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# Alzheimer disease risk genes and the age-at-onset phenotype

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## Abstract

Despite the recent identification of several novel risk genes for Alzheimer's disease (AD), little is known about their influence on the age-at-onset (AAO) of AD. The AAO is a phenotype with a heritable component distinct from disease risk and may be a useful trait to study in the context of developing interventions for delaying the onset of AD. We studied the influence of ten recently identified AD risk genes as well as *APOE* in relation to AAO in a large cohort of AD patients (N=2569). We find that the novel AD risk gene, *PICALM* exerts a small effect on the AAO of AD with earlier disease onset in risk allele carriers. In addition, we confirmed the previously reported association between the *APOE* ε4 allele and earlier disease onset. None of the other AD risk genes influenced AAO of AD. Our results suggest that besides *APOE*, other genes associated with AD risk do not exert large effects on the AAO phenotype of AD.

## Introduction

Recent large scale genome-wide association studies (GWAS) have identified several novel risk variants for Alzheimer's disease (AD) (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011). However, these single nucleotide polymorphisms (SNPs) occur commonly in the general population and exert small effect sizes, making it unlikely that they will be of clinical utility in predicting disease risk in older individuals (Seshadri et al., 2010). As the primary outcome in conventional GWAS in AD is the identification of variants associated with increased disease risk, the case versus control design in these studies largely ignores heritable variations in several other disease-related phenotypes that may be revealing of pathogenesis and of clinical utility. Examples of such potentially heritable phenotypes include clinical measures such as rates of cognitive decline (Ruiz et al., 2013), as well as those related to disease pathology such as brain atrophy

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(Furney et al., 2011; Meda et al., 2013; Potkin et al., 2009) amyloid deposition and tau accumulation/phosphorylation (Bekris et al., 2012; Han et al., 2010; Kim et al., 2011) (Cruchaga et al., 2013). Some recent studies have examined whether SNPs associated with AD risk were also related to other phenotypes such as rates of decline in memory performance and cerebrospinal fluid measures of AD pathology (Alexopoulos et al., 2011; Kauwe et al., 2011; Sweet et al., 2012).

Age-at-onset (AAO) of AD is a phenotype that is believed to be mediated by a heritable component distinct from disease risk (Dickson et al., 2008; Holmans et al., 2005). Modeling studies have suggested that upto 40% of variability in AAO of AD may be heritable (Daw et al., 1999; Daw et al., 2000; Li et al., 2002). *APOE* genotype, the most robust genetic factor associated with risk for AD only explains about 10% of variation in AAO of AD (Slooter et al., 1998) and it has been suggested that there might be several other loci with effect sizes on AAO comparable to that of *APOE*. In this study, we examined whether AD risk variants identified by recent large scale GWAS also influenced the AAO phenotype in AD patients. We also compared the relative contributions of these novel Alzheimer risk genes to *APOE* in explaining variation in AAO.

## Materials and methods

### Subjects

Data used in this analysis were derived from ‘ADC cohorts 1–3’ from the 29 National Institute on Aging (NIA)-funded Alzheimer Disease Centers (ADCs), with data coordinated by the National Alzheimer Coordinating Center (NACC). Access to the data was facilitated by the National Institute on Aging Genetics of Alzheimer’s Disease Data Storage Site (NIAGADS), a national genetics data repository that facilitates access of genotypic data to qualified investigators for the study of the genetics of late-onset Alzheimer's disease. Detailed descriptions of the ADC1, 2 and 3 cohorts are available at: https://www.alz.washington.edu/ and have previously been described in several publications (Beekly et al., 2007; Beekly et al., 2004; Morris et al., 2006; Weintraub et al., 2009).

### Genotyping and SNPs of interest

Methodological details on genotyping, data cleaning and quality control in the ADC samples have been described by Naj et al. in their recent publication reporting the identification of several novel AD risk variants in a large GWAS (Naj et al., 2011). Briefly, genotyping in the ADC1 and ADC2 samples was performed on Illumina 660 high-density SNP microarrays and in the ADC3 samples, on the Illumina OmniExpress platform. APOE genotyping was performed using SNPs rs7412 and rs429358. In this analysis, we selected the AD-risk variant SNPs reported in recent large GWAS to examine their effect on AAO of AD. These included SNPs in the following genes: *CLU* (rs11136000), *PICALM* (rs3851179), *BIN1* (rs744373), *CR1* (rs3818361), *ABCA7* (rs3764650), *MS4A6A* (rs610932), *MS4A4E* (rs670139), *EPHA1* (rs11767557), *CD33* (rs3865444) and *EXOC3L2* (rs597668).

The criteria we adopted for the selection of these specific SNPs in the current report were:

i. significant association with AD risk in a large index GWAS (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011) and,

ii. replication of the reported SNP's association with AD risk by independent GWAS and/or by meta -analysis of other GWAS data (Carrasquillo et al., 2011; Hu et al., 2011; Jun et al., 2010; Shang et al., 2013).

Where such independent replication for individual SNPs was not available in the case of *ABCA7* (rs3764640) and *MS4A6A/MS4A4E* (rs610932; rs670139), we selected these SNPs based solely on their reported association with AD risk in the index GWAS.

### Age at onset of AD

Data on the AAO of AD were collected in the ADC1–3 cohorts in two phases, as described in: https://www.alz.washington.edu/ and in previous publications (Beekly et al., 2007; Beekly et al., 2004; Morris et al., 2006; Weintraub et al., 2009). Phase-1 data were collected from ADC enrollees between 1984 and 2005. Phase-2 data were collected between 2005 to the present. Demographic details of subjects included in the analysis are shown in table-1. This analysis was restricted to Caucasian subjects with a diagnosis of AD.

### Statistical analysis

General linear models (GLM) were used with age at onset of AD as the dependent variable, and the number of AD-risk alleles of each gene as the main predictor in separate models. Other covariates included sex and the data collection phase. As the analysis was restricted to SNPs associated with increased risk of AD, our *a priori* hypothesis was that the number of risk alleles of each gene would be negatively correlated with the AAO of AD i.e. the presence of a greater number of AD risk alleles would be associated with an earlier AAO. We report our results as one-sided p-values for significance and after adjusting for multiple comparisons using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995).

## Results

Data on age-at-onset of AD was available in 2569 subjects (table-1). There were significant differences in the sex distribution between phase-1 and phase-2 samples (p=0.0008) with a slightly earlier AAO for males relative to females (1.92±0.31 years; p<.0001). The mean AAO of AD in phase-1 and phase-2 samples were 72.2±7.6 and 73.3±8.3 years respectively (p=0.0021). We therefore entered sex and data collection phase as covariates in our analysis on the effect of AD-risk genes on AAO.

Table-2 shows the AD risk allele frequencies in each of the genes studied. *APOE* ε4 carriers had a significantly lower AAO of AD than ε4 non-carriers with a decrease in 3.02 years in AAO for each unit increase in the number of ε4 alleles ($p_{FDR\text{-}adjusted}$<.0001). Moreover, APOE genotype explained 6.7% of variance in AAO of AD. Among the recently discovered Alzheimer risk genes, risk allele carriers of the AD variant of *PICALM* (rs3851179) showed a significantly lower AAO than non-carriers, with a decrease in 0.55 years in AAO of AD for each unit increase in the number of risk alleles ($p_{FDR\text{-}adjusted}$=0.0473). Variation at the

PICALM gene explained 0.24% of variance in AAO of AD. None of the SNPs in the other genes tested showed a significant effect on AAO of AD (table-3).

## Discussion

Recent large-scale GWAS have dramatically enhanced our knowledge about genetic contributions to risk for AD (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011). However, little is known about the influence of these risk variants on the age-at-onset of AD. The AAO phenotype, believed to be influenced by a strong genetic component, distinct from AD risk, may be useful to study in the context of disease-modifying treatments. Even as the recent failures of pivotal phase-III treatment trials in AD have spurred the field to target disease-modifying drugs at subjects in pre-symptomatic phases of the disease (Mullard, 2012), there is also growing recognition of the critical importance of studying strategies aimed at delaying the onset of AD (Brookmeyer et al., 1998; Duffy, 2005). Despite this, the AAO phenotype has received relatively little attention relative to disease risk in GWAS of AD.

In the current analysis, we confirmed the well-established association of *APOE* ε4 with AAO of AD (Blacker et al., 1997; Slooter et al., 1998) and also observed a small effect of the novel AD risk variant SNP in *PICALM* with AAO. None of the other AD risk genes were found to be associated with AAO of AD. It is interesting to note that a recent report suggested that the *PICALM* risk variant SNP rs3851179 also exerts a small effect on the rate of disease progression in AD patients (Ruiz et al., 2013; Sweet et al., 2012). Similar to our results in the current report, a recent study by Jones et al found that AD risk variants in the APOE and PICALM genes lower the age at onset of AD in patients with Down syndrome (Jones et al., 2013). While the precise mechanisms underlying the association between *PICALM* and AD pathogenesis are unclear, it has been suggested that *PICALM*, through regulation of clathrin-mediated endocytosis, is capable of influencing both APP processing and Aβ generation (Xiao et al., 2012). Another biological pathway relevant in the possible role of *PICALM* in AD pathogenesis may be through its influence on iron homeostasis and cell proliferation (Scotland et al., 2012). It must be noted that the AD risk variant *PICALM* SNP rs3851179 occurs at the 5′ end of *PICALM* and a recent report suggested that this variant may be in high linkage disequilibrium with rs592297, a known coding synonymous SNP that is part of an exonic splice enhancer in the gene (Schnetz-Boutaud et al., 2012).

Some methodological considerations are important to bear in mind in relation to estimating the AAO of AD. This measure is susceptible to multiple sources of error including variation in informants' abilities to detect changes in cognition as well as in physicians' assessments of symptom onset (Friedman, 1993; Schofield et al., 1995). It is plausible that inaccurate estimates of AAO and variation in methods used to derive the AAO across multiple centers may have decreased the power of our analysis to detect small effects on AAO exerted by the novel AD risk genes. In the context of GWAS in AD, designing studies with AAO as the primary phenotype of interest in large prospectively assessed cohorts of older individuals may prove challenging. However, such studies may be useful in identifying genetic components of a phenotype that may be amenable to interventions aimed at delaying the onset of AD.

In summary, besides confirming the established association of *APOE* genotype with AAO, we also find that the newly discovered AD risk gene, *PICALM*, exerts a small effect on AAO of AD. Our findings further strengthen the link between *PICALM* and AD pathogenesis. In showing that the majority of AD risk variants do not influence AAO of the disease, they also highlight the importance of studying AAO as a primary phenotype, distinct from AD risk, in GWAS of AD.

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## REFERENCES

Alexopoulos P, Guo LH, Kratzer M, Westerteicher C, Kurz A, Perneczky R. Impact of SORL1 single nucleotide polymorphisms on Alzheimer's disease cerebrospinal fluid markers. Dement Geriatr Cogn Disord. 2011; 32:164–170. [PubMed: 21997402]

Beekly DL, Ramos EM, Lee WW, Deitrich WD, Jacka ME, Wu J, Hubbard JL, Koepsell TD, Morris JC, Kukull WA. The National Alzheimer's Coordinating Center (NACC) database: the Uniform Data Set. Alzheimer Dis Assoc Disord. 2007; 21:249–258. [PubMed: 17804958]

Beekly DL, Ramos EM, van Belle G, Deitrich W, Clark AD, Jacka ME, Kukull WA. The National Alzheimer's Coordinating Center (NACC) Database: an Alzheimer disease database. Alzheimer Dis Assoc Disord. 2004; 18:270–277. [PubMed: 15592144]

Bekris LM, Millard S, Lutz F, Li G, Galasko DR, Farlow MR, Quinn JF, Kaye JA, Leverenz JB, Tsuang DW, et al. Tau phosphorylation pathway genes and cerebrospinal fluid tau levels in Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet. 2012; 159B:874–883. [PubMed: 22927204]

Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological). 1995; 57:289–300.

Blacker D, Haines JL, Rodes L, Terwedow H, Go RC, Harrell LE, Perry RT, Bassett SS, Chase G, Meyers D, et al. ApoE-4 and age at onset of Alzheimer's disease: the NIMH genetics initiative. Neurology. 1997; 48:139–147. [PubMed: 9008509]

Brookmeyer R, Gray S, Kawas C. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. Am J Public Health. 1998; 88:1337–1342. [PubMed: 9736873]

Carrasquillo MM, Belbin O, Hunter TA, Ma L, Bisceglio GD, Zou F, Crook JE, Pankratz VS, Sando SB, Aasly JO, et al. Replication of EPHA1 and CD33 associations with late-onset Alzheimer's disease: a multi-centre case-control study. Mol Neurodegener. 2011; 6:54. [PubMed: 21798052]

Cruchaga C, Kauwe JS, Harari O, Jin SC, Cai Y, Karch CM, Benitez BA, Jeng AT, Skorupa T, Carrell D, et al. GWAS of Cerebrospinal Fluid Tau Levels Identifies Risk Variants for Alzheimer's Disease. Neuron. 2013; 78:256–268. [PubMed: 23562540]

Daw EW, Heath SC, Wijsman EM. Multipoint oligogenic analysis of age-at-onset data with applications to Alzheimer disease pedigrees. Am J Hum Genet. 1999; 64:839–851. [PubMed: 10053019]

Daw EW, Payami H, Nemens EJ, Nochlin D, Bird TD, Schellenberg GD, Wijsman EM. The number of trait loci in late-onset Alzheimer disease. Am J Hum Genet. 2000; 66:196–204. [PubMed: 10631151]

Dickson MR, Li J, Wiener HW, Perry RT, Blacker D, Bassett SS, Go RC. A genomic scan for age at onset of Alzheimer's disease in 437 families from the NIMH Genetic Initiative. Am J Med Genet B Neuropsychiatr Genet. 2008; 147B:784–792. [PubMed: 18189239]

Duffy BH. The Alzheimer's Association national policy agenda. N C Med J. 2005; 66:24–26. [PubMed: 15786674]

Friedman WJ. Memory for the time of past events. Psychological Bulletin. 1993; 113:44–66.

Furney SJ, Simmons A, Breen G, Pedroso I, Lunnon K, Proitsi P, Hodges A, Powell J, Wahlund LO, Kloszewska I, et al. Genome-wide association with MRI atrophy measures as a quantitative trait locus for Alzheimer's disease. Mol Psychiatry. 2011; 16:1130–1138. [PubMed: 21116278]

Han MR, Schellenberg GD, Wang LS. Genome-wide association reveals genetic effects on human Abeta42 and tau protein levels in cerebrospinal fluids: a case control study. BMC Neurol. 2010; 10:90. [PubMed: 20932310]

Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet. 2009; 41:1088–1093. [PubMed: 19734902]

Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011; 43:429–435. [PubMed: 21460840]

Holmans P, Hamshere M, Hollingworth P, Rice F, Tunstall N, Jones S, Moore P, Wavrant DeVrieze F, Myers A, Crook R, et al. Genome screen for loci influencing age at onset and rate of decline in late onset Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet. 2005; 135B:24–32. [PubMed: 15729734]

Hu X, Pickering E, Liu YC, Hall S, Fournier H, Katz E, Dechairo B, John S, Van Eerdewegh P, Soares H. Meta-analysis for genome-wide association study identifies multiple variants at the BIN1 locus associated with late-onset Alzheimer's disease. PLoS One. 2011; 6:e16616. [PubMed: 21390209]

Jones EL, Mok K, Hanney M, Harold D, Sims R, Williams J, Ballard C. Evidence that PICALM affects age at onset of Alzheimer's dementia in Down syndrome. Neurobiol Aging. 2013

Jun G, Naj AC, Beecham GW, Wang LS, Buros J, Gallins PJ, Buxbaum JD, Ertekin-Taner N, Fallin MD, Friedland R, et al. Meta-analysis confirms CR1, CLU, and PICALM as alzheimer disease risk loci and reveals interactions with APOE genotypes. Arch Neurol. 2010; 67:1473–1484. [PubMed: 20697030]

Kauwe JS, Cruchaga C, Karch CM, Sadler B, Lee M, Mayo K, Latu W, Su'a M, Fagan AM, Holtzman DM, et al. Fine mapping of genetic variants in BIN1, CLU, CR1 and PICALM for association with cerebrospinal fluid biomarkers for Alzheimer's disease. PLoS One. 2011; 6:e15918. [PubMed: 21347408]

Kim S, Swaminathan S, Shen L, Risacher SL, Nho K, Foroud T, Shaw LM, Trojanowski JQ, Potkin SG, Huentelman MJ, et al. Genome-wide association study of CSF biomarkers Abeta1–42, t-tau, and p-tau181p in the ADNI cohort. Neurology. 2011; 76:69–79. [PubMed: 21123754]

Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet. 2009; 41:1094–1099. [PubMed: 19734903]

Li YJ, Scott WK, Hedges DJ, Zhang F, Gaskell PC, Nance MA, Watts RL, Hubble JP, Koller WC, Pahwa R, et al. Age at onset in two common neurodegenerative diseases is genetically controlled. Am J Hum Genet. 2002; 70:985–993. [PubMed: 11875758]

Meda SA, Koran ME, Pryweller JR, Vega JN, Thornton-Wells TA. Genetic interactions associated with 12-month atrophy in hippocampus and entorhinal cortex in Alzheimer's Disease Neuroimaging Initiative. Neurobiol Aging. 2013; 34:1518, e1519–e1518. [PubMed: 23107432]

Morris JC, Weintraub S, Chui HC, Cummings J, Decarli C, Ferris S, Foster NL, Galasko D, Graff-Radford N, Peskind ER, et al. The Uniform Data Set (UDS): clinical and cognitive variables and

descriptive data from Alzheimer Disease Centers. Alzheimer Dis Assoc Disord. 2006; 20:210–216. [PubMed: 17132964]

Mullard A. Sting of Alzheimer's failures offset by upcoming prevention trials. Nat Rev Drug Discov. 2012; 11:657–660. [PubMed: 22935790]

Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet. 2011; 43:436–441. [PubMed: 21460841]

Potkin SG, Guffanti G, Lakatos A, Turner JA, Kruggel F, Fallon JH, Saykin AJ, Orro A, Lupoli S, Salvi E, et al. Hippocampal atrophy as a quantitative trait in a genome-wide association study identifying novel susceptibility genes for Alzheimer's disease. PLoS One. 2009; 4:e6501. [PubMed: 19668339]

Ruiz A, Hernandez I, Ronsende-Roca M, Gonzalez-Perez A, Rodriguez-Noriega E, Ramirez-Lorca R, Mauleon A, Moreno-Rey C, Boswell L, Tune L, et al. Exploratory analysis of seven Alzheimer's disease genes: disease progression. Neurobiol Aging. 2013; 34:1310, e1311–e1317. [PubMed: 23036585]

Schnetz-Boutaud NC, Hoffman J, Coe JE, Murdock DG, Pericak-Vance MA, Haines JL. Identification and confirmation of an exonic splicing enhancer variation in exon 5 of the Alzheimer disease associated PICALM gene. Ann Hum Genet. 2012; 76:448–453. [PubMed: 22943764]

Schofield PW, Mosesson RE, Stern Y, Mayeux R. The age at onset of Alzheimer's disease and an intracranial area measurement. A relationship. Arch Neurol. 1995; 52:95–98. [PubMed: 7826282]

Scotland PB, Heath JL, Conway AE, Porter NB, Armstrong MB, Walker JA, Klebig ML, Lavau CP, Wechsler DS. The PICALM protein plays a key role in iron homeostasis and cell proliferation. PLoS One. 2012; 7:e44252. [PubMed: 22952941]

Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, Bis JC, Smith AV, Carassquillo MM, Lambert JC, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA. 2010; 303:1832–1840. [PubMed: 20460622]

Shang H, Fu J, Zhang XM, Song RR, Wang WZ. Association between EXOC3L2 rs597668 Polymorphism and Alzheimer's Disease. CNS Neurosci Ther. 2013

Slooter AJ, Cruts M, Kalmijn S, Hofman A, Breteler MM, Van Broeckhoven C, van Duijn CM. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol. 1998; 55:964–968. [PubMed: 9678314]

Sweet RA, Seltman H, Emanuel JE, Lopez OL, Becker JT, Bis JC, Weamer EA, DeMichele-Sweet MA, Kuller LH. Effect of Alzheimer's disease risk genes on trajectories of cognitive function in the Cardiovascular Health Study. Am J Psychiatry. 2012; 169:954–962. [PubMed: 22952074]

Weintraub S, Salmon D, Mercaldo N, Ferris S, Graff-Radford NR, Chui H, Cummings J, DeCarli C, Foster NL, Galasko D, et al. The Alzheimer's Disease Centers' Uniform Data Set (UDS): the neuropsychologic test battery. Alzheimer Dis Assoc Disord. 2009; 23:91–101. [PubMed: 19474567]

Xiao Q, Gil SC, Yan P, Wang Y, Han S, Gonzales E, Perez R, Cirrito JR, Lee JM. Role of phosphatidylinositol clathrin assembly lymphoid-myeloid leukemia (PICALM) in intracellular amyloid precursor protein (APP) processing and amyloid plaque pathogenesis. J Biol Chem. 2012; 287:21279–21289. [PubMed: 22539346]

## Table-1

| | N | Sex | AAO (SD) Range |
|---|---|---|---|
| Total | 2569 | 1182 (46%) male<br>1387 (54%) female | 72.6 (7.9)<br>43 – 97 |
| Phase 1 | 1708 | 746 (44%) male<br>962 (56%) female | 72.2 (7.6)<br>45 – 97 |
| Phase 2 | 861 | 436 (51%) male<br>425 (49%) | 73.3 (8.3)<br>43–96 |
| Diff p-value | | P=0.0008 | P=0.0021 |

Demographic details of subjects included in this analysis.

**Table-2**

| Gene | SNP | RAF | 0 RA | 1 RA | 2 RA | N missing |
|---|---|---|---|---|---|---|
| APOE | - | 0.41 | 854 (33.9%) | 1272 (50.5%) | 394 (15.6%) | 49 |
| CLU | rs11136000 | .62 | 361 (14.2%) | 1217 (47.7%) | 974 (38.2%) | 17 |
| PICALM | rs3851179 | .66 | 287 (11.2%) | 1169 (45.5%) | 1112 (43.3%) | 1 |
| BIN1 | rs744373 | .33 | 1144 (44.5%) | 1132 (44.1%) | 293 (11.4%) | 0 |
| CR1 | rs3818361 | .21 | 1599 (62.4%) | 841 (32.8%) | 124 (4.8%) | 5 |
| ABCA7 | rs3764650 | .10 | 2059 (80.2%) | 483 (18.8%) | 27 (1.1%) | 0 |
| MS4A6A | rs610932 | .59 | 414 (16.1%) | 1283 (50%) | 869 (33.9%) | 3 |
| MS4A4E | rs670139 | .41 | 638 (33.8%) | 944 (50.0%) | 305 (16.2%) | 682 |
| EPHA1 | rs11767557 | .83 | 87 (3.4%) | 724 (28.2%) | 1758 (68.4%) | 0 |
| CD33 | rs3865444 | .70 | 227 (8.8%) | 1103 (42.9%) | 1239 (48.2%) | 0 |
| EXOC3L2 | rs597668 | .20 | 1225 (64.9%) | 585 (31.0%) | 77 (4.1%) | 682 |

Characteristics of the Alzheimer’s disease risk genes analyzed in the study. SNP; single nucleotide polymorphism, RAF; risk allele frequency, RA; risk allele

**Table-3**

| Gene | dose effect (yrs) | Std err | p-value (1-side) | FDR Adjusted 1-sided p | Variance Explained in AAO |
|---|---|---|---|---|---|
| APOE | −3.02 | 0.22 | <.0001 | <.0001 | 6.70% |
| CLU | −0.26 | 0.23 | 0.1225 | 0.2820 | 0.068% |
| PICALM | −0.55 | 0.23 | 0.0086 | 0.0473 | 0.24% |
| BIN1 | −0.26 | 0.23 | 0.1282 | 0.2820 | 0.049% |
| CR1 | −0.26 | 0.26 | 0.1615 | 0.2961 | 0.011% |
| ABCA7 | −0.24 | 0.36 | 0.2484 | 0.3416 | 0.018% |
| MS4A6A | 0.022 | 0.22 | 0.5396 | 0.6596 | 0.013% |
| MS4A4E | −0.19 | 0.23 | 0.2045 | 0.3214 | 0.036% |
| EPHA1 | 0.36 | 0.28 | 0.9028 | 0.9028 | 0.064% |
| CD33 | 0.25 | 0.24 | 0.8573 | 0.9028 | 0.044% |
| EXOC3L2 | −0.39 | 0.28 | 0.0849 | 0.2820 | 0.099% |

General linear models (GLM) were used with age at onset of AD as the dependent variable, and the number of AD-risk alleles of each gene as the main predictor in separate models. Other covariates included sex and the data collection phase. The dose effect is interpreted as the estimated changes in AD age in years for each unit change in risk alleles.