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### Permalink

<https://escholarship.org/uc/item/6xc596k3>

### Journal

Nature Genetics, 43(5)

### ISSN

1061-4036

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### Publication Date

2011-05-01

### DOI

10.1038/ng.801

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Peer reviewed



Published in final edited form as:

Nat Genet. 2011 May ; 43(5): 436–441. doi:10.1038/ng.801.

## Common variants in *MS4A4/MS4A6E*, *CD2uAP*, *CD33*, and *EPHA1* are associated with late-onset Alzheimer's disease

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### Abstract

The Alzheimer Disease Genetics Consortium (ADGC) performed a genome-wide association study (GWAS) of late-onset Alzheimer disease (LOAD) using a 3 stage design consisting of a discovery stage (Stage 1) and two replication stages (Stages 2 and 3). Both joint and *meta*-analysis

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The Alzheimer Disease Genetics Consortium (ADGC), <http://aloi.med.upenn.edu/adgc/about/overview.html>; ADNI database, ([www.loni.ucla.edu/ADNI](http://www.loni.ucla.edu/ADNI)); ADNI investigators, [http://www.loni.ucla.edu/ADNI/Collaboration/ADNI\\_Manuscript\\_Citations.pdf](http://www.loni.ucla.edu/ADNI/Collaboration/ADNI_Manuscript_Citations.pdf); *APOE* Genotyping kit from TIB MOLBIOL, <http://www.roche-as.es/logs/LightMix> %C2%AE\_40-0445-16\_ApoE-112-158\_V080904.pdf; PLINK, <http://pngu.mgh.harvard.edu/~purcell/plink/>; PREST, <http://utstat.toronto.edu/sun/Software/Prest/>; MACH, <http://www.sph.umich.edu/csg/abecasis/mach/>; EIGENSTRAT, <http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm>; The R Project for Statistical Computing, <http://www.r-project.org/>; Package *GWAF* in R, <http://cran.r-project.org/web/packages/GWAF/index.html>; Package *gee* in R, <http://cran.r-project.org/web/packages/gee/index.html>; UCSC Genome Browser, <http://genome.ucsc.edu/>; METAL, <http://www.sph.umich.edu/csg/abecasis/Metal/>; FUGUE, <http://www.sph.umich.edu/csg/abecasis/fugue/>.

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### Competing Financial Interests

T.D.B. received licensing fees from and is on the speaker's bureau of Athena Diagnostics, Inc. M.R.F. receives research funding from BristolMyersSquibb Company, Danone Research, Elan Pharmaceuticals, Inc., Eli Lilly and Company, Novartis Pharmaceuticals Corporation, OctaPharma AG, Pfizer Inc., and Sonexa Therapeutics, Inc; Receives honoraria as scientific consultant from Accera, Inc., Astellas Pharma US Inc., Baxter, Bayer Pharmaceuticals Corporation, BristolMyersSquibb, Eisai Medical Research, Inc., GE Healthcare, Medavante, Medivation, Inc., Merck & Co., Inc., Novartis Pharmaceuticals Corp., Pfizer, Inc., Prana Biotechnology Ltd., QR Pharma, Inc., The sanofi-aventis Group, and Toyama Chemical Co., Ltd.; and is speaker for Eisai Medical Research, Inc., Forest Laboratories, Pfizer Inc. and Novartis Pharmaceuticals Corporation. A.M.G. has research funding from AstraZeneca, Pfizer and Genentech, and has received remuneration for giving talks at Pfizer and Genentech. R.C.P. is on the Safety Monitory Committee of Pfizer, Inc. (Wyeth) and a consultant to the Safety Monitoring Committee at Janssen Alzheimer's Immunotherapy Program (Elan), to Elan Pharmaceuticals, and to GE Healthcare. R.E.T. is a consultant to Eisai, Japan in the area of Alzheimer's genetics and a shareholder in, and consultant to Pathway Genomics, Inc, San Diego, CA.

analysis approaches were used. We obtained genome-wide significant results at *MS4A4A* [rs4938933; Stages 1+2, *meta*-analysis ( $P_M$ ) =  $1.7 \times 10^{-9}$ , joint analysis ( $P_J$ ) =  $1.7 \times 10^{-9}$ ; Stages 1–3,  $P_M$  =  $8.2 \times 10^{-12}$ ], *CD2AP* (rs9349407; Stages 1–3,  $P_M$  =  $8.6 \times 10^{-9}$ ), *EPHA1* (rs11767557; Stages 1–3  $P_M$  =  $6.0 \times 10^{-10}$ ), and *CD33* (rs3865444; Stages 1–3,  $P_M$  =  $1.6 \times 10^{-9}$ ). We confirmed that *CRI* (rs6701713;  $P_M$  =  $4.6 \times 10^{-10}$ ,  $P_J$  =  $5.2 \times 10^{-11}$ ), *CLU* (rs1532278;  $P_M$  =  $8.3 \times 10^{-8}$ ,  $P_J$  =  $1.9 \times 10^{-8}$ ), *BINI* (rs7561528;  $P_M$  =  $4.0 \times 10^{-14}$ ;  $P_J$  =  $5.2 \times 10^{-14}$ ), and *PICALM* (rs561655;  $P_M$  =  $7.0 \times 10^{-11}$ ,  $P_J$  =  $1.0 \times 10^{-10}$ ) but not *EXOC3L2* are LOAD risk loci<sup>1–3</sup>.

Alzheimer Disease (AD) is a neurodegenerative disorder affecting more than 13% of individuals aged 65 years and older and 30%–50% aged 80 years and older<sup>4–5</sup>. Early work identified mutations in *APP*, *PSEN1*, and *PSEN2* that cause early-onset autosomal dominant AD<sup>6–9</sup> and variants in *APOE* that affect LOAD susceptibility<sup>10</sup>. A recent GWAS identified *CRI*, *CLU*, *PICALM*, and *BINI* as LOAD susceptibility loci<sup>1–3</sup>. However, because LOAD heritability estimates are high ( $h^2 \approx 60$ – $80\%$ )<sup>11</sup>, much of the genetic contribution remains unknown.

To identify genetic variants associated with risk for AD, the ADGC assembled a discovery dataset [Stage 1; 8,309 LOAD cases, 7,366 cognitively normal controls (CNEs)] using data from eight cohorts and a ninth newly assembled cohort from the 29 NIA-funded Alzheimer Disease Centers (ADCs) (Supplementary Tables 1 and 2, Supplementary Note) with data coordinated by the National Alzheimer Coordinating Center (NACC) and samples coordinated by the National Cell Repository for Alzheimer Disease (NCRAD). For the Stage 2 replication, we used four additional datasets and additional samples from the ADCs (3,531 LOAD cases, 3,565 CNEs). The Stage 3 replication used the results of association analyses provided by three other consortia (Hollingworth *et al.*<sup>12</sup>; 7,650 LOAD cases, 25,839 mixed-age controls). For Stages 1 and 2, we used both a *meta*-analysis (*M*) approach that integrates results from association analyses of individual datasets; and a joint analysis (*J*) approach where genotype data from each study are pooled. The latter method has improved power over *meta*-analysis in the absence of between-study heterogeneity<sup>13</sup> and more direct correction for confounding sampling bias<sup>14</sup>. We were limited to *meta*-analysis for Stage 3.

Because cohorts were genotyped using different platforms, we used imputation to generate a common set of 2,324,889 SNPs. We applied uniform stringent quality control measures to all datasets to remove low-quality and redundant samples and problematic SNPs (Supplementary Tables 3, 4, and Online Methods). We performed association analysis assuming an additive model on the log odds ratio scale with adjustment for population substructure using logistic regression for case-control data and generalized estimating equations (GEE) with a logistic model for family data. Results from individual datasets were combined in the *meta*-analysis using the inverse variance method, applying a genomic control to each dataset. The joint analysis was performed using GEE and incorporated terms to adjust for population substructure and site-specific effects (Online Methods). For both approaches, we also examined an extended model of covariate adjustment that adjusted for age (age at onset or death in cases; age at exam or death in controls), sex, and number of *APOE*  $\epsilon 4$  alleles (0, 1, or 2). Genomic inflation factors ( $\lambda$ ) for both the discovery *meta*-analysis and the joint analysis and extended models were less than 1.05, indicating that there

was not substantial inflation of the test statistics (Supplementary Table 3, Supplementary Figure 1). Association findings from *meta*-analysis and joint analysis were comparable.

In Stage 1, the strongest signal was from the *APOE* region (*e.g.*, rs4420638,  $P_M = 1.1 \times 10^{-266}$ ,  $P_J = 1.3 \times 10^{-253}$ ; Supplementary Table 5). Excluding the *APOE* region, SNPs at nine distinct loci yielded a  $P_M$  or  $P_J < 10^{-6}$  (Table 1; all SNPs with  $P < 10^{-4}$  are in Supplementary Table 5). SNPs from these nine loci were carried forward to Stage 2. Five of these had not previously been associated with LOAD at a genome-wide significance level of  $P < 5.0 \times 10^{-8}$  (*MS4A*, *EPHA1*, *CD33*, *ARID5B*, and *CD2AP*). Because Hollingworth *et al.*<sup>12</sup> identified SNPs at *ABCA7* as a novel LOAD locus, we included *ABCA7* region SNPs in Stage 2 and provided the results to Hollingworth *et al.*<sup>12</sup>. For all loci in Table 1, we did not detect evidence for effect heterogeneity (Supplementary Fig. 2). One novel locus (*MS4A*) was significant in the Stage 1+2 analysis. Four other loci approached but did not reach genome-wide significance in the Stage 1+2 analyses and were carried forward to Stage 3. For three of these (*CD33*, *EPHA1*, and *CD2AP*), Stage 3 analysis strengthened evidence for association. However, Stages 2 and 3 results did not support Stage 1 results for *ARID5B* 2 (Table 2).

Stage 1+2 analysis identified the *MS4A* gene cluster as a novel LOAD locus ( $P_M = 1.7 \times 10^{-9}$ ,  $P_J = 1.7 \times 10^{-9}$ ) (Table 1, Fig. 1A). The minor allele (MAF = 0.39) was protective with identical odds ratios (ORs) from both *meta*-analysis and joint analysis ( $OR_M$  and  $OR_J = 0.88$ , 95% CI: 0.85–0.92). In the Stage 1+2 analysis, other SNPs gave smaller  $P$  values when compared to discovery SNP rs4938933, with the most significant SNP being rs4939338 ( $P_M = 2.6 \times 10^{-11}$ ,  $P_J = 4.6 \times 10^{-11}$ ;  $OR_M$  and  $OR_J = 0.87$ , 95% CI: 0.84–0.91) (Supplementary Table 5). In the accompanying manuscript<sup>12</sup>, genome-wide significant results were also obtained at the *MS4A* locus (rs670139,  $P_M = 5.0 \times 10^{-12}$ ) using an independent sample. In a combined analysis of ADGC results and those from Hollingworth *et al.*<sup>12</sup>, the evidence for this locus at rs4938933 increased to  $P_M = 8.2 \times 10^{-12}$  (Table 3:  $OR_M = 0.89$ , 95% CI: 0.87–0.92; Fig. 1A).

SNPs in the *CD2AP* locus also met our Stage 1 criteria for additional analysis (Fig. 1B). Stage 2 data modestly strengthened this association, but the results did not reach genome-wide significance. Stage 3 analysis yielded a genome-wide significance result for rs9349407 ( $P_M = 8.6 \times 10^{-9}$ ), identifying *CD2AP* as a novel LOAD locus. The minor allele (MAF = 0.27) at this SNP increased risk for LOAD ( $OR_M = 1.11$ , 95% CI: 1.07–1.15) (Table 2, Fig. 1B).

Another locus studied further in Stages 2 and 3 centered on *EPHA1*. Previous work provided suggestive evidence that this is a LOAD risk locus, although the associations did not reach genome-wide significance ( $P = 1.7 \times 10^{-6}$ )<sup>2</sup>. Here, results from Stages 1 and 2 for SNP rs11767557, located in the promoter region of *EPHA1*, reached genome-wide significance in the joint analysis. The addition of Stage 3 results increased evidence for association ( $P_M = 6.0 \times 10^{-10}$ , Table 2, Fig. 1C). The minor allele (MAF = 0.19) for this SNP is protective ( $OR_M = 0.90$ , 95% CI: 0.86–0.93). We observed no evidence for heterogeneity at this locus (Supplementary Fig. 2D, heterogeneity  $P = 0.58$ ).

In Stages 1 and 2, strong evidence for association was also obtained for SNPs in *CD33*, a gene located approximately 6Mb from *APOE*, but the results did not reach genome-wide significance. The addition of Stage 3 data confirmed that *CD33* is a LOAD risk locus (rs3865444; Stages 1–3,  $P_M = 1.6 \times 10^{-9}$ ). The minor allele (MAF = 0.30) is protective ( $OR_M = 0.91$ , 95% CI: 0.88–0.93; Tables 1,2, Fig. 1D). A single SNP (rs3826656) in the 5' region of *CD33*, was previously reported as an AD-related locus using a family-based approach as genome-wide significant ( $P = 6.6 \times 10^{-6}$ )<sup>15</sup>. We were unable to replicate this finding ( $P_M = 0.73$ ;  $P_J = 0.39$ , Stage 1 analysis for rs3826656). Though rs3826656 is only 1,348 bp from our top SNP (rs3865444), these 2 sites display only weak LD ( $r^2 = 0.13$ ).

Hollingsworth *et al*<sup>12</sup> report highly significant evidence for the association of an *ABCA7* SNP rs3764650 with LOAD ( $P_M = 4.5 \times 10^{-17}$ ) that included data from our study. In our Stage 1+2 analysis, we obtained suggestive evidence for association with *ABCA7* SNP rs3752246 ( $P_M = 5.8 \times 10^{-7}$ , and  $P_J = 5.0 \times 10^{-7}$ ), which is a missense variant (G1527A) that may alter the function of the *ABCA7* protein (see Supplementary Table 6 for functional SNPs in LD with SNPs yielding  $P_M$  or  $P_J < 10^{-4}$ ).

Our Stage 1+2 analyses also confirmed the association of previously reported loci (*BINI*, *CRI*, *CLU*, and *PICALM*) with LOAD (Table 1). For each locus, supporting evidence was  $P = 5.0 \times 10^{-8}$  in one or both types of analysis.

We also examined SNPs with statistically significant GWAS results reported by others (*GAB2*<sup>16</sup>, *PCDH11X*<sup>17</sup>, *GOLM1*<sup>18</sup>, and *MTHFDIL*<sup>19</sup>, Supplementary Table 7). Stage 1 data were used except for *PCDH11X* where Stage 1+2 data were used because Affymetrix platforms do not contain the appropriate SNP. Only SNPs in the *APOE*, *CRI*, *PICALM*, and *BINI* loci demonstrated  $P < 10^{-6}$ . For *MTHFDIL*<sup>19</sup>, at rs11754661 (previously reported  $P = 4.7 \times 10^{-8}$ ) we obtained modest independent association evidence ( $OR_M = 1.16$ , 95% CI: 1.04–1.29,  $P_M = 0.006$ ;  $OR_J = 1.19$ , 95% CI: 1.08–1.32,  $P_J = 7.5 \times 10^{-4}$ ). For the remaining sites, only nominal evidence ( $P < 0.05$ ) or no evidence was obtained. For the *GAB2* locus<sup>16</sup> at rs10793294 (previously reported  $P = 1.60 \times 10^{-7}$ ), we obtained nominal statistical significance results ( $P_M = 0.017$ ;  $P_J = 0.029$ ). The association for rs5984894 in the *PCDH11X* locus<sup>17</sup> (previously reported  $P = 3.9 \times 10^{-12}$ ), did not replicate ( $P_M = 0.89$ ,  $P_J = 0.26$ ). Likewise, findings at *GOLM1*<sup>18</sup> for rs10868366 (previously reported  $P = 2.40 \times 10^{-4}$ ) did not replicate ( $P_M = 0.71$ ;  $P_J = 0.62$ ). Another gene consistently implicated in LOAD is *SORL1*<sup>20</sup> where at rs3781835 (previously reported  $P = 0.006$ ), we obtained modest evidence for association ( $OR_M = 0.72$ , 95% CI: 0.60–0.86,  $P_M = 2.9 \times 10^{-4}$ ;  $OR_J = 0.78$ , 95% CI: 0.59–0.86;  $P_J = 3.8 \times 10^{-4}$ ).

We examined the influence of the *APOE*  $\epsilon 4$  allele on the loci in Table 1, stratified by and in interactions with *APOE*  $\epsilon 4$  allele carrier status. After adjustment, all loci had similar effect sizes to the unadjusted analyses with some showing a modest reduction in statistical significance. We previously reported evidence for a *PICALM-APOE*<sup>21</sup> interaction using a dataset that largely overlaps with the Stage 1 dataset used here. However, using the Stage 1+2 data, we do not replicate this finding or see evidence of SNP-*APOE* interactions with Table 1 loci (data not shown).

Previous work reported an association between LOAD and chromosome 19 SNP rs597668, located 7.2 kb proximal to *EXOC3L2* and 296 kb distal of *APOE*<sup>2</sup>. While we did observe a signal for this SNP (Stage 1,  $P_M = 1.5 \times 10^{-9}$ ;  $P_J = 7.7 \times 10^{-10}$ ) and other SNPs in the *EXOC2L3-MARK4* region, evidence was completely extinguished for all SNPs after adjustment for *APOE* (Online Methods, Supplementary Table 8), suggesting that signal in this region is from *APOE*.

Our observation of genome-wide significant associations at *MS4A4A*, *CD2AP*, *EPHA1*, and *CD33* extend our understanding of the genetic architecture of LOAD and confirm the emerging consensus that common genetic variation plays a significant role in the etiology of LOAD. With our findings and those by Hollingsworth *et al.*<sup>12</sup>, there are now ten LOAD susceptibility loci (*APOE*, *CRI*, *CLU*, *PICALM*, *BINI*, *EPHA1*, *MS4A*, *CD33*, *CD2AP*, and *ABCA7*). Examining the amount of genetic effect attributable to these candidate genes, the most strongly associated SNPs at each locus other than *APOE* demonstrated population attributable fractions (PAFs) between 2.72–5.97% (Supplemental Table 9), with a cumulative PAF for non-*APOE* loci estimated to be as much as 35%; however, these estimates may vary widely between studies<sup>22</sup>, and the actual effect sizes are likely to be much smaller than those estimated here because of the ‘winner’s curse’. Also the results do not account for interaction among loci, and are not derived from appropriate population-based samples.

A recent review of GWAS studies<sup>23</sup> noted that risk alleles with small effect sizes ( $0.80 < \text{OR} < 1.2$ ) likely exist for complex diseases such as LOAD but remain undetected, even with thousands of samples, because of insufficient power<sup>24</sup>. Our discovery dataset (Stage 1; 8,309 cases and 7,366 controls), was well-powered to detect associations exceeding the statistical significance threshold of  $P < 10^{-6}$  (Supplementary Table 9). If there are many loci of more modest effects, some, but not all, will likely be detected in any one study. This likely explains the genome-wide statistical significance for the *ABCA7* locus in the accompanying manuscript<sup>12</sup>, which reaches only modest statistical significance in our dataset (rs3752246;  $P_M = 1.0 \times 10^{-5}$ ,  $P_J = 1.9 \times 10^{-5}$ ). Finding additional LOAD loci will require larger studies with increased depth of genotyping to test for the effects of both common and rare variants.

## Supplementary Material

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## Acknowledgements

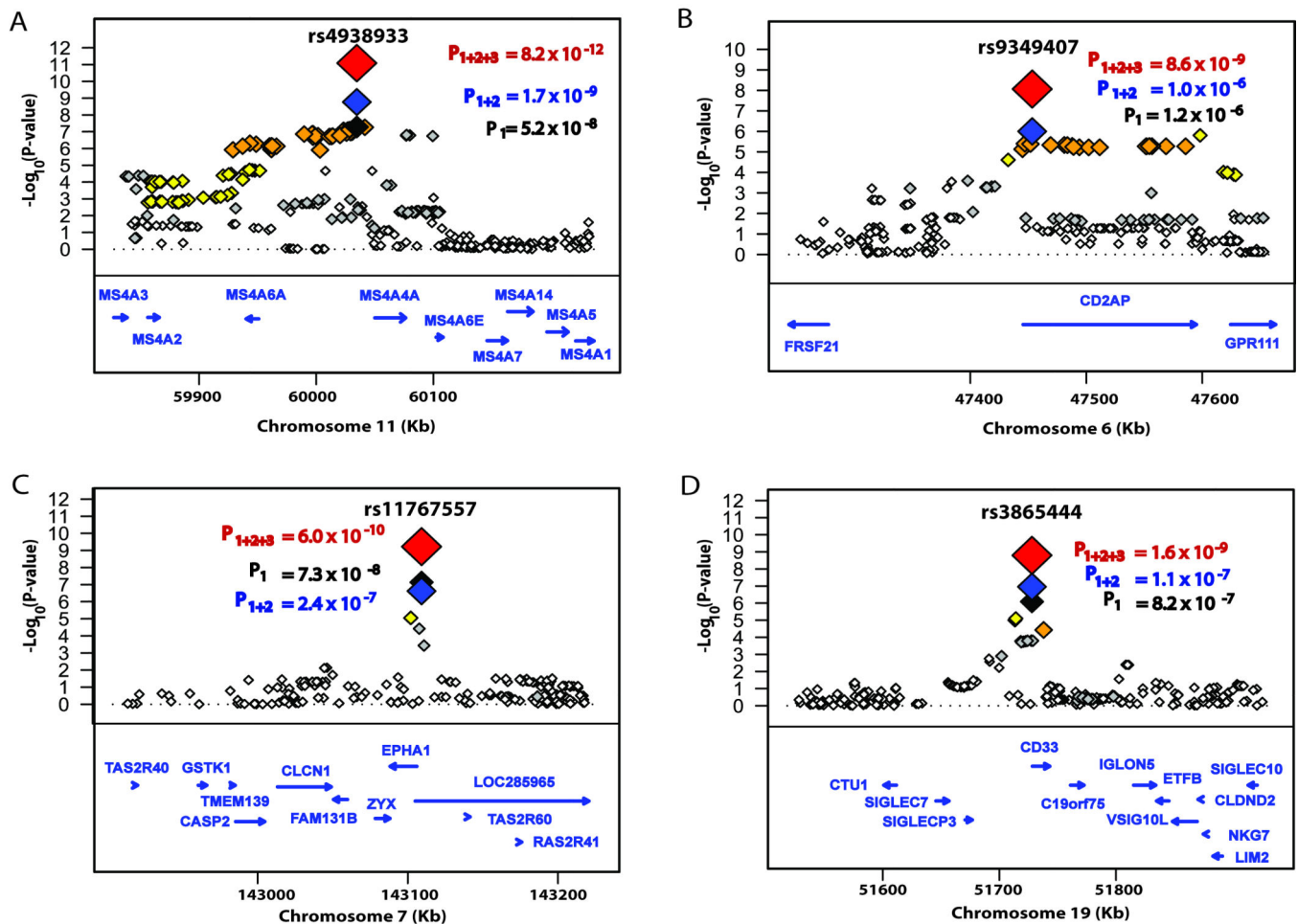
The National Institutes of Health, National Institute on Aging (NIH-NIA) supported this work through the following grants: ADGC, U01 AG032984, RC2 AG036528; NACC, U01 AG016976; NCRAD, U24 AG021886; NIA LOAD, U24 AG026395, U24 AG026390; Boston University, P30 AG013846, R01 HG02213, K24 AG027841, U01 AG10483, R01 CA129769, R01 MH080295, R01 AG009029, R01 AG017173, R01 AG025259; Columbia University, P50 AG008702, R37 AG015473; Duke University, P30 AG028377; Emory University, AG025688; Indiana University, P30 AG10133; Johns Hopkins University, P50 AG005146, R01 AG020688; Massachusetts General Hospital, P50 AG005134; Mayo Clinic, P50 AG016574; Mount Sinai School of Medicine, P50 AG005138, P01 AG002219; New York University, P30 AG08051, MO1RR00096, and UL1 RR029893; Northwestern University, P30 AG013854; Oregon Health & Science University, P30 AG008017, R01 AG026916; Rush University, P30 AG010161, R01 AG019085, R01 AG15819, R01 AG17917, R01 AG30146; University of Alabama at Birmingham, P50 AG016582, UL1RR02777; University of Arizona/TGEN, P30 AG019610, R01 AG031581, R01 NS059873; University of California, Davis, P30 AG010129; University of California, Irvine, P50 AG016573, P50, P50 AG016575, P50 AG016576, P50 AG016577; University of California, Los Angeles, P50 AG016570; University of California, San Diego, P50 AG005131; University of California, San Francisco, P50 AG023501, P01 AG019724; University of Kentucky, P30 AG028383; University of Michigan, P50 AG008671; University of Pennsylvania, P30 AG010124; University of Pittsburgh, P50 AG005133, AG030653; University of Southern California, P50 AG005142; University of Texas Southwestern, P30 AG012300; University of Miami, R01 AG027944, AG010491, AG027944, AG021547, AG019757; University of Washington, P50 AG005136, UO1 AG06781, UO1 HG004610; Vanderbilt University, R01 AG019085; and Washington University, P50 AG005681, P01 AG03991. ADNI Funding for ADNI is through the Northern California Institute for Research and Education by grants from Abbott, AstraZeneca AB, Bayer Schering Pharma AG, Bristol-Myers Squibb, Eisai Global Clinical Development, Elan Corporation, Genentech, GE Healthcare, GlaxoSmithKline, Innogenetics, Johnson and Johnson, Eli Lilly and Co., Medpace, Inc., Merck and Co., Inc., Novartis AG, Pfizer Inc, F. Hoffman-La Roche, Schering-Plough, Synarc, Inc., Alzheimer's Association, Alzheimer's Drug Discovery Foundation, the Dana Foundation, and by the National Institute of Biomedical Imaging and Bioengineering and NIA grants U01 AG024904, RC2 AG036535, K01 AG030514. We thank Creighton Phelps, Marcelle Morrison-Bogorad, and Marilyn Miller from NIA who are *ex-officio* ADGC members. Support was also from the Alzheimer's Association (LAF, IIRG-08-89720; MP-V, IIRG-05-14147) and the Veterans Affairs Administration. P.S.G.-H. is supported by Wellcome Trust, Howard Hughes Medical Institute, and the Canadian Institute of Health Research.

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**Figure 1.**

Regional association plots from the three-stage meta-analysis with LOAD.  $P_M$  values for association are shown for: (A) *MS4A* gene cluster, (B) *CD2AP*, (C) *EPHA1*, and (D) *CD33*. For each locus, the genomic position (NCBI Build 37.1) is plotted on the X-axis against  $-\log_{10}(P\text{-value})$  on the Y-axis. For the SNP with the lowest  $P$ -value at each locus in Stage 1 analyses, three  $P$ -values for association are shown:  $P_1$  meta-analysis of the ADGC Discovery (Stage 1) dataset (highlighted with a black diamond),  $P_{1+2}$  meta-analysis of the Combined ADGC Discovery and Replication (Stages 1 + 2) datasets (highlighted with a blue diamond), and  $P_{1+2+3}$  meta-analysis of the combined ADGC dataset and the external replication (Stages 1 + 2 + 3) datasets (highlighted with a red diamond). Computed estimates of linkage disequilibrium ( $r^2$ ) with the most significant SNP at each locus are shown as an orange diamond for  $r^2 \geq 0.8$ , a yellow diamond for  $0.5 \leq r^2 < 0.8$ , a grey diamond for  $0.2 \leq r^2 < 0.5$ , and a white diamond for  $r^2 < 0.2$ . Genes in each region are indicated at the bottom of each panel. The length and the direction of the arrowhead represent the scaled size and the direction of the gene, respectively.



Table 1

Genome-wide Association Results for LOAD in the ADGC Stage 1 and Stage 2 datasets

Association signals represent SNPs with the strongest associations within each locus demonstrating  $P < 10^{-6}$  in the Stage 1 dataset or in/near previously reported genes, excluding the *APOE* region (Supplementary Table 5).

SNP	CH:MB	Nearest Gene	MA	MAF	# SNPs	ADGC Discovery (Stage 1)			ADGC Replication (Stage 2)			Combined Analysis (Stages 1+2)					
						OR <sub>M</sub> (95% CI)	$P_M$	OR <sub>J</sub> (95% CI)	$P_J$	OR <sub>M</sub> (95% CI)	$P_M$	OR <sub>J</sub> (95% CI)	$P_J$	OR <sub>M</sub> (95% CI)	$P_M$	OR <sub>J</sub> (95% CI)	$P_J$
rs6701713	1:207.8	<i>CR1</i> *	A	0.20	7	1.18 1.11-1.25	$1.4 \times 10^{-8}$	1.19 1.12-1.26	$3.5 \times 10^{-9}$	1.13 1.04-1.23	0.004	1.13 1.04-1.24	0.004	1.16 1.11-1.22	$4.6 \times 10^{-10}$	1.17 1.12-1.23	$5.2 \times 10^{-11}$
rs7561528	2:127.9	<i>BIN1</i> *	A	0.35	10	1.18 1.13-1.24	$2.9 \times 10^{-11}$	1.18 1.12-1.24	$7.7 \times 10^{-11}$	1.15 1.07-1.24	$1.4 \times 10^{-4}$	1.15 1.07-1.24	$1.0 \times 10^{-4}$	1.17 1.13-1.22	$4.2 \times 10^{-14}$	1.17 1.12-1.22	$5.2 \times 10^{-14}$
rs349407	6:47.5	<i>CD2AP</i>	C	0.27	1	1.14 1.08-1.21	$1.2 \times 10^{-6}$	1.14 1.08-1.20	$5.3 \times 10^{-6}$	1.07 0.98-1.17	0.118	1.08 0.99-1.18	0.074	1.12 1.07-1.18	$1.0 \times 10^{-6}$	1.12 1.07-1.17	$2.1 \times 10^{-6}$
rs1767557	7:143.1	<i>EPHA1</i> †	C	0.19	1	0.85 0.80-0.90	$7.3 \times 10^{-8}$	0.84 0.79-0.89	$3.1 \times 10^{-8}$	0.94 0.86-1.03	0.169	0.93 0.85-1.02	0.133	0.87 0.83-0.92	$2.4 \times 10^{-7}$	0.87 0.83-0.91	$4.9 \times 10^{-8}$
rs1532278	8:27.5	<i>CLU</i> *	T	0.36	2	0.90 0.85-0.95	$5.6 \times 10^{-5}$	0.89 0.85-0.94	$2.0 \times 10^{-5}$	0.87 0.81-0.94	$2.6 \times 10^{-4}$	0.87 0.81-0.94	$2.7 \times 10^{-4}$	0.89 0.85-0.93	$8.3 \times 10^{-8}$	0.89 0.85-0.92	$1.9 \times 10^{-8}$
rs588969	10:63.6	<i>ARID5B</i>	A	0.37	0	0.88 0.84-0.93	$1.1 \times 10^{-6}$	0.88 0.84-0.93	$6.9 \times 10^{-7}$	1.05 0.97-1.13	0.234	1.05 0.98-1.13	0.189	0.93 0.89-0.97	0.001	0.93 0.89-0.97	$7.7 \times 10^{-4}$
rs938933	11:60.0	<i>MS4A4A</i>	C	0.39	22	0.88 0.84-0.92	$5.2 \times 10^{-8}$	0.87 0.83-0.92	$4.5 \times 10^{-8}$	0.90 0.84-0.97	0.005	0.90 0.84-0.97	0.004	0.88 0.85-0.92	$1.7 \times 10^{-9}$	0.88 0.85-0.92	$1.7 \times 10^{-9}$
rs561655	11:85.8	<i>PICALM</i> *	G	0.34	36	0.88 0.84-0.92	$1.2 \times 10^{-7}$	0.88 0.84-0.93	$4.6 \times 10^{-7}$	0.86 0.80-0.93	$8.4 \times 10^{-5}$	0.86 0.80-0.92	$3.7 \times 10^{-5}$	0.87 0.84-0.91	$7.0 \times 10^{-11}$	0.87 0.84-0.91	$1.0 \times 10^{-10}$
rs752246	19:1.1	<i>ABCA7</i> %	G	0.19	2	1.16 1.08-1.24	$1.0 \times 10^{-5}$	1.15 1.08-1.23	$1.9 \times 10^{-5}$	1.13 1.03-1.24	0.012	1.13 1.03-1.25	0.009	1.15 1.09-1.21	$5.8 \times 10^{-7}$	1.15 1.09-1.21	$5.0 \times 10^{-7}$
rs865444	19:51.7	<i>CD33</i> #	A	0.30	1	0.88 0.84-0.93	$8.2 \times 10^{-7}$	0.88 0.84-0.93	$1.9 \times 10^{-6}$	0.91 0.85-0.99	0.021	0.92 0.85-0.99	0.029	0.89 0.86-0.93	$1.1 \times 10^{-7}$	0.89 0.86-0.93	$2.0 \times 10^{-7}$

CH:MB, chromosome; position (in mega base pairs, build 19); MA, minor allele; MAF, minor allele frequency; # SNPs, the number of SNPs for which  $P < 1 \times 10^{-6}$  in meta-analysis from the combined analysis in Stage 1+2; OR<sub>M</sub>, odds ratio in meta-analysis;  $P_M$ ,  $P$ -value in meta-analysis; OR<sub>J</sub>, odds ratio in joint analysis;  $P_J$ ,  $P$ -value in joint analysis.

Genes with previous case-control genome-wide statistically significant associations: *CR1*\*, *CLU*\*, *PICALM*\*, *EPHA1*†. Gene with a previous association not meeting genome-wide statistical significance: *EPHA1*†. Family-based association study with reported genome-wide statistical significance: *CD33*‡.

Genes with previously published case-control association signals at  $P < 5.0 \times 10^{-8}$  are denoted with \*

the case-control locus that did not meet this level of statistical significance is denoted with †

the locus previously reported in a family-based association study as genome-wide significant with #

locus identified in Hollingworth *et al.* 12 with genome-wide significant evidence for association with. %

**Table 2**  
**Meta-Analysis of Stage 1+2 with Stage 3 (CHARGE/GERAD/EADII Consortia<sup>2</sup>) GWAS Results**

Meta-analysis using an external replication case-control sample (Stage 3) for SNPs from novel loci at which associations did not exceed the genome-wide statistical significance threshold ( $P = 5.0 \times 10^{-8}$ ) in the ADGC meta-analysis (Stage 1+2). Results for *MS4A* are also included to show association results from the ADGC and accompanying manuscript<sup>12</sup>. The external replication dataset did not include results from TGEN, ADNI, and MAYO cohorts (Supplementary Tables 1 and 2).

Gene:SNP	Cases	Controls	Total	OR <sub>M</sub> (95% CI)	P <sub>M</sub>	OR <sub>J</sub> (95% CI)	P <sub>J</sub>
<b><i>CD2AP</i>: rs9349407</b>							
ADGC	11840	10931	22771	1.12 (1.07–1.18)	$1.0 \times 10^{-6}$	1.12 (1.07–1.17)	$2.1 \times 10^{-6}$
External	6922	18896	25818	1.09 (1.03–1.15)	0.002	-	-
ADGC + External	18762	29827	48589	1.11 (1.07–1.15)	$8.6 \times 10^{-9}$	-	-
<b><i>EPHA1</i>: rs11767557</b>							
ADGC	11840	10931	22771	0.87 (0.83–0.92)	$2.4 \times 10^{-7}$	0.87 (0.83–0.91)	$4.9 \times 10^{-8}$
External	6922	24666	31588	0.91 (0.87–0.96)	$2.9 \times 10^{-4}$	-	-
ADGC + External	18762	35597	54359	0.90 (0.86–0.93)	$6.0 \times 10^{-10}$	-	-
<b><i>ARID5B</i>: rs2588969</b>							
ADGC	11840	10931	22771	0.93 (0.89–0.97)	0.001	0.93 (0.89–0.97)	$7.8 \times 10^{-4}$
External	6922	18896	25818	1.06 (1.01–1.11)	0.018	-	-
ADGC + External	18762	29827	48589	0.99 (0.95–1.02)	0.362	-	-
<b><i>MS4A4A</i>: rs4938933</b>							
ADGC	11840	10931	22771	0.88 (0.85–0.92)	$1.7 \times 10^{-9}$	0.88 (0.85–0.92)	$1.7 \times 10^{-9}$
External	6922	18896	25818	0.92 (0.88–0.97)	$5.4 \times 10^{-4}$	-	-
ADGC + External	18762	29827	48589	0.89 (0.87–0.92)	$8.2 \times 10^{-12}$	-	-
<b><i>CD33</i>: rs3865444</b>							
ADGC	11840	10931	22771	0.89 (0.86–0.93)	$1.1 \times 10^{-7}$	0.89 (0.86–0.93)	$2.0 \times 10^{-7}$
External	6922	18896	25818	0.92 (0.88–0.97)	0.002	-	-
ADGC + External	18762	29827	48589	0.91 (0.88–0.93)	$1.6 \times 10^{-9}$	-	-