Genetic Modifiers of ABCA1 Activity Interact with *APOE* Isoforms to Mediate Alzheimer's Disease Risk

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Objective: ATP-binding cassette transporter A1 (ABCA1) has been associated with Alzheimer's disease (AD), but the mechanisms by which it impacts disease risk are unknown. ABCA1 is known to bind apolipoprotein E (ApoE) and catalyze apolipoprotein lipidation. We explored whether genetic variants altering *ABCA1* function interact with *APOE* isoforms to modify AD risk.

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Methods: Using data from the Alzheimer's Disease Sequencing Project (ADSP), UK Biobank, and the Alzheimer Disease European Sequencing consortium, we assessed the impact of *ABCA1* variants on AD risk in *APOE* subgroups and tested for statistical interactions with *APOE* $\varepsilon 2$ and $\varepsilon 4$ in an all-*APOE* cohort. We first examined damaging nonsynonymous *ABCA1* variants previously associated with AD. We then constructed a measure of predicted ABCA1 activity based on HDL-associated variants and tested its association with AD risk. Finally, we explored potential pathogenic mechanisms of missense variants of interest.
Results: Damaging nonsynonymous *ABCA1* variants had differential AD risk effects between *APOE* genotype groups and exhibited interaction effects with *APOE* $\varepsilon 2$ and $\varepsilon 4$. Predicted ABCA1 activity based on HDL-associated variants was associated with reduced AD risk and interacted with *APOE* $\varepsilon 4$. Replication analyses suggested similar differences in effect size of ABCA1 variants between *APOE* groups and had concordant effect directions, although not statistically significant, in the *APOE* interaction model. *ABCA1* missense variants N1800H and E1172D were strongly associated with plasma HDL and interacted with *APOE* in AD risk. In cell-based assays, ABCA1-N1800H showed plasma membrane localization defects potentially driven by misfolding.
Interpretation: Genetic modifiers of ABCA1 activity interact with *APOE* isoforms to alter AD risk.

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Lipid metabolism has received attention as a potentially important pathway in the development of neurodegenerative disorders.[1,2] Whereas many lipid-related genes, including *APOE*, *ABCA7*, and *ABCA1*, have now been associated with Alzheimer's disease (AD), the exact role of lipid metabolism in neurodegeneration has remained elusive.[3–6]

ATP-binding cassette transporter A1 (ABCA1) is a membrane-bound lipid transporter that has been linked with AD via both common, low-impact and rare, high-impact genetic variants.[7–9] The function of ABCA1 in peripheral lipid metabolism is well characterized, as it is essential to produce high-density lipoprotein (HDL) particles in blood by catalyzing the efflux of phospholipids and cholesterol onto apolipoprotein A1 (ApoA1).[10] Loss-of-function (LoF) and certain missense variants in *ABCA1* lead to decreased HDL levels.[11] In the central nervous system (CNS), ABCA1 appears to be important in forming "HDL-like" lipoprotein nanoparticles that contain apolipoprotein E (ApoE) via a process that may share molecular features with ApoA1-based lipoprotein formation.[12,13]

Recent findings suggest that ApoE $\varepsilon 4$ pathogenicity, at least partially, results from distinct interactions in the process by which this polypeptide acquires lipids.[14–16] Although it is well established that ABCA1 is the first extracellular partner of ApoE and catalyzes the rate-liming step in the formation of ApoE-derived lipoprotein nanoparticles, the molecular basis of this process remains elusive.[12,17] Moreover, in vitro studies centered on the effect of ApoE isoforms on ABCA1-driven lipoprotein nanoparticle formation have yielded conflicting data, often depending on the model system used.[15,18–21] As no human data have characterized the relationship between *ABCA1* and *APOE* in the context of AD, understanding this interaction at a disease level could shed light on the role of these genes, and of lipoprotein metabolism more generally, in AD pathogenesis.

Building on the previously uncovered link between *ABCA1* variants and AD risk, here, we examine the possibility of a genetic interaction between *ABCA1* and *APOE* by characterizing the effect of *APOE* isoforms on the risk for AD conferred by variants affecting *ABCA1* function.

Methods

Genetic Sequencing Cohorts

As a discovery group, we analyzed subjects from the Alzheimer's Disease Sequencing Project (ADSP)[22] releases 1 through 4, comprising whole-genome sequencing (WGS) and whole-exome sequencing (WES), and UK Biobank (UKB) WES.[23] Using SNPWeights version 2.1,[24] we classified individuals in each dataset into 1 of 5 continental superpopulations defined by the 1,000 Genomes Consortium[25] and performed analyses separately in each dataset and ancestry. Pairs of individuals with at least third-degree relatedness were pruned, and thorough quality control was performed for variants and samples in each cohort. To reduce computational burden, controls in UKB were sampled at a 10:1 ratio with cases. Following this, only cohorts with sufficient power for detection of damaging nonsynonymous *ABCA1* variants, described below, were included, for a final set of cohorts including ADSP WGS (European [EUR]), ADSP WGS (African [AFR]), ADSP WES (EUR), and UKB WES (EUR).

AD Phenotyping

Data collection for ADSP and UKB has been described previously.[22,26] In ADSP, we included AD cases and healthy controls, whose phenotyping included age at onset and last known control age. In UKB, AD case status and age were set based on having a disease report in various medical records. We included AD cases throughout the age spectrum as has been done for previous genetic analyses to improve discovery power.[8,9] However, >95% of AD cases in discovery datasets had an age at onset above 55 years old.

AD Association Analysis

We performed Cox proportional hazards regression on age to AD conversion, covarying for 10 principal components, sex, and, in ADSP, sequencing platform to account for batch effects. Analyses were run in each dataset and ancestry group, then meta-analyzed using inverse-weighted variance.

Rare Variant Burden Test

We first considered a set of rare, damaging nonsynonymous variants in *ABCA1* which were previously associated with AD, comprised of LoF variants and missense variants with a REVEL score ≥ 0.75 (denoted LoF + REVEL ≥ 75).[9] For this set, variants were included if they had an allele frequency (AF) below 1% in their respective cohort and gnomAD.[27] We summed the number of alleles across selected variants for each individual (denoted "variant burden") and performed association testing on this variable. We included a cohort in the meta-analysis if it had ≥10 variant carriers.

APOE Interaction Analysis

We first calculated and compared *ABCA1* effect estimates in *APOE* genotype subgroups. We used a Bonferroni correction for the number of subgroups in each analysis to assess for statistical significance (p value cutoff = 0.05 / 4 = 0.0125). To test for an interaction, we then examined all *APOE* groups together, including 4 additional covariates: *APOE* $\varepsilon 2$ dosage; *APOE* $\varepsilon 4$ dosage; *APOE* $\varepsilon 2$ dosage × variant burden; and *APOE* $\varepsilon 4$ dosage × variant burden. We tested *APOE* interaction variables with variant burden and used the Bonferroni correction for the number of variables examined (value cutoff = 0.05 / 3 = 0.017). In the interaction analysis, we excluded individuals with an *APOE* $\varepsilon 2/\varepsilon 4$ genotype to avoid confounding by potentially conflicting effects of $\varepsilon 2$ and $\varepsilon 4$.

HDL-Weighted Burden Test

In order to construct a more empirical measure of variant impact on protein function, we estimated the effect of *ABCA1* missense variants on plasma HDL in UKB WES using BOLT-LMM.[28] We considered all missense variants with at least 10 alleles and covaried for age, sex, *APOE* $\varepsilon 2$ and $\varepsilon 4$ dosage, use of cholesterol-lowering medication, and 40 principal components. Plasma HDL values were $\log_2$-transformed. We ran 2 models: a "marginal" model testing one variant at a time for variant discovery, and a "joint" model including all variants in the locus to provide more accurate estimates of variant coefficients by accounting for linkage disequilibrium. Only *ABCA1* missense variants with 10 or more alleles were tested, and variants significantly affecting HDL levels were identified using a Bonferroni-corrected p value < 0.05 in the marginal model (correcting for the number of variants tested). We used coefficients from the joint model to construct a measure of genetically predicted ABCA1 lipid efflux activity and tested it against AD onset in the AD datasets, excluding UKB to avoid data circularity. This was done as in the rare variant burden test, except the burden variable was replaced with a weighted sum of HDL-associated *ABCA1* missense variant dosage.

Replication

As a replication group, we meta-analyzed the fifth release (R5) of ADSP along with the Alzheimer Disease European Sequencing (ADES) consortium stage 1 dataset. Data in ADES are of European ancestry and were collected as described previously[9]; tests in this dataset summed genotype posterior probability scores rather than allele counts as well as six rather than 10 principal components. For the most accurate estimation of effects, we then conducted a meta-analysis combining cohorts across the discovery and replication. In the combined meta-analysis, we tested for effect heterogeneity using Cohran's Q test implemented in the R *metafor* package.

Identification of Missense Variants of Interest

We first refined the statistical model by including only *APOE* interaction terms that were statistically significant in the combined meta-analysis. Then, for each variant included in the HDL-weighted burden test, we excluded the variant and observed changes in model fit by calculating the total difference in Akaike Information Criterion (AIC). Here, a negative ΔAIC indicated that the variant likely strengthened the model fit, whereas a positive ΔAIC indicated that the variant likely weakened the model fit. Missense variants were selected for further exploration if they had a ΔAIC beyond the commonly used cutoff of ±2.[29] Of variants of interest, we assessed those that had at least 10 allele copies in each of at least 3 cohorts for association with AD risk by themselves using the refined Cox regression model.

Cell-Based Assays of ABCA1 Variants of Interest

We transfected ABCA1 variants of interest into HEK293T and Expi293F cells for microscopy and biochemical assays. The pcDNA3.1-FLAG-ABCA1-eGFP constructs were generated using standard polymerase chain reaction (PCR) and cloning techniques with *Escherichia coli* (*E. coli*). Plasmid preparations were transfected into HEK293T or Expi293F cells and cultures were incubated for 3 to 4 days before downstream experiments. For confocal microscopy, HEK293T cells were washed, fixed, and stained with wheat germ agglutinin AlexaFlour-594

conjugate as a membrane marker and Hoechst 33342 as a nuclear stain. For purification of ABCA1-eGFP protein from Expi293F cells, the cells were pelleted, lysed by sonication, and centrifuged at high speed to isolate the membrane fraction. The membrane fraction was solubilized in a detergent solution and then separated from the insoluble fraction. Samples were electrophoresed using SDS-PAGE and then transferred to a nitrocellulose membrane for immunoblotting.

Ethics Approval and Consent to Participate

Informed consent was obtained from participants by each study's investigator. The current protocol was granted an exemption by the Stanford University Institutional Review Board (IRB) as the analyses used deidentified data.

Results

We included 67,869 individuals across 4 discovery cohorts (Table 1). We first examined the AD effect of rare LoF + REVEL ≥ 75 variants in *ABCA1* in groups defined by *APOE* genotype. Carrier frequencies of LoF + REVEL ≥ 75 *ABCA1* variants were consistent across datasets and ancestries, ranging from 1.1 to 1.4%. We replicated the finding[9] that LoF + REVEL ≥ 75 variants in *ABCA1* increase risk for AD when considering all *APOE* genotypes together (hazard ratio [HR] = 1.30, 95% confidence interval [CI] = 1.15–1.48, p value [p] = 3.9E-05). Stratifying by *APOE* genotype, LoF + REVEL ≥ 75 variants increased risk in $\varepsilon2/\varepsilon3$ and $\varepsilon3/\varepsilon3$ groups but not in $\varepsilon3/\varepsilon4$ or $\varepsilon4/\varepsilon4$ groups. AD effect sizes tended to increase with *APOE* $\varepsilon2$ alleles and decrease with *APOE* $\varepsilon4$ alleles (Fig 1A and Supplementary Table S1). Although we do not report effect estimates for *APOE* $\varepsilon2/\varepsilon2$, as there were only 4 variant carriers in this group, 3 of these were diagnosed with AD, with ages at onset 57, 79, and 78 years. Further, a univariate log-rank test combining *APOE* $\varepsilon2/\varepsilon2$ individuals from all cohorts indicated a significant difference in risk of AD onset in carriers of LoF + REVEL ≥ 75 *ABCA1* variants (p = 7.9E-09; Supplementary Fig S1).

To more robustly assess for differences in *ABCA1* effect size driven by *APOE*, we tested *ABCA1* variant burden × *APOE* isoform dosage interaction terms in a model including all *APOE* genotypes except $\varepsilon2/\varepsilon4$ (N = 66,266; N carriers = 751). In this analysis, LoF + REVEL ≥ 75 *ABCA1* variants were associated with increased AD risk by themselves; the interaction of *ABCA1* variant burden with *APOE* $\varepsilon2$ dosage was associated with increased AD risk (HR = 1.99, 95%

TABLE 1. Demographics for AD Association Cohorts

Dataset	Discovery				Replication	
	ADSP WES	ADSP WGS	ADSP WGS	UKB WES	ADSP WGS R5	ADES
Ancestry	EUR	AFR	EUR	EUR	EUR	EUR
Total, n	9,568	4,290	11,285	42,726	8,742	8,937
F, n (%)	3,991 (41.7)	1,192 (27.8)	4,798 (42.5)	23,047 (53.9)	3,711 (42.5)	5,324 (59.6)
AD cases, n (%)	4,849 (50.7)	1,456 (33.9)	6,291 (55.7)	3,950 (9.2)	1,275 (14.6)	4,966 (55.6)
Age at AD onset, mean (SD)	76.3 (8.4)	74.6 (9.0)	71.1 (11.3)	75.4 (5.3)	70.1 (12.7)	70.8 (12.5)
EOAD, n (%)[a]	444 (9.2)	188 (12.9)	1,881 (29.9)	179 (4.5)	450 (35.3)	1,774 (35.7)
Age at last HC, mean (SD)	84.6 (6.4)	74.0 (8.0)	78.8 (8.2)	71.1 (8.0)	77.3 (8.3)	77.6 (15.5)
APOE genotype, n (%)						
$\varepsilon2/\varepsilon2$	57 (0.6)	43 (1.0)	29 (0.3)	221 (0.5)	43 (0.5)	45 (0.5)
$\varepsilon2/\varepsilon3$	1,132 (11.8)	548 (12.8)	683 (6.1)	4,971 (11.6)	919 (10.5)	774 (8.7)
$\varepsilon2/\varepsilon4$	176 (1.8)	185 (4.3)	210 (1.9)	1,032 (2.4)	220 (2.5)	217 (2.4)
$\varepsilon3/\varepsilon3$	5,592 (58.4)	1,795 (41.8)	5,301 (47.0)	24,152 (56.5)	4,639 (53.1)	4,564 (51.1)
$\varepsilon3/\varepsilon4$	2,466 (25.8)	1,442 (33.6)	4,105 (36.4)	10,965 (25.7)	2,443 (27.9)	2,745 (30.7)
$\varepsilon4/\varepsilon4$	140 (1.5)	276 (6.4)	954 (8.5)	1,385 (3.2)	478 (5.5)	592 (6.6)

AD = Alzheimer's disease; ADES = Alzheimer Disease European Sequencing; ADSP = Alzheimer's Disease Sequencing Project; AFR = African; EOAD = early-onset Alzheimer's disease; EUR = European; F = female; HC = healthy control; SD = standard deviation; UKB = UK Biobank; WES = whole-exome sequencing; WGS = whole-genome sequencing.

[a]Age at AD onset < 65 years. Percentage given is out of all AD cases.

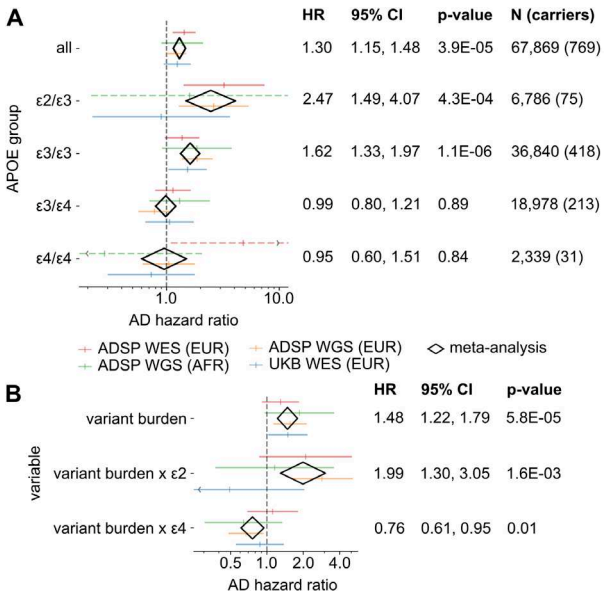

FIGURE 1: Association of the burden of LoF + REVEL ≥ 75 variants in *ABCA1* with AD in discovery cohorts. Bottom axes denote HRs with 95% CIs from Cox regression against AD onset. Solid lines reflect association results included in the meta-analysis, whereas dashed lines were excluded due to insufficient variant carriers (<10). (A) Results for regression of *ABCA1* variant burden stratified by *APOE* genotype group. (B) Results for regression of *ABCA1* variant burden and *ABCA1* variant burden × *APOE* isoform dosage terms, including all *APOE* groups. AD = Alzheimer's disease; CI = confidence interval; HR = hazard ratio. [Color figure can be viewed at www.annalsofneurology.org]

CI = 1.30–3.05; p = 1.6E-03), and the interaction of *ABCA1* variant burden with *APOE* ε4 was associated with decreased AD risk (HR = 0.76, 95% CI = 0.61, 0.95, p = 0.01; Fig 1B and Supplementary Table S1).

We next explored the association of predicted ABCA1 lipid efflux activity with AD risk. Through BOLT-LMM in 404,545 European subjects in UKB, we tested 174 *ABCA1* missense variants and identified 35 that were significantly associated with plasma HDL (p value cutoff = 0.05 / 174 = 2.87E-04; Table 2). We used a weighted sum of these variants to construct a measure of predicted ABCA1 activity, which was robustly correlated with measured HDL levels across tested ancestries in UKB WES (Supplementary Fig S2). In AD datasets, distributions of predicted ABCA1 activity were centered near zero (median = 0.0–0.014, interquartile range = 0.01–0.03 in each cohort), and more subjects had increased (28.7–84.7% in each cohort) than decreased (0.4–3.1% in each cohort) predicted ABCA1 activity from reference. Of note, the AFR cohort had a higher percentage of subjects with increased predicted ABCA1 activity (84.7%) than the EUR cohorts (28.7–35.1%; Supplementary Fig S3).

Our measure of predicted ABCA1 activity trended toward reduced AD risk when considering all *APOE* genotypes together. However, stratifying by *APOE* subgroups, *APOE* ε3/ε3 individuals showed a strong association between predicted ABCA1 activity and decreased AD risk (HR = 0.06, 95% CI = 0.02–0.17, p = 5.4E-07), and this association appeared to diminish in the presence of both ε2 and ε4 alleles. Predicted ABCA1 activity was not significantly associated with AD risk in *APOE* ε2/ε3, ε3/ε4, or ε4/ε4 groups (Fig 2A and Supplementary Table S2). An *APOE* interaction model including all *APOE* genotypes other than ε2/ε4 (N = 24,572; N carriers = 10,635) showed that predicted ABCA1 activity was associated with reduced AD risk and that the interaction of predicted ABCA1 activity with *APOE* ε4 dosage was associated with increased AD risk (HR = 11.3, 95% CI = 3.02–42.4, p = 3.2E-04). We saw no significant interaction effect of predicted ABCA1 activity with *APOE* ε2 dosage on AD risk. This suggested that, similarly to LoF + REVEL ≥ 75 variants, ε4 alleles diminished the association of predicted ABCA1 activity with reduced AD risk (Fig 2B and Supplementary Table S2).

We performed sensitivity analyses to validate the robustness of the interaction between ABCA1 variants, *APOE* ε4 dosage, and AD in the discovery group. First, we excluded UKB WES (EUR) from the test of LoF + REVEL ≥ 75 variants, as this dataset has lower diagnostic accuracy for AD than ADSP. These variants showed the same patterns of association with AD and interaction with *APOE* isoforms when including only ADSP. Second, in the HDL-weighted analysis, we explored the contributions of HDL-increasing and HDL-decreasing variants separately. Weighted sums of only HDL-increasing (n = 6) or HDL-decreasing (n = 29) ABCA1 variants showed the same patterns of association as the weighted sum including all variants, suggesting the association was driven by individuals with both increased and decreased predicted ABCA1 activity. Finally, burden tests using unweighted dosages of HDL-associated, HDL-increasing, or HDL-decreasing variants were not significantly associated with AD risk, supporting the weighting of variants based on their plasma HDL effects as a meaningful predictor of AD risk. Adding UKB WES (EUR) strengthened the association of predicted ABCA1 activity and its interaction with *APOE* ε4 with AD risk (Supplementary Table S3).

We sought independent replication of our *ABCA1-APOE* interaction results by meta-analyzing 17,679 subjects in ADSP WGS R5 and ADES (see Table 1). We focused on (1) differences in effect sizes of *ABCA1* variants between *APOE* groups; and (2) testing for an interaction in the all-*APOE* analysis. As was found in the discovery analysis, estimated AD effect sizes for LoF + REVEL ≥ 75 variants became less pronounced with increasing *APOE* ε4 alleles. LoF + REVEL ≥ 75 variants increased AD risk in the all-*APOE* cohort

TABLE 2. ABCA1 Missense Variants Associated With Plasma HDL Levels in UKB

SNV (chr:pos:ref:alt)	Protein variant	AC	AF	*p*	*β* (marginal)	SE	*β* (joint)[a]
9:104794495:T:G	N1800H	270	3.5E-04	4.5E-87	−0.361	0.018	−0.308
9:104826974:C:T	V771M	22,560	0.0290	4.3E-86	0.039	0.002	0.033
9:104824472:T:C	I883M	100,136	0.1285	3.7E-75	0.018	0.001	0.007
9:104825752:C:T	V825I	48,907	0.0628	9.2E-65	0.023	0.001	0.001
9:104831048:C:A	W590L	123	1.6E-04	8.1E-57	−0.429	0.027	−0.366
9:104798503:C:T	R1680Q	530	6.8E-04	7.5E-33	−0.154	0.013	−0.141
9:104831058:G:A	R587W	31	4.0E-05	1.1E-28	−0.569	0.054	−0.525
9:104799864:T:C	N1633S	84	1.1E-04	1.2E-23	−0.325	0.033	−0.275
9:104817351:C:G	E1172D	23,544	0.0302	5.3E-23	0.019	0.002	0.028
9:104831048:C:G	W590S	37	4.7E-05	1.7E-22	−0.476	0.050	−0.427
9:104816339:G:A	S1181F	1,417	0.0018	1.4E-18	−0.071	0.008	−0.073
9:104796190:T:C	I1749V	159	2.0E-04	1.1E-17	0.203	0.024	0.195
9:104798445:C:A	W1699C	24	3.1E-05	1.6E-16	−0.505	0.062	−0.015
9:104798504:G:A	R1680W	18	2.3E-05	6.5E-16	−0.574	0.071	−0.509
9:104884475:G:A	P85L	1,020	0.0013	2.3E-14	−0.072	0.010	−0.068
9:104812600:G:A	R1342W	16	2.1E-05	4.4E-13	−0.563	0.076	−0.497
9:104786365:T:C	T2112A	14	1.8E-05	2.5E-12	−0.582	0.081	−0.378
9:104830991:G:A	T609M	51	6.5E-05	5.6E-12	−0.274	0.042	−0.257
9:104804650:G:A	T1512M	12	1.5E-05	8.3E-12	−0.596	0.087	−0.481
9:104818830:C:A	D1099Y	12	1.5E-05	4.2E-09	−0.516	0.087	−0.451
9:104812587:C:T	G1346E	17	2.2E-05	1.2E-08	−0.408	0.074	−0.352
9:104836982:G:A	R437W	27	3.5E-05	1.7E-08	−0.301	0.058	−0.278
9:104826965:T:G	T774P	3,325	0.0043	9.0E-08	−0.027	0.005	−0.009
9:104831638:T:A	N567Y	27	3.5E-05	1.7E-07	−0.319	0.058	−0.024
9:104837095:A:G	V399A	4,169	0.0054	4.1E-07	−0.023	0.005	−0.026
9:104840293:T:C	Y347C	45	5.8E-05	4.3E-06	−0.200	0.045	−0.154
9:104821445:T:C	I964V	18	2.3E-05	7.7E-06	−0.326	0.071	−0.253
9:104816298:G:A	R1195W	35	4.5E-05	7.6E-05	−0.203	0.051	−0.150
9:104831658:A:G	I560T	17	2.2E-05	1.1E-04	−0.308	0.074	−0.059
9:104818729:C:A	K1132N	12	1.5E-05	1.2E-04	−0.336	0.087	−0.260
9:104791982:C:T	R1925Q	542	7.0E-04	1.5E-04	0.045	0.013	0.056
9:104803296:A:G	V1527A	19	2.4E-05	1.6E-04	−0.253	0.070	−0.090
9:104816337:C:T	A1182T	915	0.0012	1.7E-04	−0.036	0.010	−0.027
9:104818726:T:G	K1133N	57	7.3E-05	1.8E-04	−0.132	0.040	−0.060
9:104821421:G:A	R972W	36	4.6E-05	2.3E-04	−0.200	0.051	−0.100

Variant positions are listed for hg38.

AC = allele count; AF = allele frequency; alt = alternate allele; chr = chromosome; pos = position; ref = reference allele; SE = standard error; SNV = single nucleotide variant.

[a]Predicted HDL effects from model including all variants in a locus.

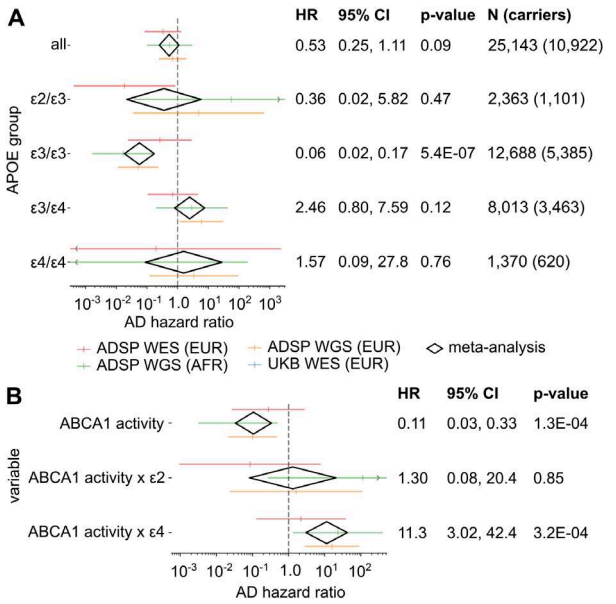

FIGURE 2: Association of predicted ABCA1 activity based on HDL-associated missense variants with AD in discovery cohorts. Bottom axes denote HRs with 95% CIs from Cox regression on a weighted sum of HDL-associated *ABCA1* missense variants against AD onset. (A) Results for regression of predicted ABCA1 activity stratified by *APOE* genotype group. (B) Results for regression of predicted ABCA1 activity and predicted ABCA1 activity × *APOE* isoform dosage terms, including all *APOE* groups. AD = Alzheimer's disease; CI = confidence interval; HR = hazard ratio. [Color figure can be viewed at www.annalsofneurology.org]

(HR = 1.66, 95% CI = 1.38–2.01, p = 9.6E-08), in *APOE* ε3/ε3 individuals, and in *APOE* ε3/ε4 individuals, whereas *APOE* ε4/ε4 subjects did not see a statistically significant increase in AD risk. The *APOE* ε2/ε3 group could not be tested due to low sample size (N carriers < 10 in both cohorts). In the *APOE* interaction model, including all *APOE* genotypes other than ε2/ε4, LoF + REVEL ≥ 75 variants increased AD risk by themselves; their interaction effect with *APOE* ε4 was not significantly associated with AD risk but showed the same effect direction as in the discovery (HR = 0.83, 95% CI = 0.62–1.11, p = 0.21), and their interaction effect with *APOE* ε2 was not statistically significant (HR = 0.74, 95% CI = 0.24–2.31, p = 0.60; Table 3).

Replication analyses validated that predicted ABCA1 activity was inversely associated with AD risk in the all-*APOE* group (HR = 0.16, 95% CI = 0.04–0.54, p = 3.4E-03) and that effect sizes became less pronounced with increasing *APOE* ε4 alleles. ABCA1 activity was associated with AD risk in *APOE* ε3/ε3, but not in APOE ε2/ε3, ε3/ε4, or ε4/ε4. In the interaction analysis, predicted ABCA1 activity was associated with decreased AD risk by itself, and the interaction of predicted ABCA1 activity with *APOE* ε4 had the same effect direction for AD risk as in the discovery but was not statistically significant (HR = 4.40, 95% CI = 0.50–38.4, p = 0.18). As in the discovery, the interaction of predicted ABCA1 activity with *APOE* ε2 was not associated with AD risk (Table 4).

We meta-analyzed discovery and replication cohorts together for the most accurate estimates of AD effects of *ABCA1* variants and to assess heterogeneity as another validation measure. In this analysis, LoF + REVEL ≥ 75 variants significantly increased AD risk in the all-*APOE* cohort, in *APOE* ε2/ε3 individuals, and in *APOE* ε3/ε3 individuals, but not in *APOE* ε3/ε4 or *APOE* ε4/ε4. The *APOE* interaction model including all *APOE* genotypes other than ε2/ε4 validated the main effect of LoF + REVEL ≥ 75 variants on AD risk and their interaction with both *APOE* ε2 and ε4. In the test of predicted ABCA1 lipid efflux activity, the combined meta-analysis showed an association between predicted ABCA1 activity and reduced AD risk in the all-*APOE* group and *APOE* ε3/ε3, but not in *APOE* ε2/ε3, ε3/ε4, or ε4/ε4. The *APOE* interaction model including all *APOE* genotypes other than ε2/ε4 validated the main effect of predicted ABCA1 activity and the association of predicted ABCA1 activity with *APOE* ε4 but showed no association of predicted ABCA1 activity with *APOE* ε2. Cohran's Q test did not find significant heterogeneity in effect sizes between cohorts for any analysis (Table 5).

We explored the contribution of each missense variant in the refined model linking predicted ABCA1 activity with AD to identify candidate variants for further study. For the refined Cox model, we removed the predicted ABCA1 activity × *APOE* ε2 term, as it was not statistically significant in the combined meta-analysis. No removal of a single variant ablated the AD association of the weighted sum or its interaction with *APOE* ε4, showing the association was not dependent on any single variant. Six missense variants had a ΔAIC ≤ −2 and thus showed a high contribution to the model: N1800H, R1342W, R1680Q, E1172D, R587W, and R437W. Meanwhile, only 2 variants had a ΔAIC ≥ 2 and thus acted to diminish the model association: G1346E and V771M (Fig 3A and Supplementary Table S4). In single-variant tests, N1800H significantly increased AD risk (HR = 2.26, 95% CI = 1.37–3.75, p = 1.5E-03) and had a near-significant interaction with *APOE* ε4 dosage (HR = 0.53, 95% CI = 0.27–1.04, p = 0.06). This variant was the most highly associated with HDL out of all missense variants due to a large decrease in HDL levels in UKB. Meanwhile, E1172D was associated with decreased AD risk (HR = 0.90, 95% CI = 0.83–0.98, p = 0.01) and significantly interacted with *APOE* ε4 dosage (HR = 1.10, 95% CI = 1.01–1.20, p = 0.03). This variant was associated with increased HDL levels in UKB. On the other hand, V771M was associated with increased

TABLE 3. Replication Analyses for the Test of LoF + REVEL ≥ 75 Variants in *ABCA1* Against AD Risk, by *APOE* Genotype

***APOE* subgroups (N; N carriers)**									
Dataset	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
	All (17,679; 264)			*ε*2/*ε*3 (N = 1,693; 17)			*ε*3/*ε*3 (9,203; 135)		
ADSP WGS R5 (EUR)	1.51	1.11, 2.06	8.9E-03	3.08[a]	0.41, 22.9[a]	0.27[a]	1.41	0.81, 2.45	0.23
ADES (EUR)	1.76	1.39, 2.22	2.4E-06	1.04[a]	0.25, 4.3[a]	0.95[a]	2.03	1.45, 2.84	3.4E-05
Meta-analysis	1.66	1.38, 2.01	9.6E-08	N/A	N/A	N/A	1.84	1.38, 2.45	3.0E-05
	*ε*3/*ε*4 (5,188; 90)			*ε*4/*ε*4 (1,070; 17)					
ADSP WGS R5 (EUR)	1.60	1.03, 2.48	0.04	1.05	0.43, 2.58	0.91			
ADES (EUR)	1.64	1.10, 2.43	0.02	1.93[a]	0.90, 4.14[a]	0.09[a]			
Meta-analysis	1.62	1.20, 2.17	1.4E-03	1.05	0.43, 2.58	0.91			

***APOE* interaction (N = 17,242; N carriers = 260)**									
	Variant burden			**Variant burden × *ε*2 dosage**			**Variant burden × *ε*4 dosage**		
Dataset	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
ADSP WGS R5 (EUR)	1.57	0.97, 2.54	0.07	1.33	0.20, 8.79	0.76	0.92	0.58, 1.48	0.74
ADES (EUR)	2.05	1.48, 2.83	1.3E-05	0.53	0.13, 2.20	0.38	0.78	0.54, 1.13	0.19
Meta-analysis	1.89	1.44, 2.47	3.5E-06	0.74	0.24, 2.31	0.60	0.83	0.62, 1.11	0.21

ADES = Alzheimer Disease European Sequencing; ADSP = Alzheimer's Disease Sequencing Project; CI = confidence interval; EUR = European; HR = hazard ratio; LoF = loss of function; N/A = not applicable; WGS = whole-genome sequencing.
[a]Result excluded from meta-analysis as N variant carriers <10.

HDL levels in UKB but was not associated with AD risk, nor did it interact with *APOE ε*4, consistent with its positive ΔAIC (Fig 3B and Supplementary Table S5).

Confocal microscopy of cultured cells transfected with variants of interest showed wild-type ABCA1 protein along with V771M and E1172D mutants, both of which were associated with increased plasma HDL levels, properly localized to the plasma membrane. Meanwhile, ABCA1-N1800H, which was associated with decreased plasma HDL levels, had poor localization to the plasma membrane. Additionally, solubilization of ABCA1 from the membrane fraction of transfected cells showed decreased levels of protein for the N1800H mutant compared to wild-type or V771M and E1172D mutants via immunoblotting of an enriched membrane fraction. Almost none of ABCA1-N1800H was pulled down from this membrane fraction via FLAG immunoprecipitation (Fig 3C–E).

Discussion

The pathogenic roles of *ABCA1* and *APOE* in AD remain unclear. Whereas genome-wide scans, such as those which discovered *ABCA1* as an AD risk locus, are useful in nominating disease-associated genes, they do not necessarily elucidate the mechanisms by which these genes play a role in disease.[8,9] Understanding these mechanisms requires follow-up analyses that place risk genes within relevant pathways and clarify the specific alterations in gene activity that lead to disease.

We identified an interaction between variants predicted to modify *ABCA1* function and *APOE* isoforms that could lend insight into the mechanisms of these two genes in AD pathogenesis. Our discovery analysis suggested varying effect sizes of damaging nonsynonymous variants in *ABCA1* on AD risk depending on *APOE* genotype, which was validated by a model supporting interaction effects with both *APOE ε*2 and *ε*4. Further, an analysis predicting ABCA1 lipid efflux activity based on HDL effects of missense variants provided additional evidence for an interaction between *ABCA1* and *APOE ε*4. Replication cohorts showed similar trends in *ABCA1* effect sizes across *APOE* subgroups, with *ε*4 tending to diminish effect magnitudes. Although formal interaction analyses were above our significance threshold in the replication,

TABLE 4. Replication Analyses for Effect of Predicted ABCA1 Activity on AD Risk, by *APOE* Genotype

***APOE* subgroups (N; N carriers)**

Dataset	HR	95% CI	*p*	HR	95% CI	*p*	HR	95% CI	*p*
	All (17,679; 7,196)			*ε2/ε3* (1,693; 717)			*ε3/ε3* (9,203; 3,783)		
ADSP WGS R5 (EUR)	0.45	0.05, 4.16	0.48	5.6E-03	2.4E-06, 13.0	0.19	0.91	0.02, 45.1	0.96
ADES (EUR)	0.10	0.02, 0.44	2.0E-03	0.19	1.1E-03, 33.1	0.53	0.03	2.8E-03, 0.26	2.0E-03
Meta-analysis	0.16	0.04, 0.54	3.4E-03	0.06	8.8E-04, 4.72	0.21	0.07	0.01, 0.46	6.2E-03
	ε3/ε4 (5,188; 2,059)			*ε4/ε4* (1,070; 427)					
ADSP WGS R5 (EUR)	0.16	0.01, 2.76	0.21	257	0.02, 3.2E06	0.25			
ADES (EUR)	0.20	0.02, 2.18	0.19	0.06	4.7E-05, 67.1	0.43			
Meta-analysis	0.18	0.03, 1.13	0.07	1.18	4.1E-03, 340	0.96			

***APOE* interaction (N = 17,242; N carriers = 7,015)**

	ABCA1 activity			ABCA1 activity × *ε2* dosage			ABCA1 activity × *ε4* dosage		
Dataset	HR	95% CI	*p*	HR	95% CI	*p*	HR	95% CI	*p*
ADSP WGS R5 (EUR)	0.22	7.8E-03, 6.35	0.38	0.04	1.5E-05, 106	0.42	3.47	0.09, 131	0.50
ADES (EUR)	0.03	3.0E-03, 0.25	1.4E-03	4.95	0.02, 1,510	0.58	5.02	0.34, 74.7	0.24
Meta-analysis	0.05	8.2E-03, 0.33	1.7E-03	0.94	0.01, 96.4	0.98	4.40	0.50, 38.4	0.18

ADES = Alzheimer Disease European Sequencing; ADSP = Alzheimer's Disease Sequencing Project; CI = confidence interval; EUR = European; HR = hazard ratio; WGS = whole-genome sequencing.

they both showed concordant effect directions with the discovery, thus adding an independent replication signal. Further, meta-analyzing all cohorts together demonstrated low effect heterogeneity, suggesting discovery and replication analyses were generally concordant. The highest heterogeneity in the interaction analyses was observed in the interaction between LoF + REVEL ≥ 75 variants and *APOE ε2* dosage. Combined with the non-significance of the interaction between predicted ABCA1 activity and *APOE ε2*, this suggests that the strongest interactions between *ABCA1* variants and *APOE* were driven by the *ε4* allele. In the combined meta-analysis, we observed that the estimated HRs for the ABCA1 activity term and its interaction term with *APOE ε4* were almost perfectly inverted (0.09 and 9.50, respectively), implying that the association of ABCA1 activity with reduced AD risk was almost entirely ablated in the presence of *APOE ε4*. Thus, carriers of *APOE ε2* and *ε3* were predicted to experience an estimated 10-fold decrease in AD risk for every doubling in ABCA1 lipid efflux activity, whereas carriers of one or more *APOE ε4* alleles experienced no detectable impacts.

Although most cohorts included were of European ancestry, our discovery analysis included one African ancestry cohort. Whereas the overall genetic architecture of AD is expected to differ between ancestry groups,[30,31] our results showed consistent effects of *ABCA1* variants between European and African ancestry individuals. LoF + REVEL ≥ 75 variants in the African ancestry cohort showed similar effect directions on AD risk as in European ancestry cohorts. Predicted ABCA1 activity in the African cohort was generally higher, likely due to higher frequencies of variants associated with increased HDL levels (see Supplementary Table S5). However, our measure of ABCA1 activity was still predictive of measured HDL levels in an independent sample of African ancestry individuals in UKB, and predicted ABCA1 activity was associated with AD by itself and in its interaction with *APOE ε4* in the African ancestry ADSP WGS cohort. The consistency of these with our general results suggests that the genetic interaction we have identified is likely relevant in African as well as European ancestry individuals.

TABLE 5. Combined Meta-Analysis of Interaction of *ABCA1* Variants With *APOE* Genotypes in AD Risk

LoF + REVEL ≥ 75 variants							
***APOE* group**	**N**	**N carriers**	**HR**	**95% CI**	***p***	I^2	**Q p***
All	85,548	1,033	1.40	1.27, 1.56	1.7E-10	20.4	0.28
ε2/ε3	6,786[a]	75	2.47	1.49, 4.07	4.3E-04	17.9	0.30
ε3/ε3	46,043	553	1.69	1.44, 1.98	1.8E-10	0.0	0.61
ε3/ε4	24,166	303	1.16	0.98, 1.37	0.09	53.6	0.06
ε4/ε4	2,817[b]	41	0.97	0.65, 1.46	0.90	0.0	0.78
***APOE* interaction**	**N = 83,508; N carriers = 1,011**						
Variable	**HR**	**95% CI**	***p***	I^2	**Q p***		
Variant burden	1.61	1.37, 1.88	2.5E-09	0.0	0.55		
Variant burden × ε2 dosage	1.76	1.18, 2.62	5.7E-03	45.5	0.10		
Variant burden × ε4 dosage	0.79	0.66, 0.94	6.8E-03	0.0	0.59		
Predicted ABCA1 activity							
***APOE* group**	**N**	**N carriers**	**HR**	**95% CI**	***p***	I^2	**Q p***
All	85,548	33,188	0.38	0.22, 0.68	9.8E-04	0.0	0.46
ε2/ε3	9,027	3,554	0.26	0.03, 2.34	0.23	22.2	0.27
ε3/ε3	46,043	17,808	0.06	0.03, 0.14	6.3E-11	0.7	0.41
ε3/ε4	24,166	9,344	1.42	0.58, 3.46	0.44	46.4	0.10
ε4/ε4	3,825	1,495	1.51	0.17, 13.2	0.71	0.0	0.73
***APOE* interaction**	**N = 83,508; N carriers = 32,369**						
Variable	**HR**	**95% CI**	***p***	I^2	**Q p***		
ABCA1 activity	0.09	0.04, 0.21	2.2E-08	0.0	0.75		
ABCA1 activity × ε2 dosage	1.76	0.20, 15.9	0.61	4.4	0.39		
ABCA1 activity × ε4 dosage	9.50	3.52, 25.7	9.0E-06	0.0	0.81		

Analyses combined discovery and replication cohorts.

ADES = Alzheimer Disease European Sequencing; ADSP = Alzheimer's Disease Sequencing Project; AFR = African; EUR = European; HR = hazard ratio; CI = confidence interval; LoF = loss of function; WES = whole-exome sequencing; WGS = whole-genome sequencing.

[a]ADSP WGS (AFR), ADSP WGS R5 (EUR), and ADES (EUR) excluded as N carriers <10.

[b]ADSP WGS (AFR), ADSP WES (EUR), and ADES (EUR) excluded as N carriers <10.

*The *p* value from Cochran's *Q* test for heterogeneity.

Importantly, although we used peripheral HDL to assess functional *ABCA1* missense variants, these results are unlikely to reflect that HDL levels themselves are causal in AD. Although some research has observed a correlation between HDL and dementia risk, Mendelian randomization analyses do not support a role for overall genetic regulation of HDL levels in AD.[32–36] We do not discount that some portion of effects may be due to systemic lipid factors; however, as we constructed our test to measure the activity of this specific lipid exporter, we interpret the association we identified as an indication that ABCA1 lipid efflux activity—likely in the CNS—is linked

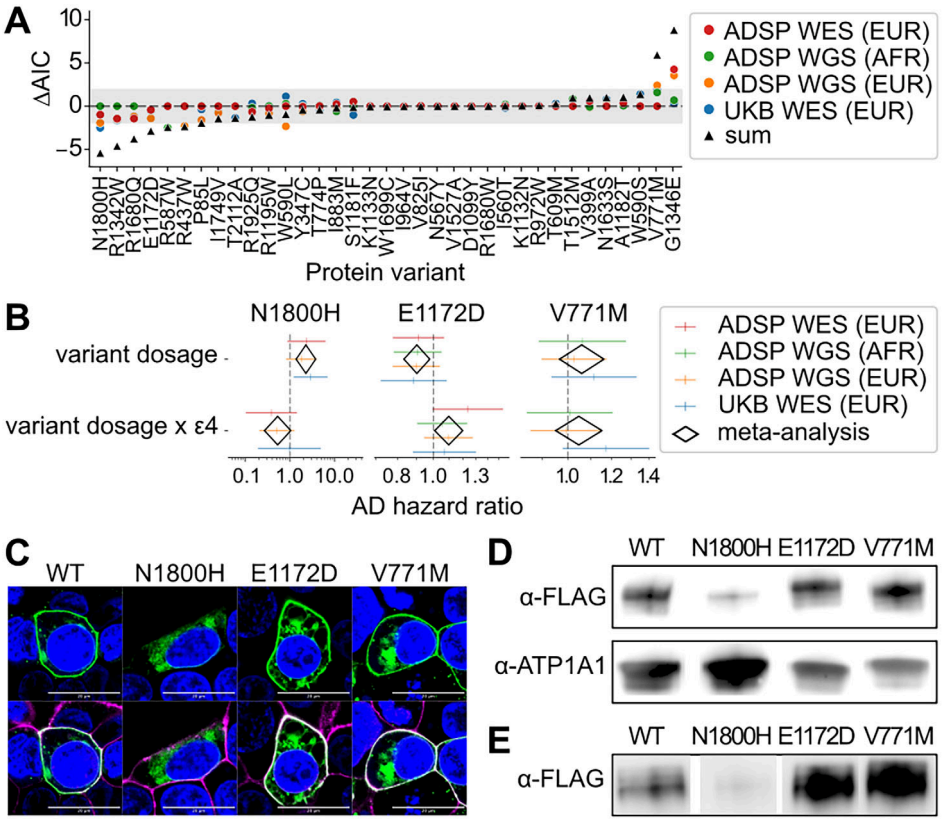

FIGURE 3: Missense variants of interest in the association of predicted ABCA1 activity with AD. (A) Contribution of each missense variant to the association of predicted ABCA1 activity and its interaction with *APOE* ε4 with AD. ΔAIC reflects the change in AIC upon the inclusion of that missense variant in the weighted sum. Variants are ordered according to the sum of their ΔAIC values across datasets. Variants were prioritized for follow-up if their ΔAIC exceeded 2 or −2, as shown by the area outside the shaded box. (B) AD association for 3 individual missense variants that had a large impact on the association between predicted ABCA1 activity and AD using the refined Cox regression model. Bottom axes denote HRs with 95% CIs from Cox regression. (C) Confocal microscopy of HEK293T cells transfected with ABCA1-eGFP in its WT form or mutated with a missense variant of interest. Hoechst 33342 nuclear stain is shown in blue, eGFP signal is shown in green, and WGA-AlexaFlour594 membrane stain in magenta. (D) Immunoblot of solubilized membrane fractions of Expi293F cells transfected with ABCA1-eGFP in its WT form or mutated with a missense variant of interest. ATP1A1 is used as a membrane-bound housekeeping protein to normalize for total protein content. White lines indicate 20 μm scale. (E) Immunoblot of solubilized membrane fractions from (D) after passing through a FLAG immunoprecipitation column. AD = Alzheimer's disease; AIC = Akaike Information Criterion; CI = confidence interval; HR = hazard ratio; WT = wild-type. [Color figure can be viewed at www.annalsofneurology.org]

to AD risk. Nonetheless, our conclusions depend on the extent to which peripheral ABCA1 lipid metabolism dynamics can be generalized to the CNS.

Collectively, our results suggest that genetically regulated ABCA1 activity is inversely correlated with AD risk in non-carriers of *APOE* ε4. The statistical ablation of an *ABCA1* risk effect by ε4 suggests that *ABCA1* and *APOE* interplay in AD pathogenesis. We put forward 2 explanations for this here. As alterations in ABCA1 function seem to be impactful mainly in ε2 and ε3 carriers without an ε4 allele, the protective functions of ApoE ε2 and ε3 relative to ε4 may be connected to ABCA1 activity levels. As ABCA1 is a lipid exporter, this supports the idea that, at least partially, the AD risk effects of ApoE protein isoforms are related to the extracellular abundance or chemistry of their corresponding lipoprotein nanoparticles in the CNS. This hypothesis is consistent with data showing that ABCA1 availability is associated with increased ApoE lipidation and reduced amyloid deposition in mouse models of AD.[37,38] On the other hand, the AD risk effect of ApoE ε4 relative to ε2 and ε3 may be partially redundant with ABCA1 activity loss. This could be explained by a reduced ability of ApoE ε4 to bind ABCA1, such that altered ABCA1 activity will have a diminished impact on AD pathogenesis in carriers of this isoform; alternatively, the ability of ABCA1 to modify HDL-like nanoparticles may be diminished due to a commensurate decrease in the abundance of these particles in carriers of *APOE* ε4. Along with a finding we have previously reported in which 2 *APOE* missense variants V236E and R251G, found in or near its lipid binding domain, protect against AD risk,[39] our results point to the relevance of ApoE lipidation dynamics in the pathogenesis of AD. Ultimately, further biochemical studies will be

required to elucidate the exact mechanisms of the interaction we identified.

We call attention to 3 missense variants in *ABCA1* that could provide an opportunity to study the biochemical correlates of the genetic link between ABCA1 activity, *APOE ε4*, and AD. Whereas N1800H was correlated with decreased plasma HDL and increased AD risk, E1172D was correlated with increased plasma HDL and decreased AD risk, and both variants had significant or near-significant statistical interactions with *APOE ε4* opposing their main effects on AD risk. These variants may be representative of the general biochemical pathway we identified among ABCA1, ApoE, and AD. On the other hand, the variant V771M was associated with increased plasma HDL levels but did not show a correlation with AD risk by itself or via an interaction with *APOE ε4*. Although the discordance in V771M may be related to variants in linkage confounding the genetic association results, it could also be explained by a pleiotropic protein-level effect, which may be contrasted with other activating missense variants such as E1172D to discern the relevant pathways of this protein in AD. As an initial exploration into the biochemical effects of the variants we identified, we found that both ABCA1 mutants associated with increased plasma HDL (E1172D and V771M) correctly localized to the plasma membrane, whereas N1800H, which was associated with decreased HDL levels, had a severe plasma membrane localization defect. Although this defect has been previously reported,[11] given that expression levels were similar across all mutants (see Supplementary Fig S5), the inability of ABCA1-N1800H to bind anti-FLAG antibodies suggests a potential misfolding mechanism. Although the mechanisms for plasma membrane insertion of transmembrane proteins are highly conserved across mammalian cells, further analysis of these variants in neuronal or astrocytic cell lines can be expected to shine light on the contribution of ABCA1 and ApoE to AD pathogenesis.

Our analysis demonstrates the relevance of genetic epistasis between *ABCA1* and *APOE* in driving AD risk. This finding motivates the study of molecular interactions between the 2 proteins and the development of therapeutics targeting the *ABCA1* gene in AD, additionally suggesting it may be important to design *ABCA1*-targeted trials with attention to *APOE* subgroup effects.

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Author Contributions

A.P.T., R.H.A., C.K., Y.L.G., J.C.L., and M.D.G. contributed to the conception and design of the study; A.P.T., R.H.A., M.M., B.G.B., M.H., J.P., D.R., P.A., C.B., C.C., J.F.D., O.D.I., J.H., H.H., G.N., S.M., M.W., A.R., R.S., J.V.S., and J.W. contributed to the acquisition and analysis of data; A.P.T., R.H.A., and D.R. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

The authors declare no conflicts of interest.

Data availability

Data for ADSP are available for approved investigators at the NIAGADS Data Sharing Service (https://dss.niagads.org/) under accession number NG00067.[40] Data for UKB are available for approved investigators at https://www.ukbiobank.ac.uk/.[41] Data from the ADES consortium are available under reasonable request to the corresponding author. Code used for analysis is deposited in a public GitHub repository at https://github.com/apenatauber/abca1-apoe-interaction and under DOI: 10.5281/zenodo.17137693.

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