fsl/) to evaluate the genotype-by-cortical Aβ deposition interaction effect on regional cortical thickness, while controlling for the effects of age, sex, handedness, and education years. Cluster-based thresholding was performed at $t > 2.3$, and clusters with a familywise error corrected $P < 0.05$ were considered significant.

2. ROS/MAP sample:

The Religious Orders Study[22] (ROS), started in 1994, and Rush Memory and Aging Project[23] (MAP), started in 1997, are longitudinal clinical-pathologic cohort studies of aging and AD. ROS enrolls older Catholic priests, nuns, and brothers from across the USA, while MAP enrolls older residents of retirement communities throughout northeastern Illinois. Both studies have similar design, and participants in both studies are dementia-free at enrollment, and agree to a detailed annual clinical evaluation and brain donation at death. The follow-up rate exceeds 90% in both studies and the autopsy rate exceeds 90% in the ROS sample and 80% in the MAP sample[24]. All participants sign an informed consent and Anatomical Gift Act. Both studies were approved by the institutional review board of Rush University Medical Center.

Global cognition scores were computed as the average of the normalized Z-scores of 17 tasks in 5 cognitive domains (episodic, semantic, and working memory, and perceptual orientation and speed)[25]. Self-declared non-Hispanic Caucasian participants were genotyped using the Affymetrix GeneChip 6.0 platform. Genomic data were imputed[26]

after quality control using MACH[27] v1.0.16a and HapMap release 22 CEU (build 36) as a reference.

Brains of deceased participants were cut into 1-cm-thick coronal slabs and immersion fixed. Multiple tissue blocks from 8 brain regions (hippocampus -CA1/subiculum-, angular gyrus, and entorhinal, superior frontal, dorsolateral prefrontal, inferior temporal, anterior cingulate, and calcarine cortices) were embedded in paraffin and sectioned at 20μm. Aβ was labeled with an N-terminus directed monoclonal antibody (10D5, courtesy Elan Pharmaceuticals; 1:1000) for up to 24 sections for each participant. Aβ deposition was quantified by automated image processing of the tissue sections[28]. In addition, tissue blocks from 5brain regions (hippocampus, and midfrontal, superior/middle temporal, inferior parietal, and entorhinal cortices) were embedded in paraffin, cut into 6μm sections, and stained using the modified Bielschowsky silver staining technique. For each of these 5 regions, neuritic and diffuse plaques were counted in the region with maximum pathological density[29]. Quantitative composite scores were computed separately for overall Aβ burden (mean percent area occupied by Aβ across regions) and neuritic and diffuse plaques (average standardized regional density) for each individual as previously described[28, 29].

Genotype-by-Aβ deposition interaction analyses in relation to global cognitive function were performed on latest available data from healthy controls, and MCI and AD patients with no other cause of cognitive impairment whose last antemortem cognitive assessment was performed within 3 years of the time of death. Participants were aged from 66 to 108 years at their latest visit, and more than one third of them were ≥90 years old (the oldest

old). Analyses were conducted while controlling for the effects of age at last cognitive assessment, sex, years of education, and study (ROS vs. MAP).

3. Statistical analysis:

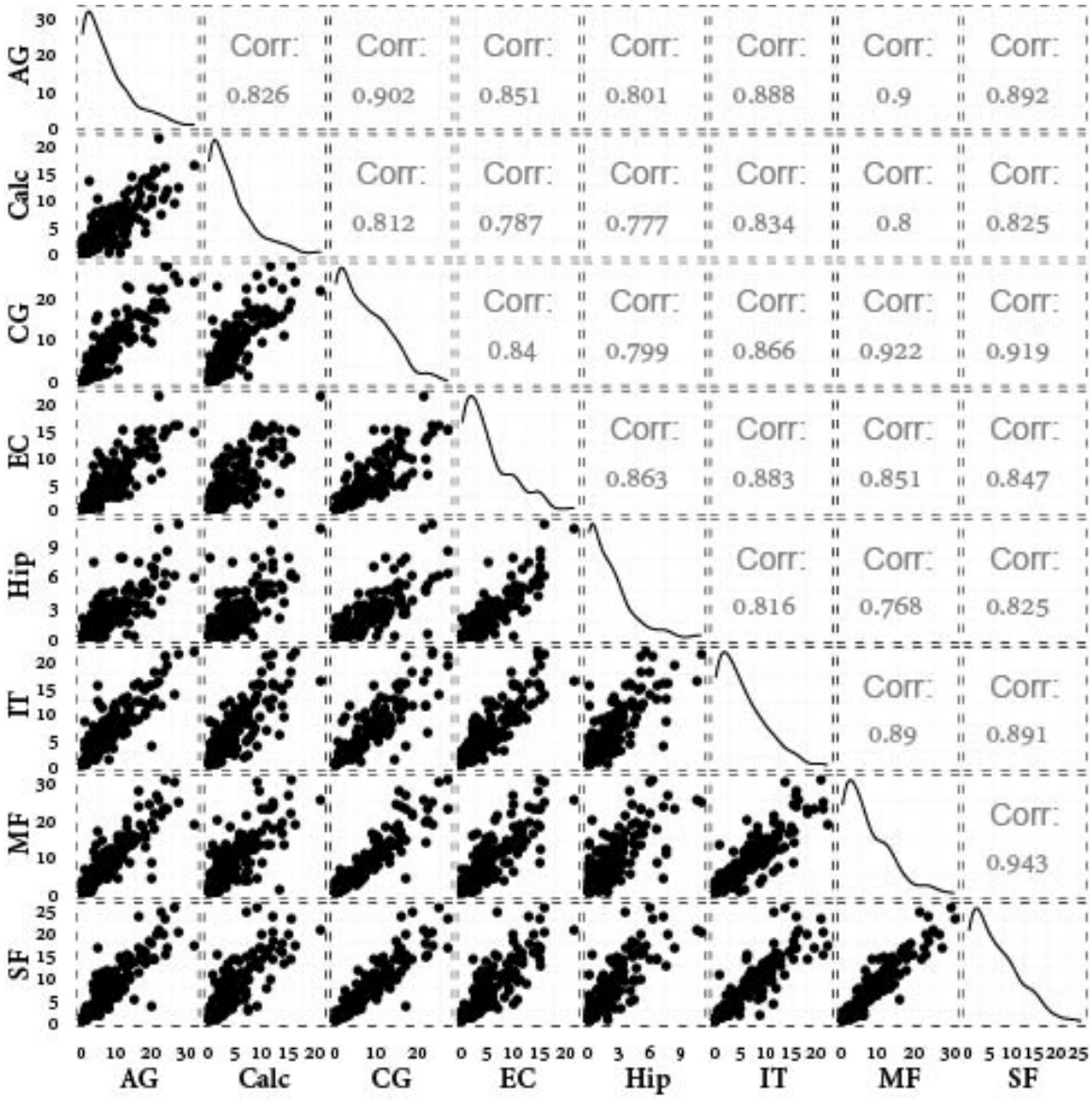
Details for genome-wide and voxel-based statistical analyses are described above. Stouffer's Z-score method was used for meta-analysis of ADNI-GO/2 and ADNI-1 results. Unless otherwise specified, all statistical analyses were conducted using general linear models in the R environment, while controlling for the effects of age, sex, and years of education, and assuming an additive mode of action for genetic variants. Model-robust standard errors were estimated using the 'sandwich' package[30] in R. Wald statistics were performed to determine significance of coefficients. Cumulative link models were used to perform statistical analyses on diagnosis (recoded as an ordinal variable: AD>late MCI>early MCI>healthy controls) using the 'ordinal' package[31] in R. CSF $A\beta_{1-42}$ levels were log-transformed prior to statistical analysis. Longitudinal analyses were performed on available ADNI-GO/2 cognitive and structural MRI longitudinal data using linear mixed-effects models with a random intercept and slope per individual using the 'lme4' package[32], and Satterthwaite's approximations of degrees of freedom were carried out using the 'lmerTest' package[33] in R. Reported p-values are two-tailed, unless otherwise specified. For analyses in the replication sets and secondary analyses, one-tailed p-values are reported given the expectation for effects in the same direction with results from the discovery set (i.e. cross-sectional ADNI-GO/2 data), as per other genome-wide association studies[34-38].

Supplementary Table1. Rs73069071-by-brain Aβ deposition interaction effect in relation to global cognitive function in ROS/MAP participants at different age thresholds (N= number of participants).

Age threshold	$P_{one\text{-}tailed}$	N
81	0.03	114
82	0.001	145
83	0.04	180
84	0.03	211
85	0.03	256
86	0.12	307
87	0.41	355
88	0.48	404
89	0.45	451
90	0.45	499
108 (the whole sample)	0.44	782

The first age group discussed above, which consists of participants 81 years or younger, is the youngest age group with more than 100 participants. The P-values for age thresholds over 90 (not shown above) are all non-significant.

Supplementary Figure1. Correlations among the regional percent areas occupied by Aβ across the 8 brain regions available for analysis from 243 ROS/MAP AD participants. AG = angular gyrus; Calc = occipital (calcarine) cortex; CG = anterior cingulate gyrus; EC = entorhinal cortex; Hip = hippocampus (CA1/subiculum); IT = inferior temporal cortex; MF = dorsolateral prefrontal; SF = superior frontal.



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