Title

Polygenic Overlap Between C-Reactive Protein, Plasma Lipids, and Alzheimer Disease

Journal

Circulation, 131(23)

ISSN

0009-7322

Authors

Desikan, Rahul S

Schork, Andrew J

Wang, Yunpeng

et al.

Publication Date

2015-06-09

DOI

10.1161/circulationaha.115.015489

Peer reviewed

Polygenic Overlap Between C-Reactive Protein, Plasma Lipids, and Alzheimer Disease

Rahul S. Desikan, MD, PhD; Andrew J. Schork, MS*; Yunpeng Wang, PhD*; Wesley K. Thompson, PhD; Abbas Dehghan, MD, PhD; Paul M Ridker, MD, MPH; Daniel I. Chasman, PhD; Linda K. McEvoy, PhD; Dominic Holland, PhD; Chi-Hua Chen, PhD; David S. Karow, MD, PhD; James B. Brewer, MD, PhD; Christopher P. Hess, MD, PhD; Julie Williams, PhD; Rebecca Sims, PhD; Michael C. O'Donovan, FRCPsych, PhD; Seung Hoan Choi, MA; Joshua C. Bis, PhD; M. Arfan Ikram, MD, PhD; Vilmundur Gudnason, MD, PhD; Anita L. DeStefano, PhD; Sven J. van der Lee, MD; Bruce M. Psaty, MD, PhD; Cornelia M. van Duijn, PhD; Lenore Launer, PhD; Sudha Seshadri, MD; Margaret A. Pericak-Vance, PhD; Richard Mayeux, MD; Jonathan L. Haines, PhD; Lindsay A. Farrer, PhD; John Hardy, PhD; Ingun Dina Ulstein, MD, PhD; Dag Aarsland, MD, PhD; Tormod Fladby, MD, PhD; Linda R. White, PhD; Sigrid B. Sando, MD, PhD; Arvid Rongve, MD, PhD; Aree Witoelar, PhD; Srdjan Djurovic, PhD; Bradley T. Hyman, MD, PhD; Jon Snaedal, MD; Stacy Steinberg, PhD; Hreinn Stefansson, PhD; Kari Stefansson, MD, PhD; Gerard D. Schellenberg, PhD; Ole A. Andreassen, MD, PhD; Anders M. Dale, PhD; for the Inflammation Working Group and International Genomics of Alzheimer's Disease Project (IGAP) and DemGene Investigators†

Received June 17, 2014; accepted April 6, 2015.

From Departments of Radiology (R.S.D., L.K.M., C.-H.C., D.S.K., J.B.B., A.M.D.), Cognitive Science (A.J.S., A.M.D.), Neurosciences (Y.W., D.H., J.B.B., A.M.D.), and Psychiatry (W.K.T., O.A.A.), University of California, San Diego, La Jolla; NORMENT, Institute of Clinical Medicine, University of Oslo, Norway (Y.W., C.-H.C., A.W., O.A.A., A.M.D., T.F.); Division of Mental Health and Addiction (Y.W., C.-H.C., A.W., O.A.A., A.M.D.), Norwegian Centre for Dementia Research, Department of Old Age Psychiatry (I.D.U.), and Department of Medical Genetics, (S.D.), Oslo University Hospital, Norway; Departments of Epidemiology (A.D., M.A.I., S.J.v.d.L., C.M.v.D.) and Radiology (M.A.I.), Erasmus Medical Center, Rotterdam, The Netherlands; Center for Cardiovascular Disease Prevention, Division of Preventative Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (P.M.R., D.I.C.); Department of Radiology and Biomedical Imaging, University of California, San Francisco (C.P.H.); Medical Research Council Centre for Neuropsychiatric Genetics and Genomics, Institute of Psychological Medicine and Clinical Neurosciences, Cardiff University School of Medicine, Wales, UK (J.W., R.S., M.C.O.); Department of Biostatistics, School of Public Health, Boston University, MA (S.H.C., A.L.D.); Department of Internal Medicine (J.C.B.) and Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology, and Health Services (B.M.P.), University of Washington, Seattle (J.C.B.); Icelandic Heart Association, Kopavogur, Iceland (V.G.); Faculty of Medicine, University of Iceland, Reykjavik (V.G., K.S.); National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA (A.L.D., S. Seshadri); Group Health Research Institute, Group Health Cooperative, Seattle, WA (B.M.P.); Laboratory of Epidemiology, Demography, and Biometry, Intramural Research Program, National Institute on Aging, Washington, DC (L.L.); Department of Neurology (S. Seshadri) and Departments of Medicine (Biomedical Genetics), Neurology, Ophthalmology, Biostatistics, and Epidemiology (L.A.F.), Boston University School of Medicine, MA; John P. Hussman Institute for Human Genomics, University of Miami, FL (M.A.P.-V.); Department of Neurology, Taub Institute on Alzheimer's Disease and the Aging Brain, and Gertrude H. Sergievsky Center, Columbia University, New York (R.M.); Department of Epidemiology and Biostatistics and Institute for Computational Biology, Case Western University, Cleveland, OH (J.L.H.); Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK (J.H.); Alzheimer's Disease Research Centre, Department of Neurobiology, Care Sciences and Society, Karolinska Institute, Stockholm, Sweden (D.A.); Centre for Age-Related Medicine, Stavanger University Hospital, Norway (D.A.); Department of Geriatric Psychiatry, Akershus University Hospital, Oslo, Norway (D.A.); Department of Neurology, Akershus University Hospital, Akershus, Norway (T.F.); Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway (L.R.W., S.B.S.); Department of Neurology, St. Olav's Hospital, Trondheim University Hospital, Trondheim, Norway (L.R.W., S.B.S.); Department of Psychiatry, Haugesund Hospital, Norway (A.R.); NORMENT, KG Jebsen Centre for Psychosis Research, Department of Clinical Science, University of Bergen, Norway (S.D.); Department of Neurology, Massachusetts General Hospital, Boston (B.T.H.); Department of Geriatric Medicine, University Hospital Reykjavik, Iceland (J.S.); deCODE Genetics, Reykjavik, Iceland (S. Steinberg, H.S., K.S.); and Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia (G.D.S.).

*A.J. Schork and Dr Wang contributed equally.

†A complete list of the Inflammation Working Group and IGAP and DemGene Investigators can be found in the online-only Data Supplement.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.115.015489/-/DC1.

Correspondence to Rahul S. Desikan, MD, PhD, and Anders M. Dale, PhD, Department of Radiology, University of California, San Diego, 8950 Villa La Jolla Dr, Ste C10, La Jolla, CA 92037-0841. E-mail rdesikan@ucsd.edu or amdale@ucsd.edu or Ole A. Andreassen, MD, PhD, KG Jebsen Centre for Psychosis Research, Bldg 49, Oslo University Hospital, Ullevål, Kirkeveien 166, PO Box 4956 Nydalen, 0424 Oslo, Norway. E-mail o.a.andreassen@medisin.uio.no

Background—Epidemiological findings suggest a relationship between Alzheimer disease (AD), inflammation, and dyslipidemia, although the nature of this relationship is not well understood. We investigated whether this phenotypic association arises from a shared genetic basis.

Methods and Results—Using summary statistics (*P* values and odds ratios) from genome-wide association studies of >200 000 individuals, we investigated overlap in single-nucleotide polymorphisms associated with clinically diagnosed AD and C-reactive protein (CRP), triglycerides, and high- and low-density lipoprotein levels. We found up to 50-fold enrichment of AD single-nucleotide polymorphisms for different levels of association with C-reactive protein, low-density lipoprotein, high-density lipoprotein, and triglyceride single-nucleotide polymorphisms using a false discovery rate threshold <0.05. By conditioning on polymorphisms associated with the 4 phenotypes, we identified 55 loci associated with increased AD risk. We then conducted a meta-analysis of these 55 variants across 4 independent AD cohorts (total: n=29 054 AD cases and 114 824 healthy controls) and discovered 2 genome-wide significant variants on chromosome 4 (rs13113697; closest gene, *HS3ST1*; odds ratio=1.07; 95% confidence interval=1.05–1.11; $P=2.86\times10^{-8}$) and chromosome 10 (rs7920721; closest gene, *ECHDC3*; odds ratio=1.07; 95% confidence interval=1.04–1.11; $P=3.38\times10^{-8}$). We also found that gene expression of *HS3ST1* and *ECHDC3* was altered in AD brains compared with control brains.

Conclusions—We demonstrate genetic overlap between AD, C-reactive protein, and plasma lipids. By conditioning on the genetic association with the cardiovascular phenotypes, we identify novel AD susceptibility loci, including 2 genome-wide significant variants conferring increased risk for AD. **(*Circulation*. 2015;131:2061-2069. DOI: 10.1161/CIRCULATIONAHA.115.015489.)**

Key Words: Alzheimer Disease ■ Genome-Wide Association Study ■ inflammation ■ lipids

Late-onset Alzheimer disease (AD) is the most common form of dementia, with an estimated prevalence of 30 million people worldwide, a number that is expected to quadruple in the next 40 years.[1] Given the absence of disease-modifying therapies and increasing awareness that symptoms develop over many years, there is significant interest in identifying effective strategies for AD prevention. Delaying dementia onset by a modest 2 years could potentially lower the worldwide prevalence of AD by >22 million cases over the next 40 years, resulting in significant societal savings.[1]

Clinical Perspective on p 2069

A growing body of evidence suggests an association between AD and potentially modifiable processes, including dyslipidemia and inflammation. In observational studies, high serum cholesterol levels have been associated with increased risk of AD,[2,3] and molecular[4] and biomarker findings[5] suggest that phospholipids may play an integral role in modulating AD-associated pathogenesis. Complement factors and activated microglia are established histopathological features in brains of AD patients,[6] and epidemiological studies in older individuals indicate that high serum levels of inflammatory proteins are associated with cognitive decline[7] and may predict dementia risk.[8] Genome-wide association studies (GWASs) in late-onset AD have replicated the established association with apolipoprotein E (*APOE*) and identified single-nucleotide polymorphisms (SNPs) implicated in lipid metabolism such as *CLU* and *ABCA7* and inflammatory processes such as *CR1* and *HLA-DRB5*.[9,10] In addition, a rare sequence variant in *TREM-2* with known anti-inflammatory function has recently been identified as conferring increased risk for AD.[11,12] Taken together, these findings suggest that processes involved with lipid metabolism and inflammation may also affect AD pathogenesis.

Combining GWASs from multiple disorders and phenotypes provides insights into genetic pleiotropy (defined as a single gene or variant being associated with >1 distinct phenotype) and could elucidate shared pathobiology. Using this approach, we have recently reported genetic overlap between a number of diseases and phenotypes and identified novel common variants associated with schizophrenia,[13,14] bipolar disorder,[13] prostate cancer,[15] hypertension,[16] and primary sclerosing cholangitis.[17] Here, we applied this method to AD, taking advantage of several large GWASs,[18–20] to identify SNPs associating with clinically diagnosed AD, C-reactive protein (CRP) levels, and plasma lipid levels (specifically triglycerides, high-density lipoprotein [HDL], and low-density lipoproteins [LDL]).

Methods

Participant Samples

We evaluated complete GWAS results in the form of summary statistics (*P* values and odds ratios [ORs]) for clinically diagnosed AD,[18] CRP levels,[19] and plasma lipid levels (triglycerides, HDL, and LDL[20]; Table 1). The CRP GWAS summary statistic data consisted of 82 725 individuals drawn from 25 studies with genotyped or imputed data at 2 671 742 SNPs (for additional details, see Reference 19). The plasma lipid GWAS summary statistic data consisted of 188 577 individuals with genotyped or imputed data at 2 508 375 SNPs (for additional details, see reference 20). We obtained publicly available AD GWAS summary statistic data from the International Genomics of Alzheimer's Disease Project (IGAP stage 1 and 2; for additional details, see the online-only Data Supplement and Reference 18). We used IGAP stage 1 as our discovery cohort, which consisted of 17 008 AD cases (mean age, 74.7±7.7 years; 59.4% female) and 37 154 controls (mean age, 76.3±8.1 years; 58.6% female) drawn from 4 different consortia across North America and Europe with genotyped or imputed data at 7 055 881 SNPs (for a description of the AD cases and controls within the IGAP stage 1 substudies, see Reference 18). To confirm our findings from IGAP stage 1, we assessed the *P* values of pleiotropic SNPs (conditional false discovery rate [FDR] <0.05; see Statistical Analysis) from the discovery analyses in 3 independent

Table 1. Summary Data From All GWASs Used in the Present Study

Disease/Trait	n	SNPs, n	Reference
AD, IGAP stages 1+2	74 046 (25 580 AD cases+48 466 controls)	7 055 881 (stage 1)+11 632 (stage 2)	Lambert et al[18]
AD, deCODE	67 817 (2470 cases+65 357 controls)	Whole-genome sequencing	Jonsson et al[12]
AD, DemGene	2015 (1004 cases+1011 controls)	693 377	N/A
TG	188 577	2 508 369	Teslovich et al[20]
LDL	188 577	2 508 375	
HDL	188 577	2 508 370	
CRP	82 725	2 671 742	Dehghan et al[19]

AD indicates Alzheimer disease; CRP, C-reactive protein; HDL, high-density lipoprotein; IGAP, International Genomics of Alzheimer's Disease Project; LDL, low-density lipoprotein; and TG, triglycerides.

AD cohorts, namely the IGAP stage 2 sample, a cohort of AD cases and controls drawn from the population of Iceland (deCODE), and a cohort of AD cases and controls drawn from the population of Norway (DemGene). The IGAP stage 2 sample consisted of 8572 AD cases (mean age, 72.5±8.1 years; 61% female) and 11 312 controls (mean age, 65.5±8.0 years; 43.3% female) of European ancestry with genotyped data at 11 632 SNPs (for additional details, see Reference 18). Clinical diagnosis of probable AD within the IGAP stage 2 cohort was established according to the *Diagnostic and Statistical Manual of Mental Disorders–3rd Edition Revised* and National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[21] The deCODE data set, drawn from the Icelandic population, included 2470 genotyped AD cases (age, 84.9±7.2 years; 65.8% female) and 65 347 genotyped controls (age, 68.8±13.7 years; 57.8% female; for additional details, see Reference 12). As previously described,[12] patients from Iceland were diagnosed with definite, probable, or possible AD on the basis of the NINCDS-ADRDA criteria[21] or according to guidelines for *International Classification of Diseases, 10th Revision* F00 and were compared with population controls. The Norwegian sample (DemGene) included 1004 cases (age, 74.1±9.6 years; 60.2% female) and 1011 controls (age, 74.6±9.3 years; 57.7% female) with genotyped data at 693 377 SNPs. Clinical diagnosis of AD and dementia within the DemGene sample was established with *International Classification of Diseases, 10th Revision* research criteria,[22] the recommendations from the National Institute on Aging-Alzheimer's Association criteria,[23] or the NINCDS-ADRDA criteria[21] (online-only Data Supplement). The relevant institutional review boards or ethics committees approved the research protocol of the individual GWAS used in the present analysis, and all human participants gave written informed consent.

For gene expression analyses, we used publicly available total RNA expression data from 1647 autopsied brain tissues (from the dorsolateral prefrontal cortex, visual cortex, and cerebellum) in 549 brains of 376 AD patients and 173 nondemented healthy controls from the Gene Expression Omnibus data set GSE44772.[24] As described previously,[24] all subjects were diagnosed at intake, and each brain underwent extensive neuropathology examination. Tissues were profiled on a custom-made Agilent 44K array of 40 638 DNA probes.

Statistical Analysis

Using recently developed statistical methods to evaluate pleiotropic effects,[13–17] we evaluated SNPs associating with AD (discovery cohort, IGAP stage 1) and CRP levels, as well as AD and plasma lipid levels. For given associated phenotypes A and B, pleiotropic enrichment of phenotype A with phenotype B exists if the proportion of SNPs or genes associated with phenotype A increases as a function of increased association with phenotype B. To assess for enrichment, we constructed fold-enrichment plots of nominal $-\log_{10}(P)$ values for all AD SNPs and for subsets of SNPs determined by the significance of their associations with CRP and plasma lipids. We also used conditional quantile-quantile plots, which are complementary to fold-enrichment plots and provide visualization of polygenic enrichment (for additional details, see the online-only Data Supplement). In fold-enrichment plots, the presence of enrichment is reflected as an upward deflection of the curve for phenotype A if the degree of deflection from the expected null line is dependent on the degree of association with phenotype B. To assess for polygenic effects below the standard GWAS significance threshold, we focused the fold-enrichment plots on SNPs with nominal $-\log_{10}(P)<7.3$ (corresponding to $P>5\times10^{-8}$). The enrichment seen can be directly interpreted in terms of true discovery rate (true discovery rate=1−FDR; for additional details, see the online-only Data Supplement).

To identify specific loci, we computed conditional FDRs.[13,14] The standard FDR framework derives from a model that assumes that the distribution of test statistics in a GWAS can be formulated as a mixture of null and nonnull effects, with true associations (nonnull effects) having more extreme test statistics, on average, than false associations (null effects). The FDR can be interpreted as the probability that an SNP is null given that its P value is as small or smaller than its observed P value. The conditional FDR is an extension of the standard FDR, which incorporates information from GWAS summary statistics of a second phenotype to adjust its significance level. The conditional FDR is defined as the probability that an SNP is null in the first phenotype given that the P values in the first and second phenotypes are as small as or smaller than the observed values. It is important to note that ranking SNPs by standard FDRs or by P values gives the same ordering of SNPs. In contrast, if the primary and secondary phenotypes are related genetically, conditional FDR reorders SNPs and results in a different ranking than that based on P values alone. We used an overall FDR threshold of <0.05, which means 5 expected false discoveries per 100 reported. Additionally, we constructed Manhattan plots based on the ranking of conditional FDR to illustrate the genomic location. In all analyses, we controlled for the effects of genomic inflation by using intergenic SNPs (see the online-only Data Supplement). Detailed information on fold-enrichment and conditional quantile-quantile plots, Manhattan plots, and conditional FDR can be found in the online-only Data Supplement and prior reports.[13–17]

For loci with conditional FDR <0.05, we performed a fixed-effects, inverse variance-weighted meta-analysis[25] across all available AD cohorts (IGAP stages 1 and 2, deCODE, and DemGene; total: n=29 054 AD cases and 114 824 healthy controls) using the R package meta (http://CRAN.R-project.org/package=meta). Briefly, the fixed-effects, inverse variance-weighted meta-analysis summarizes the combined statistical support across independent studies under the assumption of homogeneity of effects. Individual study β estimates (log ORs) are averaged, weighted by the estimated standard error.[26] The IGAP stage 1 and 2 β estimates and standard errors were obtained from the publicly available summary statistics (for additional details, see the online-only Data Supplement and the Supplementary Note in

Reference18). For the DeCODE and DemGene cohorts, β estimates and standard errors were estimated via logistic regression, predicting AD case/control status from SNP risk alleles count and adjusting for appropriate covariates, including principal components.

For the gene expression analyses, we focused on transcript expression (total RNA levels) of genes closest (within 500 kB) to the SNPs reaching genome-wide significance in our meta-analysis. Using logistic regression, we examined whether transcript expression of these genes significantly differed between AD cases and controls.

Results

We observed SNP enrichment for AD (IGAP stage 1, discovery cohort) across different levels of significance, with CRP, triglycerides, HDL, and LDL levels indicating a genetic association between AD and the 4 cardiovascular phenotypes (Figure 1). For progressively stringent *P* value thresholds for AD SNPs [ie, increasing values of nominal $-\log_{10}(P)$], we found at least 50-fold enrichment using CRP, 30-fold enrichment using triglycerides, 20-fold enrichment using HDL, and 40-fold enrichment using LDL (Figure 1). Conditional quantile-quantile plots similarly demonstrated polygenic enrichment in AD as a function of CRP and plasma lipids (Figure I in the online-only Data Supplement).

To identify AD-associated polymorphisms that are more likely to replicate, we ranked IGAP stage 1 AD SNPs conditional on their genetic association with CRP and plasma lipids (conditional FDR). We restricted our analyses to SNPs found in both IGAP stages 1 and 2 and focused on those AD variants that have not previously been described at a genome-wide significant level. At a conditional FDR <0.05, we found 55 AD susceptibility loci from IGAP stage 1 (Figure 2 and Table I in the online-only Data Supplement). For these 55 loci, we performed a meta-analysis across all available AD cohorts and found 2 novel genome-wide significant ($P<5\times10^{-8}$) loci associated with increased risk for AD (Table 2). These 2 variants are rs13113697 (chromosome 4; closest gene, *HS3ST1*; conditioning trait, triglycerides; reference allele, T; OR=1.07; 95% CI=1.05–1.11; $P=2.86\times10^{-8}$; Figures 3A and 4A) and rs7920721 (chromosome 10; closest gene, *ECHDC3*; conditioning trait, triglycerides; reference allele, G; OR=1.07; 95% CI=1.04–1.11; $P=3.38\times10^{-8}$; Figures 3B and 4B).

The meta-analysis also revealed 3 suggestive AD susceptibility loci with values of $P<1\times10^{-6}$ (Table 3 and Figure II in the online-only Data Supplement). These 3 loci are rs7396366 (on chromosome 11; closest gene, *AP2A2*; conditioning trait, CRP; reference allele, C; OR=0.94; 95% CI=0.92–0.96;

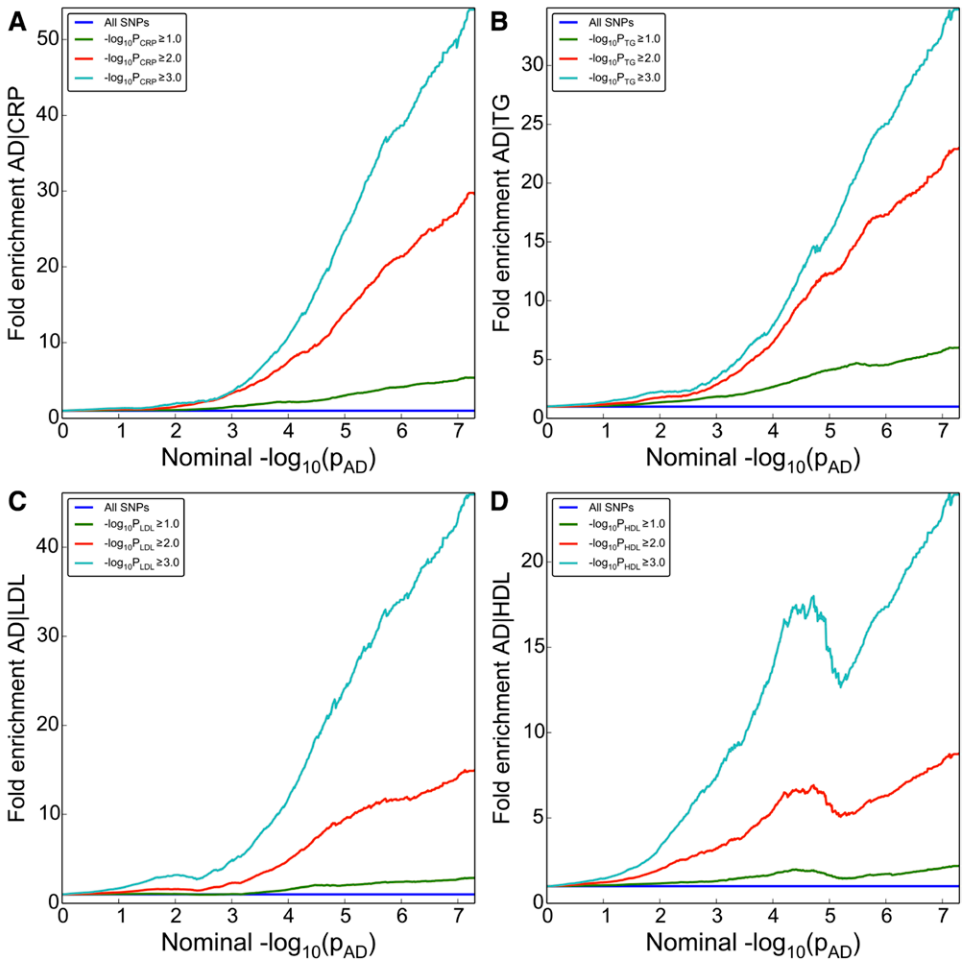

Figure 1. Fold-enrichment plots of enrichment vs nominal $-\log_{10} P$ values (corrected for inflation) in Alzheimer disease (AD) below the standard genome-wide association study threshold of $P<5\times10^{-8}$ as a function of significance of association with C-reactive protein (CRP; **A**), high-density lipoprotein (HDL; **B**), low-density lipoprotein (LDL; **C**), and triglycerides (TG; **D**) at the level of $-\log_{10}(P)\geq0$, $-\log_{10}(P)\geq1$, and $-\log_{10}(P)\geq2$ corresponding to $P\leq1$, $P\leq0.1$, and $P\leq0.01$, respectively. Blue line indicates all single-nucleotide polymorphisms (SNPs).

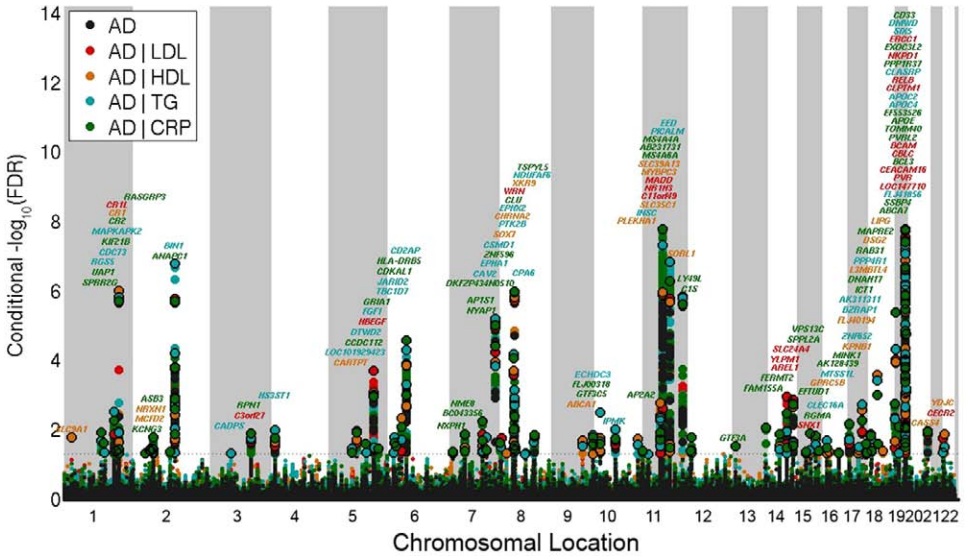

Figure 2. Conditional Manhattan plot of conditional $-\log_{10}$ (false discovery rate [FDR]) values for Alzheimer disease (AD) alone (International Genomics of Alzheimer's Disease Project [IGAP] stage 1 AD cohort; black) and AD given C-reactive protein (CRP; AD CRP; green), triglycerides (TG; AD TG; aquamarine), high-density lipoprotein (HDL; AD HDL; orange), and low-density lipoprotein (LDL; AD LDL; red). Single-nucleotide polymorphisms (SNPs) with conditional $-\log_{10}$ FDR >1.3 (ie, FDR <0.05) are shown with large points. A black line around the large points indicates the most significant SNP in each linkage disequilibrium block, and this SNP was annotated with the closest gene, which is listed above the symbols in each locus. For additional details, see the online-only Data Supplement.

P=6.8×10^{-7}), rs3131609 (on chromosome 15; closest gene, *USP50*; conditioning trait, CRP; reference allele, C; OR=0.93; 95% CI=0.91–0.96; P=7.21×10^{-7}), and rs2526378 (on chromosome 17; closest gene, *BZRAP1*; conditioning trait, triglycerides; reference allele, G; OR=0.94; 95% CI=0.92–0.96; P=2.73×10^{-7}).

We additionally evaluated the directionality of allelic effects in SNPs associated with AD and the 4 cardiovascular phenotypes (SNPs with conditional FDR <0.05). Across all 55 shared loci, we found the same direction of effect between SNPs associated with AD and CRP in 72% (18 of 25; P=0.02), HDL in 40% (4 of 10; P=0.62), LDL in 20% (1 of 5; P=0.81), and triglycerides in 40% (6 of 15; P=0.69; Table I in the online-only Data Supplement). For *HS3ST1* and *ECHD3* variants, we found an opposite direction of allelic effect between increased AD risk and triglyceride levels (Table I in the online-only Data Supplement).

We assessed whether *HS3ST1* and *ECHD3* transcript levels are altered in AD brains compared with control brains (Gene Expression Omnibus data set GSE 4472). We found significantly decreased *HS3ST1* transcript expression (standardized β coefficient=−0.09201; SE=0.01864; P=9.99×10^{-7}) and significantly increased *ECHDC3* transcript expression (standardized β coefficient=0.12715; SE=0.01829; P=8.32×10^{-12}) in AD brains compared with control brains.

Discussion

In this study, we show that polymorphisms associated with CRP and plasma lipids (triglycerides, HDL, and LDL) are also associated with increased risk for AD (genetic pleiotropy). We found that genetic enrichment in AD based on SNP association with cardiovascular phenotypes results in improved statistical power for gene discovery. By conditioning on polymorphisms associated with CRP and plasma lipid levels, we identified 55 AD susceptibility loci. In meta-analyses across 4 independent cohorts, we found that 2 of these risk variants, namely rs13113697 (on chromosome 4; closest gene, *HS3ST1*) and rs7920721 (on chromosome 10; closest gene, *ECHDC3),* were genome-wide significant. We additionally observed that *HS3ST1* and *ECHDC3* transcript expression was different in AD brains compared with control brains.

Our findings provide novel insights into the relationship among AD pathogenesis, inflammation, and dyslipidemia beyond the known loci associated with AD. We found a consistent direction of allelic effect between SNPs associated with AD risk and CRP levels, indicating overlapping pathobiology

Table 2. New Loci Reaching Genome-Wide Significance at Conditional FDR <0.05 (ORs Provided for the Reference Allele)

SNP	Position	Chromosome	Nearest Gene	Reference Allele	Associated Phenotype	Minimum Conditional FDR	IGAP Stage 1+2 *P* Value	IGAP Stage 1+2 OR (95%CI)	deCODE *P* Value	deCODE OR (95% CI)	Dem-Gene *P* Value	DemGene OR (95% CI)	Meta-Analysis *P* Value	Meta-Analysis OR (95% CI)
rs13113697	11711232	4	*HS3ST1*	T	TG	9.56E−03	5.03E−07	1.07 (1.04–1.10)	0.031	1.07 (1.01–1.14)	0.088	1.13 (0.98–1.31)	2.86E−08	1.07 (1.05–1.11)
rs7920721	11720308	10	*ECHDC3*	G	TG	4.49E−02	2.89E−07	1.07 (1.04–1.09)	0.12	1.05 (0.99–1.11)	0.08	1.12 (0.99–1.29)	3.38E−08	1.07 (1.04–1.11)

CI indicates confidence interval; FDR, false discovery rate; IGAP, International Genomics of Alzheimer's Disease Project; OR, odds ratio; SNP, single-nucleotide polymorphism; and TG, triglycerides.

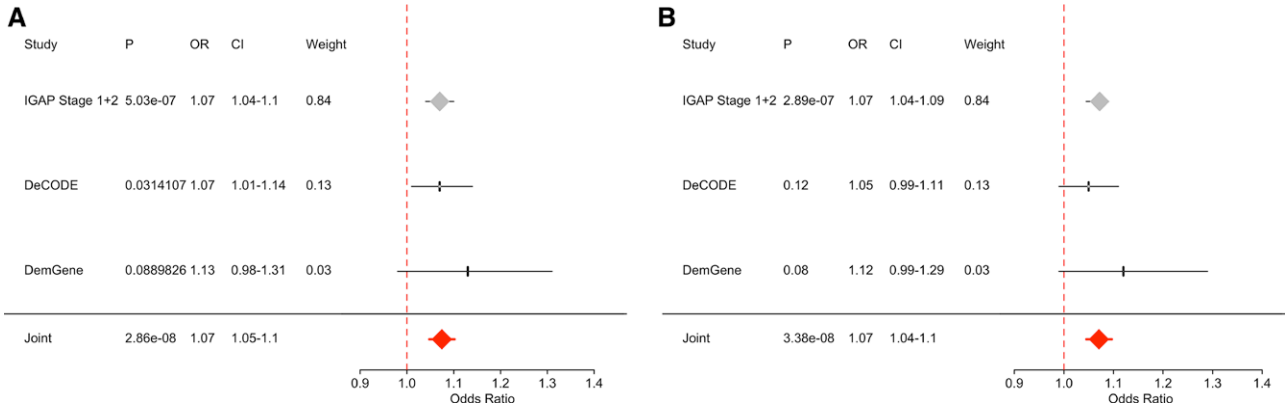

Figure 3. Forest plots for (**A**) rs13113697 on chromosome 4 and (**B**) rs7920721 on chromosome 10. CI indicates confidence interval; IGAP, International Genomics of Alzheimer's Disease Project; and OR, odds ratio.

between AD and inflammation. These results are consistent with the hypothesis that inflammatory mechanisms influence AD pathogenesis[9,27,28] and may have implications for treatment and prevention strategies in AD. On the other hand, we did not find a consistent direction of allelic effect between SNPs associated with AD risk and plasma lipid levels (LDL, HDL, and triglycerides). Additionally, for *HS3ST1* and *ECHD3* variants, we found an opposite direction of allelic effect between increased AD risk and triglycerides levels. One hypothesis for these findings is that the observed pleiotropy between AD and plasma lipids could be attributable to different haplotypes/gene alleles involving the same SNPs. Another equally plausible hypothesis is that the same haplotypes/gene alleles are involved in both AD and plasma lipids but the underlying biological mechanisms are distinct. From these findings, it seems less likely that the pleiotropic SNPs detected in this study influence AD pathogenesis via cholesterol-mediated pathways.

Unlike epidemiological studies, coheritability analyses,[29] or bivariate GWAS methods,[30] one strength of our present approach is the ability to detect genetic pleiotropy even when there is no correlation of the signed effects (mixed directionality of effect). The conditional FDR method can detect SNPs that have a nonnull effect in one trait and that also tend to have a nonnull effect in another trait, independently of directionality. Another strength of this framework is leveraging genetic signal in one phenotype to identify variants in a second phenotype that would otherwise not be detected with a single phenotype approach. We note that the conditional FDR approach allows reordering (and reranking) of SNPs based on *P* value significance in the second phenotype (eg, CRP or triglycerides), thus enabling identification of novel SNPs in the primary phenotype (eg, AD). In addition, as previously demonstrated, these genetic analysis methods result in improved sensitivity for a given specificity.[13] Using this "pleiotropic" approach, we detected 55 novel variants, indicating that genetic enrichment improves statistical power for gene discovery.

In meta-analyses, we discovered 2 GWAS significant AD susceptibility loci. The closest genes associated with

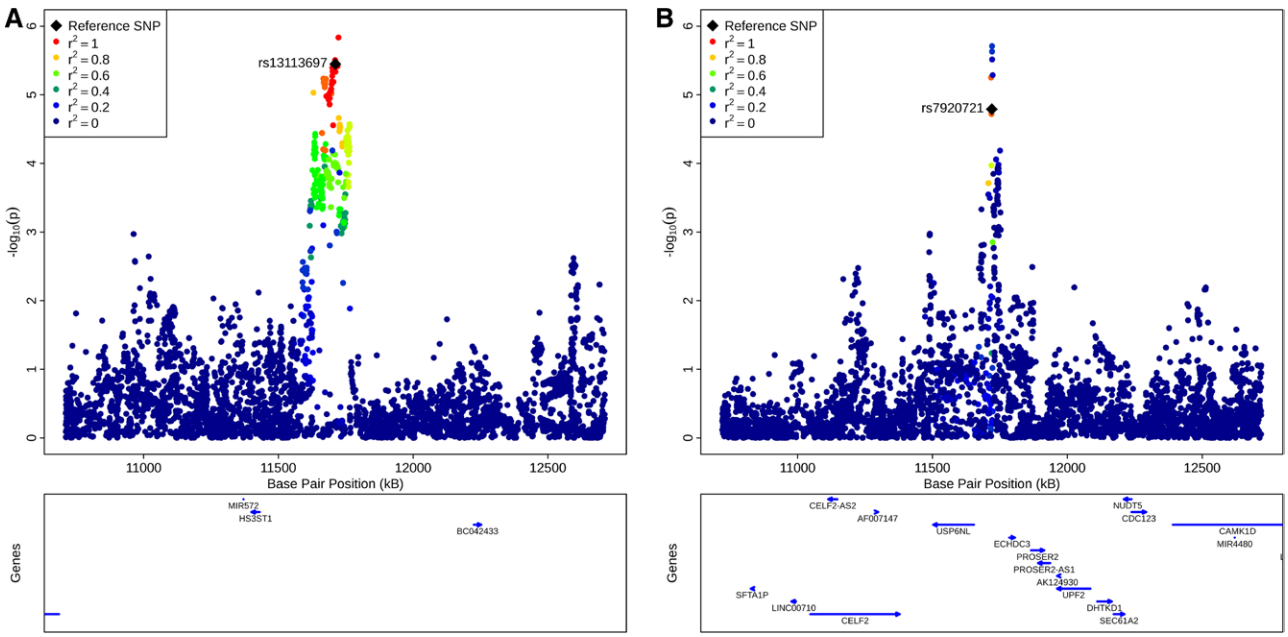

Figure 4. Regional association plots for (**A**) rs13113697 on chromosome 4 and (**B**) rs7920721 on chromosome 10. Linkage disequilibrium measured in the 1000 genomes European populations with plink version 1.07.

Table 3. SNPs Showing Suggestive Association With AD at Conditional FDR <0.05 (ORs Provided for the Reference Allele)

SNP	Position	Chromosome	Nearest Gene	Reference Allele	Associated Phenotype	Minimum Conditional FDR	IGAP Stage 1+2 *P* Value	IGAP Stage 1+2 OR (95%CI)	deCODE *P* Value	deCODE OR (95% CI)	DemGene *P* Value	DemGene OR (95% CI)	Meta-Analysis *P* Value	Meta-Analysis OR (95% CI)
rs7396366	11711232	11	*AP2A2*	C	CRP	3.91E–02	2.89E–06	0.93 (0.91–0.96)	0.22	0.96 (0.91–1.02)	0.21	0.92 (0.91–0.96)	6.80E–07	0.94 (0.92–0.96)
rs3131609	11720308	15	*USP50*	C	CRP	4.49E–02	3.90E–07	0.93 (0.90–0.96)	0.94	1.0 (0.93–1.08)	0.95	0.99 (0.86–1.15)	7.21E–07	0.93 (0.91–0.96)
rs2526378	47336320	17	*BZRAP1*	G	TG	1.83E–03	8.34E–07	0.94 (0.91–0.96)	0.50	0.98 (0.93–1.03)	9.20E–04	0.80 (0.70–0.91)	2.73E–07	0.94 (0.92–0.96)

AD indicates Alzheimer disease; CI, confidence interval; CRP, C-reactive protein; FDR, false discovery rate; IGAP, International Genomics of Alzheimer's Disease Project; OR, odds ratio; SNP, single-nucleotide polymorphism; and TG, triglycerides.

the 2 risk variants showed altered RNA levels in postmortem AD brains compared with control brains, suggesting a functional role. The first variant (rs13113697) is closest to the *HS3ST1* gene on chromosome 4 (Figure 4A), which encodes heparan sulfate glucosaminyl 3-O-sulfotransferase, an intraluminal Golgi protein enzyme with multiple biological activities.[31] The second variant (rs7920721) is closest to the *ECHDC3* gene on chromosome 10 (Figure 4B), which encodes an enzyme called enoyl CoA hydratase domain containing 3.[32] We note that by conditioning on cardiovascular traits and evaluating additional AD cohorts (deCODE and DemGene), we were able to find genome-wide significant evidence for previously[18] suggested signal close to *HS3ST1* and *ECHDC3*. At a value of $P<1.0\times10^{-6}$, we additionally found 3 suggestive variants on chromosome 11 (rs7396366; closest gene, *APA2A*), chromosome 15 (rs3131609; closest gene, *USP50*), and chromosome 17 (rs2526378; closest gene, *BZRAP1*).

It is important to note that in this study the diagnosis of AD was established clinically. Postmortem evidence from community- and population-based cohorts indicates that vascular brain injury often presents concomitantly with AD pathology and correlates with cognitive impairment above and beyond AD neuropathology.[33] It is feasible that the individuals with clinically diagnosed AD from the IGAP, deCODE, and DemGene cohorts may have concomitant vascular brain disease, which may further contribute to their cognitive decline and dementia. Therefore, an alternative interpretation of our findings is that the susceptibility loci identified in this study may increase brain vulnerability to vascular or inflammatory insults, which in turn may exacerbate the clinical consequences of AD pathological changes.

Conclusions

We found polygenic overlap among AD, CRP, and plasma lipids and leveraged this association to identify 2 novel genome-wide significant variants associated with increased AD risk. Careful and considerable effort will be required to further characterize the novel candidate genes detected in this study and to detect the functional variants responsible for the association of these loci with AD risk. Although no single common variant may be informative clinically, a combination of variants involved with inflammation or lipid metabolism may help identify older individuals at increased risk for AD. Our findings may also have implications for AD prevention trials involving anti-inflammatory agents.

Acknowledgments

We thank the IGAP for providing summary results data for these analyses.

Sources of Funding

This research was supported by grants from the National Institutes of Health (K02 NS067427, T32 EB005970, R01GM104400-01A, R01MH100351, AG033193, and U0149505), the Research Council of Norway (grants 213837, 225989, 223273, 237250/EU JPND), the South East Norway Health Authority (2013-123), the Norwegian Health Association, and the KG Jebsen Foundation. A.J. Schork was supported by National Institutes of Health grants RC2DA029475 and R01HD061414 and the Robert J. Glushko and Pamela Samuelson Graduate Fellowship. Dr Dehghan was supported by an NWO grant (veni, 916.12.154) and the Erasmus University Rotterdam Fellowship. Please see the Acknowledgments in the online-only Data Supplement for IGAP funding sources.

Disclosures

None.

References

1. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement*. 2007;3:186–191. doi: 10.1016/j.jalz.2007.04.381.
2. Shepardson NE, Shankar GM, Selkoe DJ. Cholesterol level and statin use in Alzheimer disease, I: review of epidemiological and preclinical studies. *Arch Neurol*. 2011;68:1239–1244. doi: 10.1001/archneurol.2011.203.
3. Matsuzaki T, Sasaki K, Hata J, Hirakawa Y, Fujimi K, Ninomiya T, Suzuki SO, Kanba S, Kiyohara Y, Iwaki T. Association of Alzheimer disease pathology with abnormal lipid metabolism: the Hisayama Study. *Neurology*. 2011;77:1068–1075. doi: 10.1212/WNL.0b013e31822e145d.
4. Di Paolo G, Kim TW. Linking lipids to Alzheimer's disease: cholesterol and beyond. *Nat Rev Neurosci*. 2011;12:284–296. doi: 10.1038/nrn3012.
5. Mapstone M, Cheema AK, Fiandaca MS, Zhong X, Mhyre TR, MacArthur LH, Hall WJ, Fisher SG, Peterson DR, Haley JM, Nazar MD, Rich SA, Berlau DJ, Peltz CB, Tan MT, Kawas CH, Federoff HJ. Plasma phospholipids identify antecedent memory impairment in older adults. *Nat Med*. 2014;20:415–418. doi: 10.1038/nm.3466.
6. Eikelenboom P, Hoozemans JJ, Veerhuis R, van Exel E, Rozemuller AJ, van Gool WA. Whether, when and how chronic inflammation increases the risk of developing late-onset Alzheimer's disease. *Alzheimers Res Ther*. 2012;4:15. doi: 10.1186/alzrt118.
7. Dik MG, Jonker C, Hack CE, Smit JH, Comijs HC, Eikelenboom P. Serum inflammatory proteins and cognitive decline in older persons. *Neurology*. 2005;64:1371–1377. doi: 10.1212/01.WNL.0000158281.08946.68.
8. Tan ZS, Beiser AS, Vasan RS, Roubenoff R, Dinarello CA, Harris TB, Benjamin EJ, Au R, Kiel DP, Wolf PA, Seshadri S. Inflammatory markers and the risk of Alzheimer disease: the Framingham study. *Neurology*. 2007;68:1902–1908. doi: 10.1212/01.wnl.0000263217.36439.da.
9. Jones L, Holmans PA, Hamshere ML, Harold D, Moskvina V, Ivanov D, Pocklington A, Abraham R, Hollingworth P, Sims R, Gerrish A, Pahwa JS, Jones N, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan

K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schürmann B, Heun R, Kölsch H, van den Bussche H, Heuser I, Peters O, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Hüll M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Rüther E, Carrasquillo MM, Pankratz VS, Younkin SG, Hardy J, O'Donovan MC, Owen MJ, Williams J. Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. *PLoS One*. 2010;5:e13950. doi: 10.1371/journal.pone.0013950.

10. Karch CM, Cruchaga C, Goate AM. Alzheimer's disease genetics: from the bench to the clinic. *Neuron*. 2014;83:11–26. doi: 10.1016/j.neuron.2014.05.041.
11. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, Cruchaga C, Sassi C, Kauwe JS, Younkin S, Hazrati L, Collinge J, Pocock J, Lashley T, Williams J, Lambert JC, Amouyel P, Goate A, Rademakers R, Morgan K, Powell J, St George-Hyslop P, Singleton A, Hardy J; Alzheimer Genetic Analysis Group. TREM2 variants in Alzheimer's disease. *N Engl J Med*. 2013;368:117–127. doi: 10.1056/NEJMoa1211851.
12. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, Bjornsson S, Huttenlocher J, Levey AI, Lah JJ, Rujescu D, Hampel H, Giegling I, Andreassen OA, Engedal K, Ulstein I, Djurovic S, Ibrahim-Verbaas C, Hofman A, Ikram MA, van Duijn CM, Thorsteinsdottir U, Kong A, Stefansson K. Variant of TREM2 associated with the risk of Alzheimer's disease. *N Engl J Med*. 2013;368:107–116. doi: 10.1056/NEJMoa1211103.
13. Andreassen OA, Thompson WK, Schork AJ, Ripke S, Mattingsdal M, Kelsoe JR, Kendler KS, O'Donovan MC, Rujescu D, Werge T, Sklar P, Roddey JC, Chen CH, McEvoy L, Desikan RS, Djurovic S, Dale AM; Psychiatric Genomics Consortium (PGC); Bipolar Disorder and Schizophrenia Working Groups. Improved detection of common variants associated with schizophrenia and bipolar disorder using pleiotropy-informed conditional false discovery rate. *PLoS Genet*. 2013;9:e1003455. doi: 10.1371/journal.pgen.1003455.
14. Andreassen OA, Djurovic S, Thompson WK, Schork AJ, Kendler KS, O'Donovan MC, Rujescu D, Werge T, van de Bunt M, Morris AP, McCarthy MI, Roddey JC, McEvoy LK, Desikan RS, Dale AM; International Consortium for Blood Pressure GWAS; Diabetes Genetics Replication and Meta-analysis Consortium; Psychiatric Genomics Consortium Schizophrenia Working Group. Improved detection of common variants associated with schizophrenia by leveraging pleiotropy with cardiovascular-disease risk factors. *Am J Hum Genet*. 2013;92:197–209. doi: 10.1016/j.ajhg.2013.01.001.
15. Andreassen OA, Zuber V, Thompson WK, Schork AJ, Bettella F, Djurovic S, Desikan RS, Mills IG, Dale AM; PRACTICAL Consortium; CRUK GWAS. Shared common variants in prostate cancer and blood lipids. *Int J Epidemiol*. 2014;43:1205–1214. doi: 10.1093/ije/dyu090.
16. Andreassen OA, McEvoy LK, Thompson WK, Wang Y, Reppe S, Schork AJ, Zuber V, Barrett-Connor E, Gautvik K, Aukrust P, Karlsen TH, Djurovic S, Desikan RS, Dale AM; International Consortium for Blood Pressure Genome-Wide Association Studies, Genetic Factors for Osteoporosis Consortium. Identifying common genetic variants in blood pressure due to polygenic pleiotropy with associated phenotypes. *Hypertension*. 2014;63:819–826. doi: 10.1161/HYPERTENSIONAHA.113.02077.
17. Liu JZ, Hov JR, Folseraas T, Ellinghaus E, Rushbrook SM, Doncheva NT, Andreassen OA, Weersma RK, Weismüller TJ, Eksteen B, Invernizzi P, Hirschfield GM, Gotthardt DN, Pares A, Ellinghaus D, Shah T, Juran BD, Milkiewicz P, Rust C, Schramm C, Müller T, Srivastava B, Dalekos G, Nöthen MM, Herms S, Winkelmann J, Mitrovic M, Braun F, Ponsioen CY, Croucher PJ, Sterneck M, Teufel A, Mason AL, Saarela J, Leppa V, Dorfman R, Alvaro D, Floreani A, Onengut-Gumuscu S, Rich SS, Thompson WK, Schork AJ, Næss S, Thomsen I, Mayr G, König IR, Hveem K, Cleynen I, Gutierrez-Achury J, Ricaño-Ponce I, van Heel D, Björnsson E, Sandford RN, Durie PR, Melum E, Vatn MH, Silverberg MS, Duerr RH, Padyukov L, Brand S, Sans M, Annese V, Achkar JP, Boberg KM, Marschall HU, Chazouillères O, Bowlus CL, Wijmenga C, Schrumpf E, Vermeire S, Albrecht M, Rioux JD, Alexander G, Bergquist A, Cho J, Schreiber S, Manns MP, Färkkilä M, Dale AM, Chapman RW, Lazaridis KN, Franke A, Anderson CA, Karlsen TH; UK-PSCSC Consortium; International IBD Genetics Consortium; International PSC Study Group. Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. *Nat Genet*. 2013;45:670–675. doi: 10.1038/ng.2616.
18. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B, Russo G, Thorton-Wells TA, Jones N, Smith AV, Chouraki V, Thomas C, Ikram MA, Zelenika D, Vardarajan BN, Kamatani Y, Lin CF, Gerrish A, Schmidt H, Kunkle B, Dunstan ML, Ruiz A, Bihoreau MT, Choi SH, Reitz C, Pasquier F, Cruchaga C, Craig D, Amin N, Berr C, Lopez OL, De Jager PL, Deramecourt V, Johnston JA, Evans D, Lovestone S, Letenneur L, Morón FJ, Rubinsztein DC, Eiriksdottir G, Sleegers K, Goate AM, Fiévet N, Huentelman MW, Gill M, Brown K, Kamboh MI, Keller L, Barberger-Gateau P, McGuiness B, Larson EB, Green R, Myers AJ, Dufouil C, Todd S, Wallon D, Love S, Rogaeva E, Gallacher J, St George-Hyslop P, Clarimon J, Lleo A, Bayer A, Tsuang DW, Yu L, Tsolaki M, Bossù P, Spalletta G, Proitsi P, Collinge J, Sorbi S, Sanchez-Garcia F, Fox NC, Hardy J, Deniz Naranjo MC, Bosco P, Clarke R, Brayne C, Galimberti D, Mancuso M, Matthews F, Moebus S, Mecocci P, Del Zompo M, Maier W, Hampel H, Pilotto A, Bullido M, Panza F, Caffarra P, Nacmias B, Gilbert JR, Mayhaus M, Lannefelt L, Hakonarson H, Pichler S, Carrasquillo MM, Ingelsson M, Beekly D, Alvarez V, Zou F, Valladares O, Younkin SG, Coto E, Hamilton-Nelson KL, Gu W, Razquin C, Pastor P, Mateo I, Owen MJ, Faber KM, Jonsson PV, Combarros O, O'Donovan MC, Cantwell LB, Soininen H, Blacker D, Mead S, Mosley TH Jr, Bennett DA, Harris TB, Fratiglioni L, Holmes C, de Bruijn RF, Passmore P, Montine TJ, Bettens K, Rotter JI, Brice A, Morgan K, Foroud TM, Kukull WA, Hannequin D, Powell JF, Nalls MA, Ritchie K, Lunetta KL, Kauwe JS, Boerwinkle E, Riemenschneider M, Boada M, Hiltuenen M, Martin ER, Schmidt R, Rujescu D, Wang LS, Dartigues JF, Mayeux R, Tzourio C, Hofman A, Nöthen MM, Graff C, Psaty BM, Jones L, Haines JL, Holmans PA, Lathrop M, Pericak-Vance MA, Launer LJ, Farrer LA, van Duijn CM, Van Broeckhoven C, Moskvina V, Seshadri S, Williams J, Schellenberg GD, Amouyel P; European Alzheimer's Disease Initiative (EADI); Genetic and Environmental Risk in Alzheimer's Disease; Alzheimer's Disease Genetic Consortium; Cohorts for Heart and Aging Research in Genomic Epidemiology. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet*. 2013;45:1452–1458. doi: 10.1038/ng.2802.
19. Dehghan A, Dupuis J, Barbalic M, Bis JC, Eiriksdottir G, Lu C, Pellikka N, Wallaschofski H, Kettunen J, Henneman P, Baumert J, Strachan DP, Fuchsberger C, Vitart V, Wilson JF, Paré G, Naitza S, Rudock ME, Surakka I, de Geus EJ, Alizadeh BZ, Guralnik J, Shuldiner A, Tanaka T, Zee RY, Schnabel RB, Nambi V, Kavousi M, Ripatti S, Nauck M, Smith NL, Smith AV, Sundvall J, Scheet P, Liu Y, Ruokonen A, Rose LM, Larson MG, Hoogeveen RC, Freimer NB, Teumer A, Tracy RP, Launer LJ, Buring JE, Yamamoto JF, Folsom AR, Sijbrands EJ, Pankow J, Elliott P, Keaney JF, Sun W, Sarin AP, Fontes JD, Badola S, Astor BC, Hofman A, Pouta A, Werdan K, Greiser KH, Kuss O, Meyer zu Schwabedissen HE, Thiery J, Jamshidi Y, Nolte IM, Soranzo N, Spector TD, Völzke H, Parker AN, Aspelund T, Bates D, Young L, Tsui K, Siscovick DS, Guo X, Rotter JI, Uda M, Schlessinger D, Rudan I, Hicks AA, Penninx BW, Thorand B, Gieger C, Coresh J, Willemsen G, Harris TB, Uitterlinden AG, Järvelin MR, Rice K, Radke D, Salomaa V, Willems van Dijk K, Boerwinkle E, Vasan RS, Ferrucci L, Gibson QD, Bandinelli S, Snieder H, Boomsma DI, Xiao X, Campbell H, Hayward C, Pramstaller PP, van Duijn CM, Peltonen L, Psaty BM, Gudnason V, Ridker PM, Homuth G, Koenig W, Ballantyne CM, Witteman JC, Benjamin EJ, Perola M, Chasman DI. Meta-analysis of genome-wide association studies in >80 000 subjects identifies multiple loci for C-reactive protein levels. *Circulation*. 2011;123:731–738. doi: 10.1161/CIRCULATIONAHA.110.948570.
20. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ, Johansen CT, Fouchier SW, Isaacs A, Peloso GM, Barbalic M, Ricketts SL, Bis JC, Aulchenko YS, Thorleifsson G, Feitosa MF, Chambers J, Orho-Melander M, Melander O, Johnson T, Li X, Guo X, Li M, Shin Cho Y, Jin Go M, Jin Kim Y, Lee JY, Park T, Kim K, Sim X, Twee-Hee Ong R, Croteau-Chonka DC, Lange LA, Smith JD, Song K, Hua Zhao J, Yuan X, Luan J, Lamina C, Ziegler A, Zhang W, Zee RY, Wright AF, Witteman JC, Wilson JF, Willemsen G, Wichmann HE, Whitfield JB, Waterworth DM, Wareham NJ, Waeber G, Vollenweider P, Voight BF, Vitart V, Uitterlinden AG, Uda M, Tuomilehto J, Thompson JR, Tanaka T, Surakka I, Stringham HM, Spector TD, Soranzo N, Smit JH, Sinisalo J, Silander K, Sijbrands EJ, Scuteri A, Scott J, Schlessinger D, Sanna S, Salomaa V, Saharinen J, Sabatti C, Ruokonen A, Rudan I, Rose LM, Roberts R, Rieder M, Psaty BM, Pramstaller PP, Pichler I, Perola M, Penninx BW, Pedersen NL, Pattaro C, Parker AN, Pare G, Oostra BA, O'Donnell CJ, Nieminen MS, Nickerson DA, Montgomery GW, Meitinger T, McPherson R, McCarthy MI, McArdle W, Masson D, Martin NG, Marroni F, Mangino M, Magnusson PK, Lucas G,

Luben R, Loos RJ, Lokki ML, Lettre G, Langenberg C, Launer LJ, Lakatta EG, Laaksonen R, Kyvik KO, Kronenberg F, König IR, Khaw KT, Kaprio J, Kaplan LM, Johansson A, Jarvelin MR, Janssens AC, Ingelsson E, Igl W, Kees Hovingh G, Hottenga JJ, Hofman A, Hicks AA, Hengstenberg C, Heid IM, Hayward C, Havulinna AS, Hastie ND, Harris TB, Haritunians T, Hall AS, Gyllensten U, Guiducci C, Groop LC, Gonzalez E, Gieger C, Freimer NB, Ferrucci L, Erdmann J, Elliott P, Ejebe KG, Döring A, Dominiczak AF, Demissie S, Deloukas P, de Geus EJ, de Faire U, Crawford G, Collins FS, Chen YD, Caulfield MJ, Campbell H, Burtt NP, Bonnycastle LL, Boomsma DI, Boekholdt SM, Bergman RN, Barroso I, Bandinelli S, Ballantyne CM, Assimes TL, Quertermous T, Altshuler D, Seielstad M, Wong TY, Tai ES, Feranil AB, Kuzawa CW, Adair LS, Taylor HA Jr, Borecki IB, Gabriel SB, Wilson JG, Holm H, Thorsteinsdottir U, Gudnason V, Krauss RM, Mohlke KL, Ordovas JM, Munroe PB, Kooner JS, Tall AR, Hegele RA, Kastelein JJ, Schadt EE, Rotter JI, Boerwinkle E, Strachan DP, Mooser V, Stefansson K, Reilly MP, Samani NJ, Schunkert H, Cupples LA, Sandhu MS, Ridker PM, Rader DJ, van Duijn CM, Peltonen L, Abecasis GR, Boehnke M, Kathiresan S. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010;466:707–713. doi: 10.1038/nature09270.

21. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34:939–944.
22. WHO. (World Health Organization, 1992).
23. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7:263–269. doi: 10.1016/j.jalz.2011.03.005.
24. Zhang B, Gaiteri C, Bodea LG, Wang Z, McElwee J, Podtelezhnikov AA, Zhang C, Xie T, Tran L, Dobrin R, Fluder E, Clurman B, Melquist S, Narayanan M, Suver C, Shah H, Mahajan M, Gillis T, Mysore J, MacDonald ME, Lamb JR, Bennett DA, Molony C, Stone DJ, Gudnason V, Myers AJ, Schadt EE, Neumann H, Zhu J, Emilsson V. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell*. 2013;153:707–720. doi: 10.1016/j.cell.2013.03.030.
25. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26:2190–2191. doi: 10.1093/bioinformatics/btq340.
26. Laird NM, Mosteller F. Some statistical methods for combining experimental results. *Int J Technol Assess Health Care*. 1990;6:5–30.
27. Johansson JU, Woodling NS, Wang Q, Panchal M, Liang X, Trueba-Saiz A, Brown HD, Mhatre SD, Loui T, Andreasson KI. Prostaglandin signaling suppresses beneficial microglial function in Alzheimer's disease models. *J Clin Invest*. 2015;125:350–364. doi: 10.1172/JCI77487.
28. International Genomics of Alzheimer's Disease Consortium (IGAP). Convergent genetic and expression data implicate immunity in Alzheimer's disease. *Alzheimers Dement*. 2014 Dec 20. doi: 10.1016/j.jalz.2014.05.1757.
29. Chen GB, Lee SH, Brion MJ, Montgomery GW, Wray NR, Radford-Smith GL, Visscher PM; International IBD Genetics Consortium. Estimation and partitioning of (co)heritability of inflammatory bowel disease from GWAS and immunochip data. *Hum Mol Genet*. 2014;23:4710–4720. doi: 10.1093/hmg/ddu174.
30. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, Madden PA, Heath AC, Martin NG, Montgomery GW, Goddard ME, Visscher PM. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet*. 2010;42:565–569. doi: 10.1038/ng.608.
31. Liu J, Shworak NW, Fritze LM, Edelberg JM, Rosenberg RD. Purification of heparan sulfate D-glucosaminyl 3-O-sulfotransferase. *J Biol Chem*. 1996;271:27072–27082.
32. Silbiger VN, Luchessi AD, Hirata RD, Lima-Neto LG, Cavichioli D, Carracedo A, Brión M, Dopazo J, García-García F, dos Santos ES, Ramos RF, Sampaio MF, Armaganijan D, Sousa AG, Hirata MH. Novel genes detected by transcriptional profiling from whole-blood cells in patients with early onset of acute coronary syndrome. *Clin Chim Acta*. 2013;421:184–190. doi: 10.1016/j.cca.2013.03.011.
33. Cholerton B, Larson EB, Baker LD, Craft S, Crane PK, Millard SP, Sonnen JA, Montine TJ. Neuropathologic correlates of cognition in a population-based sample. *J Alzheimers Dis*. 2013;36:699–709. doi: 10.3233/JAD-130281.

CLINICAL PERSPECTIVE

Late-onset Alzheimer disease (AD), the most common form of dementia, places a large economic and financial burden on families and society. With the aging of the US population and high costs associated with caring for cognitively impaired elderly, identifying strategies that prevent AD is of utmost importance. Cardiovascular disease (CVD) is being increasingly recognized as an important etiologic characteristic of AD. Many CVD traits such as dyslipidemia and inflammation can serve as therapeutic targets and are modifiable. Thus, focusing on the genetic and molecular overlap between CVD and AD may provide avenues to prevent or delay AD pathology. Although a number of epidemiological studies have examined the association between AD and CVD, a significant limitation of this approach is the inability to determine causal associations between CVD risk factors and AD pathobiology. Large-scale genome-wide association studies provide valuable insights into the role of specific biological pathways in disease pathogenesis, and combining genome-wide association studies from multiple disorders and phenotypes could elucidate shared pathobiology. Using data from large recent genome-wide association studies, we examined genetic overlap between AD, dyslipidemia, and inflammation. We found that genetic variants associated with increased plasma lipid and C-reactive protein levels are also associated with increased AD risk. By conditioning on genetic variants associated with the cardiovascular traits, we found novel AD susceptibility loci, including 2 genome-wide significant variants conferring increased risk for AD. Our findings provide novel insights into the relationship among AD pathogenesis, inflammation, and dyslipidemia and may have implications for AD prevention trials involving anti-inflammatory agents.