**CD33 Alzheimer’s disease locus: altered monocyte function and amyloid biology**

Elizabeth M Bradshaw1–4, Lori B Chibnik1–4, Brendan T Keenan1–2,4, Linda Ottoboni1–4, Towfique Raji1–4, Anna Tang1–4, Laura L. Rosenkrantz1–2,4, Selina Imboywa1–2,4, Michelle Lee1–2, Alina Von Korff1–2, The Alzheimer’s Disease Neuroimaging Initiative3, Martha C Morris6, Denis A Evans7, Keith Johnson3,8,10, Reisa A Sperling3,8,9,10, Julie A Schneider6, David A Bennett6 & Philip L De Jager1–4

In our functional dissection of the CD33 Alzheimer’s disease susceptibility locus, we found that the rs3865444C risk allele was associated with greater cell surface expression of CD33 in the monocytes (t_{50} = 10.06, P_{joint} = 1.3 \times 10^{-13}) of young and older individuals. It was also associated with diminished internalization of amyloid-β 42 peptide, accumulation of neuritic amyloid pathology and fibrillar amyloid on in vivo imaging, and increased numbers of activated human microglia.

Recent genome-wide studies have identified nine Alzheimer’s disease susceptibility loci, including CD33 and MS4A4/MS4A6E, which implicate the immune system in Alzheimer’s disease susceptibility1–7. Currently, the most strongly associated marker in the CD33 locus is rs3865444 (ref. 5), and higher CD33 expression levels in the brain have been associated with more advanced cognitive decline and Alzheimer’s disease8,9.

**Figure 1** The CD33 risk allele is associated with increased CD33 expression and decreased uptake. (a) The mean fluorescence intensity (MFI) of CD33 protein expression on circulating monocytes of young healthy adults was increased in individuals with the rs3865444C risk allele. There was a sevenfold greater CD33 expression (MFI) of CD33 protein expression on circulating monocytes from young healthy adults was decreased with the rs3865444C risk allele. Data shown is the change in MFI (delta MFI) between samples incubated at 4 °C and 37 °C. A, protective allele; C, risk allele. Each data point represents an individual. The horizontal line denotes the mean.

1Center for Neurologic Diseases, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, USA. 2Program in Translational NeuroPsychiatric Genomics, Institute for the Neurosciences, Department of Neurology, Brigham and Women’s Hospital, Boston, Massachusetts, USA. 3Harvard Medical School, Boston, Massachusetts, USA. 4Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts, USA. 5A full list of members is available at http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf. 6Rush Alzheimer’s Disease Center, Rush University Medical Center, Chicago, Illinois, USA. 7Rush Institute for Healthy Aging, Rush University Medical Center, Chicago, Illinois, USA. 8Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts, USA. 9Department of Neurology, Center for Alzheimer’s Research and Treatment, Brigham and Women’s Hospital, Boston, Massachusetts, USA. 10The Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Boston, Massachusetts, USA. Correspondence should be addressed to P.L.D. (pdejager@partners.org).

Received 19 March; accepted 14 May; published online 23 May 2013; doi:10.1038/nn.3435
adjusting for age, sex and batch effect (Fig. 1b); here, rs3865444C accounted for 70.4% of the variance in CD33 cell surface expression.

We found no significant interaction with Alzheimer’s disease diagnosis in the ROS and MAP subjects (z = −0.80, P = 0.43) in whom this phenotype was available. Thus, the relation of genotype and CD33 expression appears to hold throughout life, and the effect of this susceptibility allele may be involved in the earliest stages of Alzheimer’s disease. We did not find an effect of age on CD33 surface expression.

Given that myeloid cells such as infiltrating macrophages or microglia are thought to be involved in Alzheimer’s disease by phagocytosis of amyloid β, which accumulates in neuritic amyloid plaques (a neuropathologic feature of Alzheimer’s disease14), we tested the hypothesis that monocytes from subjects with the rs3865444C/CD33 risk allele have a reduced state of activation, and therefore a reduced phagocytic ability, compared with subjects with the protective allele. We found reduced uptake in circulating monocytes bearing the rs3865444C risk allele of both fluorescently labeled dextran (t55 = 3.33, P = 0.003; Fig. 1c) and amyloid β (Aβ42, t135 = 3.64, P = 0.001; Fig. 1d). We replicated this observation in an independent set of subjects (t128 = 2.18, P = 0.03; Supplementary Fig. 2). These results suggest that the alteration in uptake may be relevant to the context of Alzheimer’s disease pathology, where amyloid toxicity caused by the fibrillogenic Aβ42 form of amyloid is important. Notably, we found that, although the effect of rs3865444C was additive for CD33 expression, it may be dominant for functional traits (such as substrate uptake; Supplementary Table 2).

To investigate whether these functional consequences of the CD33 risk allele could lead to alterations of amyloid biology associated with Alzheimer’s disease, we examined two collections of older subjects (Supplementary Table 3a) that underwent positron emission tomography imaging with Pittsburgh Compound B (PiB), which measures an individual’s burden of pathogenic fibrillar amyloid. Subjects were categorized as being PiB+ or PiB− (Fig. 2a,b) and the PiB+ subjects were more likely to develop cognitive impairment and Alzheimer’s disease15. In a joint analysis of the two collections (n = 218 total), we found that rs3865444C was associated with a greater likelihood of being PiB+ (Fig. 2c,d) and, as with the internalization phenotype, the effect of rs3865444C appears to fit a dominant model (χ2(1) = 5.52, P = 0.02) better than an additive model (χ2(1) = 2.78, P = 0.1). The association held if we used the PiB burden as a quantitative trait (dominant model: z = 2.35, P = 0.02) in secondary analyses. Notably, the magnitude and direction of the association linking rs3865444C with being PiB+ remained when the analysis was limited to asymptomatic older subjects; however, the results were no longer significant (χ2(1) = 2.87, P = 0.09), possibly because of the reduced sample size (Supplementary Table 3b). Overall, these data support a role for rs3865444C in vivo amyloid accumulation in the presymptomatic phase of Alzheimer’s disease.

We then assessed the role of rs3865444C in the accumulation of neuritic amyloid plaques measured at autopsy. In ROS and MAP subjects, we found that rs3865444C is also associated with greater neuritic amyloid plaque burden (z = 2.47, P = 0.01). Furthermore, we found that rs3865444C is associated with a greater likelihood of a pathologic diagnosis of Alzheimer’s disease (χ2(1) = 6.54, P = 0.01); rs3865444C was not associated with the burden of neurofibrillary tangles (z = 1.40, P = 0.16; Supplementary Table 4). Given that these analyses were performed in individuals with measurements of CD33 expression in peripheral monocytes frozen before death, we assessed whether the level of CD33 expression in monocytes statistically mediated some of the effect of the risk allele on neuropathologic traits. We found that the effect size of the relation of rs3865444C to neuritic amyloid plaque burden was diminished by 10% and was no longer significant (z = 1.74, P = 0.08) when we added a covariate for the level of CD33 expression to the model. Thus, these two prospectively collected autopsy cohorts support a role for monocytes in mediating some of the functional consequences of rs3865444C that ultimately result in the accumulation of amyloid pathology and Alzheimer’s disease.

To further explore the role of CD33 in the brains of older subjects, we examined tissue sections from MAP subjects and found that CD33 was expressed in cells that had the morphologic attributes of microglia and macrophages (Fig. 3a–c). The CD33-expressing cells were found in the vicinity of neuritic plaques (Fig. 3a).

In older age, activated myeloid cells, such as those that express CD33, are seen in the brains of cognitively non-impaired and demented individuals16. In ROS and MAP subjects, we found that the mean proportion of terminally activated microglia/macroage (stage III microglia or macrophage) was significantly elevated relative to rs3865444C (z = 3.06, P = 0.003) in the inferior temporal lobe (which is an early target of Alzheimer’s disease–related amyloid neuropathology; Fig. 3d), and there was a suggestion that it was elevated in the posterior putamen region (z = 2.42, P = 0.017) after accounting for the testing of four hypotheses (Fig. 3e and Supplementary Table 5a). These associations remained after adjusting for a diagnosis of Alzheimer’s disease (Supplementary Table 5b). This observation is consistent with a model of Alzheimer’s disease association in which monocytes from subjects with the risk allele are less able to uptake Aβ42, which could lead to an accumulation of activated, but less functional, microglia and macrophage in the brain, where they fail to clear amyloid β plaques.

We sought to characterize the functional consequences of the CD33 Alzheimer’s disease susceptibility locus in early and late life. Our results are consistent with a recent report that decreasing CD33 expression in mice leads to enhanced Aβ42 uptake by macrophage/microglia and decreased accumulation of brain amyloid in a mouse model8. CD33 is thought to be an inhibitory lectin, and increased cell surface expression of CD33 as a result of the risk allele could lead to downregulation of myeloid cell activity, such as the reduced uptake of Aβ42. Such alterations of myeloid function throughout life could lead to the earlier accumulation of fibrillar amyloid, as measured by PiB imaging in older brains, and, ultimately, the accumulation of more neuritic amyloid pathology and a greater likelihood of meeting a diagnosis of Alzheimer’s disease on neuropathologic examination.

We observed the functional consequences of this variant in younger and presymptomatic older subjects, suggesting that the CD33 risk
allele and altered CD33 function could be involved in the very early presymptomatic phase of Alzheimer's disease, in middle or younger age. Furthermore, our data from young subjects with the risk allele suggest that previously reported Alzheimer's disease–associated alterations in immune function are not solely a feature of immune senescence in older age or a late reaction to the toxicity of amyloid and other Alzheimer's disease–related pathologies. It is not yet clear how CD33-related dysfunction of innate immune cells relates to all manifestations of Alzheimer's disease; it seems to contribute to known neuropathologic features of Alzheimer's disease, such as the accumulation of amyloid pathology, but may well be involved in other inflammatory events that are associated with Alzheimer's disease and remain unknown.

Our data are consistent with previous reports exploring the role of innate immune system dysfunction in Alzheimer's disease and identify a specific molecule, CD33, that links genetic susceptibility, altered myeloid function and amyloid pathology to an early role in the pathogenesis of Alzheimer's disease. Thus, our data suggest a new target with which to manipulate amyloid pathology and provide pertinent information that could be leveraged in algorithms with which to identify a specific molecule, CD33, that links genetic susceptibility, altered CD33 function and amyloid pathology and may influence responses to the antibody-mediated therapeutic approaches that are currently being tested in Alzheimer's disease.

METHODS

Methods and any associated references are available in the online version of the paper.

ACKNOWLEDGMENTS

The authors are grateful to the participants of the ROS, MAP, CHAP, Harvard Aging Brain Study (HAB) and Alzheimer's Disease Neuroimaging Initiative (ADNI) studies. Data used in preparation of this article were obtained from the ADNI database (http://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. We also thank the participants of the Brigham and Women's PhenoGenetic Project. This work is supported by the US National Institutes of Health (grants R01 AG031553, R01 AG030146, R01 AG17917, R01 AG15819, P30 AG10161 and R01 AG11101) and the Illinois Department of Public Health. This work was supported by grants R01 NS087305, R01 GC093080 and R01 AG043617. E.M.B. receives support from the JDRF American Diabetes Association, Boston Area Diabetes and Endocrinology Research Center and the Harvard NeuroDiscovery Center.

AUTHOR CONTRIBUTIONS

E.M.B. and P.L.D. designed and implemented the study. E.M.B. developed the experimental methods using peripheral blood mononuclear cells (PBMCs). E.M.B., I.O., T.E., J.L.R. and S.I. isolated PBMCs from the PhenoGenetic cohort and analyzed monocyte CD33 expression. E.M.B., A.T. and J.L.R. analyzed the uptake ability of monocytes. T.R., B.T.K. and L.B.C. performed the statistical analyses and assisted with the interpretation and communication of results. A.V.K., M.L. and P.L.D. coordinated the collection of blood from the PhenoGenetic cohort. R.A.S., K.J. and ADNI provided the PiB imaging data and reviewed the manuscript. R.A.S. provided the HAB blood samples. M.C.M. contributed post-mortem data on brain microglia and reviewed the manuscript. D.A.E. provided clinical data and biospecimens. J.A.S. was responsible for the microglia and Alzheimer's disease pathology data collection from the brains of deceased MAP participants and revised the manuscript. D.A.B. contributed ante-mortem biospecimens, clinical and post-mortem data, and revised the manuscript. P.L.D. conceived the study, coordinated access to all of the cohorts and wrote the manuscript with E.M.B.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.
**ONLINE METHODS**

Study subjects. Informed consent was obtained on all human subjects. All blood draws, brain autopsies, experiments and data analysis were done in compliance with protocols approved by either the Partners Human Research Committees or the Rush University Institutional Review Board.

The Brigham and Women’s Hospital PhenoGenetic Project. Peripheral venous blood was obtained from healthy control volunteers. The PhenoGenetic Project is a living tissue bank that consists of healthy subjects who are re-contactable and can therefore be recalled based on their genotype. 1,741 healthy subjects >18 years old have been recruited from the general population of Boston. They are free of chronic inflammatory, infectious and metabolic diseases. Their median age is 24, and 62.7% of subjects are women. For the CD33 expression experiments, the median age was 26.5 years (range = 20.0–50.0 years). All of the PhenoGenetic subjects used in the dextran uptake assay and 25 of the subjects used in the Aβ(42) uptake assay were also used in the CD33 staining assay; 67% of the subjects used in the Aβ(42) uptake assay were also used in the dextran uptake assay.

ROS and MAP and CHAP. Study participants were free of known dementia at enrollment, agreed to annual clinical evaluations, and signed an Anatomic Gift Act donating their brains at death. ROS enrolls Catholic priests, nuns and brothers, aged 53 or older from about 40 groups in 12 states. More than 1,150 participants have completed their baseline evaluation (87% are non-Hispanic white), and the follow-up rate of survivors and autopsy rate among the deceased both exceed 90%. MAP enrolls men and women aged 55 or older and with no evidence of dementia at baseline from assisted living facilities in the Chicago area in compliance with protocols approved by the Institutional Review Board of Rush University. More than 1,500 participants have completed their baseline evaluation (87% are non-Hispanic white). The follow-up rate of survivors exceeds 90% and the autopsy rate exceeds 80%. More detailed descriptions of ROS, MAP and CHAP can be found in prior publications11–13. The median age of subjects used in the CD33 expression experiments at sampling was 79.9 years (range = 65.8–94.8 years).

HAB. HAB is a longitudinal study of older individuals between the ages of 65 and 85 years old. Participants were evaluated with an extensive battery of functional and neuropsychological tests. All participants had a Clinical Dementia Rating of 0, performed no worse than 1.5 s.d. units below the age- and education-corrected norm on the Logical Memory IIA subset of the Wechsler Memory Scale-Revised22, and scored 26 or above on the Mini-Mental State Examination23. Participants were excluded if previously diagnosed with a neurological or psychiatric condition or if they scored >11 on the Geriatric Depression Scale24. Participants provided informed consent in accordance with protocols approved by the Partners Healthcare Institutional Review Board.

ADNI. Subjects with normal cognition, amnestic mild cognitive impairment (MCI) and mild Alzheimer’s disease were followed up with periodic neuropsychological testing, multiple imaging techniques and fluid biomarkers (http://www.loni. ucla.edu/ADNI/). Subjects (N = 96) with complete baseline clinical data sets were included in the current analysis, aged 55–89 years, in general good health or with stable medical problems at the time of screening, and had a study partner-caregiver able to provide an independent evaluation of the subject’s cognitive, behavioral and functional status. Subjects did not have substantial neurological conditions, substantial active psychiatric disorders, or alcohol or substance abuse within 2 years of screening. All subjects underwent comprehensive neuropsychological testing (assessing memory, attention, executive function, language and visuospatial function) and additional study partner questionnaires about behavior and daily functioning. All subjects had a global Clinical Dementia Rating scale31 score of 0.0 or 1. Data used in the preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (http://adni.loni. ucla.edu/). For up-to-date information, see http://www.adni-info.org/.

Genotypes. Subjects from the Brigham and Women’s Hospital PhenoGenetic Project and HAB were genotyped using DNA isolated from whole blood and the iPLEX Sequenom MassARRAY platform (genotype call rate > 95%, Hardy-Weinberg Equilibrium P > 0.001).

In the ROS, MAP, CHAP and ADNI subjects, imputed dosage values were used for analyses of the rs3865444F allele. In all four cohorts, DNA was extracted from whole blood or frozen post-mortem brain tissue. Genotype data was generated using the Affymetrix Genechip 6.0 platform at the Broad Institute’s Genetic Analysis Platform or the Translational Genomics Research Institute. Both sets of data underwent the same quality control analyses in parallel using the PLINK toolkit (http://pngu.mgh.harvard.edu/~purcell/plink/) and quality controlled genotypes were pooled. The quality control process included a principal components analysis using default parameters in EIGENSTRAT25 to identify and remove population outliers. Imputation in ROS and MAP was performed using MACH software (version 1.0.16a) and HapMap release 22 CEU (build 36). The imputation quality score for rs3865444 is 0.9963, suggesting that the single nucleotide polymorphism is imputed with a high level of confidence.

**Cells and flow cytometry analysis.** PBMCs were separated by Ficoll-Paque PLUS (GE Healthcare) gradient centrifugation. PBMCs were frozen at a concentration of 1–3 × 107 cells ml−1 in 10% DMSO (Sigma-Aldrich)/90% fetal calf serum (vol/vol, Atlanta Biologicals). After thawing, PBMCs were washed in 10 ml of phosphate-buffered saline (PBS). PBMCs were stained with antibody to human CD33 (clone AC104.3E3, Miltenyi, 1:20) or mouse IgG1 isotype (clone IF5-21FS, Miltenyi, 1:20) in PBS with 1% fetal calf serum. The monocyte gates were defined based on their distinct forward and side-scatter profile. The MFI was acquired on a FACSCalibur (BD Immunocytometry Systems) and analyzed with FlowJo software (Tree Star). An additive model was used in the analysis, adjusting for age and sex. Given the availability of genotyped samples in the phenogenetic cohort, the AA and CC genotype classes were over-sampled in Figure 1a, whereas the subjects shown in Figure 1b were not selected by genotype and represent a random sample of subjects from the CHAP, MAP and ROS cohorts, which are prospective cohorts of initially non-demented individuals.

**Uptake assays.** We tested monocyte uptake ability in circulating monocytes using two different substrates that have been deployed previously27,28. After thawing, PBMCs were washed in 10 ml PBS. Either 100,000 PBMCs per well or 50,000 purified monocytes per well were incubated with either 1 ng ml−1 FITC-labeled dextran (Sigma-Aldrich) or 15 ng ml−1 HiLyte Fluor 488–labeled Aβ(42) (AnaSpec) in 96-well polystyrene plates in HL-1 media plus 5% human serum (vol/vol) for 1 h at 37 °C or 4 °C. Cells were then washed three times with PBS and fixed in 4% paraformaldehyde (vol/vol) for 30 min. Data shown is the change in MFI (delta MFI) between samples incubated at 4 °C (where uptake is blocked) and 37 °C (where uptake occurs). A dominant model was used in the analysis, adjusting for age and sex.

Pathology, immunohistochemistry and microglia quantification. Brain tissues were removed in a standard fashion as previously described27–29. After weighing, each brain was cut into 1-cm coronal slabs using a Plexiglas jig. Slabs from one hemisphere, and those from the other hemisphere not designated for rapid freezing, were fixed for at least 3 d in 4% paraformaldehyde. We used defined landmarks to obtain at least two tissue blocks from each of the following regions: dorsolateral prefrontal cortex, middle and inferior temporal cortex, inferior parietal, hippocampus CA1/subiculum, entorhinal cortex proper, ventromedial caudate and posterior putamen. Tissue blocks were processed, embedded in paraffin, cut into either 6- or 20-μm sections, and mounted on glass slides. Neuropathologic diagnoses were made by a board-certified neuropathologist blinded to age and clinical data. Bielschowsky silver stain 6-μm sections were used to visualize neuritic plaques, diffuse plaques and neurofibrillary tangles in the frontal, temporal, parietal, entorhinal and hippocampal cortices, as previously described, for the pathologic diagnosis of Alzheimer’s disease30. A neuropathologic diagnosis of no Alzheimer’s disease, low likelihood Alzheimer’s disease, intermediate likelihood Alzheimer’s disease or high likelihood Alzheimer’s disease was given on the basis of semiquantitative estimates of neuritic plaque density as recommended by the Consortium to Establish a Registry for Alzheimer’s Disease and a neurofibrillary tangle stage by Braak and Braak as recommended by the National Institute on Aging (NIA)-Reagan criteria31. For analyses, the neuropathologic diagnosis of Alzheimer’s disease was considered absent if NIA-Reagan diagnosis was no or low and present if intermediate or high likelihood32. The density of neuritic plaques, diffuse
plagues and neurofibrillary tangles was characterized using Bielchowsky silver stain for visualization and a graticule to count total number of each in a 1-mm² area (100 magnification) of highest density. Counts for each marker were completed for each of five regions (midfrontal, medial temporal cortex, inferior frontal cortex, entorhinal cortex and hippocampus CA1/subiculum) and then converted to standardized scores using the mean of the entire deceased cohort for that region²⁹.

Immunohistochemistry for microglia was performed using an Automated Leica Bond immunostain (Leica Microsystems) and antibodies to human HLA-DR, HLA-DQ and HLA-DR (clone CR3/43, DakoCytomation, 1:100) using standard Bond epitope retrieval and detection. An investigator blinded to the clinical and pathologic data, outlined the cortical or subcortical gray region of interest on each slide using a Microbrightfield Stereology System. Stereo Investigator 8.0 software was used to place a 1,000 × 750 µm sampling grid over the region and the program was engaged to sample 4.0% of the region with a 200- × 150-µm counting frame at 400× magnification at interval grid intersection points. Using separate tags for stage 1, 2, and 3 microglia, the operator marked the microglia at each intersection point. These counts were then upweighted by the stereology software to estimate total number of microglia (stage 1, 2 and 3) in the defined area. Different stages of activation from least (stage 1) to most (stage 3) activated can be defined morphologically; when these cells become activated, their long fine processes contract and thicken and the cell body adopts a larger more rounded cellular conformation. Data from the two adjacent blocks of tissue (0.5 to 1.0 cm apart) were averaged to obtain composite average densities of microglia in each region. For CD33 staining, monoclonal antibody to human CD33 (clone PWS64, Leica Biosystems, 1:75) was used with diaminobenzidine as chromagen. The amyloid deposits were stained with an antibody to amyloid β (clone 10D5, Elan Pharmaceuticals, 1:300) using alkaline phosphatase as the chromagen.

**PiB imaging.** Amyloid burden was measured with N-methyl-[11C]-2-(4-methylaminophenyl)-6-hydroxybenzothiazole (PiB), which binds to fibrillar amyloid and was prepared at Massachusetts General Hospital as described previously³¹,³². Participants underwent PiB PET imaging as described previously³³,³⁴. Briefly, data were acquired using a Siemens/CTI ECAT HR scanner (three-dimensional mode, 63 image planes, 15.2-cm axial field of view, 5.6-mm transaxial resolution and 2.4-mm slice interval; 39 frames, 8 × 15s, 4 × 60s, 27 × 120s). After a transmission scan, 8.5–15-mCi [11C]-PiB was injected as a bolus and followed immediately by a 60-min dynamic acquisition. PET data were reconstructed and attenuation corrected, and each frame was evaluated to verify adequate count statistics and absence of head motion (interframe head motion, if present, was corrected before further processing).

The dynamic PET data were reconstructed with scatter correction using commercially available routines for three-dimensional PET data. PET data were parameterized by the DVR computed using the Logan graphical analysis technique³⁵ applied to the frame data acquired 40–60 min after injection; this method has been fully validated for PiB imaging³⁶. Time-activity curves were measured in each brain region under analysis (region of interest) and in a reference region in cerebellar cortex known to contain low levels of fibrillary amyloid. This approach has been applied to numerous PiB studies³⁶–³⁹ and yields data similar to that derived from arterial blood input methods³⁷.

For each subject, an index of PiB binding in cortical regions was calculated using the dynamic data via Logan graphical modeling in a large aggregate cortical region of interest consisting of frontal, lateral parietal and temporal, and retrosplenial cortices (the FLR region). PiB retention in the FLR region is substantial in patients with diagnosed Alzheimer’s disease and has been used as a summary measure of PiB retention in previous studies³³,³⁴,³⁹. 1.25 was the value used to separate PiB+ and PiB− in the HAB cohort, and 1.5 was used to delineate between PiB+ and PiB− in the ADNI cohort.

**Statistical analysis.** Demographic information on all data sets was presented using counts and percentages for categorical variables and means and s.d. or medians and ranges (minimum-maximum) for continuous variables. The rs3865444 single nucleotide polymorphism was coded additively relative to the C allele in all analyses. Outcomes of interest were assessed for normality using a parametric method. To correct for multiple comparisons, we performed a standard Bonferroni correction by setting the threshold of significance equal to 0.05 divided by the number of tests performed under each a priori hypothesis. Statistical analyses were performed using SAS software, version 9.3 (SAS Institute), the PLINK toolkit (http://pngu.mgh.harvard.edu/~purcell/plink/) and R, version 2.13 (http://www.r-project.org/).

**References**

21. Morris, J.C. *Neurology* **43**, 2412–2414 (1993).
22. Wechsler, D. *Wechsler Memory Scale-Revised (WMS-R)* (Psychological, San Antonio, Texas, 1987).
23. Folstein, M.F., Folstein, S.E. & McHugh, P.R. *J. Psychiatr. Res.* **12**, 189–198 (1975).
24. Yesavage, J.A. et al. *J. Psychiatr. Res.* **17**, 37–49 (1982).
25. Price, A.L. et al. *Nat. Genet.* **38**, 904–909 (2006).
26. Ciaramella, A. et al. *J. Alzheimers Dis.* **19**, 559–572 (2010).
27. Bennett, D.A. et al. *Neurology* **66**, 1837–1844 (2006).
28. Bennett, D.A., Schneider, J.A., Bienias, J.L., Evans, D.A. & Wilson, R.S. *Neurology* **64**, 834–841 (2005).
29. Schneider, J.A., Wilson, R.S., Bienias, J.L., Evans, D.A. & Bennett, D.A. *Neurology* **62**, 1148–1155 (2004).
30. Anonymous. *Neurobiol. Aging* **18**, 51–52 (1997).
31. Mathis, C.A. et al. *J. Med. Chem.* **46**, 2740–2754 (2003).
32. Klunk, W.E. et al. *Ann. Neurol.* **55**, 306–319 (2004).
33. Gomperts, S.N. et al. *Neurology* **71**, 903–910 (2008).
34. Hedges, T. et al. *Neurosci.* **29**, 12686–12694 (2009).
35. Logan, J. et al. *J. Cereb. Blood Flow Metab.* **10**, 740–747 (1990).
36. Price, J.C. et al. *J. Cereb. Blood Flow Metab.* **25**, 1528–1547 (2005).
37. Lopresti, B.J. et al. *J. Nucl. Med.* **46**, 1959–1972 (2005).
38. Fagan, A.M. et al. *Ann. Neurol.* **59**, 512–519 (2006).
39. Johnson, K.A. et al. *Ann. Neurol.* **62**, 229–234 (2007).
40. Johnson, W.E., Li, C. & Rabinovic, A. *Biostatistics* **8**, 118–127 (2007).
41. Reich, M. et al. *Nat. Genet.* **38**, 500–501 (2006).