Title
Gene-based GWAS and biological pathway analysis of the resilience of executive functioning

Journal
Brain Imaging and Behavior, 8(1)

ISSN
1931-7557

Authors
Mukherjee, Shubhabrata
Kim, Sungeun
Ramanan, Vijay K
et al.

Publication Date
2014-03-01

DOI
10.1007/s11682-013-9259-7

Peer reviewed

Published in final edited form as:
Brain Imaging Behav. 2014 March ; 8(1): 110–118. doi:10.1007/s11682-013-9259-7.

Gene-based GWAS and -biological pathway analysis of the resilience of executive functioning

Shubhabrata Mukherjee, PhD[1,*], **Sungeun Kim, PhD**[2], **Vijay K. Ramanan**[2], **Laura E. Gibbons, PhD**[1], **Kwangsik Nho, PhD**[2], **M. Maria Glymour, ScD**[3], **Nilüfer Ertekin-Taner, MD, PhD**[4], **Thomas J. Montine, MD, PhD**[1], **Andrew J. Saykin, PsyD**[2], and **Paul K. Crane, MD, MPH**[1] **for the Alzheimer's Disease Neuroimaging Initiative**

[1]Department of Medicine, University of Washington, Seattle, WA USA

[2]Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN USA

[3]Department of Society, Human Development, and Health, Harvard School of Public Health, Boston, MA USA

[4]Mayo Clinic Florida, Departments of Neurology and Neuroscience, Jacksonville, FL USA

Abstract

Resilience in executive functioning (EF) is characterized by high EF measured by neuropsychological test performance despite structural brain damage from neurodegenerative conditions. We previously reported single nucleotide polymorphism (SNP) genome-wide association study (GWAS) results for EF resilience. Here, we report gene- and pathway-based analyses of the same resilience phenotype, using an optimal SNP-set (Sequence) Kernel Association Test (SKAT) for gene-based analyses (conservative threshold for genome-wide significance = 0.05/18,123=2.8×10^{-6}) and the gene-set enrichment package *GSA-SNP* for biological pathway analyses (False discovery rate (FDR) < 0.05). Gene-based analyses found a genome-wide significant association between *RNASE13* and EF resilience (p=1.33×10^{-7}). Genetic pathways involved with dendritic/neuron spine, presynaptic membrane, postsynaptic density etc. were enriched with association to EF resilience. Although replication of these results is necessary, our findings indicate the potential value of gene- and pathway-based analyses in research on determinants of cognitive resilience.

Keywords

Memory; executive functioning; Alzheimer's disease; genes; resilience; pathways

Introduction

Cognitive impairment and dementia lead to immense personal, family, and economic devastation in the US and worldwide. Structural brain damage from vascular and degenerative diseases is common in community-based elderly and can precipitate cognitive

[*]Corresponding author: Shubhabrata Mukherjee, PhD, Box 359780, 325 Ninth Avenue, Seattle, WA 98104 USA. (206) 744-1822 phone; (206) 744-9917 fax smukherj@uw.edu.

[*]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

impairment and dementia. Covert brain infarcts (Longstreth et al. 1998; Longstreth et al. 2002; Vermeer et al. 2007) and white matter hyperintensities (WMH) (Ikram et al. 2007; Longstreth et al. 1996; Debette and Markus 2010; National Bioethics Advisory Commission) are also common findings on magnetic resonance imaging (MRI) scans of elderly people without dementia, stroke, or transient ischemic attack. The increasing prevalence of these vascular lesions occurs in parallel with rising incidence of Alzheimer's disease with age (Kukull et al. 2002). Most elderly people have pathological lesions by the time they die (Sonnen et al. 2007).

Imaging and pathology-defined lesions need not cause dementia during life (Sonnen et al. 2011). Although structural brain changes are common findings on MRI, some people appear to be resilient to cognitive deficits in the presence of these findings. People with essentially similar lesions can have dementia, no symptoms at all or anything in between.

An extensive literature addresses the gap between brain damage and retained cognitive functioning, often referred to as "cognitive reserve" (Stern 2002, 2003). Many have focused on identifying modifiable factors that can increase reserve (Scarmeas and Stern 2003). Although biological substrates of reserve have been investigated (Whalley et al. 2004), to our knowledge, the only paper on genome-wide variability associated with reserve was our earlier investigation.

Reed and colleagues (Reed et al. 2010) introduced the idea of operationalizing reserve as residual cognitive functioning after accounting for MRI-identified brain damage. Cognitive resilience describes people whose cognitive functioning is better than predicted based on their neuroimaging and demographic characteristics. We emphasize our focus on better-than-predicted cognition by using the term "resilience" (Negash et al. 2011). Most importantly, this specific phenotype can be interrogated using modern genetic investigation.

Disease-based genetic discovery is an important line of investigation that promises to improve our understanding of conditions associated with late-life cognitive losses. These on-going investigations offer the hope of identifying modifiable biological targets to reduce the burden of specific diseases. Resilience represents a complementary line of investigation that addresses why some people may be able to accumulate neuropathologic changes without expressing clinical dementia. In our previous investigation, we found that resilience was highly heritable (h^2=0.76 with a standard error of 0.44) (Mukherjee et al. 2012a).

Genome-wide association studies (GWAS) are an important first step with genome-wide single nucleotide polymorphism (SNP) data. Additional gene-based and pathway-based analyses may further contribute to understanding associations between genetic variants and phenotypes of interest. For example, Peng and colleagues (Peng et al. 2010) reported that gene-specific, rather than SNP-specific, analyses identified variants associated with several phenotypes that were not associated at genome-wide significance thresholds with any individual SNP. Genes identified in this fashion in a single cohort were confirmed by meta-analysis of data from multiple cohorts, suggesting that gene-based analyses may further illuminate SNP-based analyses for any given sample size and may also be more replicable than SNP-based analyses (Peng et al. 2010).

Beyond gene-based models, there is increasing recognition of the potential contributions of pathway-based analyses, in which variants in groups of genes that operate along a single physiologic or developmental pathway are considered together to predict the phenotype of interest (Ramanan et al. 2012b). This approach identifies pathways exhibiting an enrichment of genetic associations to the phenotype. Thus, we proposed that a three-component approach — combining SNP-, gene-, and pathway-based analyses of genome-wide SNP data

— is likely to best illuminate associations between genetic variants and phenotypes. (Peng et al. 2010). To identify additional genetic mechanisms underlying EF resilience, we complemented our previous GWAS with gene- and pathway-based analyses of high-density genotype data.

Methods

Alzheimer's Disease Neuroimaging Initiative

Data used for this study were obtained from the ADNI database (http://adni.loni.ucla.edu/). The ADNI was initiated in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations. The primary goal of ADNI-1 was to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. Michael W. Weiner, MD, VA Medical Center and University of California-San Francisco was the Principal Investigator of this initiative. This $60 million, multiyear public-private partnership involved many co-investigators from a broad range of academic institutions and private corporations. More than 800 participants, aged 55 to 90, were recruited from across more than 50 sites in the US and Canada. Longitudinal imaging data, including structural 1.5 Tesla MRI scans, were collected on the full sample. Neuropsychological and clinical assessments were collected at baseline, and at follow-up visits of six-to-twelve month intervals. Other available data used in the present analysis included *APOE* genotype and genome-wide SNP data obtained in the full ADNI-1 sample, as outlined in (Saykin et al. 2010). Further information about ADNI can be found in (Weiner et al. 2010) and at http://www.adni-info.org. The study was conducted after Institutional Review Board approval at each site. Written informed consent was obtained from all study participants, or their authorized representatives.

1.5 T MRI Neuroimaging

All participants received 1.5 Tesla structural magnetic resonance imaging (MRI). The neuroimaging methods utilized by ADNI-1 have been described in detail previously (Jack et al. 2008) utilizing calibration techniques to maintain consistent protocols across scanners and sites. Raw dicom data of T1-weighted MP-RAGE scans acquired from 1.5 Tesla scanners at baseline visits from all participants were obtained via the ADNI-1 database (http://www.loni.ucla.edu/ADNI/). In our analyses we used presence of one or more lacunes, cortical volume (summed across entorhinal cortex, fusiform, pars triangularis, caudal middle frontal, superior frontal, medial orbitofrontal, rostral, middle frontal, and lateral orbitofrontal, controlling for intracranial volume), volume of bilateral hippocampus (controlling for intracranial volume), and the natural log of WMH volume.

Psychometric composites for memory and executive functioning

ADNI-1 participants received an extensive neuropsychological assessment battery at each study visit, including several measures of memory and executive function. We applied modern psychometric theory to item-level data from the ADNI-1 neuropsychological battery to develop composite scores separately for memory (ADNI-Mem) and executive functioning (ADNI-EF). For executive functioning, we found that a bi-factor model had the best fit to the data. We extracted factor scores for the general factor defined by all of the items from Mplus v5 (Muthén and Muthén 2006); this factor score is the ADNI-EF score. For memory, we used a longitudinal single factor model to account for different versions of the ADAS-

Cog and of the Rey AVLT. We used parameters from that model to generate scores at each study visit, also using Mplus (Muthén and Muthén 2006). Further details are provided in previously published papers (Crane et al. 2012; Gibbons et al. 2012).

Genotyping and Quality Control

The ADNI-1 sample was genotyped using the Human 610-Quad BeadChip (Illumina, Inc., San Diego, CA), resulting in 620,901 SNP and copy number variant (CNV) biomarkers. The genotyping protocol followed the manufacturer's instructions and is explained in detail in (Saykin et al. 2010).

Standard quality control procedures were performed on the ADNI genotype data using PLINK v1.07 (Purcell et al. 2007). Samples were excluded based on the following criteria: (1) call rate per individual < 95%, (2) ambiguous sex identification, (3) identity check with PI_HAT> 0.125 after exclusion of individuals with no genetic consent. Markers were excluded based on the following criteria: (1) call rate per SNP < 95%, (2) Hardy-Weinberg equilibrium test in controls < 10^{-6}, (3) minor allele frequency (MAF) < 1%.

APOE was genotyped at the time of screening. The two previously established *APOE* genotype SNPs (rs429358, rs7412) that characterize the $\varepsilon 2/\varepsilon 3/\varepsilon 4$ alleles were not available on the Human610-Quad BeadChip array (Illumina, Inc., San Diego, CA). These SNPs were genotyped by PCR amplification followed by *Hha*I restriction enzyme digestion and Metaphor Gel and were available in the ADNI database (Potkin et al. 2009). They were added to the ADNI-1 genotype data based on the reported *APOE* $\varepsilon 2/\varepsilon 3/\varepsilon 4$ status before the assessment of sample quality.

To limit possible confounding effects of population ancestry, we restricted analyses to non-Hispanic Caucasian participants. To select non-Hispanic Caucasian participants in the ADNI cohort, 988 founders of HapMap (The International HapMap Project 2003) phase 3 samples were used as reference populations which consist of 11 known population and PLINK multidimensional scaling analysis was performed on the combined genotype data of the ADNI-1 and the HapMap phase 3 release 2. ADNI participants were selected if they were grouped together with the HapMap CEU (Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain (CEPH) collection) or TSI (Toscani in Italy) participants. After the QC procedure and exclusions for withdrawal of consent, 694 participants and 531,096 single nucleotide polymorphisms (SNPs) were selected for the subsequent analyses and the genotyping rate in the remaining samples was ≥0.995.

Population substructure

Population substructure was evaluated using Eigenstrat v4.2 (Price et al. 2006). The observed SNPs which passed quality control were linkage disequilibrium LD-pruned (a) in a window size of 1500 SNPs calculate pairwise LD between each pair of SNPs, b) remove one of a pair of SNPs if the LD is greater than 0.2 and then c) shift the window 150 SNPs forward and repeat the procedure) and Eigenstrat was used to derive loadings for the principal components (PCs) which were used to adjust for population substructure to minimize spurious associations and maximize power to detect true associations.

Genotype Imputation

We used the ENIGMA protocol to pre-process the genotype data(ENIGMA2 Genetics Support Team 2012). Briefly, PLINK was used to exclude SNPs on the basis of standard quality control (QC) criteria, e.g., low MAF (<0.01), poor genotype calling (call rate < 95%), 90% call rate per individual and deviations from Hardy–Weinberg equilibrium (<

10^{-6}). To obtain genotypes for SNPs not characterized on the GWAS array, genome-wide imputation was performed with Mach software (Li et al. 2010) using NCBI 1000 Genomes build 37 (UCSC hg19) as the reference panel. We took forward SNPs with imputation quality estimates of $R^2 \geq 0.50$ and performed further QC (95% call rate; 1% MAF and Hardy-Weinberg equilibrium test in controls < 10^{-6}).

GWAS analysis

We performed a GWAS on the executive functioning composite adjusting for the memory composite, demographics, Hachinski score (a measure of the vascular component of dementia (Hachinski et al. 1975), brain imaging parameters and population substructure (first three principal components) to examine the main effect of each SNP using PLINK (Purcell et al. 2007) using the 1000G imputed data. Compared to our previous analysis (Mukherjee et al. 2012a), this study utilized genotype data with substantially increased genomic coverage due to imputation.

Gene-wide analysis using SKAT-O

We mapped SNPs to genes using the b137 annotation file downloaded from the dbSNP website (http://www.ncbi.nlm.nih.gov/projects/SNP/, accessed on 11/30/2011). We assigned 3,377,248 SNPs to each of 18,123 autosomal genes according to genomic positions on the UCSC Genome Browser hg19 assembly, using the official gene boundaries as delimiters (i.e., no extended gene window was used). The joint effects of variants in each of these gene regions were tested for association with resilience using Sequential Kernel Association test (SKAT).

Burden tests are commonly used for rare variant analysis, and are more powerful when a large proportion of variants are causal and effects are in the same direction (Lee et al. 2012). SKAT is more powerful when a small proportion of variants are causal, or the effects have mixed directions (Lee et al. 2012). SKAT uses a multiple regression model to directly regress the phenotype on genetic variants in a region and on covariates, using a variance component score test to account for rare variants. SKAT is a score test, so type I error rates are protected for any choice of weights.

Both scenarios for causality and direction are plausible when the underlying biology is unknown. We used the Optimal Unified Test (SKAT-O), which uses data to adaptively estimate ρ to maximize power and minimize p-values (Lee et al. 2012) where the SKAT-O test statistic is given by:

$$Q_\rho = \rho Q_{Burden} + (1-\rho) Q_{SKAT}; 0 \leq \rho \leq 1,$$

where SKAT ($\rho = 0$) and Burden ($\rho = 1$) are special cases. This method was developed for rare variant analyses, but can be used for common variants if we specify a $\beta_{1,1}$ (uniform) weight on each of the SNPs.

Similar to the GWAS model, we performed a gene-wide analysis on the executive functioning composite adjusting for the memory composite, demographics, Hachinski score, brain imaging parameters and population substructure.

Pathway analysis

Pathway analysis of the GWAS results was performed to identify functional gene sets with important association with the resilience phenotype. We used the GSA-SNP package (Nam et al. 2010) to identify pathways with enrichment of association to EF resilience. This

software uses a competitive enrichment algorithm (Goeman and Bühlmann 2007), where the null hypothesis holds that a pathway-phenotype association is not different from all other pathway-phenotype associations under analysis. Competitive enrichment strategies are robust to the effects of genomic inflation due to population stratification or other confounding factors (Holmans 2010; Fridley and Biernacka 2011). In GSA-SNP, the significance score for each gene under analysis was calculated as the −log of the *k*-th best SNP-level *p*-value in the gene. Corresponding with the authors' recommendation (Nam et al. 2010), we selected *k=2* to limit the effects of both single, highly-significant loci and of spurious SNP-level associations on driving pathway enrichment (Ramanan et al. 2012a).

We used the Gene Ontology database to define gene sets representing biological pathways. 1454 gene sets were examined out of which 825 were biological processes, 233 were cellular components and 396 were molecular functions. Each gene set (representing a pathway) was then assessed for phenotype enrichment by the Z-statistic method (Kim and Volsky 2005), which incorporates the gene-wide significance scores and the number of genes within each set. In addition, since small pathways can exhibit spurious phenotype associations due to large single locus effects (Holmans 2010), and since large pathways are more likely to exhibit association by chance alone (Elbers et al. 2009), we restricted analyses to gene sets containing 5–200 genes. To correct for multiple hypothesis testing, we applied the False Discovery Rate (FDR) (Benjamini and Hochberg 1995) to the *p*-values generated by the enrichment algorithm (Ramanan et al. 2012a).

Results

SNP association findings

Of the 692 individuals who passed sample quality control, complete data were available for 681 of them (Table 1). The genomic inflation factor (λ) with three principal components was 1.0 for the GWAS analysis. Similar to the results reported in our earlier paper (Mukherjee et al. 2012b), the top two SNPs resided in the *RNASE13* (ribonuclease, RNase A family, 13) gene region. The top SNP in this analysis was rs3648346 (Chr 14) with an unadjusted *p*-value of 6.43×10^{-8}. rs3648348, which was reported in our earlier paper (Mukherjee et al. 2012b), was also suggestive with a *p*-value of 1.18×10^{-7}. A list of top SNPs for this analysis can be found in Table A in the Appendix.

Gene-based analyses

The most significant genes are summarized in Table 2. *RNASE13* with a *p*-value of 1.33×10^{-7} had a genome-wide level of significance ($0.05/18{,}123=2.8\times10^{-6}$). Other genes with suggestive associations included Fatty Acyl CoA Reductase 2 (*FAR2*) and Tubulin Polymerization-Promoting Protein (*TPPP2*).

Pathway analysis

We identified 111 pathways with enrichment of association to EF resilience (FDR $p<0.05$). The top 20 pathways in terms of *p*-value and FDR are presented in Table 3 and a complete list of the 111 enriched pathways are provides in Supplementary Table 1. The top pathways were related to dendritic/neuron spine, presynaptic membrane, and postsynaptic density. Some genes associated with late-onset AD such as *PTK2B, PICALM, MS4A2, APP* were present in the top 10 pathways (See Supplementary Table 1). The *p*-values for SNPs in these genes or for the genes themselves were unremarkable in the GWAS analyses (Mukherjee et al. 2012a).

Discussion

In these analyses of the executive functioning resilience phenotype, we identified one gene — *RNASE13* — with a genome-wide significant association, and found associations of pathways related to dendritic/neuron spine, presynaptic membrane, and postsynaptic density. *RNASE13* has only two SNPs in the data set, one of which (rs3748348) is observed and the other (rs3748346) is imputed. Replication analyses will be critical to determine the relevance of *RNASE13* to this phenotype.

Beyond *RNASE13*, gene-based analyses showed partial but not complete overlap with our previously reported SNP results (Mukherjee et al. 2012a). SNPs in *TPPP2* are in close LD with SNPs in *RNASE13*, as noted in our previous paper (Mukherjee et al. 2012a). None of the individual SNPs in *TPPP2* were closely associated with our phenotype (the smallest *p*-value was 2.5×10^{-5} for rs1243459). Our gene-based analyses strengthened our interest in *RNASE13* and *TPPP2*. The optimized SKAT-O approach accounts for the proportion of variants which may be causal and have mixed directions of the effects of SNPs which map to a gene. This gene-wide approach we used enabled us to summarize SNP heterogeneity (direction of effects) within each gene with a single number. This is biologically-meaningful for a complex trait like EF resilience where true associated genes are likely to have combinations of variants with heterogeneous effect sizes and directions depending on the population being analyzed. Gene-based (and pathway-based) analyses can overcome this relative limitation of SNP-based approaches.

This reduced the number of statistical tests performed and enabled us to discern signals from combinations of SNPs that were not visible when we focused on only individual SNPs as is usual with SNP-based GWAS.

Our identification of reinforcing associations between the executive functioning resilience phenotype and pathways fundamental to production and loss of neurons, fate determination, and pre- and post-synaptic structure and function raises obvious potential links between neuron biology and resilience to neurodegenerative disease. Several of these pathways have previously been implicated in LOAD, in research on the loss and alteration of dendritic spines induced by amyloid β (Aβ) in adults (Knobloch and Mansuy 2008), and in aberrations in synapse composition, shape and density induced by Aβ oligomers (Lacor et al. 2007). Synaptic proteins found in presynaptic membranes were found to be differentially affected in AD brains compared to controls which suggest differential involvement of synaptic components in AD (Shimohama et al. 1997). Brains affected with Alzheimer's disease show a reduced number of synapses, and stereological and biochemical analysis has shown that this reduction in synaptic density correlates better with cognitive decline than with the accumulation of plaques (Harel et al. 2008; Harold et al. 2009). Furthermore, expression studies indicate that there are differences in expression of genes that regulate neuron differentiation between people with AD and cognitively normal controls (Blalock et al. 2011). Further studies investigating the significance of these pathways appear warranted given the present results. Caution is certainly warranted when interpreting these results, given that this is a first report based on a relatively small sample. It will also be critical to assess disease associations with genes associated with the cognitive resilience phenotype and AD or other neurodegenerative diseases.

Gene-based and pathway-based analyses afford the opportunity to detect complementary information that would not have been easily observed from SNP-based GWAS alone. Applying these complementary approaches to standard GWAS, we were able to identify additional biological underpinnings of EF resilience.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Abbott; Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Amorfix Life Sciences Ltd.; AstraZeneca; Bayer HealthCare; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; GE Healthcare; Innogenetics, N.V.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129, K01 AG030514, and the Dana Foundation. Data management and the specific analyses reported here were supported by NIH grant R01 AG029672 (Paul Crane, PI), R13 AG030995 (Mungas), and P50 AG05136 (Montine), R01 AG032990 and P50 AG016574 (Crane), from the NIA as well as NIA R01 AG19771 (Saykin) and P30 AG10133 (Saykin/Ghetti).

References

Benjamini Y, Hochberg Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B-Methodological. 1995; 57(1): 289–300.

Blalock EM, Buechel HM, Popovic J, Geddes JW, Landfield PW. Microarray analyses of laser-captured hippocampus reveal distinct gray and white matter signatures associated with incipient Alzheimer's disease. J Chem Neuroanat. 2011; 42(2):118–126. S0891-0618(11)00074-3 [pii]. 10.1016/j.jchemneu.2011.06.007 [PubMed: 21756998]

Crane PK, Carle A, Gibbons LE, Insel P, Mackin RS, Gross A, et al. Development and assessment of a composite score for memory in the Alzheimer's Disease Neuroimaging Initiative (ADNI). Brain imaging and behavior. 201210.1007/s11682-012-9186-z

Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. [Meta-Analysis Research Support, Non-U.S. Gov't Review]. BMJ. 2010; 341:c3666.10.1136/bmj.c3666 [PubMed: 20660506]

Elbers CC, van Eijk KR, Franke L, Mulder F, van der Schouw YT, Wijmenga C, et al. Using genome-wide pathway analysis to unravel the etiology of complex diseases. Genetic Epidemiology. 2009; 33(5):419–431.10.1002/gepi.20395 [PubMed: 19235186]

ENIGMA2 Genetics Support Team. NIGMA2 IKGP Cookbook. The Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) consortium; 2012.

Fridley BL, Biernacka JM. Gene set analysis of SNP data: benefits, challenges, and future directions. [Research Support, N.I.H., Extramural Review]. Eur J Hum Genet. 2011; 19(8):837–843.10.1038/ejhg.2011.57 [PubMed: 21487444]

Gibbons LE, Carle AC, Mackin RS, Harvey D, Mukherjee S, Insel P, et al. A composite score for executive functioning, validated in Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with baseline mild cognitive impairment. Brain imaging and behavior. 201210.1007/s11682-012-9176-1

Goeman JJ, Bühlmann P. Analyzing gene expression data in terms of gene sets: methodological issues. Bioinformatics. 2007; 23(8):980–987.10.1093/bioinformatics/btm051 [PubMed: 17303618]

Hachinski VC, Iliff LD, Zilhka E, Du Boulay GH, McAllister VL, Marshall J, et al. Cerebral blood flow in dementia. Archives of neurology. 1975; 32(9):632–637. [PubMed: 1164215]

Harel A, Wu F, Mattson MP, Morris CM, Yao PJ. Evidence for CALM in directing VAMP2 trafficking. Traffic. 2008; 9(3):417–429.10.1111/j.1600-0854.2007.00694.x [PubMed: 18182011]

Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. [Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't]. Nature genetics. 2009; 41(10):1088–1093.10.1038/ng.440 [PubMed: 19734902]

Holmans P. Statistical methods for pathway analysis of genome-wide data for association with complex genetic traits. Advances in genetics. 2010; 72:141–179. citeulike-article-id:8423729. [PubMed: 21029852]

Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. [Evaluation Studies Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. PLoS genetics. 2009; 5(6):e1000529.10.1371/journal.pgen.1000529 [PubMed: 19543373]

Ikram MA, Vrooman HA, Vernooij MW, van der Lijn F, Hofman A, van der Lugt A, et al. Brain tissue volumes in the general elderly population The Rotterdam Scan Study. Neurobiol Aging. 2007

The International HapMap Project. Nature. 2003; 426(6968):789–796. nature02168 [pii]. 10.1038/nature02168 [PubMed: 14685227]

Jack CR Jr, Bernstein MA, Fox NC, Thompson P, Alexander G, Harvey D, et al. The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. J Magn Reson Imaging. 2008; 27(4):685–691. [PubMed: 18302232]

Kim SY, Volsky DJ. PAGE: parametric analysis of gene set enrichment. [Comparative Study Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S.]. BMC Bioinformatics. 2005; 6:144.10.1186/1471-2105-6-144 [PubMed: 15941488]

Knobloch M, Mansuy IM. Dendritic spine loss and synaptic alterations in Alzheimer's disease. [Research Support, Non-U.S. Gov't Review]. Molecular neurobiology. 2008; 37(1):73–82.10.1007/s12035-008-8018-z [PubMed: 18438727]

Kukull WA, Higdon R, Bowen JD, McCormick WC, Teri L, Schellenberg GD, et al. Dementia and Alzheimer disease incidence: a prospective cohort study. Arch Neurol. 2002; 59(11):1737–1746. [PubMed: 12433261]

Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, et al. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2007; 27(4):796–807.10.1523/JNEUROSCI.3501-06.2007 [PubMed: 17251419]

Lee S, Wu MC, Lin X. Optimal tests for rare variant effects in sequencing association studies. Biostatistics. 2012; 13(4):762–775. kxs014 [pii]. 10.1093/biostatistics/kxs014 [PubMed: 22699862]

Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genetic epidemiology. 2010; 34(8):816–834.10.1002/gepi.20533 [PubMed: 21058334]

Longstreth WT Jr, Bernick C, Manolio TA, Bryan N, Jungreis CA, Price TR. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: the Cardiovascular Health Study. Arch Neurol. 1998; 55(9):1217–1225. [PubMed: 9740116]

Longstreth WT Jr, Dulberg C, Manolio TA, Lewis MR, Beauchamp NJ Jr, O'Leary D, et al. Incidence, manifestations, and predictors of brain infarcts defined by serial cranial magnetic resonance imaging in the elderly: the Cardiovascular Health Study. Stroke. 2002; 33(10):2376–2382. [PubMed: 12364724]

Longstreth WT Jr, Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, et al. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke. 1996; 27(8):1274–1282. [PubMed: 8711786]

Mukherjee S, Kim S, Gibbons LE, Nho K, Risacher SL, Glymour MM, et al. Genetic architecture of resilience of executive functioning. Brain Imaging Behav. 2012a10.1007/s11682-012-9184-1

Mukherjee S, Kim S, Gibbons LE, Nho K, Risacher SL, Glymour MM, et al. Genetic architecture of resilience of executive functioning. Brain imaging and behavior. 2012b10.1007/s11682-012-9184-1

Muthén, L.; Muthén, B. Mplus users guide. Los Angeles, CA: Muthen and Muthen; 2006. Version 4.1 ed

Nam D, Kim J, Kim SY, Kim S. GSA-SNP: a general approach for gene set analysis of polymorphisms. [Research Support, Non-U.S. Gov't]. Nucleic acids research. 2010; 38(Web Server issue):W749–754.10.1093/nar/gkq428 [PubMed: 20501604]

National Bioethics Advisory Commission. [Accessed May 7 2007] http://www.georgetown.edu/research/nrcbl/nbac/

Negash S, Bennett DA, Wilson RS, Schneider JA, Arnold SE. Cognition and neuropathology in aging: multidimensional perspectives from the Rush Religious Orders Study and Rush Memory And Aging Project. [Review]. Current Alzheimer research. 2011; 8(4):336–340. [PubMed: 21222592]

Peng G, Luo L, Siu H, Zhu Y, Hu P, Hong S, et al. Gene and pathway-based second-wave analysis of genome-wide association studies. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. European Journal of Human Genetics. 2010; 18(1):111–117.10.1038/ejhg.2009.115 [PubMed: 19584899]

Potkin SG, Guffanti G, Lakatos A, Turner JA, Kruggel F, Fallon JH, et al. Hippocampal atrophy as a quantitative trait in a genome-wide association study identifying novel susceptibility genes for Alzheimer's disease. PLoS One. 2009; 4(8):e6501.10.1371/journal.pone.0006501 [PubMed: 19668339]

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. [Research Support, Non-U.S. Gov't]. Nature genetics. 2006; 38(8):904–909.10.1038/ng1847 [PubMed: 16862161]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3): 559–575. [PubMed: 17701901]

Ramanan VK, Kim S, Holohan K, Shen L, Nho K, Risacher SL, et al. Genome-wide pathway analysis of memory impairment in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort implicates gene candidates, canonical pathways, and networks. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. Brain imaging and behavior. 2012a; 6(4):634–648.10.1007/s11682-012-9196-x [PubMed: 22865056]

Ramanan VK, Shen L, Moore JH, Saykin AJ. Pathway analysis of genomic data: concepts, methods, and prospects for future development. [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S. Review]. Trends in genetics: TIG. 2012b; 28(7):323–332.10.1016/j.tig.2012.03.004 [PubMed: 22480918]

Reed BR, Mungas D, Farias ST, Harvey D, Beckett L, Widaman K, et al. Measuring cognitive reserve based on the decomposition of episodic memory variance. Brain: a journal of neurology. 2010; 133(Pt 8):2196–2209.10.1093/brain/awq154 [PubMed: 20591858]

Saykin AJ, Shen L, Foroud TM, Potkin SG, Swaminathan S, Kim S, et al. Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: Genetics core aims, progress, and plans. Alzheimers Dement. 2010; 6(3):265–273. S1552-5260(10)00082-8 [pii]. 10.1016/j.jalz.2010.03.013 [PubMed: 20451875]

Scarmeas N, Stern Y. Cognitive reserve and lifestyle. J Clin Exp Neuropsychol. 2003; 25(5):625–633. [PubMed: 12815500]

Shen L, Kim S, Risacher SL, Nho K, Swaminathan S, West JD, et al. Whole genome association study of brain-wide imaging phenotypes for identifying quantitative trait loci in MCI and AD: A study of the ADNI cohort. Neuroimage. 2010; 53(3):1051–1063. S1053-8119(10)00064-9 [pii]. 10.1016/j.neuroimage.2010.01.042 [PubMed: 20100581]

Shimohama S, Kamiya S, Taniguchi T, Akagawa K, Kimura J. Differential involvement of synaptic vesicle and presynaptic plasma membrane proteins in Alzheimer's disease. [Research Support, Non-U.S. Gov't]. Biochemical and biophysical research communications. 1997; 236(2):239–242.10.1006/bbrc.1997.6940 [PubMed: 9240416]

Sonnen JA, Larson EB, Crane PK, Haneuse S, Li G, Schellenberg GD, et al. Pathological correlates of dementia in a longitudinal, population-based sample of aging. Ann Neurol. 2007; 62(4):406–413. [PubMed: 17879383]

Sonnen JA, Santa Cruz K, Hemmy LS, Woltjer R, Leverenz JB, Montine KS, et al. Ecology of the aging human brain. [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. Archives of neurology. 2011; 68(8):1049–1056.10.1001/archneurol.2011.157 [PubMed: 21825242]

Stern Y. What is cognitive reserve? Theory and research application of the reserve concept. J Int Neuropsychol Soc. 2002; 8(3):448–460. [PubMed: 11939702]

Stern Y. The concept of cognitive reserve: a catalyst for research. J Clin Exp Neuropsychol. 2003; 25(5):589–593. [PubMed: 12815497]

Vermeer SE, Longstreth WT Jr, Koudstaal PJ. Silent brain infarcts: a systematic review. [Research Support, N.I.H., Extramural Review]. Lancet neurology. 2007; 6(7):611–619.10.1016/S1474-4422(07)70170-9 [PubMed: 17582361]

Weiner MW, Aisen PS, Jack CR Jr, Jagust WJ, Trojanowski JQ, Shaw L, et al. The Alzheimer's disease neuroimaging initiative: progress report and future plans. Alzheimers Dement. 2010; 6(3):202–211. e207. [PubMed: 20451868]

Whalley LJ, Deary IJ, Appleton CL, Starr JM. Cognitive reserve and the neurobiology of cognitive aging. Ageing Res Rev. 2004; 3(4):369–382. [PubMed: 15541707]

Appendix

Table A

Top SNPs for GWAS of executive functioning resilience

CHR	SNP	Base Position	Minor Allele	Beta	*P*-value	Nearest Gene
14	rs3748346	21501095	C	.21	6.43×10^{-8}	*RNASE13*
14	rs3748348	21501195	A	.20	1.18×10^{-7}	*RNASE13*
14	rs112249116	75566463	T	.45	5.74×10^{-7}	*NEK9*
14	rs72736225	75564726	A	.45	5.74×10^{-7}	*NEK9*
14	rs7143599	75613178	T	.45	5.81×10^{-7}	*TMED10*
14	rs72736242	75588320	A	.45	5.81×10^{-7}	*NEK9*
14	rs72736209	75540434	G	.45	8.71×10^{-7}	*ZC2HC1C*
14	rs72734300	75530066	T	.45	8.71×10^{-7}	*ACYP1*
14	rs72736204	75535153	A	.45	8.71×10^{-7}	*ZC2HC1C*
14	rs68179743	75507657	A	.44	1.12×10^{-6}	*MLH3*
6	rs6933137	166142202	G	.53	4.05×10^{-6}	*PDE10A*
6	rs6908907	166141724	A	.53	4.05×10^{-6}	*PDE10A*
6	rs11962446	166153398	A	.52	4.33×10^{-6}	*PDE10A*
6	rs59751101	166165863	G	.52	4.33×10^{-6}	*PDE10A*
6	rs59392157	166167530	C	.52	4.33×10^{-6}	*PDE10A*
6	rs6921426	166149261	T	.52	4.33×10^{-6}	*PDE10A*
6	rs11970244	166174747	C	.52	4.42×10^{-6}	*PDE10A*
12	rs10771511	29437217	C	-.16	6.62×10^{-6}	*FAR2*
2	rs16987794	20748761	C	.23	7.88×10^{-6}	*HS1BP3*
6	rs6912825	166160068	T	.50	8.58×10^{-6}	*PDE10A*

Table 1

Description of participants included in the analyses

Baseline diagnosis	NC[a]	MCI[a]	AD[a]	Total
# of participants	189	330	162	681
Sex: (M/F)	105/84	214/116	89/73	408/273
Age	76.0 (4.9)	75.0 (7.4)	75.8 (7.4)	75.5 (6.8)
Education (years)	16.1 (2.7)	15.8 (3.0)	14.9 (3.1)	15.6 (2.9)
ADNI-Mem	0.96 (0.5)	−0.09 (0.6)	−0.87 (0.6)	0.02 (0.9)
ADNI-EF	0.72 (0.7)	0.01 (0.8)	−0.92 (0.8)	−0.01 (1.0)
Hachinski score	0.54 (0.7)	0.61 (0.7)	0.67 (0.7)	0.60 (0.7)
Stroke/Infarcts (Y/N)	17/172	28/302	13/149	58/623
Cerebral cortex thickness (mm)	−186.29 (19.6)	−196.18 (21.1)	−202.27 (22.4)	−194.88 (21.8)
Hippocampal volume (cm^3)	0.43 (0.4)	−0.06 (0.5)	−0.41 (0.5)	−0.01 (0.6)
White matter hyperintensities volume (cm^3)[b]	−1.56 (1.6)	−1.39 (1.7)	−0.85 (1.6)	−1.31 (1.7)

[*] All summary statistics for variables are reported as Mean (S.D) unless otherwise noted

[a] NC → Normal Controls; MCI → Mild Cognitive Impairment patients; AD → Alzheimer's Disease cases

[b] White matter hyperintensities was natural log-transformed

Table 2

Top 10 Genes for resilience using *SKAT-O* (in terms of *p*-values).

Gene	Chr	# SNPs mapped to the gene	*SKAT-O Beta(1,1) P*-value
RNASE13	14	2	1.33×10^{-7}
FAR2	12	189	3.72×10^{-5}
TPPP2	14	2	9.60×10^{-5}
MLH3	14	13	1.15×10^{-4}
BIRC3	11	4	1.30×10^{-4}
GDPD1	17	14	1.31×10^{-4}
GTF3C5	9	35	1.50×10^{-4}
TLE2	19	15	2.19×10^{-4}
FECH	18	56	2.49×10^{-4}
STXBP5L	3	1064	2.58×10^{-4}

Table 3

Top canonical pathways (False Discovery Rate < 0.0001) for resilience of executive functioning.

Pathway (gene set) name	Set size[a]	Uncorrected *P*-value
Dendritic/Neuron spine	151 (131)	1.27×10^{-12}
Presynaptic membrane	47 (42)	8.68×10^{-11}
Postsynaptic density/Dendritic spine head	111 (100)	2.00×10^{-10}
Calcium ion transmembrane transporter activity	109 (104)	3.75×10^{-10}
Ras guanyl-nucleotide exchange factor activity	93 (86)	4.67×10^{-10}
Rho guanyl-nucleotide exchange factor activity	75 (68)	9.33×10^{-10}
Calmodulin binding	161 (150)	1.11×10^{-9}
Calcium channel activity	92 (89)	1.89×10^{-9}
Guanyl-nucleotide exchange factor activity	167 (151)	2.91×10^{-9}
Cell adhesion molecule binding	52 (46)	5.06×10^{-9}
Synaptic membrane	204 (179)	9.49×10^{-9}
Divalent inorganic cation transmembrane transporter activity	129 (120)	3.21×10^{-8}
Transmembrane receptor protein tyrosine phosphatase activity	18 (18)	3.53×10^{-8}
Regulation of phospholipase C activity	65 (61)	5.22×10^{-8}
Voltage-gated ion channel activity	177 (156)	5.41×10^{-8}
Positive regulation of phospholipase C activity	64 (60)	5.54×10^{-8}
Chloride channel activity	72 (60)	8.36×10^{-8}
Axon part	121 (106)	8.48×10^{-8}
Synapse organization	83 (71)	1.03×10^{-7}
cAMP metabolic process	32 (30)	1.38×10^{-7}

[a]Entries are displayed as: number of genes in the set (number of genes from the GWA data)