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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analysis approaches were used. We obtained genome-wide significant results at MS4A4A [rs4938933; Stages 1+2, meta-analysis (PM) = 1.7 × 10^{-9}, joint analysis (PJ) = 1.7 × 10^{-9}; Stages 1–3, PM = 8.2 × 10^{-12}], CD2AP (rs9349407; Stages 1–3, PM = 8.6 × 10^{-9}), EPHA1 (rs11767557; Stages 1–3 PM = 6.0 × 10^{-10}, and CD33 (rs3865444; Stages 1–3, PM = 1.6 × 10^{-9}). We confirmed that CR1 (rs6701713; PM = 4.6 × 10^{-10}, PJ = 5.2 × 10^{-11}), CLU (rs1532278; PM = 8.3 × 10^{-8}, PJ = 1.9 × 10^{-8}), BIN1 (rs7561528; PM = 4.0 × 10^{-14}, PJ = 5.2 × 10^{-14}), and PICALM (rs561655; PM = 7.0 × 10^{-11}, PJ = 1.0 × 10^{-10}) but not EXOC3L2 are LOAD risk loci.

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\(^4\–^5\). Early work identified mutations in APP, PSEN1, and PSEN2 that cause early-onset autosomal dominant AD\(^6\–^9\) and variants in APOE that affect LOAD susceptibility\(^10\). A recent GWAS identified CR1, CLU, PICALM, and BIN1 as LOAD susceptibility loci\(^1\–^3\). However, because LOAD heritability estimates are high (\(h^2 \approx 60–80\%\))\(^11\), 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.\(^12\); 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\(^13\) and more direct correction for confounding sampling bias\(^14\). 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 ε4 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

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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 \(P \leq 5.0 \times 10^{-8}\) (MS4A, EPHA1, CD33, ARID5B, and CD2AP). Because Hollingworth et al.\(^{12}\) 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.\(^{12}\). 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 (\(OR_M = 0.88\), 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 (\(P_M = 2.6 \times 10^{-11}\), \(P_J = 4.6 \times 10^{-11}\); \(OR_M = 0.87\), 95% CI: 0.84–0.91) (Supplementary Table 5). In the accompanying manuscript\(^{12}\), 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.\(^{12}\), 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 (\(P = 1.7 \times 10^{-6}\))^2. 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 ($P = 6.6 \times 10^{-6}$) \[15\]. 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 ($r^2 = 0.13$).

Hollingworth et al \[12\] 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 (BIN1, CR1, CLU, and PICALM) with LOAD (Table 1). For each locus, supporting evidence was $P \leq 5.0 \times 10^{-8}$ in one or both types of analysis.

We also examined SNPs with statistically significant GWAS results reported by others (GAB2\[16\], PCDH11X\[17\], GOLM1\[18\], and MTHFD1L \[19\], 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, CR1, PICALM, and BIN1 loci demonstrated $P < 10^{-6}$. For MTHFD1L\[19\], 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\[16\] 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\[17\] (previously reported $P = 3.9 \times 10^{-12}$), did not replicate ($P_M = 0.89$, $P_J = 0.26$). Likewise, findings at GOLM1\[18\] 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\[20\] 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 ε4 allele on the loci in Table 1, stratified by and in interactions with APOE ε4 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\[21\] 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. 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., there are now ten LOAD susceptibility loci (APOE, CR1, CLU, PICALM, BIN1, 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, 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 noted that risk alleles with small effect sizes (0.80 < OR < 1.2) likely exist for complex diseases such as LOAD but remain undetected, even with thousands of samples, because of insufficient power. 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, 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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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 \( P \leq 1 \times 10^{-6} \) 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_M (95% CI) \( P_M \) | OR_J (95% CI) \( P_J \) | OR_M (95% CI) \( P_M \) | OR_J (95% CI) \( P_J \) |
| rs701713 | 1:207.8 | CR1*          | A  | 0.20 | 7      | 1.18 (1.11–1.25) 1.4\times 10^{-6} 1.19 | 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 | 5.2\times 10^{-11} |
| rs561528 | 2:127.9 | BIN1*         | A  | 0.35 | 10     | 1.18 (1.13–1.24) 2.9\times 10^{-11} 1.18 | 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 | 5.2\times 10^{-14} |
| rs349407 | 6:47.5 | CD2AP         | C  | 0.27 | 1      | 1.14 (1.08–1.21) 1.2\times 10^{-6} 1.14 | 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 | 2.1\times 10^{-6} |
| rs767557 | 7:143.1 | EPHA1*        | C  | 0.19 | 1      | 0.85 (0.80–0.90) 7.3\times 10^{-6} 0.84 | 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 | 4.9\times 10^{-8} |
| rs332278 | 8:27.5 | CLU*          | T  | 0.36 | 2      | 0.90 (0.85–0.95) 5.6\times 10^{-5} 0.89 | 0.87 (0.81–0.94) 0.19 | 0.87 (0.81–0.94) 0.133 | 0.89 (0.85–0.93) 8.3\times 10^{-8} 0.89 | 1.9\times 10^{-8} |
| rs588969 | 10:63.6 | ARID5B        | A  | 0.37 | 0      | 0.88 (0.84–0.93) 1.1\times 10^{-6} 0.88 | 0.87 (0.81–0.94) 0.19 | 0.87 (0.81–0.94) 0.133 | 0.93 (0.89–0.97) 0.001 0.93 | 7.7\times 10^{-4} |
| rs938933 | 11:60.0 | MS4A4A        | C  | 0.39 | 22     | 0.88 (0.84–0.92) 5.2\times 10^{-5} 0.87 | 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 | 1.7\times 10^{-9} |
| rs561655 | 11:85.8 | PICALM*       | G  | 0.34 | 36     | 0.88 (0.84–0.92) 1.2\times 10^{-7} 0.88 | 0.86 (0.80–0.93) 0.86 | 0.86 (0.80–0.92) 3.7\times 10^{-5} 0.87 | 0.84 (0.84–0.91) 7.0\times 10^{-11} 0.87 | 1.0\times 10^{-10} |
| rs572246 | 19:11  | ABCA7%        | G  | 0.19 | 2      | 1.16 (1.10–1.24) 1.0\times 10^{-5} 1.15 | 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 | 5.0\times 10^{-7} |
| rs865444 | 19:51.7 | CD33*         | A  | 0.30 | 1      | 0.88 (0.84–0.93) 8.2\times 10^{-7} 0.88 | 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 | 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 \( P \leq 1 \times 10^{-6} \) in meta-analysis from the combined analysis in Stage 1+2; OR_M, odds ratio in meta-analysis; \( P_M \), \( P \)-value in meta-analysis; OR_J, odds ratio in joint analysis; \( P_J \), \( P \)-value in joint analysis.

Genes with previous case-control genome-wide statistically significant associations: CR1*, CLU 1,3, PICALM*, BIN1*. Gene with a previous association not meeting genome-wide statistical significance: EPHA1‡. Family-based association study with reported genome-wide statistical significance: CD3315.

Genes with previously published case-control association signals at \( P \leq 5.0 \times 10^{-8} \) are denoted with *.

Genes previously reported in a family-based association study as genome-wide significant with ‡.

Genes identified in Hollingworth et al.12 with genome-wide significant evidence for association with †.
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. The external replication dataset did not include results from TGEN, ADNI, and MAYO cohorts (Supplementary Tables 1 and 2).

| Gene/SNP       | Cases | Controls | Total | $OR_M$ (95% CI) | $P_M$  | $OR_J$ (95% CI) | $P_J$  |
|----------------|-------|----------|-------|----------------|--------|----------------|--------|
| **CD2AP**: rs9349407 |       |          |       |                |        |                |        |
| 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) | $8.6 \times 10^{-9}$ | -              | -      |
| **EPHA1**: rs11767557 |       |          |       |                |        |                |        |
| 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) | $6.0 \times 10^{-10}$ | -              | -      |
| **ARID5B**: rs2588969 |       |          |       |                |        |                |        |
| 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  | -              | -      |
| **MS4A4A**: rs4938933 |       |          |       |                |        |                |        |
| 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) | $8.2 \times 10^{-12}$ | -              | -      |
| **CD33**: rs3865444 |       |          |       |                |        |                |        |
| 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) | $1.6 \times 10^{-9}$ | -              | -      |