

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.

View this article online at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/ana.78303). DOI: 10.1002/ana.78303

Received Oct 16, 2025, and in revised form Jun 2, 2026. Accepted for publication Jun 18, 2026.

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Additional supporting information can be found in the online version of this article.

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* $\epsilon 2$ and $\epsilon 4$ 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* $\epsilon 2$ and $\epsilon 4$. Predicted *ABCA1* activity based on HDL-associated variants was associated with reduced AD risk and interacted with *APOE* $\epsilon 4$. 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.

ANN NEUROL 2026;100:737–750

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 $\epsilon 4$ 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-limiting 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 Consortium²⁵ 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 (

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 non-synonymous 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* $\epsilon 2$ dosage; *APOE* $\epsilon 4$ dosage; *APOE* $\epsilon 2$ dosage $\times$ variant burden; and *APOE* $\epsilon 4$ dosage $\times$ 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* $\epsilon 2/\epsilon 4$ genotype to avoid confounding by potentially conflicting effects of $\epsilon 2$ and $\epsilon 4$.

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* $\epsilon 2$ and $\epsilon 4$ dosage, use of cholesterol-lowering medication, and 40 principal components. Plasma HDL values were log₂-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 previously⁹; 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 Cochran'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 Δ AIC indicated that the variant likely strengthened the model fit, whereas a positive Δ AIC indicated that the variant likely weakened the model fit. Missense variants were selected for further exploration if they had a Δ AIC 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

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 finding⁹ 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 $\epsilon 2/\epsilon 3$ and $\epsilon 3/\epsilon 3$ groups but not in $\epsilon 3/\epsilon 4$ or $\epsilon 4/\epsilon 4$ groups. AD effect sizes tended to increase with *APOE* $\epsilon 2$ alleles and decrease with *APOE* $\epsilon 4$ alleles (Fig 1A and Supplementary Table S1). Although we do not report effect estimates for *APOE* $\epsilon 2/\epsilon 2$, 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* $\epsilon 2/\epsilon 2$ 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 $\times$ *APOE* isoform dosage interaction terms in a model including all *APOE* genotypes except $\epsilon 2/\epsilon 4$ (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* $\epsilon 2$ 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)

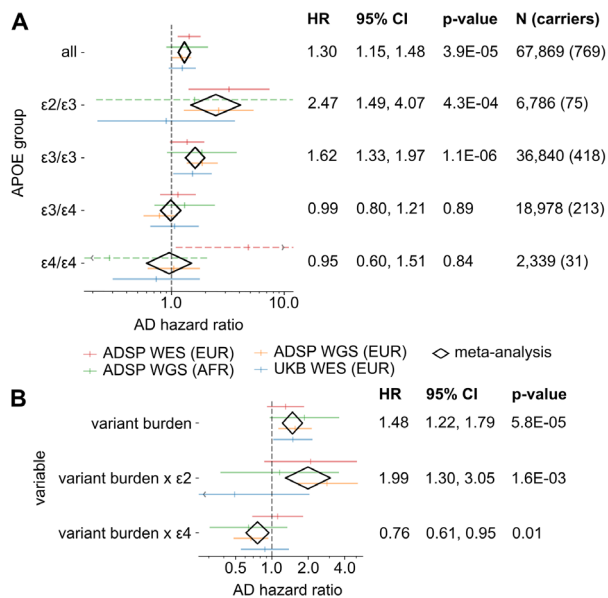


FIGURE 1: Association of the burden of LoF + REVEL ≥ 75 variants in ABCA1 with AD in discovery cohorts. Bottom axes denote HRs with 95% CIs from Cox regression against AD onset. Solid lines reflect association results included in the meta-analysis, whereas dashed lines were excluded due to insufficient 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 $\times$ 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 $\epsilon 4$ 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 $\epsilon 3/\epsilon 3$ 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 $\epsilon 2$ and $\epsilon 4$ alleles. Predicted ABCA1 activity was not significantly associated with AD risk in APOE $\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 4$, or $\epsilon 4/\epsilon 4$ groups (Fig 2A and Supplementary Table S2). An APOE interaction model including all APOE genotypes other than $\epsilon 2/\epsilon 4$ (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 $\epsilon 4$ dosage was associated with increased AD risk (HR

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

SNV (chr:pos:ref:alt)	Protein variant	AC	AF	<i>p</i>	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				

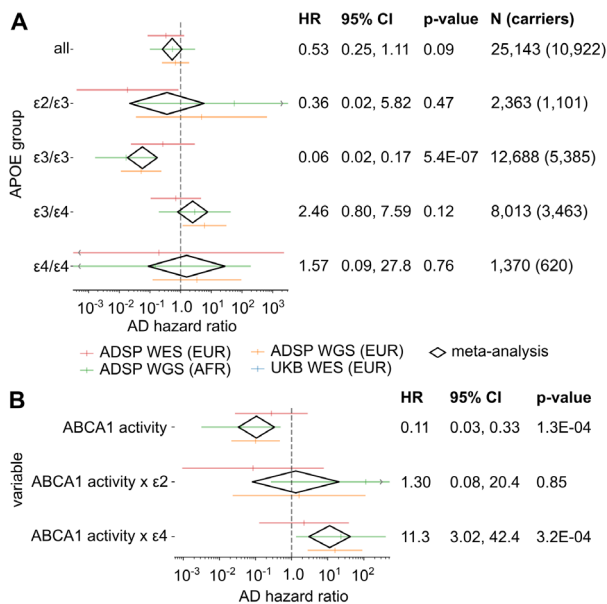


FIGURE 2: Association of predicted ABCA1 activity based on HDL-associated missense variants with AD in discovery cohorts. Bottom axes denote HRs with 95% CIs from Cox regression on a weighted sum of HDL-associated ABCA1 missense variants against AD onset. (A) Results for regression of predicted ABCA1 activity stratified by APOE genotype group. (B) Results for regression of predicted ABCA1 activity and predicted ABCA1 activity $\times$ 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 $\epsilon 3/\epsilon 3$ individuals, and in APOE $\epsilon 3/\epsilon 4$ individuals, whereas APOE $\epsilon 4/\epsilon 4$ subjects did not see a statistically significant increase in AD risk. The APOE $\epsilon 2/\epsilon 3$ 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 $\epsilon 2/\epsilon 4$, LoF + REVEL ≥ 75 variants increased AD risk by themselves; their interaction effect with APOE $\epsilon 4$ 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 $\epsilon 2$ 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 $\epsilon 4$ alleles. ABCA1 activity was associated with AD risk in APOE $\epsilon 3/\epsilon 3$, but not in APOE $\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 4$, or $\epsilon 4/\epsilon 4$. In the interaction analysis, predicted ABCA1 activity was associated with decreased AD risk by itself, and the interaction of predicted ABCA1 activity with APOE $\epsilon 4$ 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 $\epsilon 2$ 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 $\epsilon 2/\epsilon 3$ individuals, and in APOE $\epsilon 3/\epsilon 3$ individuals, but not in APOE $\epsilon 3/\epsilon 4$ or APOE $\epsilon 4/\epsilon 4$. The APOE interaction

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

<i>APOE</i> subgroups (N; N carriers)									
Dataset	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
	All (17,679; 264)			$\epsilon 2/\epsilon 3$ (N = 1,693; 17)			$\epsilon 3/\epsilon 3$ (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
	$\epsilon 3/\epsilon 4$ (5,188; 90)			$\epsilon 4/\epsilon 4$ (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			
<i>APOE</i> interaction (N = 17,242; N carriers = 260)									
Dataset	Variant burden			Variant burden $\times$ $\epsilon 2$ dosage			Variant burden $\times$ $\epsilon 4$ dosage		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
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				

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	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
	All (17,679; 7,196)			$\epsilon 2/\epsilon 3$ (1,693; 717)			$\epsilon 3/\epsilon 3$ (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
	$\epsilon 3/\epsilon 4$ (5,188; 2,059)			$\epsilon 4/\epsilon 4$ (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)

Dataset	ABCA1 activity			ABCA1 activity $\times \epsilon 2$ dosage			ABCA1 activity $\times \epsilon 4$ dosage		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
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.

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

LoF + REVEL ≥ 75 variants							
<i>APOE</i> group	N	N carriers	HR	95% CI	<i>p</i>	<i>I</i>²	Q <i>p</i>[*]
All	85,548	1,033	1.40	1.27, 1.56	1.7E-10	20.4	0.28
$\epsilon 2/\epsilon 3$	6,786 ^a	75	2.47	1.49, 4.07	4.3E-04	17.9	0.30
$\epsilon 3/\epsilon 3$	46,043	553	1.69	1.44, 1.98	1.8E-10	0.0	0.61
$\epsilon 3/\epsilon 4$	24,166	303	1.16	0.98, 1.37	0.09	53.6	0.06
$\epsilon 4/\epsilon 4$	2,817 ^b	41	0.97	0.65, 1.46	0.90	0.0	0.78
<i>APOE</i> interaction							
N = 83,508; N carriers = 1,011							
Variable	HR	95% CI	<i>p</i>	<i>I</i>²	Q <i>p</i>[*]		
Variant burden	1.61	1.37, 1.88	2.5E-09	0.0	0.55		
Variant burden $\times \epsilon 2$ dosage	1.76	1.18, 2.62	5.7E-03	45.5	0.10		
Variant burden $\times \epsilon 4$ dosage	0.79	0.66, 0.94	6.8E-03	0.0	0.59		
Predicted <i>ABCA1</i> activity							
<i>APOE</i> group	N	N carriers	HR	95% CI	<i>p</i>	<i>I</i>²	Q <i>p</i>[*]
All	85,548	33,188	0.38	0.22, 0.68	9.8E-04	0.0	0.46
$\epsilon 2/\epsilon 3$	9,027	3,554	0.26	0.03, 2.34	0.23	22.2	0.27
$\epsilon 3/\epsilon 3$	46,043	17,808	0.06	0.03, 0.14	6.3E-11	0.7	0.41
$\epsilon 3/\epsilon 4$	24,166	9,344	1.42	0.58, 3.46	0.44	46.4	0.10
$\epsilon 4/\epsilon 4$	3,825	1,495	1.51	0.17, 13.2	0.71	0.0	0.73
<i>APOE</i> interaction							

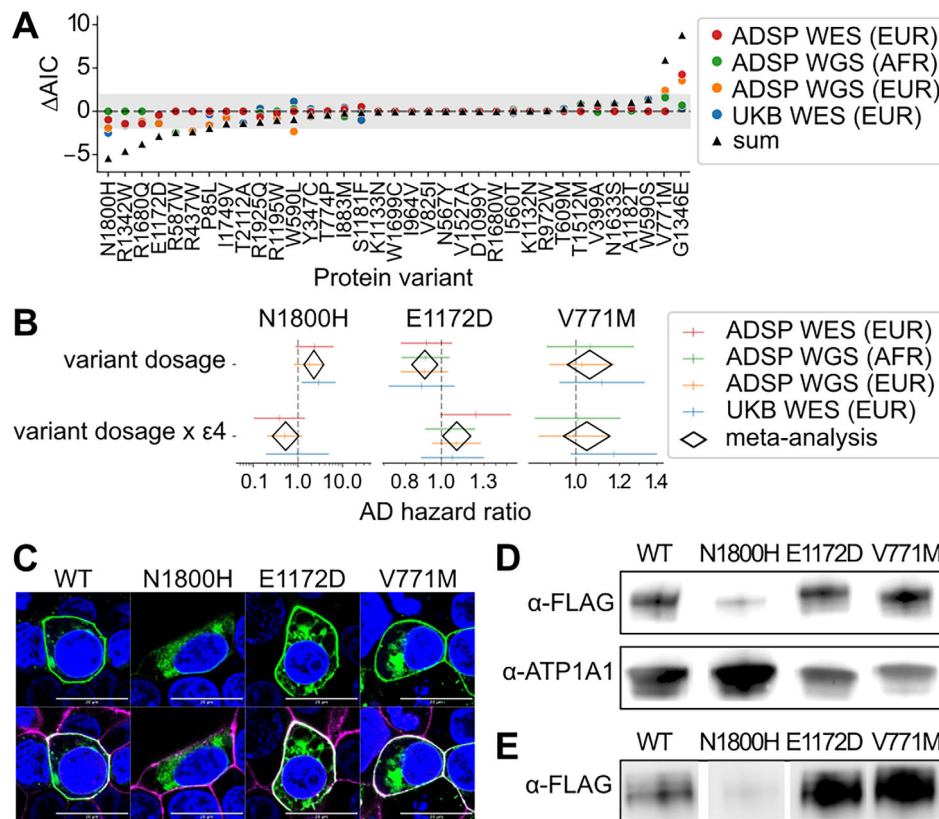


FIGURE 3: Missense variants of interest in the association of predicted ABCA1 activity with AD. (A) Contribution of each missense variant to the association of predicted ABCA1 activity and its interaction with *APOE* $\epsilon 4$ with AD. Δ AIC reflects the change in AIC upon the inclusion of that missense variant in the weighted sum. Variants are ordered according to the sum of their Δ AIC values across datasets. Variants were prioritized for follow-up if their Δ AIC 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-AlexaFluor594 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 μ m 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* $\epsilon 4$. The statistical ablation of an *ABCA1* risk effect by $\epsilon 4$ 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 $\epsilon 2$ and $\epsilon 3$ carriers without an $\epsilon 4$ allele, the protective functions of ApoE $\epsilon 2$ and $\epsilon 3$ relative to $\epsilon 4$ 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 $\epsilon 4$ relative to $\epsilon 2$ and $\epsilon 3$ may be partially redundant with ABCA1 activity loss. This could be explained by a reduced ability of ApoE $\epsilon 4$ 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* $\epsilon 4$. 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* $\epsilon 4$, 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* $\epsilon 4$ 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* $\epsilon 4$. 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.

Acknowledgments

This study was supported by NIH grants R35AG072290 and P30AG066515, as well as a grant from Wu Tsai Neurosciences Institute Seed Grants Program, accessible at [https://neuroscience.stanford.edu/our-science/funded-](https://neuroscience.stanford.edu/our-science/funded-projects/structural-and-mechanistic-analysis-protein-protein-interface-between-abca1-and-apoe-potential-therapeutic-target-alzheimers-disease)

[projects/structural-and-mechanistic-analysis-protein-protein-interface-between-abca1-and-apoe-potential-therapeutic-target-alzheimers-disease](https://neuroscience.stanford.edu/our-science/funded-projects/structural-and-mechanistic-analysis-protein-protein-interface-between-abca1-and-apoe-potential-therapeutic-target-alzheimers-disease). This research was supported in part by a training grant from NIH Cellular and Molecular Training Grant (NIGMS, grant number 5T32GM007276). This research has been conducted using the UK Biobank Resource under Application Number 45420. UK Biobank is generously supported by its founding funders the Wellcome Trust and UK Medical Research Council, as well as the Department of Health, Scottish Government, the Northwest Regional Development Agency, British Heart Foundation and Cancer Research UK. Data for this study were prepared, archived, and distributed by the National Institute on Aging Alzheimer's Disease Data Storage Site (NIAGADS) at the University of Pennsylvania (U24-AG041689), funded by the National Institute on Aging. We thank the ADSP investigators and participants for providing data supporting this study. Further acknowledgements for use of ADSP data can be found in the Supplementary Acknowledgments. The investigators would like to thank UK Dementia Research Institute and Moondance Dementia Research Laboratory, the Department of Psychological Medicine at Cardiff University for providing data used as part of the ADES consortium. We thank all the study participants, their families, the participating medical staff, general practitioners, pharmacists, and all laboratory personnel involved in patient diagnosis, blood collection, blood biobanking, DNA preparation, and sequencing. The work in this paper was carried out on the Cartesius supercomputer, which is embedded in the Dutch national e-infrastructure with the support of the SURF Cooperative. Computing hours were granted in 2016, 2017, 2018, and 2019 to H. Holstege by the Dutch Research Council (project name: 100-plus; project nos. 15318 and 17232). ADES data was generated using funding obtained by the following study cohorts: ADES-FR, AgeCoDe-UKBonn; Barcelona SPIN; AC-EMC; ERF and Rotterdam; ADC-Amsterdam; 100-plus study; EMIF-90+; Control Brain Consortium; PERADES.

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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