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Genome-Wide Association Studies of ARIA From the Aducanumab Phase 3 ENGAGE and EMERGE Studies

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Abstract

Background and Objectives

Amyloid-related imaging abnormalities (ARIA) were the most common adverse events reported in the phase 3 ENGAGE and EMERGE trials of aducanumab, an anti-amyloid monoclonal antibody. *APOE* ε4 carrier status has been shown to increase risk of ARIA in prior trials of aducanumab and other anti-amyloid therapies; however, the remainder of the human genome has not been evaluated for ARIA risk factors. Therefore, we sought to determine in a hypothesis-free manner whether genetic variants beyond *APOE* influence risk of ARIA in aducanumab-treated patients.

Methods

We performed genome-wide association studies (GWAS) of ARIA in participants in the ENGAGE and EMERGE trials. Participants had mild cognitive impairment due to Alzheimer disease or mild Alzheimer disease dementia and were amyloid-positive on PET scans. All participants underwent regular MRI monitoring to detect and diagnose ARIA.

Results

Of the 3,285 participants in the intent-to-treat population, this analysis included 1,691 with genotyping array data who received at least one dose of aducanumab with at least one post-baseline MRI. All participants in the study cohort were of European ancestry; 51% were female. The mean age was 70.3 years. 31% had ARIA-E, 19% had ARIA-H microhemorrhage, and 14% had ARIA-H superficial siderosis. We identified one genome-wide significant ($p < 5.0 \times 10^{-8}$) association at the chromosome 19 locus encompassing *APOE*. The *APOE* association with ARIA was stronger in ε4/ε4 homozygotes (OR = 4.28, 4.58, 7.84; $p < 2.9 \times 10^{-14}$ for ARIA-E, ARIA-H microhemorrhage, and ARIA-H superficial siderosis, respectively) than in ε3/ε4 heterozygotes (OR = 1.74, 1.46, 3.14; $p \leq 0.03$). We found greater odds of radiographically severe ARIA (OR = 7.04–24.64, $p \leq 2.72 \times 10^{-5}$) than radiographically mild ARIA (OR = 3.19–5.00, $p \leq 1.37 \times 10^{-5}$) among ε4/ε4 homozygotes. *APOE* ε4 was also significantly associated with both symptomatic (ε4/ε4 OR = 3.64–9.52; $p < 0.004$) and asymptomatic (ε4/ε4 OR = 4.20–7.94, $p < 1.7 \times 10^{-11}$) cases, although among ARIA cases, *APOE* did not appear to modulate symptomatic status. No other genome-wide significant associations were found.

Discussion

We identified a strong, genome-wide significant association between *APOE* and risk of ARIA. Future, larger studies may be better powered to detect associations beyond *APOE.* These findings indicate that *APOE* is the strongest genetic risk factor of ARIA incidence, with implications for patient management and risk-benefit treatment decisions.

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Glossary

ARIA = amyloid-related imaging abnormality; **ARIA-E** = ARIA with edema; **ARIA-H** = ARIA with hemorrhage; **CAA** = cerebral amyloid angiopathy; **GWAS** = genome-wide association studies; **HWE** = Hardy-Weinberg equilibrium; **LTE** = long-term extension; **MAF** = minor allele frequency; **PCs** = principal components; **PRS** = polygenic risk score; **QC** = quality control; **VCF** = variant call file; **VQSR** = Variant Quality Score Recalibration.

Trial Registration Information

Both trials (ENGAGE [221AD301]: NCT02477800 and EMERGE [221AD302]: NCT02484547) were registered in June 2015 at clinicaltrials.gov and enrolled patients from August 2015 to July 2018.

Introduction

Amyloid-related imaging abnormalities (ARIA) refer to a range of findings detected on brain MRI that are associated with the use of monoclonal antibodies targeting amyloid beta (Aβ) in patients with Alzheimer disease.[1] ARIA can occur with edema or sulcal effusion (ARIA-E) or hemorrhage (ARIA-H) characterized by hemosiderin deposition, which occurs with microhemorrhage or with superficial siderosis.[1] Most ARIA cases are asymptomatic[2] (e.g., for ARIA-E, 74% of 10 mg/kg aducanumab,[3] 78% of donanemab,[4] 78% of lecanemab,[5] >80% of gantenerumab,[6] 85% of bapineuzumab *APOE* ε4 carriers[7] were asymptomatic). Clinical manifestations of symptomatic cases range from headaches (47% in phase 3 trials of aducanumab[3]) to, in rare instances, seizures (<1%), the majority of which are transient (83% of ARIA-E resolved radiographically within 16 weeks[3]).

The biological underpinnings of ARIA remain poorly understood, but several nonexclusive mechanistic hypotheses implicating cerebral amyloid angiopathy (CAA) have been developed to explain the occurrence of ARIA after treatment with anti-Aβ antibodies. Direct clearance of CAA, followed by disruption of the vascular integrity and vascular leakage, is often cited as the primary cause of ARIA.[8] Mobilization of amyloid cleared from the parenchymal plaques into an already dysfunctional perivascular drainage system, because of the presence of CAA, has also been suggested to potentially play a role. In addition, the current therapeutic antibodies, having full effector function and the ability to engage microglial cells, might also trigger and/or exacerbate CAA-related perivascular inflammation.[9,10]

ARIA was the most common adverse event in the phase 3 clinical trials of aducanumab, which has been approved by the US Food and Drug Administration for the treatment of Alzheimer disease under the accelerated approval pathway.[3,11] ARIA has been observed in other anti-amyloid antibody therapies including bapineuzumab,[7] crenezumab,[12] donanemab,[4] ponezumab,[13] solanezumab,[14] gantenerumab,[6] and lecanemab.[5] In clinical trials of these therapies, ARIA-E incidence ranged from 0.7% in ponezumab to 35.2% in aducanumab while ARIA-H incidence ranged from 4.9% in crenezumab and solanezumab to 19.1% in aducanumab.[15]

A widely reported risk factor of ARIA is carriage of the *APOE* ε4 allele, which is also found to be the strongest risk factor of late-onset Alzheimer disease.[16] In addition, the *APOE* ε4 genotype has been shown to influence ARIA risk in a dose-dependent manner based on carriage of one (heterozygote) or 2 (homozygote) copies of the ε4 allele.[5,6,17,18] It is not known, however, whether *APOE* ε4 is the primary genetic driver of ARIA risk or whether other genetic factors, including AD risk variants such as those found in *TREM2*,[16] influence risk of aducanumab-related ARIA. The ENGAGE and EMERGE trials represent a unique opportunity to investigate genetic factors beyond *APOE* because they are the largest phase 3 pool of safety data of a monoclonal antibody to date. We therefore conducted a genome-wide association study (GWAS) to determine, in a hypothesis-free manner, the genetic associations with ARIA among aducanumab-treated patients in the ENGAGE and EMERGE trials.

Methods

Cohort Description

A full description of the ENGAGE and EMERGE trials (N = 3,285) has been published previously.[3,11] Briefly, ENGAGE and EMERGE were 2 phase 3 clinical trials which evaluated safety and efficacy of aducanumab, in participants with mild cognitive impairment due to Alzheimer disease and early Alzheimer disease dementia. Trial participants were aged 50–85 years, with confirmed Aβ pathology by amyloid-positive PET scans. The main inclusion and exclusion criteria are presented in eTable 1 (links.lww.com/WNL/D269).[19,20] Participants were recruited from 348 sites in 20 countries.[11] Participants were randomized 1:1:1 to low-dose (3 mg/kg for *APOE* ε4 carriers, 6 mg/kg for *APOE* ε4 noncarriers), high-dose (before protocol version 4: 6 mg/kg for *APOE* ε4 carriers, 10 mg/kg for *APOE* ε4 noncarriers; after protocol version 4: 10 mg/kg regardless of *APOE* ε4 status), and placebo groups over 76 weeks, with a long-term extension (LTE) period for up to an additional 5 years.

The analyses presented here included all the participants in the ENGAGE and EMERGE studies who received at least one infusion of aducanumab (at any dose, either during the placebo-controlled period or switched to aducanumab during the LTE period) and who consented for genetic testing and had at least one post-baseline MRI assessment.

Standard Protocol Approvals, Registrations, and Patient Consents

The original trials were conducted in accordance with the Declaration of Helsinki and the International Conference for Harmonisation and Good Clinical Practice guidelines and were approved by ethics committees or institutional review boards at each site. Study participants provided written informed consent.

ARIA

A detailed description of ARIA assessment has been published previously.[3] Briefly, ARIA was detected using MRI and evaluated by a centralized reader with expertise in the diagnosis of ARIA. MRIs were performed for all participants at baseline and at weeks 14, 22, 30, 42, 54, 66, and 78 of the placebo-controlled period; regular monitoring continued during the LTE period. If ARIA was detected, additional MRI scans were conducted every 4 weeks until ARIA was resolved or stabilized. ARIA was categorized as ARIA with edema or effusion (ARIA-E) or as ARIA with hemorrhage (ARIA-H), characterized either by microhemorrhage or superficial siderosis. ARIA-H macrohemorrhage was also identified, but was not included in this genetic analysis due to very small sample size (N = 4 in the genetic cohort).

All forms of ARIA were categorized by radiographic severity. ARIA-E was considered mild (single edematous region <5 cm), moderate (a single region 5–10 cm or multiple regions summing up < 10 cm), or severe (any region >10 cm). Similarly, ARIA-H microhemorrhage cases were classified by the number of new (incident) microhemorrhages, including mild (N = 1–4), moderate (N = 5–9), or severe (N ≥ 10) microhemorrhages. ARIA-H superficial siderosis was classified by the number of new (incident) areas of superficial siderosis: mild (N = 1), moderate (N = 2), and severe (N > 2 incident areas). In addition, ARIA cases were classified as symptomatic or asymptomatic based on participant self-report.

Genotyping Data

Two thousand eight hundred and eighty two participants provided consent for genetic research related to aducanumab. Of these, 2,408 had sufficient DNA concentrations for genotyping. All samples were genotyped on the Illumina Infinium Global Screening Array-24v3.0 at the Broad Institute (Cambridge, MA).

Standard quality control (QC) metrics were applied to samples and genetic variants. Variants were excluded if they had high levels of missingness (>0.98; N = 28,024), low minor allele frequency (MAF <0.02; N = 187,930), or were out of Hardy-Weinberg equilibrium (HWE $>10^{-50}$; N = 18,934). Samples were excluded if they had high levels of missingness (>2%; N = 2), sex inconsistencies (N = 37), or excess heterozygosity (>6 SD from the mean; N = 14). Samples were also evaluated for relatedness and unexpected duplicates. All duplicates (pihat >0.9; N = 16) and one member of each related pair (pihat >0.4; N = 3) were excluded.

We conducted principal component analysis to determine genetic ancestry and generate principal components (PCs) to control for as covariates in the GWAS. We did not analyze participants of non-European ancestries (N = 271) because the small number of samples of other ancestries prevented stratified analysis or multiancestry approaches. We used SmartPCA in EIGENSTRAT to identify outliers (N = 63) and then further excluded PC1 >0.01 to remove a distinct cluster primarily comprising self-reported Asian race (N = 194) and finally excluded self-reported race other than "White" or "Not Reported" (N = 14). To determine the number of PCs to include as covariates in our genetic models, we used Tracy-Widom (TW) statistics.[16] PCs were included if TW p was < 0.05 that accounted for ≥1% of variance. This resulted in a list of 12 PCs; however, we chose to include the top 10 PCs as covariates to limit the loss of power incurred with an increasing number of variables in a model. We excluded samples with discordant *APOE* genotypes between targeted genotyping results generated during trial screening and *APOE* genotypes generated by exome sequencing (N = 11).

Post-QC genotypes were imputed to the 1,000-genome reference panel using the Michigan Imputation server.[21] We removed imputed variants with low imputation scores (R^2 < 0.3), low-frequency variants (MAF <0.01), and variants out of HWE ($>10^{-50}$), leaving a total of 9.5 million SNPs in 2,054 samples for analysis.

Exome Sequencing Data

Samples underwent exome sequencing at the Broad Institute (Cambridge, MA). First, libraries were constructed, which included library preparation, hybrid capture, sequencing with 150bp paired-end reads, and sample identification QC check. Libraries were then sequenced using paired-end sequencing on an Illumina platform. The Laboratory Picard bioinformatics pipeline was implemented, with a goal of 85% of targets with >20× coverage. The Broad Institute provided jointly called variant call files (VCFs) generated using GATK best practices.[22] This process converted raw CRAM files to FASTQ files, followed by mapping to a reference sequence, producing BAM files. Variants were then called using HaploypteCaller to produce VCFs. Individual VCFs were merged and jointly called.

After transfer of jointly called files to a secure server, we performed sample-level and variant-level QC. The GATK pipeline generates a quality score for each variant, referred to as Variant Quality Score Recalibration (VQSR). This variable is generated through a machine learning method which incorporates multiple-variant QC metrics to determine whether a variant is likely to pass or fail quality control.[22] We first filtered out variants which failed VQSR (N = 334,149). After

manual review of additional variant QC metrics, we excluded variants with Fisher strand bias >40, strand odds ratio >3, mapping quality rank sum < −5, mapping quality rank sum >5, read position rank sum < −4, quality by depth <2, mapping quality <40, and depth across samples >2× the mean (147,000; N = 259,516). Individual analyses also excluded variants which were out of Hardy-Weinberg equilibrium (HWE $>10^{-50}$). We also excluded samples with a low call rate (<0.80; N = 1), samples with sex inconsistencies (N = 40), and unexpected duplicates (N = 16). Because genotyping data (which includes variants across the genome) can more accurately evaluate relatedness and genetic ancestry than exome sequencing data (which includes variants only in the coding region), we excluded one sample from each pair identified as related using the genotyping data (N = 3) and non-European ancestry samples identified using the genotyping data (N = 290) and *APOE* inconsistencies (N = 11). Post-QC exome sequencing data resulted in >560,000 variants and 2,053 samples for analysis.

Statistical Analysis

To maximize sample size, analyses were based on the aducanumab-treated period, consisting of both the placebo-controlled period of each study (for participants who received aducanumab in the placebo-controlled period) and the LTE period (for all participants who received at least one dose). Analyses included only participants on aducanumab treatment, with genetic data which passed QC, and at least one post-baseline MRI. For the logistic models analyzing association with ARIA risk, all participants with available data were included in the risk set as they contribute information to the model. The logistic model results, therefore, can be interpreted as the increased odds of experiencing ARIA-E compared with not experiencing ARIA-E, regardless of other events (with a corresponding interpretation for ARIA-H).

For both genome-wide and exome-wide analyses, we ran 2 models. Model 1 controlled for age at study baseline, sex, study (ENGAGE or EMERGE), last dose level before the ARIA event or censor, and genetic ancestry measured by PCs 1–10. To identify genetic variants independent of *APOE*, Model 2 adjusted for all covariates used in Model 1, with the addition of the 2 SNPs defining *APOE* genotype, rs429358-C and rs7412-T. We ran analyses for any ARIA; any ARIA-H; and separately for ARIA-E, ARIA-H microhemorrhage, and ARIA-H superficial siderosis. In addition, we stratified by symptomatic status and radiographic severity. ARIA-H can occur with or without ARIA-E; therefore, we performed secondary analyses among isolated ARIA-H cases. All analyses were run in PLINK2.

APOE genotypes were calculated using the SNPs rs429358-C and rs7412-T, and logistic regression was run evaluating the association between each form of ARIA and the *APOE* genotype, using the so-called neutral *APOE* genotype ε3/ε3 category as the reference group, using the same covariates as in the GWAS Model 1. For analyses stratifying ARIA outcomes by radiographic severity or symptomatic status, we excluded the ε2/ε3 and ε2ε4 genotypes because the small number of participants in those groups led to potential model convergence issues. Logistic regression evaluating association between *APOE* genotype and ARIA as well as plots were generated with R (version 4.1.1). Kaplan-Meier plots were produced using SAS software version 9.4 (SAS Institute). We additionally ran gene-based analyses in an attempt to increase power from the single-variant exome-wide analysis. We used the R package SKAT to run SKAT-O tests, after annotating variants with MAF < 0.01 using Variant Effect Predictor.[23]

To determine whether genetic variants associated with Alzheimer disease across the genome contributed to risk of ARIA, we ran a polygenic risk score (PRS) analysis. The PRS was constructed using GWAS summary statistics from Schwartzentruber et al.,[24] applying a clumping and thresholding approach, including variants with $p < 0.1$ and excluding the *APOE* region (chr19:44.4–46.5 Mb).[25] The PRS was then inverse rank normal transformed. We performed linear regression for the association between each type of ARIA and the Alzheimer disease PRS, controlling for number of *APOE* ε2 alleles, number of *APOE* ε4 alleles, age, sex, study, last dose level before the ARIA event or censor, and the first 10 PCs.

Data Availability

While the data described in this article are not publicly available, the authors and Biogen are supportive of data sharing. Biogen has established processes to share protocols, clinical study reports, study-level data, and deidentified patient-level data. These data and materials will be made available to qualified scientific researchers in support of the objective(s) in their approved, methodologically sound research proposal. Proposals should be submitted through Vivli (vivli.org/). To gain access, data requestors will need to sign a data sharing agreement. For general inquiries, please contact datasharing@biogen.com. Biogen's data sharing policies and processes are detailed on the website biogentrialtransparency.com/.

Results

Of the participants who underwent genome-wide genotyping and passed QC metrics (N = 2,054), 1,691 were also treated with aducanumab and had at least one post-baseline MRI. Of these, 529 had ARIA-E, 324 had ARIA-H microhemorrhage, 229 had ARIA-H superficial siderosis, and 1,047 experienced none of these events (Table 1). Incidences of all types of ARIA were similar in this genetic cohort compared with the full cohort, as were demographic characteristics, with the exception of race and region.[3] Incidences of all ARIA types were similar between ENGAGE and EMERGE trials. Sex was evenly split across ARIA-E and ARIA-H microhemorrhage; however, of those who experienced ARIA-H superficial siderosis, 40% were female. The mean age was similar. A greater proportion of participants homozygous for *APOE* ε4 experienced ARIA, as was seen in the full cohort.

Table 1 Demographics of ENGAGE and EMERGE Participants With ARIA and Genetic Data

	ARIA-E (N = 529)	ARIA-H microhemorrhage (N = 324)	ARIA-H superficial siderosis (N = 229)	ARIA-H (any) (N = 454)	ARIA (any) (N = 644)	None[a] (N = 1,047)	Full genetic cohort (N = 1,691)
Study							
ENGAGE No. (%)	264 (50)	153 (47)	122 (53)	222 (49)	313 (49)	491 (47)	804 (48)
EMERGE No. (%)	265 (50)	171 (53)	107 (47)	232 (51)	331 (51)	556 (53)	887 (52)
Sex, % female	267 (50)	167 (52)	91 (40)	212 (47)	322 (50)	535 (51)	857 (51)
Age, mean (SD)	70.1 (7.4)	70.9 (7.2)	70.4 (7.0)	70.7 (7.3)	70.5 (7.4)	70.1 (7.4)	70.3 (7.4)
***APOE* genotype, n (%)**							
ε2/ε2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ε2/ε3	9 (2)	3 (1)	4 (2)	7 (2)	12 (2)	24 (2)	36 (2)
ε2/ε4	13 (2)	6 (2)	4 (2)	8 (2)	15 (2)	19 (2)	34 (2)
ε3/ε3	86 (16)	54 (17)	22 (10)	67 (15)	112 (17)	354 (34)	466 (28)
ε3/ε4	258 (49)	144 (44)	113 (49)	219 (48)	315 (49)	527 (50)	842 (50)
ε4/ε4	163 (31)	117 (36)	86 (38)	1,553 (34)	190 (30)	123 (12)	313 (19)
Dose at ARIA or censor, n (%)							
1	2 (<1)	2 (1)	1 (<1)	—	—	—	—
3	300 (57)	188 (58)	122 (53)	—	—	—	—
6	106 (20)	51 (16)	39 (17)	—	—	—	—
10	121 (23)	83 (26)	67 (29)	—	—	—	—
Radiographic severity, n (%)				—	—	—	
Mild	155 (29)	237 (73)	113 (49)				
Moderate	304 (57)	41 (13)	62 (27)				
Severe	70 (13)	46 (14)	54 (24)				
Symptomatic ARIA	133 (25)	39 (12)	33 (14)	—	—	—	—

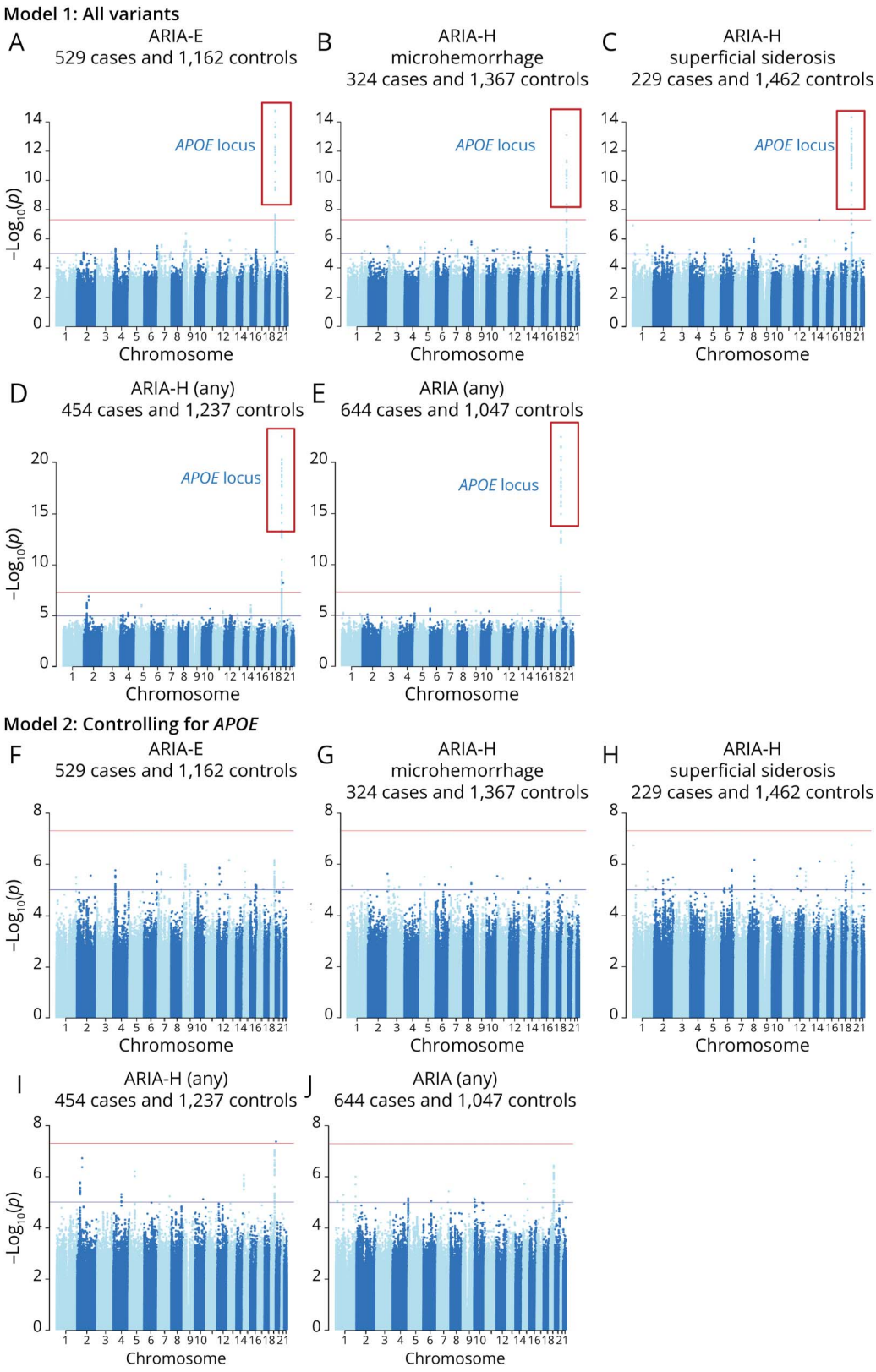
All participants were of European ancestry and from geographic regions Europe/Canada/Australia and the United States.
[a] None = no ARIA-E, ARIA-H microhemorrhage, or ARIA-H superficial siderosis.

In the GWAS, the most significant association was a locus in the *APOE* region, which was strongly and genome-wide significantly associated with risk of ARIA overall and with all 3 types of ARIA analyzed as well as any ARIA-H (Figure, Table 2). There was no evidence of genomic inflation (lambda <1.03, eFigure 1, links.lww.com/WNL/D269). When conditioning on the *APOE* genotype, no additional variants were genome-wide significant, indicating a single independent hit in this locus. The association with ARIA occurred in an *APOE* ε4 dose-dependent manner: participants homozygous for *APOE* ε4 exhibited 4.28 greater odds of experiencing ARIA-E, 4.58 greater odds of experiencing ARIA-H microhemorrhage, and 7.84 greater odds of experiencing ARIA-H superficial siderosis when compared with ε3/ε3 homozygotes (Table 2). There was a less pronounced effect of carrying one copy of the ε4 allele (ε2/ε4 or ε3/ε4) in ARIA-E (OR = 1.74–2.60), ARIA-H microhemorrhage (OR = 1.46–1.63), and ARIA-H superficial siderosis (OR = 2.64–3.14). We did not find a protective effect of the *APOE* ε2 allele in a small, underpowered group of ε2/ε3 carriers (N = 36; p = 0.12–0.69). Time to first ARIA analysis showed similar results to the main analysis (eFigure 2). Analysis of targeted *APOE* genotyping data from the full cohort showed similar results (eTable 2).

To further investigate the effect of *APOE* on risk of ARIA, we stratified by radiographic severity and symptomatic/asymptomatic status. *APOE* ε4/ε4 genotypes were significantly associated with mild, moderate, and severe radiographic ARIA (Table 3). The effect was stronger among ε4/ε4 homozygotes than ε3/ε4 heterozygotes and showed a larger effect in severe (ε4/ε4 OR = 7.04–24.64, $p \leq 2.38 \times 10^{-6}$) vs mild (ε4/ε4 OR = 3.19–5.00, $p \leq 1.37 \times 10^{-5}$) cases.

APOE was also associated with both symptomatic (ε4/ε4 OR = 3.64–9.52; $p < 0.004$) and asymptomatic (ε4/ε4 OR = 4.20–7.94, $p < 1.7 \times 10^{-11}$) ARIA (Table 4). These

Figure Manhattan Plots for GWAS for ARIA-E, ARIA-H Microhemorrhage, ARIA-H Superficial Siderosis, and Any ARIA



All ARIA outcomes show a strong peak in the *APOE* locus (Model 1) and no additional genome-wide significant loci (as indicated by horizontal red line). Model 2, controlling for *APOE*-tagging SNPs, rs429358_C and rs7412_T, resulted in no genome-wide significant hits in any ARIA analysis, indicating a single independent peak within this locus. ARIA = amyloid-related imaging abnormality.

Table 2 Association of *APOE* Genotypes With ARIA[a,b]

APOE genotype	ARIA-E (N = 529 vs 1,162 controls)				ARIA-H microhemorrhage (N = 324 vs 1,367 controls)				ARIA-H superficial siderosis (N = 229 vs 1,462 controls)			
	OR	95% CI	RR[c]	*p* Value	OR	95% CI	RR[c]	*p* Value	OR	95% CI	RR[c]	*p* Value
ε2/ε3	1.47	0.66–3.28	1.37	0.34	0.78	0.23–2.65	0.81	0.69	2.47	0.79–7.7	2.15	0.12
ε2/ε4	2.60	1.23–5.50	2.07	0.01	1.63	0.64–4.17	1.47	0.31	2.64	0.84–8.29	2.27	0.10
ε3/ε4	1.74	1.30–2.31	1.55	0.0002	1.46	1.03–2.06	1.35	0.03	3.14	1.94–5.09	2.59	3.2E-06
ε4/ε4	4.28	3.04–6.02	2.81	6.2E-17	4.58	3.09–6.78	2.85	2.9E-14	7.84	4.67–13.19	4.66	7.8E-15

[a] Values are compared with the ε3/ε3 reference group.
[b] Models controlled for age at study baseline, sex, study (ENGAGE or EMERGE), last dose level before the ARIA event or censor, and genetic ancestry measured by PCs 1–10.
[c] RR was calculated from OR using the equation RR = OR/(1−p_0 + (p_0 × OR)), where p_0 is the baseline risk defined as ε3/ε3 incidence (16% for ARIA-E, 17% for ARIA-H microhemorrhage, 10% for ARIA-H with superficial siderosis).

associations showed a stronger effect in *APOE* ε4/ε4 homozygotes vs ε3/ε4 heterozygotes. However, in an analysis among only those who experienced ARIA, no association was seen for *APOE* when comparing symptomatic vs asymptomatic cases ($p > 0.05$; Table 4).

There was no significant association between *APOE* and isolated ARIA-H either microhemorrhage or superficial siderosis, although sample sizes were small, and hence, analyses were underpowered (Table 5). Odds ratios trended in a similar direction toward ARIA-H combined with ARIA-E.

After controlling for the *APOE* locus, we found no additional genome-wide significant associations independent of the *APOE* region (Figure). Similarly, the exome-wide association study found no significant, high-confidence associations beyond the *APOE* locus (eFigure 3, links.lww.com/WNL/D269). Gene-based tests did not identify additional significant signals associated with the ARIA traits (eTable 3). Note that no *APOE* SNPs met the allele frequency threshold of MAF <0.01, thus *APOE* was not tested in the gene-based analysis.

The PRS analysis revealed a significant association between increased polygenic risk of Alzheimer disease and all 3 types of ARIA (ARIA-E: beta = 0.14, $p = 0.04$; ARIA-H microhemorrhage: beta = 0.20, $p = 0.01$; ARIA-H with superficial siderosis beta = 0.20, $p = 0.03$; eTable 4, links.lww.com/WNL/D269).

Table 3 Association of *APOE* Genotypes With ARIA Stratified by Radiographic Severity[a,b]

ARIA-E												
	Mild (N = 146 vs 1,114 controls)				Moderate (N = 295 vs 1,114 controls)				Severe (N = 66 vs 1,114 controls)			
APOE genotype	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value
ε3/ε4	1.74	1.11–2.71	1.55	0.02	1.70	1.18–2.46	1.53	0.005	2.09	0.88–4.98	1.78	0.09
ε4/ε4	3.37	1.95–5.82	2.44	1.37E-05	4.19	2.75–6.39	2.77	2.89E-11	7.04	2.83–17.53	3.58	2.72E-05
ARIA-H microhemorrhage												
	Mild (N = 230 vs 1,306 controls)				Moderate (N = 39 vs 1,306 controls)				Severe (N = 46 vs 1,306 controls)			
APOE genotype	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value
ε3/ε4	1.33	0.91–1.93	1.26	0.14	2.23	0.72–6.9	1.84	0.16	2.60	0.72–9.37	2.04	0.14
ε4/ε4	3.19	2.06–4.93	2.32	1.83E-07	8.27	2.49–27.52	3.70	0.001	21.40	5.99–76.42	4.79	2.38E-06
ARIA-H with superficial siderosis												
	Mild (N = 108 vs 1,400 controls)				Moderate (N = 60 vs 1,400 controls)				Severe (N = 53 vs 1,400 controls)			
APOE genotype	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value
ε3/ε4	33.30	1.77–6.15	2.68	0.0002	2.11	0.89–5.01	1.90	0.09	6.48	1.5–28.04	4.18	0.01
ε4/ε4	5.00	2.48–10.08	3.57	6.76E-06	8.17	3.34–19.96	4.76	4.05E-06	24.64	5.64–107.68	7.32	2.06E-05

[a] Values are compared with the ε3/ε3 reference group.
[b] Models controlled for age at study baseline, sex, study (ENGAGE or EMERGE), last dose level before the ARIA event or censor, and genetic ancestry measured by PCs 1–10. ε2/ε3 and ε2/ε4 genotypes were excluded from these analyses because small sample sizes in these groups led to potential model convergence issues.

Table 4 Association of *APOE* Genotypes With ARIA Stratified by Symptomatic ARIA[a,b]

ARIA-E												
	Symptomatic (N = 125 vs 1,114 controls)				Asymptomatic (N = 382 vs 1,114 controls)				Symptomatic vs asymptomatic (N = 125 vs 382)			
APOE genotype	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value
ε3/ε4	1.79	1.04–3.06	1.59	0.03	1.70	1.24–2.35	1.53	0.001	1.10	0.6–2.03	1.09	0.75
ε4/ε4	3.64	1.97–6.73	2.56	3.8E-05	4.56	3.13–6.63	2.90	2.2E-15	0.79	0.41–1.54	0.82	0.49
ARIA-H microhemorrhage												
	Symptomatic (N = 38, vs 1,306 controls)				Asymptomatic (N = 277 vs 1,306 controls)				Symptomatic vs asymptomatic (N = 38 vs 277)			
APOE genotype	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value
ε3/ε4	1.03	0.37–2.88	1.02	0.96	1.50	1.04–2.16	1.38	0.03	0.58	0.19–1.74	0.62	0.33
ε4/ε4	7.41	2.73–20.08	3.55	8.3E-05	4.20	2.76–6.37	2.72	1.7E-11	1.44	0.5–4.11	1.34	0.50
ARIA-H with superficial siderosis												
	Symptomatic (N = 32 vs 1,400 controls)				Asymptomatic (N = 189 vs 1,400 controls)				Symptomatic vs asymptomatic (N = 32 vs 189)			
APOE genotype	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value	OR	95% CI	RR	*p* Value
ε3/ε4	4.81	1.08–21.35	3.48	0.04	3.01	1.81–5.00	2.51	2.1E-05	2.16	0.43–11.00	1.94	0.35
ε4/ε4	9.52	2.03–44.61	5.14	0.004	7.94	4.59–13.73	4.69	1.2E-13	1.27	0.24–6.77	1.24	0.78

[a] Values are compared with the ε3/ε3 reference group.
[b] Models controlled for age at study baseline, sex, study (ENGAGE or EMERGE), last dose level before the ARIA event or censor, and genetic ancestry measured by PCs 1–10. E2/ε3 and ε2/ε4 genotypes were excluded from these analyses because small sample sizes in these groups led to potential model convergence issues.

Discussion

In this genome-wide assessment of genetic risk factors of ARIA, we identified a robust association between genetic variants at the *APOE* locus and risk of developing aducanumab-related ARIA-E and ARIA-H, with no additional associations observed beyond this region. The strong association observed at *APOE* in this analysis is consistent with observations from other amyloid-lowering therapies, which also found an elevated incidence of ARIA among *APOE* ε4 carriers[5,6,17,18] and in a recent systematic review of ARIA.[26] A phase 2 study of bapineuzumab found a hazard ratio of 3.8 for *APOE* ε4 heterozygotes and 7.15 for *APOE* ε4 homozygotes for ARIA-E.[17] A phase 3 study of solanezumab did not show a significantly increased incidence of ARIA-E among ε4 heterozygotes.[18] Similarly, a phase 3 study of gantenerumab showed that both ARIA-E and ARIA-H occurred more frequently among *APOE* ε4 heterozygotes vs noncarriers and among *APOE* ε4 homozygotes vs *APOE* ε4 heterozygotes.[6] In a phase 3 trial of lecanemab, ARIA-E was more frequent among homozygotes (32.6%) than heterozygotes (10.9%) or noncarriers (5.4%) in the treatment arm. A similar pattern was seen for ARIA-H (homozygotes: 39%, heterozygotes 14%, noncarrier: 11.9%).[5] In addition, in the phase 3 trials of aducanumab, more *APOE* ε4 carriers experienced aducanumab-related ARIA than noncarriers (35.9% of *APOE* ε4 heterozygous and 66% of *APOE* ε4 homozygotes experienced ARIA).[3,11] Similarly, in a phase 3 study of donanemab, among treated participants ARIA-E occurred in 15.7%

Table 5 Association of *APOE* Genotypes With Isolated ARIA-H and ARIA-H Combined With Other ARIA[a,b,c]

	ARIA-H microhemorrhage						ARIA-H superficial siderosis					
	Isolated			Combined with ARIA-E			Isolated			Combined with ARIA-E		
	(N = 84 vs 1,537 controls)			(N = 231 vs 1,390 controls)			(N = 29 vs 1,592 controls)			(N = 192 vs 1,429 controls)		
APOE genotype	OR	95% CI	*p* Value	OR	95% CI	*p* Value	OR	95% CI	*p* Value	OR	95% CI	*p* Value
ε3/ε4	1.05	0.6–1.84	0.85	1.66	1.09–2.54	0.02	1.88	0.66–5.35	0.24	3.45	2.02–5.92	6.42E-06
ε4/ε4	1.69	0.86–3.32	0.13	5.55	3.51–8.77	2.16E-13	2.66	0.8–8.81	0.11	8.90	5.03–15.77	6.75E-14

[a] Values are compared with the ε3/ε3 reference group.
[b] Models controlled for age at study baseline, sex, study (ENGAGE or EMERGE), last dose level before the ARIA event or censor, and genetic ancestry measured by PCs 1–10. E2/ε3 and ε2/ε4 genotypes were excluded from these analyses because small sample sizes in these groups led to potential model convergence issues.
[c] Isolated ARIA-H is defined as ARIA-H in patients who experienced no ARIA-E during the aducanumab-treated period; all other ARIA-H is considered to be combined with ARIA-E (regardless of whether the ARIA-E and ARIA-H events were contemporaneous).

of *APOE* ε4 noncarriers, 22.8% of *APOE* ε4 heterozygotes, and 40.6% of *APOE* ε4 homozygotes.[27]

In addition, this study highlights the importance of reporting heterozygosity by genotype when evaluating its association with ARIA, given the substantially larger effects observed among ε4 homozygotes vs heterozygotes in contrast to earlier publications addressing ARIA in aducanumab, which reported *APOE* primarily by the presence or absence of ε4 alleles,[3,11] masking differences between the groups. Notably, from a patient care perspective, the same laboratory genotyping test is used to determine the number of *APOE* ε4 alleles a patient carries as that used to determine whether the patient is an *APOE* ε4 carrier or not. Thus, this information is readily available for individuals who have *APOE* carrier status tested and does not require more sophisticated or further testing for number of *APOE* ε4 alleles.

Our evaluation of genetic risk factors of ARIA across the full genome did not detect genome-wide significant associations beyond the *APOE* locus. However, we observed a significant association between polygenic risk of Alzheimer disease and ARIA, indicating that additional genetic variants beyond *APOE* likely contribute toward ARIA risk. Although this study represents the largest published GWAS of ARIA to date, it is still underpowered to detect more modest associations. For example, we were well-powered (~80% power) to detect a relative risk of 1.6 for ARIA-E, 1.8 for ARIA-H microhemorrhage, and 2.0 for ARIA-H superficial siderosis for common variants with allele frequencies of 0.3 (eTable 5, links.lww.com/WNL/D269). This allowed us to detect the strong association with the *APOE* locus. We were underpowered to detect associations of more rare variants or smaller effect sizes of the range often observed in large GWAS. Larger studies in the future may detect additional associations, shedding light on the biological mechanisms contributing toward ARIA. For example, studies with 1,000–2,000 cases would be well-powered to detect moderate effect sizes for common variants (eTable 5). This may be achieved through meta-analysis of multiple amyloid-targeting clinical trials. For potential clinical risk prediction, our results indicate that no other loci in the genome are associated with ARIA at a comparable magnitude with *APOE*; thus, *APOE* remains the strongest genetic candidate for prospective clinical utility.

Potential limitations of this study include the following considerations. Although treatment randomization was stratified dichotomously by *APOE* ε4 carrier status (i.e., carrier or noncarrier), the number of copies of ε4 was not a stratification factor, leaving potential for imbalance across planned dose groups. For some types of events (severe ARIA-H superficial siderosis in particular), the estimated odds ratios may be sensitive to the small number of events in the ε3/ε3 reference group. The genetic population included only those with European ancestry in the United States, Europe, Canada, and Australia; the results may not generalize to other regions or ancestral groups.

ARIA can occur both with and without symptoms and with varying degrees of radiographic severity.[15] The clinical relevance and long-term outcomes associated with ARIA is yet to be determined. Our study indicated that *APOE* is associated with both symptomatic and asymptomatic ARIA but that *APOE* is not predictive of symptomatic vs asymptomatic ARIA. Nonetheless, we found significant associations between *APOE* ε4 genotype and all levels of radiographic severity in ARIA, with larger effect sizes for the radiographically severe ARIA than for radiographically mild ARIA.

ARIA represents the most common adverse event for aducanumab, and *APOE* genotype provides a useful indication of ARIA risk. Given the large number of patients with Alzheimer disease who carry *APOE* ε4 alleles (46%–52% heterozygotes, 15%–19% homozygotes in the UK Biobank and BioFINDER cohorts (internal analysis) and ENGAGE and EMERGE), a large proportion of patients with AD may be affected by ARIA. Indeed, the recently published Appropriate Use Guidelines call for incorporating *APOE* genotyping into clinical care.[28]

This work highlights the value genetics can add to providing additional information to guide clinical treatment decisions. Although not yet standard in clinical settings, targeted genotyping of *APOE* is relatively inexpensive, highly accurate, and consistent over time, making it an attractive biomarker for patient risk stratification.

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Disclosure

S.J. Loomis is an employee of Biogen, and may hold stocks/stock options in Biogen; R. Miller is a former employee of Biogen, and may hold stocks/stock options in Biogen; C. Castrillo-Viguera is a former employee of Biogen, and may hold stocks/stock options in Biogen; K. Umans is a former employee of Biogen, and may hold stocks/stock options in Biogen; W. Cheng is an employee of Biogen, and may hold stocks/stock options in Biogen; J. O'Gorman is an employee of Biogen, and may hold stocks/stock options in Biogen; R. Hughes is an employee of Biogen, and may hold stocks/stock options in Biogen; S. Budd Haeberlein is a former employee of Biogen, and may hold stocks/stock options in Biogen; C.D. Whelan is a former employee of Biogen, and may hold stocks/stock options in Biogen. Go to Neurology.org/N for full disclosures.

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