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Meta-Analysis confirms *CR1*, *CLU*, and *PICALM* as Alzheimer's disease risk loci and reveals interactions with *APOE* genotypes

Dr. Gyungah Jun, PhD, Dr. Adam C. Naj, PhD, Dr. Gary W. Beecham, PhD, Dr. Li-San Wang, PhD, Ms. Jacqueline Buros, BS, Mr. Paul J. Gallins, MS, Dr. Joseph D. Buxbaum, PhD, Dr. Nilufer Ertekin-Taner, MD, PhD, Dr. M. Daniele Fallin, PhD, Dr. Robert Friedland, MD, Dr. Rivka Inzelberg, MD, Dr. Patricia Kramer, PhD, Dr. Ekaterina Rogava, PhD, Dr. Peter St George-Hyslop, MD, FRCP, ADGC, Ms. Laura B. Cantwell, MPH, Dr. Beth A. Dombroski, PhD, Dr. Andrew J. Saykin, PsyD*, Dr. Eric M. Reiman, MD, Dr. David A. Bennett, MD, Dr. John C. Morris, MD, Dr. Kathryn L. Lunetta, PhD, Dr. Eden R. Martin, PhD, Dr. Thomas J. Montine, MD, PhD, Dr. Alison M. Goate, DPhil, Dr. Deborah Blacker, MD, Dr. Debby W. Tsuang, MD, Mr. Duane Beekly, BS, Dr. L. Adrienne Cupples, PhD, Dr. Hakon Hakonarson, MD, PhD, Dr. Walter Kukull, PhD, Dr. Tatiana M. Foroud, PhD, Dr. Jonathan Haines, PhD, Dr. Richard Mayeux, MD, Dr. Lindsay A. Farrer, PhD, Dr. Margaret A. Pericak-Vance, PhD, and Dr. Gerard D. Schellenberg, PhD

Abstract

Objectives—To determine whether genotypes at *CLU*, *PICALM*, and *CR1* confer risk for Alzheimer's disease (AD) and whether risk for AD associated with these genes is influenced by *APOE* genotypes.

Design—Association study of AD and *CLU*, *PICALM*, *CR1* and *APOE* genotypes.

Setting—Academic research institutions in the United States, Canada, and Israel.

Participants—7,070 AD cases, 3,055 with autopsies, and 8,169 elderly cognitively normal controls, 1,092 with autopsies from 12 different studies, including Caucasians, African Americans, Israeli-Arabs, and Caribbean Hispanics.

Results—Unadjusted, *CLU* [odds ratio (OR) = 0.91, 95% confidence interval (CI) = 0.85 – 0.96 for single nucleotide polymorphism (SNP) rs11136000], *CR1* (OR = 1.14, CI = 1.07 – 1.22, SNP rs3818361), and *PICALM* (OR = 0.89, CI = 0.84 – 0.94, SNP rs3851179) were associated with AD in Caucasians. None were significantly associated with AD in the other ethnic groups. *APOE* ϵ 4 was significantly associated with AD (ORs from 1.80 to 9.05) in all but one small Caucasian cohort and in the Arab cohort. Adjusting for age, sex, and the presence of at least one *APOE* ϵ 4 allele greatly reduced evidence for association with *PICALM* but not *CR1* or *CLU*. Models with the main SNP effect, *APOE* ϵ 4 (+/-), and an interaction term showed significant interaction between *APOE* ϵ 4 (+/-) and *PICALM*.

Conclusions—We confirm in a completely independent dataset that *CR1*, *CLU*, and *PICALM* are AD susceptibility loci in European ancestry populations. Genotypes at *PICALM* confer risk predominantly in *APOE* ϵ 4-positive subject. Thus, *APOE* and *PICALM* synergistically interact.

Corresponding author: Gerard D. Schellenberg, Ph.D., Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Room 609B Stellar-Chance Laboratories, 422 Curie Boulevard, Philadelphia, PA 19104-6100, Phone; (215) 746-4580, FAX; (215) 898-9969, gerardsc@mail.med.upenn.edu.

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INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, affecting 5% of the population over 65 years and 30–50% over 80 years. Substantial progress was made identifying genes for rare forms of early-onset AD^{1–4} and this early success significantly contributed to biologic study on AD mechanisms and more recently multiple drug discovery approaches. Late-onset AD, the common form of the disease, has been more difficult to solve with apolipoprotein E (*APOE*) being the only confirmed susceptibility locus⁵. The combination of high-density genotyping methods, large well-characterized AD and control populations, and statistical methods to evaluate population stratification now provide the tools to identify additional genes contributing to AD risk.

Recently, two genome-wide association studies (GWAS) reported evidence that variations in *CLU* (encoding Clusterin), *PICALM* (encoding the Phosphatidylinositol Binding Clathrin Assembly protein), and *CRI* (encoding Complement Component (3b/4b) Receptor 1), confer genetic risk for AD^{6–7}. Evidence for these three loci reached genome wide significance in samples consisting of 5,964 cases and 10,188 controls (*PICALM* and *CLU*) and 5,887 cases and 8,508 controls (*CRI* and *CLU*). To analyze the role of these genes in AD risk, the Alzheimer's Disease Genetics Consortium (ADGC) performed a meta analysis using GWAS data for 15,239 subjects from 9 Northern European Whites cohorts and 5 cohorts that included African Americans, Israeli Arabs, and Caribbean Hispanics (Table 1). Genotypes for *CRI*, *CLU*, and *PICALM* were analyzed for association with AD using cohorts that are completely independent of those originally used to identify these 3 loci as AD susceptibility factors. The controls used are all elderly (age > 60 years). We also examined the interaction of *APOE* with *CRI*, *CLU*, and *PICALM* on AD risk.

METHODS

SUBJECTS

All cohorts are described in more detail in the supplementary material provide online. The **National Institute on Aging (NIA) Alzheimer's Disease Center (ADC)** subjects were ascertained, evaluated, and sampled by the clinical and neuropathology cores of the 29 NIA-funded ADCs (Table 1). Subject data data collection is coordinated by the National Alzheimer's Coordinating Center (NACC). DNA from these samples for genotyping was prepared by the National Cell Repository for Alzheimer's Disease (NCRAD). The **Alzheimer's Disease Neuroimaging Initiative (ADNI)** subjects are AD cases and controls ascertained for neuroimaging, biomarker, and genetic studies. Data used here were generated as previously described⁸ and obtained from the ADNI database (www.loni.ucla.edu/ADNI). The **Collaborative Aging and Memory Project (CAMP)** subjects are from the Amish communities of central Ohio and northern Indiana^{9–10}. The **Columbia University (CU)** subjects are a Hispanic cohort described in detail elsewhere¹¹. The **Framingham Heart Study (FHS)** is a single-site, community-based, ongoing cohort study described elsewhere^{12–14}. Phenotype and genome-wide association study (GWAS) data were from dbGaP website (<http://www.ncbi.nlm.nih.gov/gap>). The **Johns Hopkins University (JHU) subjects are from the Genetic and Environmental Risk Factors for Alzheimer's disease among African Americans (GenerAAtions) Study** identified through the electronic claims database of the Henry Ford Health System. The **MIRAGE Study** is a family-based genetic epidemiological study of AD in which AD cases and unaffected sibling controls were enrolled at 17 clinical centers in the United States, Canada, Germany, and Greece¹⁵. The **NIA-LOAD Family Study**¹⁶ cohort are families with two or more affected siblings with LOAD and unrelated, non-demented control subjects similar in age and ethnic background. One case per family was selected and controls were determined to be cognitively normal after an in-person neurological examination and were not related to a study participant. The

Oregon Health and Science University (OHSU) were recruited from aging research cohorts at 10 NIA-funded ADCs and do not overlap with other ADGC samples. The **TGEN** dataset is a publicly available sample of AD cases and controls (<http://www.tgen.org/research/index.cfm?pageid=1065>¹⁷). The **University of Miami/Vanderbilt University/Mt. Sinai School of Medicine (UM/VU/MSSM)** were new and previously published^{18–22} subjects ascertained at the University of Miami, Vanderbilt University and Mt. Sinai School of Medicine. The **Wadi Ara** dataset are from an inbred Arab community in northern Israel^{23–26}.

GENOTYPING

The cohorts used were genotyped either on Illumina or Affymetrix SNP arrays (Table 2). We selected 17 SNPs from *CR1*, *CLU*, and *PICALM* that were recently reported to be significantly associated with AD in two large GWA studies^{6–7} (Table 3). Additional genotypes were obtained using an Applied Biosystems' (ABI) TaqMan Assays including genotypes for rs7982. Genotyping for the *APOE* $\epsilon 2/\epsilon 3/\epsilon 4$ alleles was performed as described in the supplementary material provided online.

ANALYSIS

The analysis included only individuals with a censoring age of 60 years or older. The age used for cases was that most closely approximating the age of disease onset. For some cohorts, age-at-onset was ascertained while for others, only age-at-ascertainment was available. For some autopsied subjects, only age-at-death was available and was used as the censoring age. For all studies, the age used for controls was the age of last exam or death. (see also supplementary material provided online).

Imputation procedure—We imputed genotypes for all SNPs within 10Kb of the three genes using the Markov Chain haplotyping (MaCH) software²⁷ to obtain a common set of SNPs across all datasets. We imputed SNPs from both HapMap releases II and III and retained those with pairwise linkage disequilibrium (LD; $r^2 > 0.50$) for further analysis (see also the supplementary material online for more detail and for data cleaning protocols).

Population Substructure—To determine if population substructure existed in the different datasets, we used 30,000 – 100,000 SNPs with minor allele frequency (MAF) > 0.25 and minimal between-SNP linkage disequilibrium ($r^2 < 0.20$) sampled at random from the autosomes, and analyzed with the STRUCTURE software package^{28–29}. To account for population substructure in association analyses, EIGENSTRAT³⁰ was used on each cohort to generate loadings from principal components analysis on the sampled SNPs sampled (see also supplementary material online).

Statistical Analysis—Genotyped and imputed SNPs were tested for association with AD using a logistic generalized linear model (GLM) in case-control datasets and a logistic generalized estimating equation (GEE) in family-based datasets. Genotyped SNPs were coded as 0, 1, or 2 according to the number of minor alleles under the additive genetic model, whereas *APOE* was coded as 0 or 1 according to the presence or absence of the $\epsilon 4$ allele. For imputed SNPs, a quantitative estimate between 0 and 2 for the dose of the minor allele were used to incorporate the uncertainty of the imputation estimates. Regression models for each SNP without covariates were evaluated for comparison with results from the original reports^{6–7}. Additional models containing all permutations of covariates for age, gender and *APOE* $\epsilon 4$ status were also tested. Formal tests of interaction between the SNPs and *APOE* were assessed by including the main effects and an interaction term. Regression models were evaluated using the R package³¹. Heterogeneity among odds ratios was assessed using Cochran's Q , which was calculated as the weighted sum of squared

differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method. Q is distributed as a χ^2 with k (number of studies) minus 1 degrees of freedom. The I^2 statistic^{32–33} describes the percentage of variation across studies that is due to heterogeneity rather than chance and is calculated as follows: $I^2 = 100\% \times (Q - df) / Q$. I^2 is an intuitive and simple expression of the inconsistency of studies' results. Unlike Q it does not inherently depend upon the number of studies considered. SNP association results obtained from individual datasets were combined by meta-analysis using the inverse variance method implemented in the software package METAL (<http://www.sph.umich.edu/csg/abecasis/Metal/index.html>). An additive model was assumed and the association results across datasets were combined by summing the regression coefficients weighted by the inverse variance of the coefficients. The meta-analysis P-value of the association was estimated by the summarized test statistic.

RESULTS

To analyze the role *CR1*, *CLU*, and *PICALM* in AD risk, the ADGC performed a meta-analysis using phenotypes and GWAS data from 12 different cohorts (Table 1). The ADGC is a collaborative network in the United States that includes the 29 NIA-funded ADCs and numerous AD genetics investigators who are working to identify genes responsible for AD. Of 7,070 AD cases examined, 3,055 of had autopsy documentation of AD. Of the 8,169 cognitively normal elderly subjects (age >60) examined, 1,155 had autopsies documenting absence of significant AD neuropathology. The cohorts used included unrelated Caucasian cases and controls from the following sources: the NIA-funded ADCs, ADNI^{8, 34}, UM/VU/MSSM^{18–21}, TGEN¹⁷, and OHSU³⁵. Caucasian cases and controls from the following family-based studies were also included: the MIRAGE Study¹⁵, FHS^{13–14, 36}, NIA-LOAD, and CAMP^{9–10}. Populations not of Caucasian descent included African American subjects from several ADCs, a community-based (Detroit) study of AD, and the MIRAGE study¹⁵; Caribbean Hispanics from Manhattan, the Dominican Republic, and Puerto Rico; and members of a genetically isolated Arab community in Wadi Ara, Israel^{23–26}.

In each dataset, we evaluated association of AD with SNPs in or near *CR1*, *CLU*, and *PICALM* that were genotyped on various platforms or imputed (Table 2). Results were combined across datasets using a meta-analysis approach (Table 3). We analyzed each racial/ethnic group separately. In Caucasians, the largest group ($n = 5,935$ cases, 7,034 controls), we found significant evidence of association with multiple SNPs at each locus. In the unadjusted analyses, we obtained an odds ratio (OR) of 0.91 with a 95% confidence interval (CI) of (0.85 – 0.96) for *CLU* SNP rs11136000, which is comparable to the effect-size reported previously for the same SNP (ORs = 0.86⁷ and 0.91⁶). For the *CR1* SNP rs3818361, we obtained an OR of 1.14 (CI = 1.07 – 1.22) compared to the previous report of 1.19⁷. *PICALM* SNP rs3851179 had an OR of 0.89 (CI = 0.84 – 0.94) compared to 0.86 observed previously⁶. None of the SNPs were significantly associated with AD in any of the other ethnic groups analyzed together or separately, possibly due to small sizes of these groups (1,135 cases and 1,135 controls, Supplementary eTable 1).

We also examined the influence of *APOE* on the associations of the three genes with AD, since *APOE* is a known AD susceptibility locus in most ethnic groups^{5, 37} and several *APOE* genotypes have been reported to modify disease expression in persons with rare mutations in presenilin 1 (*PSEN1*)³⁸, presenilin 2 (*PSEN2*)³⁹, and the amyloid precursor protein (*APP*)^{39–40} genes. For the 13 cohorts where *APOE* genotype data were available, presence of one or more *APOE* $\epsilon 4$ alleles was significantly associated with AD (ORs ranging from 1.80 to 9.05) in all groups except the Amish and Israeli-Arabs (Table 4). We next re-evaluated the association of AD with the *CR1*, *CLU* and *PICALM* SNPs in the Caucasian cohorts adjusting for age, sex, and the presence of at least one *APOE* $\epsilon 4$ allele and

found greatly reduced evidence for association with *PICALM* after adjustment (Table 3, Supplementary eTable 2), an effect that is attributable primarily to *APOE* $\epsilon 4$ (eTable 2). To explore this effect further, we analyzed the association of *CRI*, *CLU*, and *PICALM* SNPs with AD in subgroups stratified by the presence (+) or absence (-) of the *APOE* $\epsilon 4$ allele. This analysis revealed that the association with *CLU* is evident only among $\epsilon 4$ (-) subjects, whereas the association with *PICALM* is evident only among $\epsilon 4$ (+) subjects (Table 5). Analysis of models containing terms for the main effects of each SNP and *APOE* $\epsilon 4$ (+/-), and an interaction term showed significant evidence of interaction for *APOE* $\epsilon 4$ (+/-) and seven of the nine *PICALM* SNPs with indications of a synergistic effect of these two genes on AD risk (Table 5 and Supplementary eTable 3). Interactions of *CRI* and *CLU* SNPs with *APOE* $\epsilon 4$ (+/-) were not statistically significant.

COMMENTS

Using a large multi-center dataset of AD cases and controls, we confirm that *CRI*, *CLU* and *PICALM* are AD susceptibility loci in European ancestry populations. The ORs we get for each is similar to those obtained in the original discovery cohort, suggesting that these estimates of risk are quite accurate for the Caucasian AD population, reflecting in part the large size of the cohorts used⁶⁻⁷. Clearly a large dataset is required to replicate these small-effect loci. We were unable to replicate the association of these 3 genes in the African-American, Arab, and Hispanic populations. However, further analysis is merited in these racial/ethnic groups using larger cohorts.

While this manuscript was being prepared for publication, a GWAS on AD was reported by Seshadri *et al.*⁴¹. There was some overlap in that study and ours in that the TGEN and Framingham cohorts are used in both studies. However, whereas Seshadri *et al.* used only prospectively diagnosed AD cases (n=52) and unrelated controls (n=2,091) from the Framingham Study, we included these subjects as well as prevalent and newly diagnosed cases and related controls yielding a total sample of 197 AD cases and 2,392 controls. Both studies independently confirm that *CLU* and *PICALM* are AD susceptibility genes. A primary difference between the 2 studies is that here we confirm *CRI* as an AD locus while Seshadri *et al.*⁴¹ obtained only nominal support for *CRI*.

The cohorts used here have several features worth mentioning in the context of GWAS for AD. First, the cohorts have a large number of autopsies in the cases (3,055). Because the gold standard for diagnosis is neuropathologic confirmation of AD pathology, using autopsied cases reduces etiologic heterogeneity. Second, the controls used here were elderly, of comparable age to case onset ages, and were cognitively normal. Since these subjects lived to a comparable age to cases without developing AD, the case-control contrast should be more robust than if young controls are used. In addition, cases and controls will be comparably censored at other non-AD loci responsible for common diseases of the elderly that are unrelated to AD. Third, the cohorts used here were not involved in the initial discovery of *CLU*, *CRI* and *PICALM* and thus represent a completely independent replication dataset. This is critical in terms of evaluating evidence that these genes are truly AD risk loci. The ideal controls for an AD GWAS would be subjects who were cognitively normal at death, had autopsy documentation that plaque load and tangle distribution did not reach criteria for AD pathology, and who were elderly. In autopsy series of older cognitively normal subjects, most have some NFTs and some non-neuritic, and possibly sparse neuritic amyloid deposits, but do not reach the accepted threshold for AD, although about a third of these normal subjects do meet neuropathologic criteria for AD⁴²⁻⁴⁵. In autopsy series of MCI subjects, up to two thirds of subjects have AD-level neuropathology⁴⁶. These findings give rise to the hypothesis that amyloid deposition and tangle formation begin before cognitive decline becomes detectable, an idea strengthened by recent biomarker and amyloid

imaging work⁴⁷. Thus in persons without dementia, a fraction, mostly those with MCI, will develop AD within a few years and this conversion rate increases with the age of the population, decreasing the contrast between cases and controls and reducing power. To minimize the potential confounding effect of MCI, we excluded them from these analyses and emphasized 1,155 controls with autopsy information (Table 1).

When we examined the interaction *CRI*, *CLU* and *PICALM*, and *APOE* genotypes, we detected synergy between *APOE* and *PICALM* but not with *CRI* or *CLU*. Our results show that the *PICALM* association is predominantly in subjects carrying the *APOE* ϵ 4 allele. Consistent with conclusions from previous studies showing interaction of *APOE* with *PSEN1*³⁸, *PSEN2*³⁹, and *APP*^{39–40}, our results suggest that the *APOE* and *PICALM* gene products participate in a common pathogenic pathway leading to AD. Since *PSEN1*, *PSEN2*, and *APP* are all involved in A β production, *PICALM* may also participate in this process though a more indirect involvement cannot be ruled out and the biology of these interactions remains to be determined. We did not detect an interaction of *APOE* with *CRI* or *CLU*, though this could be due to sample size, which was not large enough to detect very weak interactions. Also, since the *APOE* effect on AD risk is much stronger in young case populations³⁷, the age structure of our study and of others may not be optimal for detecting these interactions.

Our study and those from other consortia^{6,7,56} show that AD susceptibility loci can be identified by GWAS. Initial AD GWAS had sample sizes that, in comparison to those from the large consortia, were modest and inadequately powered to detect the small effect loci replicated here^{19, 48–53}. As sample sizes increase, as in other complex disorders, we expect additional loci to be identified.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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#: ADGC members

Steven E. Arnold, MD; Clinton T. Baldwin, PhD; Robert Barber, PhD; Thomas Beach, MD, PhD; Eileen H. Bigio, MD; Thomas D. Bird, MD; Adam Boxer, MD, PhD; James R. Burke, MD, PhD; Nigel Cairns, PhD FRCPath; Steven L. Carroll, MD, PhD; Helena C. Chui, MD; David G. Clark, MD; Carl W. Cotman, PhD; Jeffrey L. Cummings, MD; Charles DeCarli, MD; Ramon Diaz-Arrastia, MD, PhD; Malcolm Dick, PhD; Dennis W. Dickson, MD; William G. Ellis, MD; Kenneth B. Fallon, MD; Martin R. Farlow, MD; Steven Ferris, PhD; Matthew P. Frosch, MD, PhD; Douglas R. Galasko, MD; Marla Gearing, PhD; Daniel H. Geschwind, MD, PhD; Bernardino Ghetti, MD; Sid Gilman, MD FRCP; Bruno Giordani, PhD; Jonathan Glass, MD; Neill R. Graff-Radford, MD; Robert C. Green, MD; John H. Growdon, MD; Ronald L. Hamilton, MD; Lindy E. Harrell, MD, PhD; Elizabeth Head, PhD; Lawrence S. Honig, MD, PhD; Christine M. Hulette, MD; Bradley T. Hyman, MD, PhD; Gregory A. Jicha, MD, PhD; Lee-Way Jin, MD, PhD; Nancy Johnson, PhD; Jason Karlawish, MD; Anna Karydas, BA; Jeffrey A. Kaye, MD; Ronald Kim, MD; Edward H. Koo, MD; Neil W. Kowall, MD; James J. Lah, MD, PhD; Allan I. Levey, MD, PhD; Andrew Lieberman, MD, PhD; Oscar L. Lopez, MD; Wendy J. Mack, PhD; William Markesbery, MD; Daniel C. Marson, MD, PhD; Frank Martiniuk, PhD; Eliezer Masliah, MD; Ann C. McKee, MD; Marsel Mesulam, MD; Joshua W. Miller, PhD; Bruce L. Miller, MD; Carol A. Miller, MD; Joseph E. Parisi, MD; Daniel P. Perl, MD; Elaine Peskind, MD; Ronald C. Petersen, MD, PhD; Wayne Poon, PhD; Joseph F. Quinn, MD; Murray Raskind, MD; Barry Reisberg, MD; John M. Ringman, MD; Erik D. Roberson, MD, PhD; Roger N. Rosenberg, MD; Mary Sano, PhD; Julie A. Schneider, MD; Lon S. Schneider, MD; William Seeley, MD; Michael L. Shelanski, MD, PhD; Charles D. Smith, MD; Salvatore Spina, MD; Robert A. Stern, PhD; Rudolph E. Tanzi, PhD; John Q. Trojanowski, MD, PhD; Juan C. Troncoso, MD; Viviana M. Van Deerlin, MD, PhD; Harry V. Vinters, MD; Jean Paul Vonsattel, MD; Sandra Weintraub, PhD; Kathleen A. Welsh-Bohmer, PhD; Randall L. Woltjer, MD, PhD; Steven G. Younkin, MD, PhD.

Author Affiliations

Departments of Medicine (Genetics Program) (Dr. Jun, Ms. Buros, and Drs. Farrer and Baldwin), Ophthalmology (Dr. Jun), Biostatistics (Drs. Jun, Lunetta and Cupples), Neurology (Drs. Farrer, Green, Kowall, McKee and Stern), Genetics & Genomics and Epidemiology (Drs. Farrer and Green), and Pathology (Drs. Kowall and McKee), Boston University, Massachusetts; The John P. Hussman Institute for Human Genomics (Drs. Naj and Beecham, Mr. Gallins, and Dr. Pericak-Vance), and Dr. John T. Macdonald Foundation Department of Human Genetics (Drs. Beecham, Martin and Pericak-Vance), University of Miami, Florida; Departments of Pathology and Laboratory Medicine (Dr. Wang, Ms. Cantwell, Drs. Dombroski, Schellenberg, Trojanowski, and Van Deerlin), Psychiatry (Dr. Arnold) and Medicine (Dr. Karlawish), University of Pennsylvania School of Medicine, Philadelphia; Departments of Psychiatry (Drs. Buxbaum, Perl and Sano), Neuroscience (Drs. Buxbaum and Perl), Genetics and Genomic Sciences (Dr. Buxbaum), and Pathology (Dr. Perl), Mount Sinai School of Medicine, New York, New York; Departments of Neuroscience (Drs. Ertekin-Taner and Dickson), Neurology (Drs. Ertekin-Taner and Graff-Radford), and Pharmacology (Dr. Younkin), Mayo Clinic Jacksonville, Florida; Departments of Epidemiology (Dr. Fallin) and Pathology (Dr. Troncoso), Johns Hopkins

University, Baltimore, Maryland; Department of Neurology, University of Louisville, Kentucky (Dr. Friedland); Sheba Medical Center, Departments of Neurology and Medicine, Tel Aviv University, Israel (Dr. Inzelberg); Departments of Neurology (Drs. Kramer, Kaye and Quinn), Molecular & Medical Genetics (Dr. Kramer), Biomedical Engineering (Dr. Kaye), and Pathology (Dr. Woltjer), Oregon Health & Science University, Portland, Oregon; Centre for Research in Neurodegenerative Diseases, Department of Medicine, University of Toronto, Ontario, Canada (Drs. Rogaeva and St George-Hyslop); Cambridge Institute for Medical Research, Department of Clinical Neurosciences, University of Cambridge, United Kingdom (Dr. St George-Hyslop); Departments of Radiology and Imaging Sciences (Dr. Saykin), Medical and Molecular Genetics (Drs. Saykin, Foroud), Neurology (Dr. Farlow), and Pathology and Laboratory Medicine (Drs. Ghetti and Spina), Indiana University, Indianapolis; Arizona Alzheimer's Consortium and Banner Alzheimer's Institute and Neurogenomics Division, Translational Genomics Research Institute and Department of Psychiatry, University of Arizona, University of Arizona, Phoenix (Dr. Reiman); Civin Laboratory for Neuropathology, Banner Sun Health Research Institute, Sun City, Arizona (Dr. Beach); Rush Alzheimer's Disease Center (Dr. Bennett) and Departments of Neurological Sciences (Drs. Bennett and Schneider) and Pathology (Dr. Schneider), Rush University Medical Center, Chicago, Illinois; Departments of Neurology (Dr. Morris), Pathology and Immunology (Drs. Morris and Cairns) and Psychiatry (Dr. Goate), Washington University, St. Louis, Missouri; Department of Pathology (Dr. Montine), Psychiatry and Behavioral Sciences (Drs. Tsuang, Peskind and Raskind), National Alzheimer's Coordinating Center (Mr. Beekly and Dr. Kukull), Departments of Epidemiology (Dr. Kukull), Neurology (Dr. Bird), University of Washington, Seattle; Departments of Psychiatry and Epidemiology (Dr. Blacker), C.S. Kubik Laboratory for Neuropathology (Dr. Frosch), Departments of Neurology (Drs. Growdon, Hyman, and Tanzi), Massachusetts General Hospital, Charlestown, Massachusetts; Center for Applied Genomics, Children's Hospital of Philadelphia, Pennsylvania (Dr. Hakonarson); Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee (Dr. Haines); Sergievsky Center (Dr. Mayeux), Taub Institute (Drs. Mayeux, Honig and Vonsattel), and Departments of Neurology (Dr. Honig), and Pathology (Dr. Shelanski), Columbia University, New York, New York; Departments of Pharmacology and Neuroscience (Dr. Barber) and Neurology (Drs. Diaz-Arrastia and Rosenberg), University of Texas Southwestern, Fort Worth, Texas; Departments of Pathology (Dr. Bigio), Psychiatry and Behavioral Sciences (Dr. Johnson), and Cognitive Neurology and Alzheimer's Disease Center (Drs. Mesulam and Weintraub), Northwestern University, Chicago, Illinois; Departments of Neurology University of California San Francisco (Dr. Boxer, Ms. Karydas, and Drs. Miller and Seeley); Departments of Neurology (Drs. Clark, Harrell, Marson and Roberson), and Pathology (Drs. Carroll, Fallon), University of Alabama at Birmingham; Departments of Medicine (Drs. Burke and Welsh-Bohmer), Pathology (Dr. Hulette), and Psychiatry & Behavioral Sciences (Dr. Welsh-Bohmer), Duke University, Durham, North Carolina; Departments of Neurology (Drs. Chui and Schneider), Preventive Medicine (Dr. Mack), Pathology (Dr. Miller), and Psychiatry (Dr. Schneider), University of Southern California, Los Angeles; Institute for Memory Impairments and Neurological Disorders (Drs. Cotman, Dick, and Poon), and Departments of Molecular and Biomedical Pharmacology (Dr. Head), and Pathology and Laboratory Medicine (Dr. Kim), University of California Irvine; Department of Neurology (Drs. Cummings, Ringman and Vinters), Neurogenetics Program (Dr. Geschwind), and Department of Pathology & Laboratory Medicine (Dr. Vinters), University of California Los Angeles; Departments of Neurology (Dr. DeCarli) and Pathology and Laboratory Medicine (Drs. Ellis, Jin and Miller), University of California Davis; Departments of Psychiatry (Drs. Ferris and Reisberg) and Medicine (Dr. Martiniuk), and Alzheimer's Disease Center (Dr. Reisberg), New York University, New York; Departments of Neurosciences (Drs. Galasko, Koo and Masliah) and Pathology (Dr. Masliah), University of California San Diego; Department of Pathology and

Laboratory Medicine and Emory Alzheimer's Disease Center (Dr. Gearing), and Departments of Neurology (Drs. Glass, Lah and Levey), and Pathology (Dr. Glass), Emory University, Atlanta, Georgia; Departments of Neurology (Dr. Gilman), Psychiatry (Dr. Giordani), and Pathology (Dr. Lieberman), University of Michigan, Ann Arbor; Departments of Pathology (Neuropathology) (Dr. Hamilton) and Neurology (Dr. Lopez), University of Pittsburgh, Pennsylvania; Departments of Neurology (Drs. Jicha, Markesbery and Smith) and Pathology (Dr. Markesbery), University of Kentucky, Lexington; Departments of Anatomic Pathology, and Laboratory Medicine and Pathology (Dr. Parisi) and Neurology (Dr. Petersen) Mayo Clinic Rochester, New York.

Author Contributions

Study concept and design: Bennett, Blacker, Buxbaum, Ertekin-Taner, Farrer, Foroud, Goate, Haines, Hakonarson, Kramer, Kukull, Martin, Mayeux, Montine, Morris, Pericak-Vance, Reiman, Saykin, Schellenberg, and Tsuang. *Acquisition of data:* Arnold, Baldwin, Barber, Beach, Beekly, Bennett, Bigio, Bird, Boxer, Burke, Cairns, Cantwell, Carroll, Chui, Clark, Cotman, Cummings, DeCarli, Diaz-Arrastia, Dick, Dickson, Dombroski, Ellis, Fallin, Fallon, Farlow, Ferris, Foroud, Friedland, Frosch, Galasko, Gearing, Geschwind, Ghetti, Gilman, Giordani, Glass, Graff-Radford, Green, Growdon, Haines, Hakonarson, Hamilton, Harrell, Head, Honig, Hulette, Hyman, Inzelberg, Jicha, Jin, Johnson, Karlawish, Karydas, Kaye, Kim, Koo, Kowall, Lah, Levey, Lieberman, Lopez, Mack, Markesbery, Marson, Martiniuk, Masliah, Mayeux, McKee, Mesulam, Miller, Miller, Miller, Parisi, Perl, Peskind, Petersen, Poon, Quinn, Raskind, Reiman, Reisberg, Ringman, Roberson, Rogaeva, Rosenberg, Sano, Saykin, Schellenberg, Schneider, Schneider, Seeley, Shelanski, Smith, Spina, St George-Hyslop, Stern, Tanzi, Trojanowski, Troncoso, Van Deerlin, Vinters, Vonsattel, Weintraub, Welsh-Bohmer, Woltjer, and Younkin. *Analysis and interpretation of data:* Beecham, Buros, Cupples, Farrer, Gallins, Haines, Jun, Kramer, Kukull, Lunetta, Martin, Montine, Morris, Naj, Pericak-Vance, Schellenberg, Tsuang, and Wang. *Drafting of the manuscript:* Beecham, Bennett, Buros, Buxbaum, Ertekin-Taner, Farrer, Foroud, Gallins, Haines, Jun, Kramer, Mayeux, Morris, Naj, Pericak-Vance, Reiman, Saykin, Schellenberg, Wang. *Critical revision of the manuscript for important intellectual content:* Arnold, Baldwin, Barber, Beach, Beecham, Beekly, Bennett, Bigio, Bird, Blacker, Boxer, Burke, Buros, Buxbaum, Cairns, Cantwell, Carroll, Chui, Clark, Cotman, Cummings, Cupples, DeCarli, Diaz-Arrastia, Dick, Dickson, Dombroski, Ellis, Ertekin-Taner, Fallin, Fallon, Farlow, Farrer, Ferris, Foroud, Friedland, Frosch, Galasko, Gallins, Gearing, Geschwind, Ghetti, Gilman, Giordani, Glass, Goate, Graff-Radford, Green, Growdon, Haines, Hakonarson, Hamilton, Harrell, Head, Honig, Hulette, Hyman, Inzelberg, Jicha, Jin, Johnson, Jun, Karlawish, Karydas, Kaye, Kim, Koo, Kowall, Kramer, Kukull, Lah, Levey, Lieberman, Lopez, Lunetta, Mack, Markesbery, Marson, Martin, Martiniuk, Masliah, Mayeux, McKee, Mesulam, Miller, Miller, Miller, Montine, Morris, Naj, Parisi, Pericak-Vance, Perl, Peskind, Petersen, Poon, Quinn, Raskind, Reiman, Reisberg, Ringman, Roberson, Rogaeva, Rosenberg, Sano, Saykin*, Schellenberg, Schneider, , Schneider, Seeley, Shelanski, Smith, Spina, St George-Hyslop, Stern, Tanzi, Trojanowski, Troncoso, Tsuang, Van Deerlin, Vinters, Vonsattel, Wang, Weintraub, Welsh-Bohmer, Woltjer, and Younkin. *Statistical analysis:* Beecham, Buros, Cupples, Farrer, Gallins, Haines, Jun, Lunetta, Naj, Pericak-Vance, and Wang. *Obtained funding:* Bennett, Blacker, Farrer, Foroud, Goate, Haines, Hakonarson, Kukull, Mayeux, Montine, Morris, Pericak-Vance, Reiman, Saykin, Schellenberg, Tsuang, and Wang. *Administrative, technical, and material support:* Arnold, Baldwin, Barber, Beach, Beekly, Bigio, Bird, Boxer, Burke, Buros, Cairns, Cantwell, Carroll, Chui, Clark, Cotman, Cummings, DeCarli, Diaz-Arrastia, Dick, Dickson, Dombroski, Ellis, Fallin, Fallon, Farlow, Ferris, Friedland, Frosch, Galasko, Gallins, Gearing, Geschwind, Ghetti, Gilman, Giordani, Glass, Graff-Radford, Green, Growdon, Hakonarson, Hamilton, Harrell, Head, Honig, Hulette, Hyman, Inzelberg, Jicha, Jin,

Johnson, Karlawish, Karydas, Kaye, Kim, Koo, Kowall, Kukull, Lah, Levey, Lieberman, Lopez, Mack, Markesbery, Marson, Martiniuk, Masliah, McKee, Mesulam, Miller, Miller, Miller, Parisi, Perl, Peskind, Petersen, Poon, Quinn, Raskind, Reisberg, Ringman, Roberson, Rogaeva, Rosenberg, Sano, Schellenberg, Schneider, Schneider, Seeley, Shelanski, Smith, Spina, Stern, St George-Hyslop, Tanzi, Trojanowski, Troncoso, Van Deerlin, Vinters, Vonsattel, Weintraub, Welsh-Bohmer, Woltjer, and Younkin. *Study supervision:* Beekly, Cantwell, Farrer, Kukull, Pericak-Vance, Schellenberg.

Table 1

Sample Description

Cohort	Number of Cases	Number of autopsies	Mean onset age (SD)	Number of Controls	Number of autopsies	Mean age at last exam (SD)	Total	Percent of Ethnic group
Caucasian Subjects								
ADC	1,595	1,421	73 (7.7)	553	134	77 (8.7)	2,148	17%
ADNI	286	0	74 (8.1)	195	0	78 (5.4)	481	4%
CAMP	127	0	79 (7.9)	105	0	76 (7.8)	232	2%
FHS	197	0	83 (6.4)	2,392	0	73 (7.5)	2,589	20%
UM/VU/MSSM	1,170	370	74 (7.7)	1,169	75	74 (7.6)	2,339	18%
MIRAGE	560	0	71 (6.5)	790	0	72 (7.1)	1,350	10%
NIA LOAD	993	367	72 (6.9)	884	45	76 (8.4)	1,877	14%
OHSU	187	215	87 (7.3)	429	461	86 (7.2)	616	5%
TGEN	820	613	80 (8.3)	517	377	83 (8.9)	1,337	10%
Totals	5,935	2,986		7,034	1,092		12,969	100%
African American Subjects								
ADC	61	61	75 (7.0)	63	63	76 (6.2)	124	14%
JHU	221	0	77 (6.6)	186	0	78 (6.6)	407	45%
MIRAGE	180	0	70 (8.9)	200	0	71 (10.0)	380	42%
Totals	462	61		449	63		911	100%
Arab Subjects								
Wadi Ara	124	0	78 (7.9)	142	0	72 (6.0)	266	100%
Caribbean Hispanic Subjects								
Columbia	549	8	80 (8.0)	544	0	79 (6.4)	1,093	100%
All Ethnic Groups								
Totals	7,070	3,055		8,169	1,155		15,239	

SD: standard deviation; ADC: Alzheimer Disease Centers cohort; ADNI: Alzheimer Disease Neuroimaging Initiative cohort; FHS.: Framingham Heart Study cohort; UM/VU/MSSM: University of Miami/Vanderbilt University/Mt. Sinai School of Medicine cohort; MIRAGE: Multi Institutional Research on Alzheimer's Genetic Epidemiology cohort; NIA LOAD: National Institute on Aging Late-onset Alzheimer Disease cohort; OHSU: Oregon Health Sciences University cohort; TGEN: Translational Genomics Research Institute cohort; JHU: Johns Hopkins University cohort. Additional information on all cohorts is provided in the supplementary materials supplied online.

Table 2

GWAS genotyping platform, numbers of SNPs genotyped and imputed, and *APOE* genotype distribution for the study samples

Cohort	Genotyping platform	CRI, CLU & PICALM SNPs	
		Number genotyped [†]	Number Imputed [‡]
Caucasian Subjects			
ADC	Illumina 660Quad	11	6
ADNI	Illumina 610Quad	10	6
CAMP	Affymetrix 6.0	16	0
FHS	Affymetrix 5.0	3	13
UM/VU/MSSM	Illumina 550, 610Quad, 1M, 1M-duo; Affymetrix 6.0	17	0
MIRAGE	Illumina 660Quad	8	8
NIA LOAD	Illumina 610Quad	11	6
OHSU	Illumina 370K	9	6
TGEN	Affymetrix 500K	3	12
African American Subjects			
ADC	Illumina 660Quad	10	5
JHU	Illumina 660Quad	10	4
MIRAGE	Illumina 660Quad	8	7
Arab Subjects			
Wadi Ara	Illumina 660Quad	9	5
Caribbean Hispanic Subjects			
Columbia	Illumina 650Y	10	0

Abbreviations are as in Table 1.

[†]The number of genotyped SNPs includes SNPs on the genotyping platform and SNPs genotyped individually by TaqMan or other techniques (see supplementary material online).

[‡]The number of imputed SNPs reflects the number satisfying predetermined quality thresholds (R-squared > 0.5).

Table 3
Meta-analysis results for association of AD with SNPs in *CRI*, *CLU*, and *PICALM* in Caucasians

SNP	MA	MAF	Unadjusted			adjusted age sex & APOE			Effect direction: unadjusted/adjusted									
			OR	95% CI	P [‡]	OR	95% CI	P [‡]	ADC	ADNI	CAMP	FHS	UMVU/MSSM	MIRAGE	NIA LOAD	OHSU	TGEN	
<i>CRI</i>																		
rs3818361	A	0.26	1.14	1.07 – 1.22	6.1×10 ⁻⁵	1.15	1.07 – 1.24	0.0002	+/-	++	-/-	++	++	++	++	++	++	++
rs6701713	A	0.26	1.14	1.07 – 1.22	8.8×10 ⁻⁵	1.15	1.07 – 1.24	0.0002	+/-	++	-/-	++	++	++	++	++	++	++
rs1408077	A	0.26	1.14	1.07 – 1.22	0.0001	1.16	1.07 – 1.25	0.0002	++	++	-/-	++	++	++	++	++	++	++
<i>CLU</i>																		
rs7012010	C	0.39	1.10	1.03 – 1.17	0.0025	1.10	1.02 – 1.17	0.0081	++	++	??	+/-	++	++	++	++	-/-	++
rs3087554	C	0.16	1.00	0.92 – 1.09	0.92	0.98	0.89 – 1.08	0.71	-/-	-/+	++	++	-/-	++	++	++	++	??
rs11136000	T	0.43	0.91	0.85 – 0.96	0.0007	0.92	0.86 – 0.98	0.0096	-/-	-/-	-/-	-/+	-/+	-/+	-/-	-/-	-/-	-/+
rs9331888	G	0.25	0.99	0.92 – 1.06	0.76	0.99	0.91 – 1.07	0.74	-/-	-/-	++	++	-/-	++	++	++	++	-/-
rs7982	T	0.38	0.87	0.81 – 0.94	0.0002	0.89	0.83 – 0.97	0.0046	-/-	??	-/-	??	-/-	-/-	-/-	-/-	??	??
<i>PICALM</i>																		
rs532470	G	0.49	1.06	1.00 – 1.11	0.048	1.02	0.96 – 1.09	0.47	-/-	-/-	++	++	++	++	++	++	-/-	++
rs592297	C	0.20	0.92	0.86 – 0.99	0.02	0.96	0.89 – 1.04	0.33	-/-	++	-/-	-/-	-/+	-/-	-/+	-/+	++	-/-
rs677909	C	0.40	0.88	0.83 – 0.94	3.3×10 ⁻⁵	0.94	0.88 – 1.00	0.056	-/-	++	-/+	-/-	-/-	-/-	-/-	-/+	++	-/-
rs636848	G	0.24	1.02	0.96 – 1.08	0.6	1.00	0.93 – 1.07	0.98	-/-	-/-	++	++	-/-	-/+	++	-/+	-/-	++
rs541458	C	0.39	0.88	0.83 – 0.93	2.6×10 ⁻⁵	0.94	0.88 – 1.00	0.048	-/-	++	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
rs561655	G	0.29	0.89	0.84 – 0.94	3.4×10 ⁻⁵	0.92	0.87 – 0.99	0.017	-/-	++	-/-	-/+	-/+	-/+	-/+	-/+	-/+	-/+
rs543293	A	0.36	0.88	0.83 – 0.93	2.3×10 ⁻⁵	0.92	0.86 – 0.98	0.015	-/-	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
rs7941541	G	0.28	0.89	0.83 – 0.95	0.0007	0.95	0.88 – 1.03	0.21	-/-	-/+	-/+	-/+	-/+	-/+	-/+	-/+	??	??
rs3851179	T	0.35	0.89	0.84 – 0.94	3.9×10 ⁻⁵	0.93	0.87 – 0.99	0.026	-/-	-/+	-/+	++	-/+	-/+	-/+	-/+	-/+	-/+

MA: minor allele; MAF: weighted-average minor allele frequency; OR: odds ratio; 95% CI: 95% confidence interval; P: meta-analysis P value; ?: no data. Other abbreviations are the same as in Table 1.

[‡] P-values and odds ratios estimated under an additive model using logistic regression without covariates (unadjusted) and with covariates (adjusted for age, gender, and APOE) in a meta-analysis of nine Caucasian cohorts comprising 5,935 cases and 7,034 cognitively normal controls. Generalized Linear Models were used to estimate case-control data, and Generalized Estimating Equations were used to estimate family-based data.

Table 4

APOE genotype and allele frequencies, and odds ratios for association of $\epsilon 4$ with Alzheimer's Disease

Cohort	Subject status	n	% $\epsilon 4$ positive	<i>APOE</i> Genotype frequencies (n/total)										Association of <i>APOE</i> $\epsilon 4$ with AD [†]		
				2/2	2/3	2/4	3/3	3/4	4/4	4	3	2	1	OR	95% CI	P Value
Caucasian Subjects																
ADC	Cases	1,582	68.0	0.00	0.03	0.02	0.29	0.49	0.16	0.03	0.55	0.42	5.22	(4.21 – 6.46)	9.3×10 ⁻⁵²	
	Controls	540	28.2	0.01	0.14	0.01	0.57	0.27	0.01	0.08	0.77	0.15				
ADNI	Cases	286	67.7	0.00	0.02	0.03	0.3	0.47	0.18	0.02	0.55	0.43	4.50	(3.17 – 6.40)	5.1×10 ⁻¹⁷	
	Controls	195	26.7	0.01	0.11	0.02	0.62	0.23	0.02	0.07	0.79	0.14				
CAMP	Cases	123	36.6	0.00	0.1	0.02	0.54	0.27	0.08	0.06	0.72	0.22	1.20	0.70 – 2.07	5.1×10 ⁻⁰¹	
	Controls	102	31.7	0.00	0.11	0.02	0.58	0.28	0.02	0.06	0.77	0.17				
FHS	Cases	183	35.5	0.02	0.07	0.03	0.56	0.3	0.03	0.07	0.74	0.19	2.10	1.52 – 2.89	5.4×10 ⁻⁰⁶	
	Controls	2,284	20.8	0.00	0.13	0.02	0.66	0.17	0.02	0.08	0.81	0.12				
UM/YU/MSSM	Cases	1,162	59.4	0.00	0.04	0.02	0.37	0.43	0.15	0.03	0.60	0.37	4.45	3.78 – 5.24	4.7×10 ⁻⁷¹	
	Controls	1,137	23.2	0.01	0.12	0.02	0.64	0.2	0.02	0.08	0.80	0.12				
MIRAGE	Cases	559	58.1	0.00	0.04	0.03	0.37	0.41	0.14	0.04	0.60	0.36	1.80	1.56 – 2.07	1.2×10 ⁻¹⁵	
	Controls	788	39.5	0.00	0.08	0.02	0.52	0.31	0.07	0.05	0.72	0.23				
NIA LOAD	Cases	985	75.6	0.00	0.02	0.02	0.22	0.55	0.19	0.02	0.51	0.47	9.05	7.34 – 11.17	6.1×10 ⁻⁹⁴	
	Controls	881	25.5	0.01	0.14	0.03	0.59	0.21	0.01	0.09	0.77	0.14				
OHSU	Cases	186	40.3	0.00	0.09	0.05	0.51	0.32	0.03	0.07	0.72	0.22	2.30	1.62 – 3.24	2.4×10 ⁻⁰⁶	
	Controls	421	21.2	0.00	0.17	0.02	0.62	0.18	0.01	0.09	0.80	0.11				
TGEN	Cases	819	61.5	0.00	0.03	0.04	0.35	0.43	0.15	0.04	0.58	0.38	4.75	3.78 – 5.96	6.9×10 ⁻⁴¹	
	Controls	517	21.5	0.03	0.12	0.02	0.63	0.19	0.01	0.10	0.79	0.11				
African American Subjects																
ADC	Cases	61	70.5	0.00	0.07	0.02	0.23	0.54	0.15	0.04	0.53	0.43	3.92	2.00 – 7.67	6.7×10 ⁻⁰⁵	
	Controls	60	34.4	0.02	0.13	0.1	0.5	0.23	0.02	0.13	0.68	0.18				
JHU	Cases	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
	Controls	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
MIRAGE	Cases	180	69.4	0.00	0.03	0.03	0.28	0.49	0.17	0.03	0.54	0.43	2.17	1.65 – 2.85	2.4×10 ⁻⁰⁸	
	Controls	199	48.2	0.00	0.08	0.04	0.44	0.39	0.06	0.06	0.67	0.27				

Cohort	Subject status	n	% ε4 positive	APOE Genotype frequencies (n/total)							Association of APOE ε4 with AD [†]				
				2/2	2/3	2/4	3/3	3/4	4/4	2	3	4	OR	95% CI	P Value
Arab Subjects															
Wadi Ara	Cases	73	6.8	0.00	0.00	0.00	0.93	0.07	0	0.00	0.97	0.03	2.87	0.54 – 15.26	0.217
	Controls	80	2.5	0.00	0.00	0.00	0.98	0.03	0	0.00	0.99	0.01			
Caribbean Hispanic Subjects															
Columbia	Cases	549	40.4	0.01	0.07	0.03	0.52	0.31	0	0.06	0.71	0.23	2.16	1.67 – 2.81	4.9×10 ⁻⁰⁹
	Controls	544	23.9	0.01	0.12	0.02	0.64	0.20	0	0.08	0.80	0.13			

Abbreviations are listed in Tables 1 and 3.

Association of AD with *CRI*, *CLU*, and *PICALM* SNPs stratified by *APOE* $\epsilon 4$ carrier status and testing statistical interaction with *APOE* $\epsilon 4$ carrier status in Caucasian cohorts

Table 5

Gene/SNP	<i>APOE</i> $\epsilon 4$ (-) [†]			<i>APOE</i> $\epsilon 4$ (+) [†]			SNP* <i>APOE</i> interaction [‡]		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
<i>CRI</i>									
rs3818361	1.10	1.02 – 1.19	0.0170	1.14	1.03 – 1.26	0.0120	1.01	0.99 – 1.03	0.2800
rs6701713	1.10	1.01 – 1.19	0.0210	1.14	1.03 – 1.26	0.0110	1.01	0.99 – 1.04	0.2800
rs1408077	1.06	1.00 – 1.12	0.0360	1.15	1.03 – 1.27	0.0099	1.06	0.97 – 1.16	0.1900
<i>CLU</i>									
rs7012010	1.10	1.00 – 1.20	0.0430	1.05	1.00 – 1.10	0.0640	1.03	0.94 – 1.12	0.5100
rs3087554	1.01	0.90 – 1.14	0.8800	1.00	0.84 – 1.18	0.9700	1.00	0.82 – 1.22	1.0000
rs11136000	0.91	0.84 – 0.98	0.0150	0.93	0.84 – 1.03	0.1700	0.98	0.92 – 1.06	0.6500
rs9331888	1.03	0.93 – 1.14	0.5300	0.92	0.80 – 1.05	0.1900	0.89	0.77 – 1.04	0.1400
rs7982	0.87	0.79 – 0.97	0.0092	0.92	0.81 – 1.05	0.2200	1.06	0.91 – 1.24	0.4800
<i>PICALM</i>									
rs522470	0.99	0.92 – 1.08	0.8900	1.12	1.01 – 1.24	0.0300	1.11	0.98 – 1.25	0.1000
rs592297	1.04	0.97 – 1.11	0.3200	0.90	0.79 – 1.03	0.1200	0.85	0.73 – 1.00	0.0480
rs677909	0.99	0.91 – 1.08	0.8000	0.86	0.77 – 0.96	0.0062	0.86	0.75 – 0.98	0.0260
rs636848	0.96	0.88 – 1.06	0.4400	1.07	0.95 – 1.21	0.2700	1.07	0.92 – 1.23	0.3900
rs541458	0.99	0.91 – 1.08	0.8100	0.86	0.77 – 0.96	0.0066	0.86	0.75 – 0.98	0.0270
rs561655	0.97	0.89 – 1.06	0.5000	0.83	0.75 – 0.93	0.0009	0.82	0.73 – 0.93	0.0024
rs543293	1.00	0.92 – 1.09	0.9800	0.83	0.74 – 0.93	0.0011	0.81	0.71 – 0.93	0.0026
rs7941541	0.98	0.90 – 1.08	0.7300	0.90	0.79 – 1.02	0.0990	0.89	0.79 – 0.99	0.0360
rs3851179	0.99	0.91 – 1.07	0.7300	0.86	0.77 – 0.95	0.0034	0.84	0.74 – 0.95	0.0068

Abbreviations are as in Table 3.

[†] Meta-analysis P-values and odds ratios estimated under an additive model using logistic regression without covariates among subjects with no *APOE* $\epsilon 4$ alleles [*APOE* $\epsilon 4$ (-)] and among individuals with at least one *APOE* $\epsilon 4$ alleles (*APOE* $\epsilon 4$ (+)).

[‡] Meta-analysis P-values and ORs for the interaction term (SNP**APOE* interaction) were evaluated using logistic regression under an additive model including terms for the two main effects (SNP minor allele dosage and the presence of at least one *APOE* $\epsilon 4$ allele) and their interaction.