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Discovery of gene-gene interactions across multiple independent datasets of Late Onset Alzheimer Disease from the Alzheimer Disease Genetics Consortium

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Abstract

Late-onset Alzheimer disease (LOAD) has a complex genetic etiology, involving locus heterogeneity, polygenic inheritance and gene-gene interactions; however, the investigation of interactions in recent GWAS has been limited. We used a biological knowledge-driven approach to evaluate gene-gene interactions for consistency across thirteen datasets from the Alzheimer Disease Genetics Consortium. Fifteen SNP-SNP pairs within three gene-gene combinations were identified: *SIRT1* x *ABCB1, PSAP x PEBP4, and GRIN2B x ADRA1A.* Additionally, we extend a previously identified interaction from an endophenotype analysis between *RYR3 x CACNA1C.* Finally, *post hoc* gene expression analyses of the implicated SNPs further implicate *SIRT1* and *ABCB1*, and implicate *CDH23* which was most recently identified as an AD risk locus in an epigenetic analysis of AD. The observed interactions in this manuscript highlight ways in which genotypic variation related to disease may depend on the genetic context in which it occurs. Further, our results highlight the utility of evaluating genetic interactions to explain additional variance in AD risk and identify novel molecular mechanisms of AD pathogenesis.

Keywords

gene-gene interactions; epistasis; Alzheimer disease; Biofilter

INTRODUCTION

Alzheimer disease (AD) has a strong yet complex genetic etiology and has already demonstrated allelic and locus heterogeneity and polygenic inheritance. It is possible that additional complexity, including gene-gene interactions, is also involved in the etiology of AD. Although rare mutations in multiple genes can affect early onset AD, only common variation in *APOE* has a large effect on the more common late onset form of AD (LOAD). Recent genome-wide association studies in LOAD have identified up to 21 additional novel genetic loci for AD, including genes from multiple pathways, such as beta-amyloid processing and clearance, calcium signaling and extracellular matrix (Naj et al., 2011; Lambert et al., 2013). Other than *APOE*, the identified genetic loci have very modest effects, and in total the known genetic influences in LOAD still explain only about 33% of the broad-sense heritability (Ridge, Mukherjee, Crane, & Kauwe, 2013), which has been estimated to be 60–80% (Gatz, Reynolds, & Fratiglioni, 2006; So, Gui, Cherny, & Sham, 2011). One possible source of additional heritability is gene-gene interactions. Known loci could further influence disease risk through interactions with each other, as well as with other as yet unknown genetic factors. Also, novel loci with no detectable independent main effect on LOAD risk could interact with each other to significantly increase risk.

To date, the investigation of gene-gene interactions in LOAD has been pursued almost exclusively using a hypothesis-driven, candidate gene approach. Arosio et al. (2004) reported an interaction between variants in the pro-inflammatory cytokine genes IL6 and IL10, and Mateo et al. (2006) reported an interaction between the dopamine beta-hydroxylase gene (DBH) and each of the two cytokine genes *IL1A* and *IL6* (Arosio et al., 2004; Mateo et al., 2006). The Epistasis Project was able to replicate both of these findings in LOAD (Combarros et al., 2010). Interactions between variants in the transferrin gene

(*TF*) and the hemochromatosis gene (HFE) also have been identified and replicated in multiple cohorts for association with LOAD (Robson et al., 2004; Kauwe et al., 2010). An interaction between the insulin gene (*INS*) and the peroxisome proliferator-activated receptor alpha gene (*PPARα*) has been reported in Northern but not Southern Europeans (Kolsch et al., 2012; Heun et al., 2012). Risk for LOAD and vascular dementia reportedly vary according to the interaction of genotypes in the *MTHFR* and *IL6* genes (Mansoori et al., 2012).

Even in hypothesis-free genome-wide association studies (GWAS) of Alzheimer's disease, when testing of gene-gene interactions has been incorporated, it has been restricted to interactions between *APOE* and other risk loci with known main effect associations. Belbin et al. (2011) investigated interactions among 21 LOAD candidate and confirmed risk genes, including *APOE*, *BIN1*, *CLU*, *CR1* and *PICALM* but failed to detect any interactions with disease status or age-at-onset that were significant after correction for multiple testing (Belbin et al., 2011). Similarly, Carrasquillo et al. (2011) failed to identify significant interactions between variants in *BIN1* and other LOAD risk genes, including *APOE*, *CLU*, *CR1* and *PICALM* (Carrasquillo et al., 2011).

In this study, we aimed to identify novel gene-gene interactions that demonstrated association with LOAD across multiple independent datasets. We used a network-based approach to discovery, utilizing prior biological knowledge about LOAD candidate genes—the pathways in which they participate and the genes with which they are related or are known to interact—to guide initial selection of gene-gene models for investigation (Bush, Dudek, & Ritchie, 2009). We also utilized a meta-analysis approach by which we could evaluate the consistency of each identified SNP x SNP interaction across the thirteen independent data sources while correcting for the total number of comparisons evaluated. Finally, we performed a comprehensive analysis of two gene-gene pairs that were previously identified in projects by our research group leveraging endophenotypes of Alzheimer's disease in order to validate the observed effects in case-control datasets.

MATERIALS AND METHODS

Datasets and Quality Control Procedures

Study data consisted of subjects from thirteen datasets available through the Alzheimer's Disease Genetics Consortium (ADGC), including: the Adult Changes in Thought (ACT); the National Institute on Aging Alzheimer Disease Centers (ADC1, ADC2, ADC3); the Alzheimer's Disease Neuroimaging Initiative (ADNI); Oregon Health & Science University (OHSU); Rush University Religious Orders Study/Memory and Aging Project (ROSMAP); Translational Genomics Research Institute series 2 (TGEN2); University of Miami/ Vanderbilt University/Mt.Sinai School of Medicine (UM/VU/MMSM); and Washington University (WashU). All subjects were recruited under protocols approved by the appropriate Institutional Review Boards.

After quality control, the combined dataset included samples from 7,758 LOAD cases and 6,724 cognitively normal elder (CNE) controls. For most of the cohorts, LOAD cases met NINCDS-ADRDA criteria for probable or definite LOAD with age at onset greater than 60

years, and clinically-defined CNEs had a documented MMSE, CASI or 3MS score in the normal range. The only exceptions were TGEN2 and ADNI. The TGEN2 dataset comprised clinically- and neuropathologically-characterized brain donors, 668 with LOAD and 365 CNEs without dementia or significant LOAD pathology. The samples were obtained from 21 different National Institute on Aging-support LOAD Center brain banks and from the Miami Brain Bank as previously described (Reiman et al., 2007; Liang et al., 2011; Caselli et al., 2007; Webster et al., 2009). Additional samples from other brain banks in the United States, United Kingdom and the Netherlands were obtained in the same manner. The Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset comprised 268 LOAD cases and 173 CNEs with neuroimaging support for diagnosis. In the ADNI cohort, LOAD subjects were between the ages of 55–90 years old, had an MMSE score of 20–26 inclusive, met NINCDS/ADRDA criteria for probable LOAD (McKhann et al., 1984), and had an MRI consistent with the diagnosis of LOAD at the most recent follow-up. Table 1 presents descriptive statistics for each of the datasets.

Genotyping

Samples were genotyped at different stages of recruitment on the Affymetrix 6 (UM/VU/MSSM), Affymetrix 1M (TGEN2), Illumina 610 (ADNI, OHSU, UM/VU/MSSM), Illumina 660 (ACT, ADC1, ADC2, WashU), Illumina OmniExpress (ADC3), and Illumina IM (ROSMAP, UM/VU/MSSM). Each dataset was independently imputed using IMPUTE2 with 1000 Genomes Phase 2 samples of European ancestry. Since we were primarily interested in discovering novel gene-gene interactions and not those that modify risk of the major LOAD gene, *APOE*, we excluded SNPs within 50kb of *APOE*.

Quality Control Procedures

Quality control procedures were applied to each dataset separately. Genotype data were cleaned by applying a 98% threshold for genotyping efficiency and a minimum minor allele frequency of 0.10. SNPs not in Hardy-Weinberg equilibrium in controls ($P < 10^{-6}$) were excluded. Subjects for whom reported and genetic sex were inconsistent were identified by analysis of X-chromosome SNPs using PLINK (Purcell et al., 2007) and were excluded from further analysis.

Statistical Analysis

We used a biological knowledge-driven approach (Biofilter; Bush et al., 2009) to select SNP-SNP models with *a priori* evidence that their genes or protein products interact or participate in common biological pathways or processes. Biofilter 2.0 merges information from 13 independently curated annotation databases, including dbSNP, NCBI Entrez Gene, BioGRID, Molecular Interaction database (MINT), PharmGKB, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, NetPath, Protein Family database (Pfam), ORegAnno, UCSC and the NHGRI GWAS Catalog, into one Library of Knowledge Integration (LOKI) database (Pendergrass et al., 2013). Biofilter will accept a candidate gene list and then create a network of gene-gene combinations for which there are at least two LOKI sources suggesting the genes are related or are likely to interact in some manner (e.g., at the protein-protein or biological pathway level). Biofilter will also take as

input the list of genetic variants available in the dataset to be analyzed and will map these variants to genes in the network it built based on strict gene boundaries, a specified distance on either side of the gene boundaries, or the structure of linkage disequilibrium around genes. We chose to map variants to all genes within 50kb of the gene boundaries.

In our analysis, we began by selecting all genes previously implicated in AD genetic association studies. From these sources we identified 1,297 genes. We then used Biofilter to reduce the list down to genes within networks whose edges had an implication index of at least two for further investigation. This left a total of 825 genes (see Supplementary Table 1), comprising 43,376 SNPs.

We analyzed each SNP-SNP pair for interaction effects related to risk for LOAD using an additive encoding of alleles. We utilized the INTERSNP software (http://intersnp.meb.uni-bonn.de/) for genome-wide interaction analysis to test for the significance of the additive interaction term in a logistic regression model (Herold, Steffens, Brockschmidt, Baur, & Becker, 2009). To evaluate the results of this large number of tests, we used a multiple step procedure. First, because it was not computationally feasible to print out all pairs analyzed, we generated a list of the most significant 100,000 SNP-SNP interactions for each dataset. Second, we removed intragenic SNP-SNP pairs where the SNPs were annotated to the same gene. Third, we removed those pairs for which there were less than three observations in the lowest SNP x SNP contingency table cell, which could lead to spurious associations. That is, if there were fewer than three individuals with a given genotype combination in the 3×3 genotype matrix, we would remove that SNP-SNP pair from the analysis. Finally, we then performed a meta-analysis on the remaining 1,191,392 SNP-SNP pairs across all thirteen datasets.

Our correction for multiple comparisons was based on the effective number of independent SNP-SNP pairs evaluated. There were 43,376 SNPs selected for analysis after applying Biofilter. By accounting for linkage disequilibrium using the standard approach in PLINK (--indep 50 5 2) with a Variance Inflation Factor (VIF) of 2 ($r^2 > 0.5$), we determined there were approximately 3,626 independent loci, comprising 6,541,638 independent, *intergenic* SNP-SNP interactions evaluated. Therefore, when applying the Bonferroni procedure, our threshold for statistical significance was set to $\alpha = 7.64 \times 10^{-9}$. Additionally, in order to be considered statistically significant, a given SNP-SNP pair had to be present (and available for testing and subsequent meta-analysis) in at least six out of thirteen datasets and could not demonstrate evidence of heterogeneity across data sources (heterogeneity p-value > 0.05). *Post hoc* analyses adjusting for age, sex, and population principal components were also performed to ensure that these potential confounding factors were not driving any observed association. Finally, we report all SNP x SNP interactions as ‘suggestive’ that passed a less conservative correction accounting for the total number of pairs analyzed in the meta-analysis ($\alpha = 4.20 \times 10^{-8}$). These interaction results may warrant future investigation and are reported in our supplemental materials.

Validation of Genetic Interactions Identified using Endophenotypes of AD

Previous work from our group leveraging amyloid imaging and brain volume data from ADNI identified two gene-gene interactions: *RYR3 x CACNA1C* (Koran, Hohman, &

Thornton-Wells, 2014) and *SYNJ2 x PIK4A* (Koran, Hohman, Meda, & Thornton-Wells, 2014). The SNP selection procedure and coding for those previous analyses differed significantly from the procedure implemented in the present analysis, however we wanted to fully evaluate whether these gene interactions are associated with case-control status. We selected all SNPs within these genes from the genome browser database (http://genome.ucsc.edu/cgi-bin/hgGateway) and used the same statistical approach outlined above, except we used a dominant allele coding to align with the previous analyses. In the case of the *CACNA1C* x *RYR3* interaction analysis, we identified 1,800 SNPs across the two genes after applying the same genotype filtering outlined above with the exception that we used a minor allele frequency (MAF) filter of 0.05 and a dominant encoding to be consistent with the previously reported analyses. We included all covariates mentioned above in our analysis. After accounting for linkage disequilibrium (VIF = 2) there were approximately 10,086 independent, intergenic SNP-SNP combinations evaluated (Bonferroni corrected $\alpha = 5 \times 10^{-6}$). In the case of the *SYNJ2* x *PIK4A* analysis, we identified 620 SNPs across the two genes. After accounting for linkage disequilibrium, there were approximately 2,112 independent, intergenic SNP-SNP combinations evaluated (Bonferroni corrected $\alpha = 2.37 \times 10^{-5}$).

Post hoc Expression Quantitative Trait Loci (eQTL) Analysis

We leveraged the genotype tissue expression (GTEx; http://www.gtexportal.org/home/) project database, the seeQTL expression quantitative trait loci (eQTL) searchable database of human expression QTLs (http://www.bios.unc.edu/research/genomic_software/seeQTL/), and publically available gene expression data from the ROS/MAP dataset calculated from prefrontal cortex tissue and made available through the Accelerating Medicines Partnership AD project (https://www.synapse.org/#!Synapse:syn2580853/wiki/). These sources were used to assess cis eQTLs for genes proximal to the implicated SNP. At the time of analysis, there were 13 tissues available in GTEx and 2 tissues available in the see QTL database in addition to the prefrontal cortex sample available through AMP-AD. Therefore, we corrected all eQTL p-values for the number of tissues evaluated using the Bonferroni procedure (Bonferroni corrected $\alpha = 0.003$). Finally, we used the AMP-AD data to determine whether the observed genes were differentially expressed in the prefrontal cortex of AD cases v. controls when covarying for age and sex.

RESULTS

After removing intragenic SNP pairs and pairs with low contingency cell counts, a total of 1,191,392 pairs were analyzed in a meta-analysis across the thirteen datasets. Ten SNP-SNP combinations within the sirtuin 1 (*SIRT1*) and ATP-binding cassette sub-family B (MDR/TAP), member 1 (*ABCB1*) loci remained statistically significant when correcting for multiple comparisons. When adjusting for age, sex, and population PCs, three additional SNP-SNP pairs within the phosphatidylethanolamine-binding protein 4 (*PEBP4*) and the prosaposin (*PSAP*) loci, and one additional SNP-SNP pair within the glutamate receptor, ionotropic, N-methyl D-aspartate 2B (*GRIN2B*) and the adrenoceptor alpha 1A (*ADRA1A*) loci reached statistical significance. The most significant SNP-SNP pairs within these loci are presented in Table 2 and are described in greater detail below. All SNP-SNP

combinations and their main effects are reported in Supplementary Table 2. An additional 68 SNP-SNP pairs and the annotated genes are also included in Supplementary Table 2 as results suggestive of (but not reaching) significance. The most significant SNP-SNP pair within the *SIRT1* x *ABCB1* interaction was between rs34104788 and rs4728700 (OR=1.36, $p=2.7\times10^{-9}$) and was present in 9 out of the 13 datasets.

The forest plot for this SNP-SNP pair is presented in Figure 1 and the Manhattan plot is presented in Figure 2 using Locus Zoom (Pruim et al., 2010). The most significant SNP-SNP pair within the *PEBP4* x *PSAP* interaction was between rs2466176 and rs762571 (OR=1.25, $p=1.1\times10^{-9}$) and was present in 12 out of 13 datasets (Figure 3). The most significant SNP-SNP pair within the *GRIN2B x ADRA1A* interaction was between rs564830 and rs1805474 (OR=0.77, $p=6.67\times10^{-9}$) and was present in 10 out of 13 datasets (Figure 4).

Validation of Genetic Interactions Identified using Endophenotypes of AD

In our validation analyses, we observed a statistically significant association between 4 SNP-SNP pairs in the *RYR3* and *CACNA1C* genes when correcting for multiple comparisons (Supplementary Table 3); however we did not observe any effects in the *SYNJ2* x *PIK4A* gene pair when correcting for multiple comparisons.

Post Hoc Expression Quantitative Trait Loci Analysis

We observed statistically significant associations between rs4728700 and *ABCB1* in skeletal tissue (p =0.002), rs762571 and *CDH23* in monocytes ($p=1\times10^{-63}$), and rs2466176 and uncharacterized *LOC101929237* in prefrontal cortex tissue ($p=7\times10^{-11}$). Although we did not observe an eQTL association for rs34104788, we did observe another SNP in weak LD with that one (rs11596401, $r^2 = 0.33$, $D'=1$) as an eQTL in monocytes ($p=1\times10^{-9}$). We did not observe eQTL associations for rs1805474 or rs564830. In case-control expression analyses, *GRIN2B* was not available in the AMP-AD dataset, so we corrected for the 6 genes evaluated (including *CDH23* from the eQTL results above). When correcting for multiple comparisons, *CDH23* showed significantly elevated expression in AD relative to controls (p=0.0002). A nominal elevation was observed for *SIRT1* (p=0.02) and a nominal reduction was observed for *PEBP4* (p=0.03) and *PSAP* (p=0.03).

DISCUSSION

Using a biological knowledge-driven approach, we were able to identify SNP-SNP interactions that showed a consistent signal across multiple independent data sources. The 15 SNP-SNP pairs identified were within three gene-gene combinations. Below we summarize the possible relevance of these gene pairs to Alzheimer's disease risk and progression. We also provide validation of a previously identified genetic interaction between *RYR3* and *CACNA1C*, highlighting the utility of leveraging endophenotypes of AD in smaller datasets and reemphasizing the important role of calcium homeostasis in the pathogenesis of AD.

The interaction between intronic SNPs within *SIRT1* and *ABCB1* appears to modify AD risk through alterations in amyloid clearance. *SIRT1* has been shown to suppress amyloid-beta production (Donmez, Wang, Cohen, & Guarente, 2010). It has been suggested that this

suppression occurs through modulations in the production of α-secretase, which may also be the mechanism by which caloric restriction relates to AD resilience in model systems (Qin et al., 2006). *ABCB1* on the other hand, plays a crucial role in Aβ clearance (Kuhnke et al., 2007). It is highly expressed at the blood brain barrier and transports Aβ out of the brain and into the bloodstream (Elali & Rivest, 2013). However, a recent study failed to show an association between *ABCB1* polymorphisms and CSF levels of Aβ-42, suggesting that variance in this gene alone is unlikely to account for substantial variation in AD risk (Kohen et al., 2011). What is more interesting is the potential interaction between these genes. Activation of *SIRT1* increases the expression of *ABCB1* in cancer cell lines, leading to an increased drug efflux (Wang & Chen, 2013). Similarly, activation of *SIRT1* in AD brains may promote amyloid clearance through increased expression of *ABCB1* at the blood brain barrier. Thus, the rather modest effect of genetic variation in either of these genes, perhaps associated with a moderate reduction in amyloid risk, may be multiplicative when genetic variation in both genes results in a substantial increase in Aβ clearance. The *post hoc* gene expression analysis suggests that the observed association is, indeed, likely acting through *SIRT1* and *ABCB1*. However, additional functional work is needed to validate the suggestive results reported herein.

The interaction between intronic SNPs within *PSAP* and *PEBP4* is particularly interesting when considering the results of the *post hoc* expression analysis. Rs762571 appears to be a strong cis-eQTL for cadherin-related 23 which encodes a calcium dependent cell adhesion glycoprotein involved in sterocilia organization. It is not surprising, therefore, that *CDH23* has been implicated in hearing loss and Usher syndrome (Kowalski, Pawelczyk, Rajkowska, Dudarewicz, & Sliwinska-Kowalska, 2014; Miyagawa, Nishio, & Usami, 2012; Nakanishi et al., 2010). Interesting, methylation signals within the *CDH23* loci have been implicated in AD previously (Lord & Cruchaga, 2014), although the potential mechanism remains somewhat unclear. Epithelial cadherin (encoded by *CDH1*) has been shown to bind to presenilin-1, which ultimately regulates cadherin function and cell-cell adhesion (Baki et al., 2001). Similarly, neural cadherin has been implicated in amyloid-β release via an interaction with presenilin-1. However, it is unclear whether the implicated *CDH23* effect acts through a comparable amyloid pathway. The SNP within *PEBP4* is a strong eQTL suggesting a potential functional role; however, the eQTL is for an overlapping uncharacterized locus (*LOC101929237*), which is a long non-coding antisense RNA within the *PEBP4* locus. This SNP also showed a modest association with *PEBP4* in the prefrontal cortex (p=0.01) in the same direction as that observed with *LOC101929237*, but ultimately it is unclear whether the observed association with AD is driven by *PEBP4* directly, by *PEBP4* through *LOC101929237*, or by a different locus regulated by *LOC101929237*.

The final observed interaction between intronic SNPs within *GRINB2* and *ADRA1A* were not supported by cis-eQTL results, leaving open the possibility that these SNPs may be acting through other genes. However, both of these genes have some potential relevance to AD. *GRIN2B* has been implicated in AD previously and is thought to act through alterations in NMDA receptor activity (Andreoli et al., 2014). The alpha(1)-adrenoceptor has been shown to prevent memory deficits in transgenic mouse models (Katsouri et al., 2013). Relevant to the observed interaction, there also appears to be a synergistic effect of NMDA

receptors and the alpha(1)-adrenoceptors on spatial navigation performance, leaving open the possibility that the observed association between *GRIN2B* and *ADRA1A* is driven by downstream alterations in NMDA and adrenoceptor activity relevant to the amyloid cascade (Riekkinen, Stefanski, Kuitunen, & Riekkinen, 1996). However, future functional analysis is warranted to further investigate this pathway.

Validation of Previously Identified Interactions

This study leveraged data from thirteen independent datasets and a knowledge-based variable selection technique to identify biologically plausible genetic interactions. Previous work in our lab has used a comparable approach to identify genetic interactions in relation to specific endophenotypes of Alzheimer's disease (Koran, Hohman, Meda, & Thornton-Wells, 2014; Koran et al., 2014), and here we successfully validated the previously identified *RYR3-CACNA1C* interaction in relation to AD. As previously concluded, it appears that genes along the amyloid-calcium axis interact to confer risk for AD.

It is interesting that we did not observe interactions among the previously identified AD GWAS SNPs even though they were included in our analyses. Previous work in our lab focusing specifically on the ten most significant SNPs identified in AD GWAS studies found very weak interaction effects in relation to amyloid deposition (Hohman, Koran, & Thornton-Wells, 2013). The present results provide additional evidence that genetic interactions among previously identified AD loci are unlikely to account for additional variance in AD risk. However, our results also suggest that genetic interactions exist within the pathways identified by previously reported risk loci. Future work performing full genome wide interaction analyses may shed additional light on this topic and clarify whether genetic interactions in other pathways confer additional risk.

The meta-analysis technique applied in the current analysis sought to balance statistical power with the desire for replication; however analyzing all thirteen datasets individually did reduce power to detect interaction effects in some of the smaller data sets such as OHSU, ADNI, and WASHU. Moreover, this approach limited our ability to evaluate less frequent variants given the low expected cell counts in contingency tables for variants with a MAF < 0.10 in these smaller data sources. While the meta-analysis approach gives us confidence in our observed interaction effects, follow-up analyses further replicating these interactions and evaluating the functional role of the SNPs involved will be necessary in order to better understand the mechanism of these interactions.

The results of this project provide additional evidence that genetic interactions likely explain some of the missing heritability in AD. However, given the small effect sizes observed, comparable to those of single marker analyses, it seems unlikely that interactions can explain all of the missing heritability. Future work will seek to perform full genome-wide interaction analyses to better understand the breadth of interaction effects in AD.

In conclusion, using a biological knowledge-driven approach aimed at identifying consistent SNP x SNP interactions across thirteen independent datasets, we were able to identify a number of gene-gene models with biologically plausible mechanisms of action. These models build on the substantial literature on common variants associated with AD, and

highlight the potential utility of applying large scale genetic interaction models to better understand disease risk and progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We use a biologically-driven, network-based approach for interaction analysis
- We conduct a meta-analysis of SNP interactions across thirteen ADGC datasets
- We identify 15 significant SNP interactions across three gene pairs
- Genetic interactions explain variability in AD risk beyond single variant effects

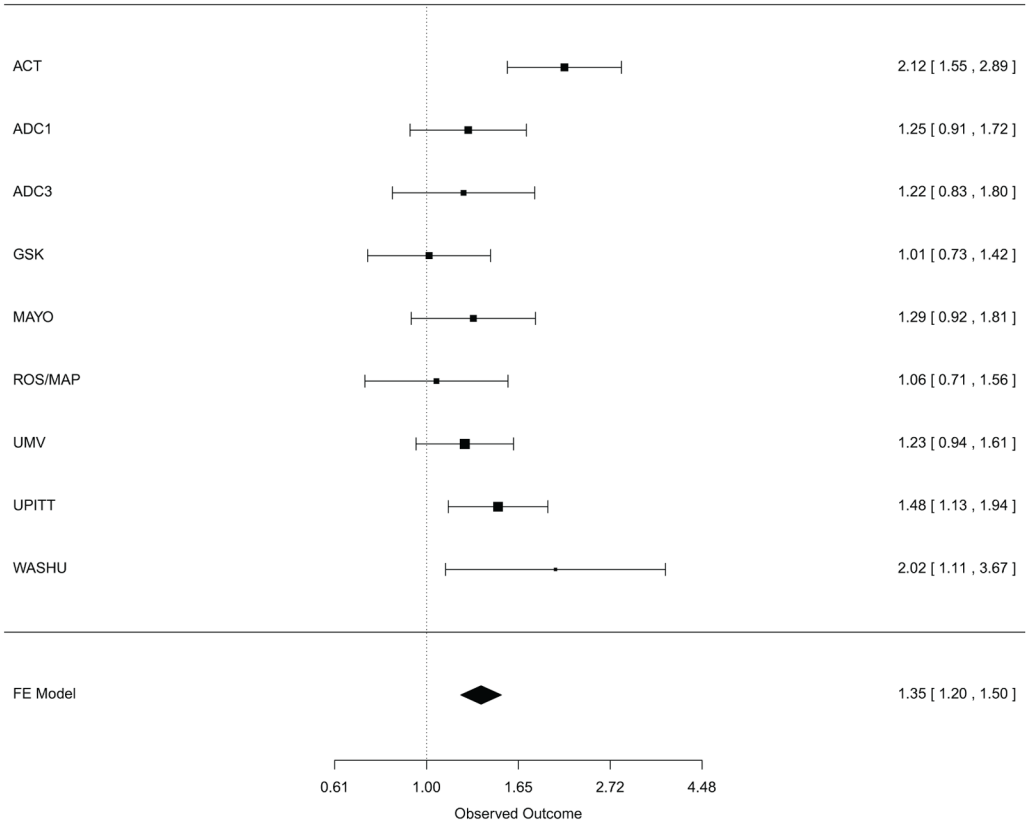

Figure 1.

Each dataset is presented along the Y-axis, and the odds ratio is presented along the y-axis. For each dataset, the square represents the odds ratio and the confidence band represents the 95% confidence interval around the odds ratio. The fixed effects odds ratio is presented as a diamond at the bottom of the graph. The width of the diamond represents the 95% confidence interval of the fixed effects odds ratio.

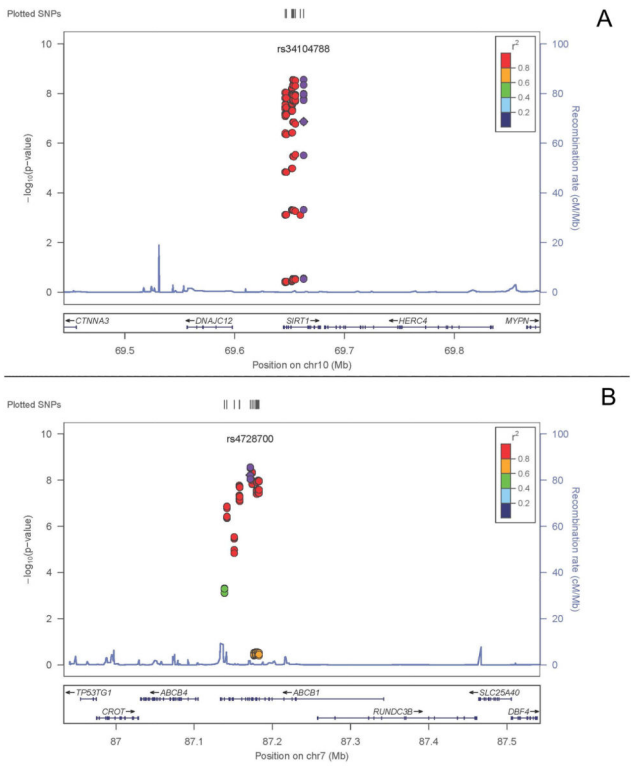

Figure 2.

This figure provides the interaction p-value for the SNP-SNP pairs between SIRT1 (panel A) and ABCB1 (panel B) that were meta-analyzed in the final step of the interaction analysis. Each point in panel A has a corresponding point in panel B, as each point represents a single SNP-SNP interaction across these genes. The chromosome position is presented along the x-axis. The –log10 p-value is presented along the Y axis. Points are colored by their linkage disequilibrium to the most significant SNP in the gene.

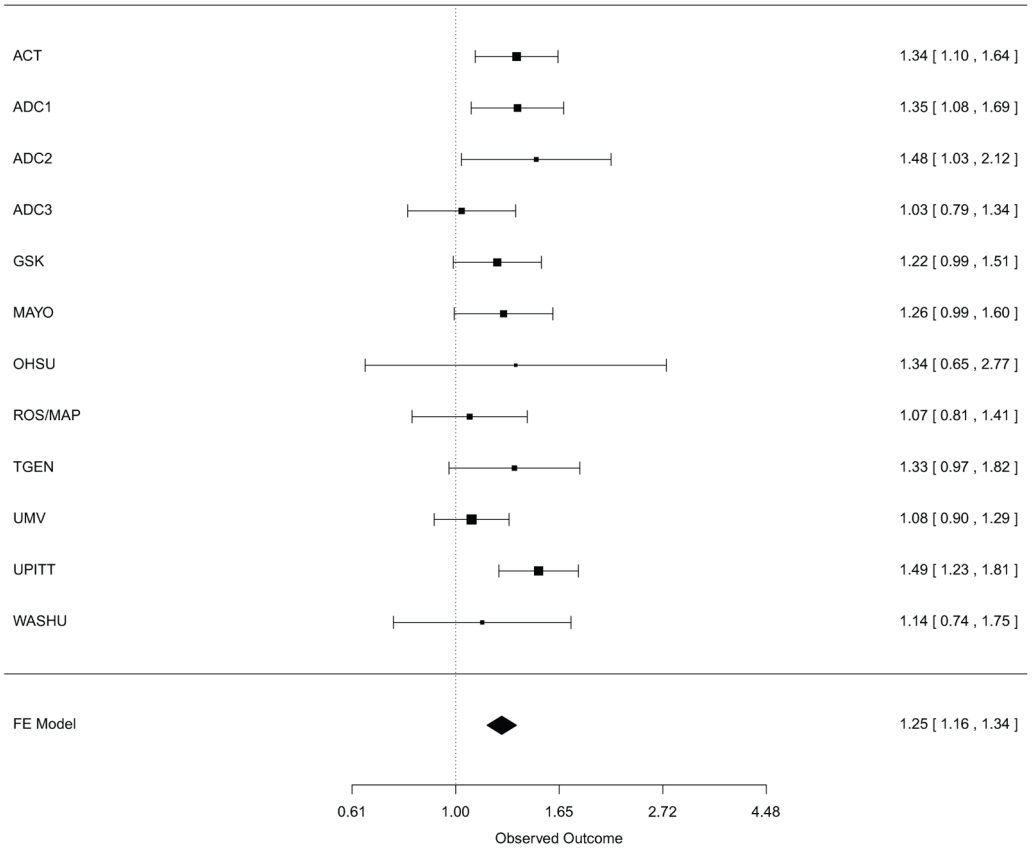

Figure 3.

Each dataset is presented along the x-axis, and the odds ratio is presented along the y-axis. For each dataset, the square represents the odds ratio and the confidence band represents the 95% confidence interval around the odds ratio. The fixed effects odds ratio is presented as a diamond at the bottom of the graph. The width of the diamond represents the 95% confidence interval of the fixed effects odds ratio.

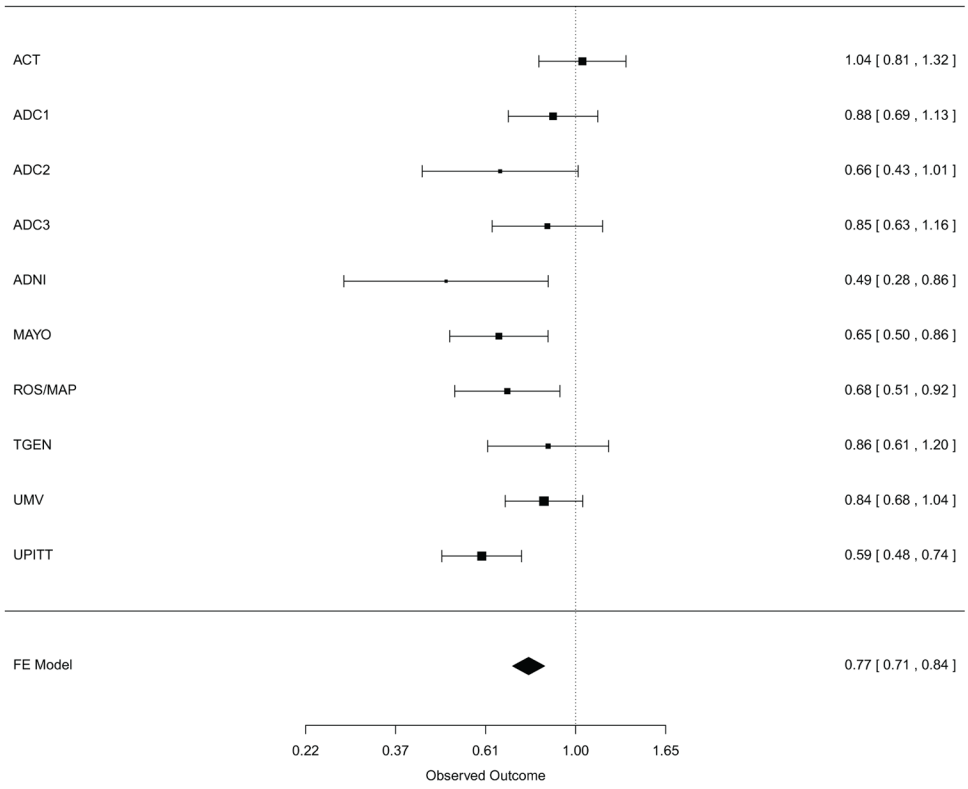

Figure 4.

Each dataset is presented along the x-axis, and the odds ratio is presented along the y-axis. For each dataset, the square represents the odds ratio and the confidence band represents the 95% confidence interval around the odds ratio. The fixed effects odds ratio is presented as a diamond at the bottom of the graph. The width of the diamond represents the 95% confidence interval of the fixed effects odds ratio.

Table 1

Demographics and Summary Statistics for the thirteen ADCG Datasets included in analyses.

	LOAD Cases				Controls			
Cohort	**N**	**Median AOO**	**% Female**	**% *APOE* ε4 carriers**	**N**	**Median AOE***	**% Female**	**% *APOE* ε4 carriers**
ACT	532	84	62	45	1571	81	55	22
ADC1	1549	72	54	68	512	76	59	30
ADC2	727	74	51	62	156	76	68	29
ADC3	894	76	55	62	586	74	63	25
ADNI	268	76	42	68	173	78	40	25
GSK	652	74	57	64	712	74	64	24
MAYO	647	75	58	66	1025	73	51	28
OHSU	132	86	62	44	153	85*	55	16
ROSMAP	295	86	71	38	769	82	72	18
TGEN2	668	81	65	65	365	80*	38	23
UM/VU/MSSM	2354	74	65	58	2252	74	61	23
UPITT	1160	73	63	58	827	75	63	20
Wash U	339	73	57	54	187	77	60	27

AOO = Age of Onset for Alzheimer's Disease

AOE = Age of last Exam (when Control status was determined)

* Median age of death is reported for the OHSU and TGEN2 cohorts because these are autopsy-based cohorts.

Table 2

Meta-Analysis SNP x SNP Models Surviving Correction for Multiple Comparisons. Most significant SNP-SNP pair for each gene-gene combination is presented.

Locus 1			Locus 2			No Covariates	Covariates
RefSeq#	Position Chr:bp*	Gene	RefSeq#	Position Chr:bp*	Gene	Fixed Effects Meta-Analysis Odds Ratio, P-value [95% CI]	Fixed Effects Meta-Analysis Odds Ratio, P-value [95% CI]
rs34104788	chr10:69662460-69662960	*SIRT1*	rs4728700	chr7:87171409-87171909	*ABCB1*	**OR=1.36, p=2.7×10^{-9} [1.23, 1.50]**	OR=1.35, p=1.4×10^{-7} [1.20, 1.50]
rs762571	chr10:73594249-73594749	*PSAP*	rs2466176	chr8:22737790-22738290	*PEBP4*	OR=1.20, p=2.9×10^{-8} [1.13, 1.28]	**OR=1.25, p=1.0×10^{-9} [1.16, 1.34]**
rs1805474	chr12:13741900-13742400	*GRIN2B*	rs564830	chr8:26694540-26695040	*ADRA1A*	OR=0.82, p=1.0×10^{-6} [0.76, 0.89]	**OR=0.77, p=6.7×10^{-9} [0.71, 0.84]**

*Chromosome and base pair positions are reported for 1000 Genomes project data, which uses the GRCh37 build.