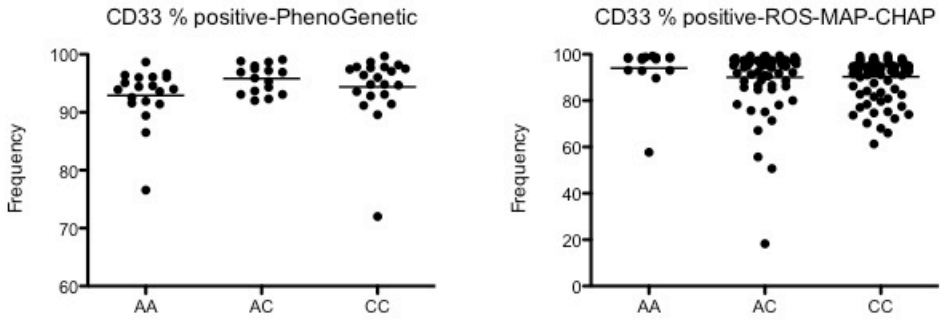
Supplementary Material

CD33 Alzheimer's disease locus: Altered monocyte function and amyloid biology

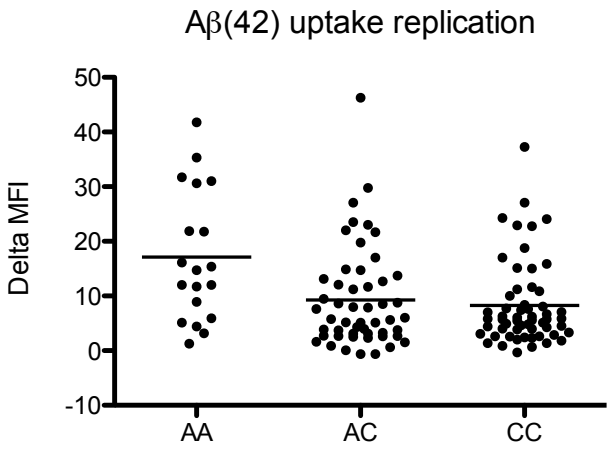
Bradshaw EM[1,2,3], Chibnik LB[1,2,3,4], Keenan BT[1,2,4], Ottoboni L[1,2,4], Raj, T[1,2,3,4], Tang A[1,2,4], Rosenkrantz LL[1,2,4], Imboywa S[1,2,4], Lee M[1,2], Von Korff A[1,2], The Alzheimer's Disease Neuroimaging Initiative[7], Morris MC[5], Evans DA[6], Johnson K[3,9,10], Sperling RA[3,7,9,10], Schneider JA[5], Bennett DA[5], De Jager PL[1,2,3,4].

[1]Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA.
[2] Program in Translational NeuroPsychiatric Genomics, Institute for the Neurosciences, Departments of Neurology and Psychiatry, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, NRB168, Boston, MA 02115
[3] Harvard Medical School, Boston, MA 02115
[4] Program in Medical and Population Genetics, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142
[5]Rush Alzheimer's Disease Center, Rush University Medical Center, 600 S Paulina St., Chicago, IL 60612
[6]Rush Institute for Healthy Aging, Rush University Medical Center,1645 W. Jackson Blvd., Chicago 60612
[7]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_Authorship_List.pdf.
[8]Department of Neurology, Center for Alzheimer's Research and Treatment, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, MA 02115, USA
[9]Department of Neurology, Massachusetts General Hospital, Boston, MA, USA
[10]The Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Boston, MA, USA

Bradshaw, et al. Supplemental Figure 1



Supplemental Figure 1: There is no difference in the frequency of CD33 positive monocytes from subjects separated by genotype. The frequency of CD33 positive cells was determines for the PhenoGenetic cohort (a) and the ROS-MAP-CHAP cohorts (b) used for determining CD33 MFI. No significant difference was seen between rs3865444 genotypes ($p = 0.21$ and 0.43 respectively). Each circle represents an individual. The line represents the mean.



Supplemental Figure 2: Replication of the *CD33* risk allele being associated with decreased A-beta uptake by monocytes. The delta MFI of FITC-labeled Aβ(42) uptake by monocytes from healthy adults was measured and compared between subjects of different genotypes (N=128). All subjects were different from the subjects reported in Figure 2. 33 of these subjects are from the PhenoGenetic collection, and 94 come from the Harvard Aging Brain study. A dominant model was used in the analysis, adjusting for age and sex. Each circle represents an individual.

Supplemental Table 1a. Demographic characteristics† of the BWH PhenoGenetic project subjects used in different experiments					
	PhenoGenetic subjects				
	CD33 Discovery	CD33 Replication	Dextran	Aβ	Aβ Replication
sample size	32	19	30	32	128
age at sampling	26.5 [20.0-50.0]	34.0 [20.0-54.0]	30.5 [20.0-54.0]	27.0 [20.0-53.0]	68.75 [20.0-86.50]
female	20 (67%)	5 (26%)	14 (47%)	25 (78%)	70 (55%)

†Summary Statistics presented as median [min - max] or count (%)

Supplemental Table 1b. Demographic characteristics of the ROS, MAP and CHAP subjects for the CD33			
	ROS	MAP	CHAP
sample size	91	60	24
age at sampling	78.3 (5.8)	84.0 (5.0)	78.6 (8.7)
female	48 (53%)	34 (57%)	15 (63%)
AD (pathological diagnosis)	56 (62%)	40 (67%)	0 (0%)

Supplemental Table 2. Analysis for the rs3865444[C] risk allele in monocyte functional assays									
	Dextran uptake			Aβ42 uptake			Aβ42 uptake replication		
	n	Median (Q1-Q3)	p-value*	n	median (Q1-Q3)	p-value*	n	median (Q1-Q3)	p-value*
rs3865444, additive									
AA	10	13.7 (8.0 - 19.6)	t(29) = 2.66 0.01	9	28.2 (18.4 - 43.4)	t(31) = -3.27 0.003	19	16.1 (5.9-35.4)	t(124) = -1.74 0.08
AC	9	3.3 (-0.8 - 9.2)		12	13.9 (9.3 - 18.3)		52	5.7 (2.9-14.3)	
CC	11	3.5 (0.7 - 6.6)		11	11.8 (8.6 - 17.0)		57	5. 8 (3.3-10.0)	
rs3865444, dominant									
AA	10	13.7 (8.0 - 19.6)	t(29) = 3.33 0.003	9	28.2 (18.4 - 43.4)	t(31) = 3.64 0.001	19	16.1 (5.9-35.4)	t(124) = 2.18, 0.03
AC/CC	20	5.3 (0.7 - 7.9)		23	12.7 (8.9 - 17.2)		109	5.7 (3.1-12.7)	

* p-value from ANOVA with dextran uptake values or square root transformed Aβ42 uptake values, controlling for age and batch (for Aβ42 uptake)

Supplemental Table 3a. Demographic characteristics† of the Harvard Aging Brain study and ADNI subjects used in PiB imaging experiments		
	PiB imaging subjects	
	HAB	ADNI
sample size	122	96
age at sampling	72.1 [65.0-86.5]	75.7 [55.2-89.9]
female	69 (57%)	33 (34%)
MCI	0 (0%)	60 (63%)
AD	0 (0%)	19 (20%)

†Summary Statistics presented as median [min - max] or count (%)

Supplemental Table 3b. Analysis for the rs3865444[C] risk allele and PiB		
	β estimate (SE) [p-value]	
	Total	Asymptomatic
sample size	218	139
rs3865444, additive for C		
PiB positive vs negative	0.39 (0.24) [$X^2(1)=2.78$, p=0.10]	0.46 (0.31) $X^2(1)=2.23$, [p=0.14]
continuous PiB	0.09 (0.04) [Z=1.22, p=0.22]	0.05 (0.03) [Z=1.32, p=0.19]
rs3865444, dominant for C		
PiB positive vs negative	1.85 (0.79) [$X^2(1)=5.52$, p=0.02]	1.82 (1.07) $X^2(1)=2.87$, [p=0.09]
continuous PiB	0.19 (0.08) [Z=2.35, p=0.02]	0.12 (0.08) [Z=1.48, p=0.14]

Supplemental Table 4: Association between rs3865444[C] and Neuropathological phenotypes (n=151)			
Phenotype	**Association with rs3865444[C]**	**p-value[1]**	**p-value[2]**
Neuritic amyloid plaques[3]	Yes	Z=2.47, p=0.01	Z=1.74, p=0.08
Pathologic diagnosis of AD[4]	Yes	$X^2(1)=6.53$, p=0.01	$X^2(1)=3.99$, p=0.05
Neurofibrillary tangles[3]	No	Z=1.40, p=0.16	Z=0.13, p=0.90

[1] adjusted for age at death and sex

[2] adjusted for age at death, sex and cd33 surface expression

[3] ANOVA with square-root transformed values

[4] Logistic regression

Supplemental Table 5a. Demographic characteristics of the MAP subjects for the macrophage/microglia analyses	
sample size	172
age at sampling	89.8 [74.8-101.6]
age at death	89.8 [74.8-101.6]
education	15.0 [8.0-22.0]
female	112 (65%)
CHAP	0 (0%)
ROS	0 (0%)
MAP	172 (100%)
AD	60 (35%)
MCI	47 (27%)
NIA - Reagan criteria	
No-AD	29 (17%)
Low	74 (43%)
Intermediate	67 (39%)
High	2 (1%)

Supplemental Table 5b. Analysis for the rs3865444^{C} risk allele in activated microglia/macrophage infiltrate					
		additive		**dominant for C**	
Variable (Region) †	**n**	**Beta (95% CI)**	**p-value*‡**	**Beta**	**p-value***
Inferior Temporal Lobe	168	0.336 (0.110,0.551)	Z=3.06 0.003	0.570	Z=2.11 0.036
Mid-Frontal	172	0.156 (-0.091,0.405)	Z=1.24 0.216	0.505	Z=1.65 0.102
Posterior Putamen	166	0.253 (0.047,0.458)	Z=2.42 0.017	0.281	Z=1.06 0.290
Ventral Medial Caudate	160	0.058 (-0.142,0.255)	Z=0.58 0.563	0.382	Z=1.56 0.121

* p-value for additive from GLM, p-value for dominant from Wilcoxon rank sum

†Outcome for each region was square-root transformed for normality

‡Models adjusted for age at death