JAMA Neurology | Original Investigation

Association of Rare *APOE* Missense Variants V236E and R251G With Risk of Alzheimer Disease

Yann Le Guen, PhD; Michael E. Belloy, PhD; Benjamin Grenier-Boley, MSc; Itziar de Rojas, MSc; Atahualpa Castillo-Morales, PhD; Iris Jansen, PhD; Aude Nicolas, PhD; Céline Bellenguez, PhD; Carolina Dalmasso, PhD; Fahri Küçükali, PhD; Sarah J. Eger, BA; Katrine Laura Rasmussen, MD, PhD; Jesper Qvist Thomassen, PhD; Jean-François Deleuze, PhD; Zihuai He, PhD; Valerio Napolioni, PhD; Philippe Amouyel, MD, PhD; Frank Jessen, MD; Patrick G. Kehoe, PhD; Cornelia van Duijn, PhD; Magda Tsolaki, MD, PhD; Pascual Sánchez-Juan, MD, PhD; Kristel Sleegers, MD, PhD; Martin Ingelsson, MD, PhD; Giacomina Rossi, PhD; Mikko Hiltunen, PhD; Rebecca Sims, PhD; Wiesje M. van der Flier, PhD; Alfredo Ramirez, MD, PhD; Ole A. Andreassen, MD, PhD; Ruth Frikke-Schmidt, MD, PhD; Julie Williams, PhD; Agustín Ruiz, MD, PhD; Jean-Charles Lambert, PhD; Michael D. Greicius, MD; Members of the EADB, GR@ACE, DEGESCO, DemGene, GERAD, and EADI Groups

IMPORTANCE The *APOE* ε2 and *APOE* ε4 alleles are the strongest protective and risk-increasing, respectively, genetic variants for late-onset Alzheimer disease (AD). However, the mechanisms linking *APOE* to AD—particularly the apoE protein's role in AD pathogenesis and how this is affected by *APOE* variants—remain poorly understood. Identifying missense variants in addition to *APOE* ε2 and *APOE* ε4 could provide critical new insights, but given the low frequency of additional missense variants, AD genetic cohorts have previously been too small to interrogate this question robustly.

OBJECTIVE To determine whether rare missense variants on *APOE* are associated with AD risk.

DESIGN, SETTING, AND PARTICIPANTS Association with case-control status was tested in a sequenced discovery sample (stage 1) and followed up in several microarray imputed cohorts as well as the UK Biobank whole-exome sequencing resource using a proxy-AD phenotype (stages 2 and 3). This study combined case-control, family-based, population-based, and longitudinal AD-related cohorts that recruited referred and volunteer participants. Stage 1 included 37 409 nonunique participants of European or admixed European ancestry, with 11 868 individuals with AD and 11 934 controls passing analysis inclusion criteria. In stages 2 and 3, 475 473 participants were considered across 8 cohorts, of which 84 513 individuals with AD and proxy-AD and 328 372 controls passed inclusion criteria. Selection criteria were cohort specific, and this study was performed a posteriori on individuals who were genotyped. Among the available genotypes, 76 195 were excluded. All data were retrieved between September 2015 and November 2021 and analyzed between April and November 2021.

MAIN OUTCOMES AND MEASURES In primary analyses, the AD risk associated with each missense variant was estimated, as appropriate, with either linear mixed-model regression or logistic regression. In secondary analyses, associations were estimated with age at onset using linear mixed-model regression and risk of conversion to AD using competing-risk regression.

RESULTS A total of 544 384 participants were analyzed in the primary case-control analysis; 312 476 (57.4%) were female, and the mean (SD; range) age was 64.9 (15.2; 40-110) years. Two missense variants were associated with a 2-fold to 3-fold decreased AD risk: *APOE* ε4 (R251G) (odds ratio, 0.44; 95% CI, 0.33-0.59; $P = 4.7 \times 10^{-8}$) and *APOE* ε3 (V236E) (odds ratio, 0.37; 95% CI, 0.25-0.56; $P = 1.9 \times 10^{-6}$). Additionally, the cumulative incidence of AD in carriers of these variants was found to grow more slowly with age compared with noncarriers.

CONCLUSIONS AND RELEVANCE In this genetic association study, a novel variant associated with AD was identified: R251G always coinherited with ε4 on the *APOE* gene, which mitigates the ε4-associated AD risk. The protective effect of the V236E variant, which is always coinherited with ε3 on the *APOE* gene, was also confirmed. The location of these variants confirms that the carboxyl-terminal portion of apoE plays an important role in AD pathogenesis. The large risk reductions reported here suggest that protein chemistry and functional assays of these variants should be pursued, as they have the potential to guide drug development targeting *APOE*.

JAMA Neurol. 2022;79(7):652-663. doi:10.1001/jamaneurol.2022.1166
Published online May 31, 2022.

Editorial page 649

Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: Authors and collaborators of the EADB, GR@ACE, DEGESCO, DemGene, GERAD, and EADI Groups appear at the end of the article.

Corresponding Author: Yann Le Guen, PhD, Department of Neurology and Neurological Sciences, Stanford University, 290 Jane Stanford Way, E265, CA 94305-5090 (yleguen@stanford.edu).

Late-onset Alzheimer disease (AD) is a highly polygenic neurodegenerative disorder with, to date, 75 risk loci associated with AD risk.[1] Most of the common single-nucleotide variants (SNVs) at these loci only contribute a small amount to an individual's risk of AD,[2] with the exception of the *APOE* ε2 and ε4 missense variants that are associated with substantially decreased[3] and increased AD risk,[4] respectively. It is estimated that 25% of the genetic variance of AD can be attributed to *APOE* ε2 and *APOE* ε4.[5] Despite the outsized role of these 2 common *APOE* alleles, more than 25 years after the initial studies linking them to AD, their role in pathogenesis remains ill-defined. Human studies have shown that ε4 speeds and ε2 slows the age-related misprocessing of β-amyloid, although how this occurs at the molecular level remains uncertain.[6,7] Even the most basic question, does ε4 act via a loss-of-function or gain-of-function mechanism, remains a point of contention.[8] Loss-of-function variants on *APOE* are exceedingly rare, and the sole case report describing a compound heterozygote with 2 loss-of-function variants involved a patient who was too young to be informative.[9] The study of additional missense variants on *APOE* may also help to answer this critical question and further elucidate the role of *APOE* in AD. In addition to ε2 and ε4, the only common missense variant (with a minor allele frequency [MAF] greater than 1%) is Arg145Cys (R145C), an African-ancestry variant always found coinherited with *APOE* ε3, which we have shown increases risk for AD.[10] The Arg136Ser (R136S) Christchurch variant has recently been posited to play a protective role in early-onset AD related to *PSEN1* variants, but this study had no statistical genetics support as it was based on data from a single patient.[11] Finally, strong functional evidence has been marshalled recently to support a protective role for the Val236Glu (V236E) variant, although this was based on data from an earlier case-control study with only approximately 9000 participants,[12,13] likely underpowered to provide firm estimates of disease risk.

On this background, we aimed to investigate, at large scale, the association of rare missense variants on *APOE* with AD risk. We used the Alzheimer's Disease Sequencing Project (ADSP) whole-genome sequencing (WGS) and whole-exome sequencing (WES) data as our discovery sample (stage 1) and sought to replicate significant variants (stages 2 and 3) in multiple cohorts using microarray data imputed on the Trans-Omics for Precision Medicine (TOPMed) reference panel (National Institutes of Health),[14] or by using directly sequenced and genotyped variants from a large Danish general prospective population cohort,[15] as well as using the proxy-AD phenotype[1] in the UK Biobank WES data. After filtering, 3 variants, Leu28Pro (L28P), Val236Glu (V236E), and Arg251Gly (R251G), were tested for their association with AD risk after adjusting for ε2 and ε4 dosages. In complementary analyses, we assessed these associations in an *APOE*-stratified approach to account for the complete linkage disequilibrium of these variants with either the ε2, ε3, or ε4 allele. In secondary analyses, combining stage 1 and 2 data sets, we tested their association with age at onset in individuals with AD and with risk of conversion to AD using competing-risk regression.

Key Points

Question Are *APOE* missense variants, other than the common *APOE* alleles ε2 and ε4, associated with Alzheimer disease (AD) risk?

Findings In this genetic association study including 544 384 participants, multiple studies including 67 896 individuals with AD, 28 484 with proxy-AD, and 340 306 healthy controls were meta-analyzed. Two rare missense variants (*APOE* ε3 [V236E] and *APOE* ε4 [R251G]) substantially reduced the risk of AD (by more than 60% and more than 50%, respectively).

Meaning Single amino acid alterations of the *APOE* ε3 and *APOE* ε4 isoforms can result in substantial risk reduction for AD.

Methods

Participants and Sources of Data

Participants or their caregivers provided written informed consent in the original studies. The current study protocol was granted an exemption by the Stanford University Institutional Review Board because the analyses were carried out on deidentified, off-the-shelf data; therefore, additional informed consent was not required. For stage 1 and stage 2, phenotypic information and genotypes were obtained from publicly released genome-wide association study data sets assembled by the Alzheimer's Disease Genetics Consortium (ADGC) and derived from WES and WGS data generated by the ADSP, with phenotype and genotype ascertainment described elsewhere.[16-20] The cohorts' queried accession numbers, as well as the sequencing technology or SNV genotyping platforms are described in eTables 1 and 2 in Supplement 1. Information about stage 3, which included external replication cohorts and UK Biobank, is provided in the eMethods in Supplement 1. Briefly, these included European Alzheimer's Disease DNA Biobank (EADB) core, European Alzheimer's Disease Initiative (EADI), Genetic and Environmental Risk in Alzheimer's Disease Consortium (GERAD), Norwegian Dementia Genetics Network (DemGene), and Genome Research at Fundació Alzheimer Center Barcelona (GR@ACE)/Dementia Genetics Spanish Consortium (DEGESCO) cohorts for which phenotype, genotype quality control, and imputation have already been described in Bellenguez et al[1]; and the Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS) *APOE* sequencing and genotyping were described in Rasmussen et al.[15] The following sections describe quality control procedures and ancestry determination applied to the ADSP and ADGC samples, respectively, used as stage 1 and stage 2. This study followed the Strengthening the Reporting of Genetic Association Studies (STREGA) reporting guideline. UK Biobank WES data were analyzed under Application Number 45420.

Quality Control Procedures

Prior to ancestry, principal components, and relatedness determination, in each cohort platform, variants were excluded based on genotyping rate (less than 95%), MAF less than 1%, and Hardy-Weinberg equilibrium in controls ($P < 10^{-6}$) using

PLINK version 1.9.[21] gnomAD[22] database-derived information was used to filter out SNVs that met one of the following exclusion criteria[23,24]: (1) located in a low-complexity region, (2) located within common structural variants (MAF greater than 1%), (3) multiallelic SNVs with MAF greater than 1% for at least 2 alternate alleles, (4) located within a common insertion or deletion, (5) having any flag different than PASS (passed all variant filters) in gnomAD version 3, and (6) having potential probe variants. The latter are defined as SNVs for which the probe may have variable affinity owing to the presence of other SNV(s) within 20 base pairs and with MAF greater than 1%. Individuals with more than 5% genotype missingness were excluded. Duplicate individuals were identified with KING (Kinship-based Inference for GWAS)[25] and their clinical, diagnostic, and pathological data (including age at onset of cognitive symptoms, age at examination for clinical diagnosis, age at last examination, and age at death), as well as sex, race, and *APOE* genotype were cross-referenced across cohorts. Duplicate entries with irreconcilable phenotype or discordant sex were flagged for exclusion. For individuals with duplicated genotype in sequencing and imputed data, the sequencing entry was used in the stage 1 discovery set and the imputed entry was not included in the stage 2 replication set. To apply the PC-AiR and PC-Relate methods, we simply considered the intersection of the variants passing quality control in both ADSP WES and ADSP WGS in the discovery set and similarly the intersection of the variants across cohorts genotyping platform in the replication set.

Ancestry Determination

For each cohort, we first determined the ancestry of each individual with SNPWeights version 2 (Harvard)[26] using reference populations from the 1000 Genomes Consortium.[27] By applying an ancestry percentage cutoff greater than 75%, the samples were stratified into 5 super populations: South Asian, East Asian, United States, African, and European individuals and an admixed group composed of individuals not passing the 75% cutoff in any single ancestry (eTable 3 in Supplement 1).[10,23] Since the *APOE* missense variants of interest L28P, V236E, and R251G are too rare to assess reliably in non-European ancestry populations (eTable 4 in Supplement 1), we restricted our analysis to European and admixed European individuals. Admixed European individuals were also included in the main analysis and were part of the admixed group defined above and had at least 15% European ancestry. We performed sensitivity analyses in increments of 30%, including admixed European individuals at 45% and 75% cutoffs. The latter corresponding to the super population threshold.

Imputation

Each cohort genotyping platform was imputed on the TOPMed imputation server per ancestry group to obtain an imputation quality (R^2) per ancestry group. We retained cohorts with R^2 greater than 0.70 at rs199768005 for the V236E analyses and at rs267606661 for the R251G analyses. As there was no significant association signal for rs769452 (L28P) in the stage 1 primary analysis, we did not check its imputation quality in stage 2 samples.

APOE Genotype Ascertainment

We directed specific attention to the genotyping of the SNVs determining the main *APOE* genotype (rs429358 and rs7412), rs769452-C (*APOE* [L28P]), rs199768005-A (*APOE* [V236E]), and rs267606661-G (*APOE* [R251G]) and followed the procedure described in Le Guen et al.[10] Note that Leu28Pro (L28P), Val236Glu (V236E), and Arg251Gly (R251G) are also sometimes referred to as *L46P*, *V254E*, and *R269G*, respectively, when the first 18 codons of *APOE* encoding a signal peptide are included.

Samples Analyzed

Our discovery sample (stage 1) was composed of individuals of European and admixed European ancestry from the ADSP WES and WGS, corresponding to 11 868 individuals with AD and 11 934 cognitively normal controls (**Table 1**). eFigure 1 in Supplement 1 provides a flowchart of the filtering steps leading to the inclusion of these individuals and describes how these data sets were combined. To build a replication sample (stage 2) for V236E and R251G, we queried for individuals of European and admixed European ancestry in all the publicly available microarray genetic data sets that we had access to at the time of the study in July 2021 (Table 1). These data sets are largely part of the ADGC, and as such, this replication will be referred to hereafter as the ADGC replication in stage 2. After quality control and duplicate removal, 7768 individuals with AD and 8059 controls remained in the ADGC replication sample. eTable 5 in Supplement 1 presents the demographic characteristics of the remaining individuals with AD and cognitively unimpaired controls. In stage 3, we pursued additional replication in external data sets (not publicly available) and in the UK Biobank WES using the proxy-AD phenotype (Table 1; eMethods in Supplement 1). Overall, the external replications included 36 393 individuals with AD and 150 943 controls, and the UK Biobank replication included 28 484 individuals with proxy-AD and 157 436 controls. Across cohorts reported in Table 1, the *APOE* genotype were split as follows: ε2/ε2, 0.5%; ε2/ε3, 10.4%; ε3/ε3, 54.5%; ε2/ε4, 2.5%; ε3/ε4, 27.6%; ε4/ε4, 4.4%.

Study Design and Statistical Analysis

In our analysis, we only considered missense variants with a minor allele count greater than 10 in any *APOE* main genotype groups in our next-generation sequencing discovery (stage 1) to avoid outlier-confounded effect size estimates.[28] Three *APOE* missense variants were retained for further analyses: L28P, V236E, and R251G (eTable 4 in Supplement 1). The V236E variant is always coinherited with *APOE* ε3, and the L28P and R251G are always coinherited with *APOE* ε4 (eTable 6 in Supplement 1). Two variants are coinherited when they are on the same chromosome copy and close enough to each other that a meiotic crossover event never occurs between them. We thus developed 2 complementary approaches to take into account these linkage disequilibrium structures. In primary analyses, we estimated the AD risk associated with L28P, V236E, and R251G on case-control diagnoses using linear mixed-model regression (stages 1 and 2 and UK Biobank) and logistic regression model (stage 3), adjusted for ε2 and ε4 dosages, in

Table 1. Demographic Characteristics per *APOE* Genotype[a]

Sample	Diagnosis	Individuals, No.	APOE ε2/ε2			APOE ε2/ε3			APOE ε3/ε3			APOE ε2/ε4			APOE ε3/ε4			APOE ε4/ε4		
			Total, No.	Female, No. (%)	Age, mean (SD), y	Total, No.	Female, No. (%)	Age, mean (SD), y	Total, No.	Female, No. (%)	Age, mean (SD), y	Total, No.	Female, No. (%)	Age, mean (SD), y	Total, No.	Female, No. (%)	Age, mean (SD), y	Total, No.	Female, No. (%)	Age, mean (SD), y
ADSP	CN	11 934	73	40 (54.8)	82.6 (8.3)	1481	924 (62.4)	83.0 (8.0)	7429	4636 (62.4)	82.3 (8.1)	195	137 (70.3)	79.8 (8.9)	2561	1590 (62.1)	79.7 (8.2)	195	123 (63.1)	76.6 (7.5)
	AD	11 868	29	17 (58.6)	82.5 (6.9)	583	369 (63.3)	80.1 (9.7)	5313	3236 (60.9)	77.0 (10.1)	258	158 (61.2)	75.3 (8.2)	4919	2853 (58.0)	73.2 (8.5)	766	406 (53.0)	67.9 (8.1)
ADGC	CN	8059	56	26 (46.4)	79.1 (10.2)	978	629 (64.3)	76.2 (9.5)	4795	2968 (61.9)	74.5 (9.4)	209	132 (63.2)	73.8 (10.1)	1847	1143 (61.9)	71.4 (10.1)	174	106 (60.9)	68.7 (9.3)
	AD	7768	10	6 (60.0)	72.5 (8.2)	323	181 (56.0)	75.8 (10.4)	2494	1586 (63.6)	74.7 (10.5)	237	150 (63.3)	75.7 (8.8)	3258	2059 (63.2)	73.0 (8.6)	1446	830 (57.4)	69.7 (7.2)
EADB core	CN	21 160	121	72 (59.5)	68.6 (13.2)	2503	1457 (58.2)	66.8 (15.1)	13 365	7725 (57.8)	67.0 (14.5)	396	220 (55.6)	66.7 (13.3)	4390	2445 (55.7)	66.3 (13.6)	385	212 (55.1)	64.2 (12.6)
	AD	19 873	27	14 (51.9)	76.4 (11.7)	877	522 (59.5)	74.2 (11.2)	8285	5128 (61.9)	72.9 (11.0)	435	287 (66.0)	73.2 (10.7)	8003	5042 (63.0)	71.7 (9.7)	2246	1289 (57.4)	67.6 (8.8)
GR@ACE	CN	8539	33	19 (57.6)	53.1 (17.6)	858	448 (52.2)	57.5 (18.7)	6005	3009 (50.1)	56.7 (18.0)	99	49 (49.5)	56.7 (17.6)	1459	727 (49.8)	56.7 (17.6)	85	37 (43.5)	54.9 (14.8)
	AD	7355	16	14 (84.6)	84.6 (3.5)	389	274 (70.4)	81.4 (8.1)	3840	2703 (70.4)	80.9 (7.9)	115	84 (73.0)	78.7 (7.4)	2590	1808 (69.8)	78.7 (7.4)	405	262 (64.7)	74.8 (7.3)
EADI	CN	6331	38	20 (52.6)	82.6 (7.5)	772	457 (59.2)	81.0 (7.5)	4247	2582 (60.8)	80.1 (7.7)	109	66 (60.6)	78.8 (7.1)	1106	655 (59.2)	79.0 (7.6)	59	42 (71.2)	77.1 (6.7)
	AD	2397	7	6 (85.7)	79.3 (6.0)	128	88 (68.8)	78.0 (10.8)	1078	704 (65.3)	76.5 (10.6)	71	42 (59.2)	73.4 (8.8)	888	586 (66.0)	72.6 (9.2)	225	146 (64.9)	68.1 (7.0)
GERAD	CN	7007	47	26 (55.3)	49.3 (11.0)	853	427 (50.1)	51.5 (12.6)	4127	2142 (51.9)	50.9 (11.9)	180	93 (51.7)	49.8 (10.9)	1627	843 (51.8)	49.9 (10.9)	173	86 (49.7)	49.9 (11.0)
	AD	2989	10	6 (60.0)	81.2 (9.7)	140	88 (62.9)	79.3 (11.3)	1092	677 (62.0)	79.3 (9.6)	90	57 (63.3)	80.4 (7.6)	1306	838 (64.2)	77.7 (8.9)	351	219 (62.4)	74.2 (8.4)
DemGene	CN	5911	32	11 (34.4)	68.7 (11.2)	685	336 (49.1)	69.2 (12.4)	3236	1540 (47.6)	68.9 (11.0)	167	76 (45.5)	70.6 (10.6)	1595	769 (48.2)	67.3 (10.5)	196	87 (44.4)	64.7 (11.0)
	AD	1687	5	2 (40.0)	74.0 (1.4)	72	42 (58.3)	71.6 (10.6)	537	359 (66.9)	73.7 (9.6)	43	31 (72.1)	75.4 (7.0)	769	512 (66.6)	72.2 (8.4)	261	161 (61.7)	69.3 (8.1)
CCHS and CGPS	CN	101 995	705	387 (54.9)	57.0 (13.2)	12 818	7063 (55.1)	57.6 (13.6)	57 115	31 299 (54.8)	57.5 (13.4)	2936	1627 (55.4)	56.8 (13.0)	25 616	14 063 (54.9)	56.7 (12.8)	2778	1600 (57.6)	55.3 (12.7)
	AD	2092	12	6 (50.0)	72.6 (5.3)	129	69 (53.5)	73.3 (8.4)	844	496 (58.8)	73.3 (8.4)	70	43 (61.4)	71.2 (8.0)	821	512 (62.4)	70.9 (8.0)	216	123 (56.9)	68.8 (7.9)

Abbreviations: AD, Alzheimer disease; ADGC, Alzheimer's Disease Genetic Consortium; ADSP, Alzheimer's Disease Sequencing Project; CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; CN, cognitively normal; DemGene, Norwegian Dementia Genetics Network; EADB, European Alzheimer's Disease DNA Biobank; EADI, European Alzheimer's Disease Initiative; GERAD, Genetic and Environmental Risk in Alzheimer's Disease Consortium; GR@ACE, Genome Research at Fundació Alzheimer Center Barcelona.

[a] UK Biobank demographic characteristics are not reported in this table since cases correspond to proxy-AD phenotype mostly relying on self-report of first-degree relatives' diagnosis without age at onset being specified.

addition to the covariates described below for all analyses. The adjustment by the common ε3 and ε4 *APOE* alleles is necessary because the rare variants tested here are always coinherited with either the ε3 or ε4 *APOE* allele. In complementary analyses, we also estimated the AD risk associated with V236E and R251G stratified by their associated common *APOE* allele genotype. V236E was assessed in *APOE* ε3/ε3 and R251G was assessed in the *APOE* ε3/ε4 stratum. An association was considered significant in stage 1 if it reached a Bonferroni-corrected *P* value threshold of .017 (.05/3) in the model adjusted for ε2 and ε4 dosages; all *P* values were 2-tailed. L28P was not associated with AD risk in this model and was not studied further.

Sample sizes and demographic characteristics for the stratified analyses are shown in eTable 5 in Supplement 1. In sensitivity analyses, we estimated AD risk associations for different European ancestry inclusion thresholds. In secondary analyses, combining stages 1 and 2 data sets, we estimated the influence of significant stage 1 variants on age at onset in AD cases using linear mixed-model regression and risk of conversion to AD using competing-risk regression. In secondary analyses, associations were considered significant when passing the nominal *P* value threshold of .05. The case-control and age-at-onset analyses used linear mixed-model regression available through the GENESIS package version 3.12.[29] Multivariate competing-risk regression and cumulative incidence estimation were implemented using the *cmprsk* package version 2.2.[30] In this time-to-event analysis, failure events were defined as age at onset for individuals who developed AD (conversion to AD) and age at death for controls. Controls without reported death were right-censored at age at last visit. Left censoring was set at age 50 years, and younger individuals were excluded from the analysis. All statistical analyses were adjusted for sex and 4 genetic principal components estimated with the PC-AiR method[31] implemented in GENESIS. Linear mixed-model analyses were additionally covaried by a sparse genetic association matrix estimated with the PC-Relate method[32] implemented in GENESIS. Case-control analyses were not adjusted for age given that correcting for age when individuals with AD are younger than controls leads to the model incorrectly inferring the age effect on AD risk, resulting in statistical power loss.[23]

Case-control analyses in stage 3, external replication cohorts and proxy-AD phenotype in UK Biobank, were implemented to be consistent with the stage 1 primary analyses. Exact model/analysis details are described in the eMethods in Supplement 1. For the ADSP/ADGC cohorts, all statistical analyses were performed in R version 4.0.2 (The R Foundation). All meta-analyses were implemented with a fixed-effect inverse variance-weighted design implemented in the *metafor* R package version 3.0.2.[33]

Results

A total of 544 384 participants were analyzed in the primary case-control analysis; 312 476 (57.4%) were female, and the mean (SD; range) age was 64.9 (15.2; 40-110) years. In stage 1 primary analyses, V236E (rs199768005-A) and R251G (rs267606661-G) were associated with a 4-fold to 5-fold decreased AD risk in nonstratified analyses adjusted for ε2 and ε4 dosages (V236E: odds ratio [OR], 0.23; 95% CI, 0.09-0.56; $P = .001$; R251G: OR, 0.20; 95% CI, 0.08-0.49; $P = 3.7 \times 10^{-4}$) (**Figure 1**; **Table 2**). Similarly, in *APOE*-stratified analyses, V236E was associated with a 3-fold decreased AD risk in individuals with ε3/ε3 (OR, 0.31; 95% CI, 0.12-0.82; $P = .02$), and R251G was associated with a 5-fold decreased AD risk in individuals with ε3/ε4 (OR, 0.17; 95% CI, 0.06-0.48; $P = 7.8 \times 10^{-4}$) (Table 2). The L28P variant (rs769452-C) was not associated with AD risk in the nonstratified analyses (OR, 1.12; 95% CI, 0.77-1.62; $P = .56$). As such, it was not investigated further.

In stages 2 and 3, across multiple replication cohorts, the effects of V236E and R251G in nonstratified analyses were concordant and both were significantly associated with AD risk (V236E: OR, 0.42; 95% CI, 0.27-0.66; $P = 2.0 \times 10^{-4}$; R251G: OR, 0.48; 95% CI, 0.35-0.66; $P = 5.8 \times 10^{-6}$). The overall meta-analysis (Figure 1; Table 2) provides robust effect size estimate for these 2 variants and confirmed their association with a 2-fold to 3-fold decreased AD risk (V236E: OR, 0.37; 95% CI, 0.25-0.56; $P = 1.9 \times 10^{-6}$; R251G: OR, 0.44; 95% CI, 0.33-0.59; $P = 4.7 \times 10^{-8}$). Similar results were obtained in *APOE*-stratified meta-analyses (Table 2; eFigure 2 in Supplement 1). We further estimated the odds per *APOE* genotype group, using individuals with ε3/ε3 who did not carry V236E as the reference (ie, OR of individuals with *APOE* ε3/ε3 equals 1), by meta-analyzing the ADSP discovery and ADGC replication cohorts. Compared with the reference ε3/ε3 group, the ε3/ε3 (V236E) and ε3/ε4 (R251G) groups had AD risk lower than or similar to the ε2/ε3 group (**Figure 2**).

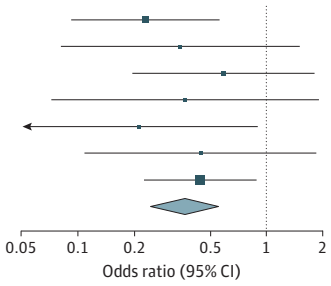
Results of sensitivity analyses evaluating different European ancestry cutoffs are shown in eTable 8 and eFigure 3 in Supplement 1. Briefly, the results remained unchanged when selecting individuals with admixed ancestry with at least 45% European ancestry or when restricting the analysis to individuals with European ancestry (75% cutoff). We note that the ORs in the combined ADSP/ADGC data sets for V236E and R251G remain unchanged at different ancestry cutoffs. For example, using an ancestry cutoff at 75%, the nonstratified meta-analysis yielded an OR of 0.27 (95% CI, 0.12-0.58; $P = 8.6 \times 10^{-4}$) for V236E compared with an OR of 0.26 (95% CI, 0.12-0.56; $P = 5.4 \times 10^{-4}$) using a cutoff of 15%. Similar observations were made for the R251G variant. As additional supplementary analyses, we assessed the effect of the inclusion of all dementia (rather than AD specifically) in the CCHS and CGPS data set, and we estimated the significance without including UK Biobank. Overall, the significance of the results slightly improved when including a broader dementia category (R251G: OR, 0.44; 95% CI, 0.33-0.59; $P = 3.5 \times 10^{-8}$) (eTable 9 in Supplement 1). While removing UK Biobank proxy-AD phenotype samples reduced the significance of our results slightly, the ORs became slightly more protective (R251G: OR, 0.39; 95% CI, 0.27-0.56; $P = 1.2 \times 10^{-7}$) (eTable 10 in Supplement 1).

In secondary analyses, including data from stages 1 and 2, we considered the meta-analysis of ADSP/ADGC samples

Figure 1. Association of V236E and R251G With Alzheimer Disease (AD) Risk Across All Cohorts

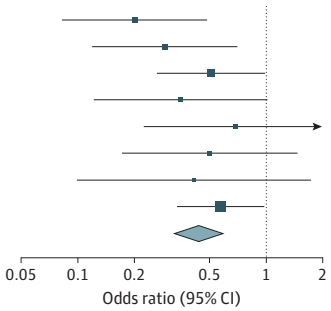
A *APOE* V236E

Source	Odds ratio (95% CI)
ADSP (WGS and WES)	0.23 (0.09-0.56)
ADGC (TOPMed imputed)	0.35 (0.08-1.51)
EADB core (TOPMed imputed)	0.59 (0.19-1.80)
GERAD (TOPMed imputed)	0.37 (0.07-1.90)
DemGene (TOPMed imputed)	0.21 (0.05-0.90)
CCHS and CGPS (APOE sequencing and genotyping)	0.45 (0.11-1.84)
UK Biobank proxy-AD (WES)	0.45 (0.23-0.89)
Summary estimate ($P = 1.9 \times 10^{-6}$)	0.37 (0.25-0.56)



B *APOE* R251G

Source	Odds ratio (95% CI)
ADSP (WGS and WES)	0.20 (0.08-0.49)
ADGC (TOPMed imputed)	0.29 (0.12-0.70)
EADB core (TOPMed imputed)	0.51 (0.26-0.99)
GR@ACE (TOPMed imputed)	0.35 (0.12-1.01)
EADI (TOPMed imputed)	0.68 (0.22-2.09)
GERAD (TOPMed imputed)	0.50 (0.17-1.47)
CCHS and CGPS (APOE sequencing and genotyping)	0.41 (0.10-1.72)
UK Biobank proxy-AD (WES)	0.57 (0.34-0.98)
Summary estimate ($P = 4.7 \times 10^{-8}$)	0.44 (0.33-0.59)



Forest plots show the results for the non–*APOE*-stratified analyses adjusted by ε2 and ε4 dosages. eFigure 2 in Supplement 1 presents equivalent forest plots for these 2 variants in the *APOE*-stratified sensitivity analyses, showing consistent findings. ADGC indicates Alzheimer's Disease Genetic Consortium; ADSP, Alzheimer's Disease Sequencing Project; CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; DemGene, Norwegian Dementia Genetics Network; EADB, European Alzheimer's Disease DNA Biobank; EADI, European Alzheimer's Disease Initiative; GERAD, Genetic and Environmental Risk in Alzheimer's Disease Consortium; GR@ACE, Genome Research at Fundació Alzheimer Center Barcelona; TOPMed, Trans-Omics for Precision Medicine; WES, whole-exome sequencing; WGS, whole-genome sequencing.

(eTable 5 in Supplement 1). In non-*APOE* stratified analyses adjusted for ε2 and ε4 dosages (eTable 7 in Supplement 1), V236E carriers had a mean age at AD onset 10.5 years older than non-carriers (β = 10.64; 95% CI, 1.78-19.49; $P = .02$) and slower incidence with age (hazard ratio [HR], 0.30; 95% CI, 0.12-0.76; $P = .01$). While R251G's association with age at onset was not significant (β = 0.97; 95% CI, −2.96 to 4.91; $P = .63$), its association with reduced AD incidence with age was just nominally significant (HR, 0.67; 95% CI, 0.46-0.97; $P = .04$). In *APOE*-stratified analyses (eTable 7 in Supplement 1), a similar association of V236E with age at AD onset was observed in individuals with ε3/ε3 (β = 10.93; 95% CI, 1.06-20.81; $P = .03$). R251G carriers had a mean age at AD onset 6 years older than noncarriers of ε3/ε4, but this association was not significant (β = 6.04; 95% CI, −0.71 to 12.79; $P = .08$). The competing risk results emphasized that the cumulative incidence of AD in participants with ε3/ε3 grows slower with age in individuals carrying the V236E variant (HR, 0.40; 95% CI, 0.17-0.97; $P = .04$) and similarly in participants with ε3/ε4 carrying the R251G variant (HR, 0.26; 95% CI, 0.13-0.54; $P = 2.9 \times 10^{-4}$).

Discussion

We have shown that 2 missense variants V236E and R251G are each associated with a more than 2-fold reduction in AD risk (Figure 2). These variants have an allele frequency of less than 0.1% in gnomAD version 3.1, even when restricting this frequency estimate to individuals of European ancestry (eTable 4 in Supplement 1). Because of their rarity and linkage disequilibrium with the common *APOE* ε3 and ε4 alleles, they have not been identified in prior genome-wide association studies.[1] The protective effect of V236E has already been reported in a smaller prior study focused on *APOE*[13] and was suggestive in a population-based study,[15] but we validated this finding here in a large-scale genomic study and provide an improved estimate of its effect size. To our knowledge, the association of R251G with AD risk has not been previously reported. This variant, carried on the same haplotype as ε4, is the first *APOE* variant found to mitigate the AD risk attributable to the ε4 isoform of the apoE protein. Notably, having R251G in association with *APOE* ε4 results in a risk estimate similar to *APOE* ε2, as shown in Figure 2 where *APOE* ε3/ε4 (R251G) and *APOE* ε2/ε3 have an equivalent OR.

Regarding potential mechanisms driving these associations, it is notable that these 2 variants are on apoE's C-terminal domain. The common *APOE* ε2 and *APOE* ε4 alleles are located on the N-terminal domain of the protein near the receptor-binding region. Their outsized role in AD risk has, understandably, focused attention on the N-terminal domain and the differential capacity of these alleles to, for example, bind apoE's receptors.[34,35] The current results add support to studies suggesting that the C-terminal domain is also of critical importance for AD pathogenesis.[36-38] R251G is located within apoE's lipid-binding region (amino acid residues 244 to 272), while V236E is adjacent to this region.[8] A 2021 publication[12] provided evidence for the protectiveness of V236E against AD pathology and explored the functional mechanism support-

Table 2. Association of V236E and R251G With Alzheimer Disease (AD) Risk[a]

	AD case-control regression (nonstratified)				AD case-control regression (*APOE* stratified)			
Sample	Individuals, No.	MAC	OR (95% CI)	*P* value	Individuals, No.	MAC	OR (95% CI)	*P* value
V236E[b]								
ADSP	23 427	20	0.23 (0.09-0.56)	.001	12 604	17	0.31 (0.12-0.82)	.02
ADGC imputed	11 652	10	0.35 (0.08-1.51)	.16	5741	10	0.40 (0.10-1.57)	.19
EADB core	41 033	27.17	0.59 (0.19-1.80)	.34	21 650	21.28	0.53 (0.15-1.92)	.30
GERAD	9996	17.72	0.37 (0.07-1.90)	.18	5219	9.43	0.77 (0.10-6.06)	.78
DemGene	7598	58.68	0.21 (0.05-0.90)	.009	3773	35.88	0.56 (0.13-2.46)	.40
CCHS and CGPS	104 084	240	0.45 (0.11-1.84)	.23	57 955	191	0.18 (0.01-2.97)	.27
UK Biobank proxy-AD	185 741	277	0.45 (0.23-0.89)	.02	109 120	219	0.47 (0.21-1.04)	.06
Meta-analysis	383 531	650.57	0.37 (0.25-0.56)	1.9×10^{-6}	216 062	503.59	0.43 (0.27-0.69)	4.4×10^{-4}
R251G[c]								
ADSP	23 314	26	0.20 (0.08-0.49)	3.7×10^{-4}	7335	18	0.17 (0.06-0.48)	7.8×10^{-4}
ADGC imputed	14 134	29	0.29 (0.12-0.70)	.006	4630	16	0.19 (0.07-0.54)	.002
EADB core	41 033	59.16	0.51 (0.26-0.99)	.049	12 393	40.27	0.34 (0.15-0.76)	.008
GR@ACE	15 894	21.27	0.35 (0.12-1.01)	.049	4049	17.81	0.22 (0.06-0.77)	.01
EADI	8728	19.21	0.68 (0.22-2.09)	.49	1994	13.32	1.14 (0.32-4.04)	.84
GERAD	9996	23.17	0.50 (0.17-1.47)	.18	2933	16.82	0.57 (0.18-1.88)	.34
CCHS and CGPS	104 087	105	0.41 (0.10-2.72)	.23	26 437	75	0.33 (0.05-2.43)	.28
UK Biobank proxy-AD	185 735	335	0.57 (0.34-0.98)	.04	43 820	262	0.67 (0.36-1.22)	.19
Meta-analysis	402 921	617.81	0.44 (0.33-0.59)	4.7×10^{-8}	103 591	459.22	0.41 (0.29-0.57)	3.2×10^{-7}

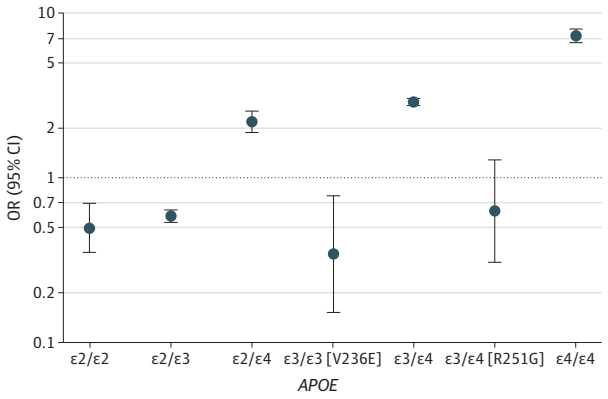
Abbreviations: ADGC, Alzheimer's Disease Genetic Consortium; ADSP, Alzheimer's Disease Sequencing Project; CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; DemGene, Norwegian Dementia Genetics Network; EADB, European Alzheimer's Disease DNA Biobank; EADI, European Alzheimer's Disease Initiative; GERAD, Genetic and Environmental Risk in Alzheimer's Disease Consortium; GR@ACE, Genome Research at Fundació Alzheimer Center Barcelona; MAC, minor allele count; OR, odds ratio.

[a] The significance of their association with AD risk was equivalent in nonstratified analyses adjusted by *APOE* ε2 and ε4 dosages and in *APOE*-stratified analysis considering the main *APOE* genotype group with the most carriers for each variant, namely ε3/ε3 and ε3/ε4 for V236E and R251G, respectively.

[b] For V236E, all *APOE* alleles are used in nonstratified analyses and the ε3/ε3 alleles only in *APOE*-stratified analyses.

[c] For R251G, all *APOE* alleles are used in nonstratified analyses and the ε3/ε4 alleles only in *APOE*-stratified analyses.

Figure 2. Risk Equivalence of *APOE* ε3/ε3 (V236E) and *APOE* ε3/ε4 (R251G) to ε2/ε3 Carriers



Alzheimer disease risk per *APOE* genotype was compared with the *APOE* ε3/ε3 reference group (ie, odds ratio [OR] for *APOE* ε3/ε3 equals to 1), meta-analyzing results from the Alzheimer's Disease Genetic Consortium and Alzheimer's Disease Sequencing Project cohorts (stages 1 and 2). eFigure 3 in Supplement 1 presents equivalent results at different inclusion cutoffs for European ancestry.

ing its protective role. The lipid-binding region, with its abundance of nonpolar residues, is thought to be a region that can foster oligomerization.[39-41] Switching a nonpolar valine for an acidic glutamic acid might be predicted to reduce the hydrophobicity of this region and reduce its tendency to oligomerize. Notably, the authors showed reduced levels of insoluble β-amyloid and apoE aggregates in the brain of V236E carriers compared with noncarriers.[12] In 5×FAD mice, they observed that *APOE* ε3 (V236E) reduced Aβ deposition, plaque-associated immune response, and neuritic dystrophy around amyloid plaques.[12] Chemically, they noted that *APOE* ε3 (V236E) primarily remains as a monomer and is less likely to form oligomers compared with the canonical *APOE* ε3 allele.[12] This propensity of V236E to reduce apoE aggregation was also observed when this variant was introduced on an *APOE* ε4 allele. It is worth noting, however, that V236E also appears to increase dimerization (see Figure S10[12]), which may affect apoE's ability to bind to its receptors.[42-44]

Given that R251G is located squarely in the lipid-binding region of the protein, it is possible that R251G confers a protective effect by reducing apoE's ability to form insoluble oligomers. The switch from a charged arginine amino acid to a nonpolar glycine might, however, be expected to increase rather than decrease oligomerization. Changes in this region could also enhance apoE ε4's ability to bind lipids rendering it more like ε3 or ε2 in this capacity.[45] Alternatively, the introduction of glycine could disrupt the α helix structure

of the C-terminal impacting apoE ε4's hypothesized N-terminal-C-terminal domain interaction.[34,35] In any case, pending protein chemistry experiments exploring potential structural and functional changes, the mechanism underlying the substantial protective effect of R251G remains to be elucidated.

Limitations

Our study has several limitations. The V236E association was not genome-wide significant. We included the UK Biobank data set that does not include a direct clinical diagnosis of AD. Because of the paucity of variant carriers of non-European ancestries, we did not assess these variants in other ancestries (although they can be found in African American individuals and admixed Latino individuals based on gnomAD estimates; eTable 4 in Supplement 1). These caveats point to the need for further confirmation of these variants as available AD data sets grow and become more ancestrally diverse.

Conclusions

Our work was performed on, to our knowledge, the largest available sample to date for *APOE* ε3 (V236E) and *APOE* ε4 (R251G). These findings validate the protective effect of the V236E variant and has uncovered a novel protective missense variant on *APOE* ε4. Each variant had a substantial association with reducing the risk of AD. While some compelling functional data suggest that V236E confers protection by reducing oligomerization of apoE, there are alternative mechanisms that merit consideration (increasing dimerization, for one). The protective mechanism of R251G remains unexplored, but finding a single amino acid substitution that renders the *APOE* -ε4 allele protective supports the idea that *APOE* ε4-specific treatments are worth exploring.[46,47] We anticipate that the findings reported here will spark additional mechanistic work on apoE's role in AD pathogenesis.

ARTICLE INFORMATION

Accepted for Publication: March 16, 2022.

Published Online: May 31, 2022. doi:10.1001/jamaneurol.2022.1166

Author Affiliations: Department of Neurology and Neurological Sciences, Stanford University, Palo Alto, California (Le Guen, Belloy, Eger, He, Greicius); Institut du Cerveau, Paris Brain Institute, Paris, France (Le Guen); University of Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167-RID-AGE Facteurs de risque et déterminants moléculaires des maladies liées au vieillissement, Lille, France (Grenier-Boley, Nicolas, Bellenguez, Amouyel, Lambert); Research Center and Memory Clinic Fundació ACE, Institut Català de Neurociències Aplicades, Universitat Internacional de Catalunya, Barcelona, Spain (de Rojas, Ruiz); Networking Research Center on Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain (de Rojas, Sánchez-Juan, Ruiz); UKDRI@Cardiff, School of Medicine, Cardiff University, Cardiff, United Kingdom (Castillo-Morales, Williams); Alzheimer Center Amsterdam, Department of Neurology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, the Netherlands (Jansen, van der Flier); Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, Vrije University, Amsterdam, the Netherlands (Jansen); Department of Psychiatry and Psychotherapy, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany (Dalmasso, Jessen, Ramirez); Estudios en Neurociencias y Sistemas Complejos (ENyS) CONICET-HEC-UNAJ, Universidad Nacional Arturo Jauretche, Florencio Varela, Argentina (Dalmasso); Complex Genetics of Alzheimer's Disease Group, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium (Küçükali, Sleegers); Laboratory of Neurogenetics, Born-Bunge Institute, Antwerp, Belgium (Küçükali, Sleegers); Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium (Küçükali, Sleegers); Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark (Rasmussen, Thomassen, Frikke-Schmidt); Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark (Rasmussen, Frikke-Schmidt); Université Paris-Saclay, CEA, Centre National de Recherche en Génomique Humaine, Evry, France (Deleuze); Quantitative Sciences Unit, Department of Medicine, Stanford University, Palo Alto, California (He); School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy (Napolioni); German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany (Jessen, Ramirez); Cluster of Excellence Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany (Jessen, Ramirez); Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom (Kehoe); Department of Epidemiology, ErasmusMC, Rotterdam, the Netherlands (van Duijn); Nuffield Department of Population Health Oxford University, Oxford, United Kingdom (van Duijn); 1st Department of Neurology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece (Tsolaki); Alzheimer's Centre Reina Sofia-CIEN Foundation, Madrid, Spain (Sánchez-Juan); Department of Public Health and Caring Sciences / Geriatrics, Uppsala University, Uppsala, Sweden (Ingelsson); Krembil Brain Institute, University Health Network, Toronto, Ontario, Canada (Ingelsson); Department of Medicine and Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, Canada (Ingelsson); Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy (Rossi); Institute of Biomedicine, University of Eastern Finland, Joensuu, Kuopio, Finland (Hiltunen); Division of Psychological Medicine and Clinical Neuroscience, School of Medicine, Cardiff University, Cardiff, United Kingdom (Sims, Williams); Department of Neurodegenerative Diseases and Geriatric Psychiatry, University Hospital Bonn, Bonn, Germany (Ramirez); Department of Psychiatry and Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, UT Health San Antonio, The University of Texas Health Science Center at San Antonio (Ramirez); NORMENT Centre, Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway (Andreassen); Institute of Clinical Medicine, University of Oslo, Oslo, Norway (Andreassen).

Authors of the EADB, GR@ACE, DEGESCO, DemGene, GERAD, and EADI Groups: Beatrice Arosio, PhD; Luisa Benussi, PhD; Anne Boland, PharmD, PhD; Barbara Borroni, MD; Paolo Caffarra, PhD; Delphine Daian, MSc; Antonio Daniele, MD, PhD; Stéphanie Debette, MD, PhD; Carole Dufouil, PhD; Emrah Düzel, MD, PhD; Daniela Galimberti, PhD; Vilmantas Giedraitis, PhD; Timo Grimmer, PhD; Caroline Graff, MD; Edna Grünblatt, PhD; Olivier Hanon, MD; Lucrezia Hausner, PhD; Stefanie Heilmann-Heimbach, PhD; Henne Holstege, PhD; Jakub Hort, MD, PhD; Deckert Jürgen, MD; Teemu Kuulasmaa, MSc; Aad van der Lugt, PhD; Carlo Masullo, MD; Patrizia Mecocci, MD, PhD; Shima Mehrabian, PhD; Alexandre de Mendonça, PhD; Susanne Moebus, PhD; Benedetta Nacmias, PhD; Gael Nicolas, MD, PhD; Robert Olaso, PhD; Goran Papenberg, PhD; Lucilla Parnetti, MD, PhD; Florence Pasquier, MD, PhD; Oliver Peters, MD; Yolande A. L. Pijnenburg, MD; Julius Popp, MD; Innocenzo Rainero, MD, PhD; Inez Ramakers, PhD; Steffi Riedel-Heller, MD; Nikolaos Scarmeas, MD, PhD; Philip Scheltens, MD, PhD; Norbert Scherbaum, MD; Anja Schneider, MD; Davide Seripa, PhD; Hilkka Soininen, MD, PhD; Vincenzo Solfrizzi, MD, PhD; Gianfranco Spalletta, MD, PhD; Alessio Squassina, PhD; John van Swieten, MD; Thomas J. Tegos, MD, PhD; Lucio Tremolizzo, MD, PhD; Frans Verhey, MD, PhD; Martin Vyhnalek, MD, PhD; Jens Wiltfang, MD; Mercè Boada, MD, PhD; Pablo García-González, MSc; Raquel Puerta, MSc; Luis M. Real, PhD; Victoria Álvarez, PhD; María J. Bullido, PhD; Jordi Clarimon, PhD; José María García-Alberca, MD, PhD; Pablo Mir, MD, PhD; Fermin Moreno, MD, PhD; Pau Pastor, MD, PhD; Gerard Piñol-Ripoll, MD, PhD; Laura Molina-Porcel, MD, PhD; Jordi Pérez-Tur, PhD; Eloy Rodríguez-Rodríguez, MD, PhD; Jose Luís Royo, PhD; Raquel Sánchez-Valle, MD, PhD; Martin Dichgans, MD; Dan Rujescu, MD.

Affiliations of Authors of the EADB, GR@ACE, DEGESCO, DemGene, GERAD, and EADI Groups: Research Center and Memory Clinic Fundació ACE, Institut Català de Neurociències Aplicades,

Universitat Internacional de Catalunya, Barcelona, Spain (Boada, García-González, Puerta); Networking Research Center on Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain (Boada, García-González, Bullido, Clarimon, García-Alberca, Mir, Moreno, Pérez-Tur, Rodríguez-Rodríguez); Alzheimer Center Amsterdam, Department of Neurology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, the Netherlands (Holstege, Pijnenburg, Scheltens); Université Paris-Saclay, CEA, Centre National de Recherche en Génomique Humaine, Evry, France (Boland, Daian, Olaso); German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany (Schneider); 1st Department of Neurology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece (Tegos); Department of Public Health and Caring Sciences / Geriatrics, Uppsala University, Uppsala, Sweden (Giedraitis); Institute of Biomedicine, University of Eastern Finland, Joensuu, Kuopio, Finland (Kuulasmaa); Department of Neurodegenerative Diseases and Geriatric Psychiatry, University Hospital Bonn, Bonn, Germany (Schneider); Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy (Arosio); Molecular Markers Laboratory, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy (Benussi); Centre for Neurodegenerative Disorders, Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy (Borroni); Unit of Neurology, University of Parma, Parma, Italy (Caffarra); Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy (Daniele); Neurology Unit, IRCCS Fondazione Policlinico Universitario A. Gemelli, Rome, Italy (Daniele); University Bordeaux, Inserm, Bordeaux Population Health Research Center, Bordeaux, France (Debette); Department of Neurology, Bordeaux University Hospital, Bordeaux, France (Debette); Inserm, Bordeaux Population Health Research Center, UMR 1219, University of Bordeaux, ISPED, CIC 1401-EC, Université de Bordeaux, Bordeaux, France (Dufouil); CHU de Bordeaux, Pole santé publique, Bordeaux, France (Dufouil); German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany (Düzel); Institute of Cognitive Neurology and Dementia Research (IKND), Otto-von-Guericke University, Magdeburg, Germany (Düzel); Neurodegenerative Diseases Unit, Fondazione IRCCS Ca' Granda, Ospedale Policlinico, Milan, Italy (Galimberti); Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy (Galimberti); Technical University of Munich, School of Medicine, Klinikum rechts der Isar, Department of Psychiatry and Psychotherapy, Munich, Germany (Grimmer); Unit for Hereditary Dementias, Theme Aging, Karolinska University Hospital-Solna, Stockholm, Sweden (Graff); Department of Child and Adolescent Psychiatry and Psychotherapy, Psychiatric University Hospital Zurich, University of Zurich, Zurich, Switzerland (Grünblatt); Neuroscience Center Zurich, University of Zurich and ETH Zurich, Zurich, Switzerland (Grünblatt); Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland (Grünblatt); Université de Paris, EA 4468, APHP, Hôpital Broca, Paris, France (Hanon); Department of Geriatric Psychiatry, Central Institute of Mental Health Mannheim, Faculty Mannheim, University of Heidelberg, Heidelberg, Germany (Hausner); Institute of Human Genetics, University of Bonn, School of Medicine, University Hospital Bonn, Bonn, Germany (Heilmann-Heimbach); Department of Clinical Genetics, VU University Medical Centre, Amsterdam, the Netherlands (Holstege); Memory Clinic, Department of Neurology, Charles University, 2nd Faculty of Medicine and Motol University Hospital, Prague, Czech Republic (Hort, Vyhnalek); International Clinical Research Center, St Anne's University Hospital Brno, Brno, Czech Republic (Hort, Vyhnalek); Department of Psychiatry, Psychosomatics and Psychotherapy, Center of Mental Health, University Hospital of Würzburg, Würzburg, Germany (Jürgen); Department of Radiology and Nuclear Medicine, ErasmusMC, Rotterdam, the Netherlands (van der Lugt); Institute of Neurology, Catholic University of the Sacred Heart, Rome, Italy (Masullo); Institute of Gerontology and Geriatrics, Department of Medicine and Surgery, University of Perugia, Perugia, Italy (Mecocci); Clinic of Neurology, UH Alexandrovska, Medical University Sofia, Sofia, Bulgaria (Mehrabian); Faculty of Medicine, University of Lisbon, Lisbon, Portugal (de Mendonça); Institute for Urban Public Health, University Hospital of University Duisburg-Essen, Essen, Germany (Moebus); Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy (Nacmias); IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy (Nacmias); Normandie Université, UNIROUEN, Inserm U1245 and CHU Rouen, Department of Genetics and CNR-MAJ, Rouen, France (Nicolas); Aging Research Center, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet and Stockholm University, Stockholm, Sweden (Papenberg); Centre for Memory Disturbances, Lab of Clinical Neurochemistry, Section of Neurology, University of Perugia, Perugia, Italy (Parnetti); Université de Lille, Inserm 1172, CHU Clinical and Research Memory Research Centre (CMRR) of Distalz, Lille, France (Pasquier); German Center for Neurodegenerative Diseases (DZNE), Berlin, Germany (Peters); Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Psychiatry and Psychotherapy, Berlin, Germany (Peters); Old Age Psychiatry, Department of Psychiatry, Lausanne University Hospital, Lausanne, Switzerland (Popp); Department of Geriatric Psychiatry, University Hospital of Psychiatry Zürich, Zurich, Switzerland (Popp); Institute for Regenerative Medicine, University of Zürich, Zurich, Switzerland (Popp); Department of Neuroscience "Rita Levi Montalcini," University of Torino, Torino, Italy (Rainero); Maastricht University, Department of Psychiatry and Neuropsychologie, Alzheimer Center Limburg, Maastricht, the Netherlands (Ramakers, Verhey); Institute of Social Medicine, Occupational Health and Public Health, University of Leipzig, Leipzig, Germany (Riedel-Heller); 1st Department of Neurology, Aiginition Hospital, National and Kapodistrian University of Athens, Medical School, Athens, Greece (Scarmeas); Taub Institute for Research in Alzheimer's Disease and the Aging Brain, The Gertrude H. Sergievsky Center, Department of Neurology, Columbia University, New York, New York (Scarmeas); LVR-Hospital Essen, Department of Psychiatry and Psychotherapy, Medical Faculty, University of Duisburg-Essen, Essen, Germany (Scherbaum); Laboratory for Advanced Hematological Diagnostics, Department of Hematology and Stem Cell Transplant, "Vito Fazzi" Hospital, Lecce, Italy (Seripa); Institute of Clinical Medicine - Neurology, University of Eastern Finland, Kuopio, Finland (Soininen); Interdisciplinary Department of Medicine, Geriatric Medicine and Memory Unit, University of Bari Aldo Moro, Bari, Italy (Solfrizzi); Laboratory of Neuropsychiatry, IRCCS Santa Lucia Foundation, Rome, Italy (Spalletta); Department of Psychiatry and Behavioral Sciences, Baylor College of Medicine, Houston, Texas (Spalletta); Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy (Squassina); Department of Neurology, ErasmusMC, Rotterdam, the Netherlands (van Swieten); Neurology, "San Gerardo" Hospital, Monza and University of Milano-Bicocca, Milan, Italy (Tremolizzo); Department of Psychiatry and Psychotherapy, University Medical Center Goettingen, Goettingen, Germany (Wiltfang); German Center for Neurodegenerative Diseases (DZNE), Goettingen, Germany (Wiltfang); Neurosciences and Signaling Group, Institute of Biomedicine (iBiMED), Department of Medical Sciences, University of Aveiro, Aveiro, Portugal (Wiltfang); Unidad Clínica de Enfermedades Infecciosas y Microbiología, Hospital Universitario de Valme, Sevilla, Spain (Real); Depatamento de Especialidades Quirúrgicas, Bioquímica e Inmunología, Facultad de Medicina, Universidad de Málaga, Málaga, Spain (Real, Royo); Laboratorio de Genética, Hospital Universitario Central de Asturias, Oviedo, Spain (Álvarez); Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain (Álvarez); Centro de Biología Molecular Severo Ochoa (UAM-CSIC), Universidad Autónoma de Madrid, Madrid, Spain (Bullido); Instituto de Investigacion Sanitaria 'Hospital la Paz' (IdIPaz), Madrid, Spain (Bullido); Department of Neurology, II B Sant Pau, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain (Clarimon); Alzheimer Research Center & Memory Clinic, Andalusian Institute for Neuroscience, Málaga, Spain (García-Alberca); Unidad de Trastornos del Movimiento, Servicio de Neurología y Neurofisiología, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain (Mir); Department of Neurology, Hospital Universitario Donostia, San Sebastian, Spain (Moreno); Neurosciences Area, Instituto Biodonostia, San Sebastian, Spain (Moreno); Fundació Docència i Recerca MútuaTerrassa, Terrassa, Spain (Pastor); Memory Disorders Unit, Department of Neurology, Hospital Universitari Mutua de Terrassa, Terrassa, Spain (Pastor); Unitat Trastorns Cognitius, Hospital Universitari Santa Maria de Lleida, Lleida, Spain (Piñol-Ripoll); Institut de Recerca Biomedica de Lleida (IRBLLeida), Lleida, Spain (Piñol-Ripoll); Neurological Tissue Bank (Biobanc), Hospital Clinic IDIBAPS, Barcelona, Spain (Molina-Porcel); Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Department, Hospital Clinic, Barcelona, Spain (Molina-Porcel); Unitat de Genètica Molecular, Institut de Biomedicina de València-CSIC, Valencia, Spain (Pérez-Tur); Unidad Mixta de Neurologia Genètica, Instituto de Investigación Sanitaria La Fe, Valencia, Spain (Pérez-Tur); Neurology Service, Marqués de Valdecilla University Hospital (University of

Cantabria and IDIVAL), Santander, Spain (Rodríguez-Rodríguez); Alzheimer's Disease and Other Cognitive Disorders Unit, Service of Neurology, Hospital Clínic of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain (Sánchez-Valle); Institute for Stroke and Dementia Research (ISD), University Hospital, Ludwig Maximilian University of Munich, Munich, Germany (Dichgans); German Center for Neurodegenerative Diseases (DZNE), Munich, Germany (Dichgans); Munich Cluster for Systems Neurology (SyNergy), Munich, Germany (Dichgans); Medical University of Vienna, Department of Psychiatry and Psychotherapy, Vienna, Austria (Rujescu).

Author Contributions: Drs Le Guen and Greicius had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Le Guen and Belloy are co–first authors.
Study concept and design: Le Guen, Belloy, Arosio, Dichgans, Dufouil, Galimberti, Boada, Nacmias, Rainero, van Swieten, Tegos, Jessen, van Duijn, Tsolaki, Greicius.
Acquisition, analysis, or interpretation of data: Le Guen, Belloy, Grenier-Boley, de Rojas, Castillo-Morales, Jansen, A. Nicolas, Bellenguez, Dalmasso, Küçükali, Eger, Álvarez, Benussi, Boland, Borroni, Bullido, Caffarra, Clarimon, Daian, Daniele, Debette, Deleuze, Dichgans, Dufouil, Düzel, García-Alberca, García-González, Giedraitis, Grimmer, Graff, Grünblatt, Hanon, Hausner, Heilmann-Heimbach, Holstege, Hort, Jürgen, Kuulasmaa, van der Lugt, Masullo, Mecocci, Mehrabian, de Mendonça, Mir, Moebus, Moreno, G. Nicolas, Olaso, Papenberg, Parnetti, Pasquier, Pastor, Peters, Pijnenburg, Piñol-Ripoll, Popp, Molina-Porcel, Puerta Fuentes, Pérez-Tur, Ramakers, Rasmussen, Real, Riedel-Heller, Rodríguez-Rodríguez, Royo, Rujescu, Scarmeas, Scheltens, Scherbaum, Schneider, Seripa, Soininen, Solfrizzi, Spalletta, Squassina, Sánchez-Valle, Thomassen, Tremolizzo, Verhey, Vyhnalek, Wiltfang, He, Napolioni, Amouyel, Kehoe, Sánchez-Juan, Sleegers, Ingelsson, Rossi, Hiltunen, Sims, van der Flier, Ramirez, Andreassen, Frikke-Schmidt, Williams, Ruiz, Lambert, Greicius.
Drafting of the manuscript: Le Guen, Belloy, Dichgans, García-Alberca, Kuulasmaa, Olaso, Piñol-Ripoll, van Swieten, Jessen, Hiltunen, Greicius.
Critical revision of the manuscript for important intellectual content: Le Guen, Belloy, Grenier-Boley, de Rojas, Castillo-Morales, Jansen, A. Nicolas, Bellenguez, Dalmasso, Küçükali, Eger, Álvarez, Arosio, Benussi, Boland, Borroni, Bullido, Caffarra, Clarimon, Daian, Daniele, Debette, Deleuze, Dichgans, Dufouil, Düzel, Galimberti, García-Alberca, García-González, Giedraitis, Grimmer, Graff, Grünblatt, Hanon, Hausner, Heilmann-Heimbach, Holstege, Hort, Jürgen, van der Lugt, Masullo, Mecocci, Mehrabian, de Mendonça, Boada, Mir, Moebus, Moreno, Nacmias, G. Nicolas, Papenberg, Parnetti, Pasquier, Pastor, Peters, Pijnenburg, Popp, Molina-Porcel, Puerta Fuentes, Pérez-Tur, Rainero, Ramakers, Rasmussen, Real, Riedel-Heller, Rodríguez-Rodríguez, Royo, Rujescu, Scarmeas, Scheltens, Scherbaum, Schneider, Seripa, Soininen, Solfrizzi, Spalletta, Squassina, Sánchez-Valle, Tegos, Thomassen, Tremolizzo, Verhey, Vyhnalek, Wiltfang, He, Napolioni, Amouyel, Kehoe, van Duijn, Tsolaki, Sánchez-Juan, Sleegers, Ingelsson, Rossi, Sims, van der Flier, Ramirez, Andreassen, Frikke-Schmidt, Williams, Ruiz, Lambert, Greicius.
Statistical analysis: Le Guen, Belloy, Grenier-Boley, Jansen, A. Nicolas, Bellenguez, Rasmussen, Tegos, Thomassen, He, Frikke-Schmidt, Ruiz, Greicius.
Obtained funding: Le Guen, Belloy, Debette, Dufouil, Düzel, Graff, Holstege, Jürgen, Scarmeas, Spalletta, Sánchez-Valle, He, Jessen, Sánchez-Juan, Sleegers, Hiltunen, Sims, Ramirez, Andreassen, Frikke-Schmidt, Williams, Ruiz, Lambert, Greicius.
Administrative, technical, or material support: Eger, Álvarez, Arosio, Benussi, Boland, Daian, Düzel, Galimberti, García-Alberca, García-González, Giedraitis, Grimmer, Graff, Grünblatt, Hausner, Heilmann-Heimbach, Holstege, Jürgen, Kuulasmaa, Moebus, G. Nicolas, Olaso, Popp, Molina-Porcel, Real, Rodríguez-Rodríguez, Royo, Schneider, Soininen, Solfrizzi, Thomassen, Tremolizzo, Verhey, He, Tsolaki, Ingelsson, Sims, Ramirez, Andreassen, Frikke-Schmidt, Williams, Ruiz, Lambert, Greicius.
Study supervision: Arosio, Caffarra, Daniele, Deleuze, Düzel, García-Alberca, Jürgen, Masullo, Mehrabian, Mir, Nacmias, Parnetti, Pastor, Piñol-Ripoll, Rainero, Riedel-Heller, Rujescu, Scheltens, Solfrizzi, van Swieten, He, Napolioni, Jessen, Kehoe, Sleegers, Ruiz, Lambert, Greicius.

Conflict of Interest Disclosures: Dr Belloy reported grants from Alzheimer's Association during the conduct of the study. Dr Küçükali reported grants from the Research Foundation Flanders and grants from The University of Antwerp Research Fund during the conduct of the study. Dr Borroni reported personal fees from Alector, Wave Life Sciences, and Denali outside the submitted work. Dr Debette reported grants from Leducq Foundation, European Research Council Starting Grant, French National Research Agency, Horizon 2020, and EU Joint Programme—Neurodegenerative Disease Research during the conduct of the study. Dr Dufouil reported grants from Fondation Plan Alzheimer, French Ministry of Research, GE Healthcare, and Avid Radiopharmaceuticals/Lilly during the conduct of the study. Dr Düzel reported grants from German Research Foundation, German Federal Ministry of Education and Research, and Human Brain Project as well as personal fees from Roche, Biogen, and neotiv GmbH outside the submitted work. Dr Grimmer reported personal fees from AbbVie, Alector, Anavex, Biogen, Eli Lilly, Functional Neuromodulation, Grifols, Iqvia, Life Molecular Imaging, Novo Nordisk, Noselab, NuiCare, Roche Diagnostics, Roche Pharma, Schwabe, Toyama, UCB, and Vivoryon outside the submitted work. Dr Graff reported grants from Swedish Medical Research Council, ALF Medicine Region Stockholm, Swedish Demensfonden, Alzheimer Foundation, Karolinska Institutet StratNeuro, and Swedish Brain Foundation during the conduct of the study. Dr Hanon reported personal fees from Bayer, Servier, Pfizer, AstraZeneca, Bristol Myers Squibb, and Boehringer outside the submitted work. Dr Heilmann-Heimbach reported personal fees from Life & Brain GmbH during the conduct of the study. Dr Hort reported stock options from Alzheon outside the submitted work. Dr Jürgen reported grants from Vogel Foundation during the conduct of the study; grants from German Research Foundation, German Federal Ministry of Education and Research, Bavarian Secretary of Commerce, and European Union outside the submitted work. Dr Boada reported personal fees from Grifols, Araclon Biotech, Roche, Biogen, Lilly, Merck, Zambon, Novo Nordisk, Bioiberica, Eisai, Servier, and Schwabe as well as grants from Grifols, La Caixa, Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas, and European Federation of Pharmaceutical Industries and Associations outside the submitted work. Dr Moreno reported grants from Instituto de Salud Carlos III and Tau Consortium outside the submitted work. Dr Peters reported grants from Roche, Biogen, Eisai, and Vivoryon as well as personal fees from Roche, Biogen, Eisai, and Griffols outside the submitted work. Dr Popp reported grants from Swiss National Research Foundation during the conduct of the study. Dr Molina-Porcel reported institutional research fees from Biogen outside the submitted work. Dr Ramakers reported grants from Janssen Pharmaceuticals outside the submitted work. Dr Scarmeas reported grants from Alzheimer's Association, European Social Fund, European Strategic Partnership Agreement Program and Ministry for Health Greece during the conduct of the study. Dr Scheltens reported part-time employment from Lump Sum; grants from Alzheimer's Drug Discovery Foundation and Cure Alzheimer; is a principal investigator for UCB, Toyama, and Alzheon; and serves on the steering committee of Vivoryon and NOVO outside the submitted work. Dr Schneider reported grants from Cure Alzheimer Foundation, Verum Foundation, and MjFF Foundation outside the submitted work. Dr Spalletta reported grants from Italian Ministry of Health during the conduct of the study. Dr Sánchez-Valle reported personal fees from Wave Pharmaceuticals and Ionis-Biogen as well as grants from Biogen and Sage Pharmaceuticals outside the submitted work. Dr Wiltfang reported personal fees from Abbott, Biogen, Boehringer-Ingelheim, Immungenetics, Janssen, Lilly, Merck Sharp & Dohme, Pfizer, Roche Pharma, Actelion, Amgen, Beejing Yibai Science and Technology, and Roboscreen outside the submitted work; and had a patent for PCT/EP 2011 001724 issued and a patent for PCT/EP 2015 052945 issued. Dr Amouyel reported personal fees from Fondation Alzheimer and Genoscreen Biotech outside the submitted work. Dr Jessen reported grants from German Center for Neurodegenerative Diseases (DZNE) during the conduct of the study. Dr Sleegers reported grants from Alzheimer Research Foundation Belgium, Research Foundation Flanders, and University of Antwerp Research Fund during the conduct of the study. Dr Ingelsson reported personal fees from BioArctic AB. Dr van der Flier reported grants from Netherlands Organisation for Health Research and Development (ZonMW) European Alzheimer's Disease DNA Biobank during the conduct of the study; grants from Alzheimer Nederland, Hersenstichting, CardioVascular Onderzoek Nederland, Health-Holland, Topsector Life Sciences & Health, Alzheimer & Neuropsychiatrie Foundation, Philips, Biogen MA, Novartis-NL, Life-MI, Avid Radiopharmaceuticals, Roche BV, Fujifilm, Combinostics, Gieskes-Strijbis, Dioraphte, Edwin Bouw, Equilibrio, and Pasman outside the submitted work; has performed contract research for Biogen MA and Boehringer Ingelheim; has been an invited speaker at Boehringer Ingelheim, Biogen MA, Danone, Eisai, WebMD Neurology (Medscape), and Springer Healthcare; is consultant to Oxford Health Policy Forum CIC, Roche, and Biogen MA; participated in advisory boards of Biogen MA and Roche; was associate editor of Alzheimer, Research

and Therapy in 2020/2021; and is associate editor at Brain. Dr Ramirez reported grants from Bundesministerium für Bildung und Forschung and German Research Foundation (Deutsche Forschungsgemeinschaft) during the conduct of the study. Dr Andreassen reported grants from KG Jebsen Stiftelsen, South East Norway Health Authority, Research Council of Norway, and European Union Horizon 2020 during the conduct of the study as well as personal fees from HealthLytix, Lundbeck, and Sunovion outside the submitted work. Dr Ruiz reported grants from Grifols, Fundación Bancaria La Caixa, Instituto de Salud Carlos III, Joint Programming for Neurological Disease—Precision Medicine Program, European Union Innovative Medicine Initiative, and EuroNanoMed, personal fees from Landsteiner Genmed; nonfinancial support from Araclon Biotech and Prevail Therapeutics; and has contracts with Roche, Cortexyme, Janssen outside the submitted work. Dr Greicius reported grants from the National Institutes of Health during the conduct of the study. No other disclosures were reported.

Funding/Support: This work was supported by the National Institute of Health and National Institute of Aging grants AG060747 (Dr Greicius), AG066206 (Dr He), AG066515 (Drs He and Greicius), the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie (grant agreement No. 890650; Dr Le Guen), the Alzheimer's Association (AARF-20-683984; Dr Belloy), and the Iqbal Farrukh and Asad Jamal Fund, a grant from the EU Joint Programme–Neurodegenerative Disease Research (European Alzheimer DNA BioBank, European Alzheimer's disease DNA Biobank; Joint Programming for Neurological Disease). Inserm UMR1167 is also funded by the Inserm, Institut Pasteur de Lille, Lille Métropole Communauté Urbaine, and the French government's LabEx DISTALZ program (Development of Innovative Strategies for a Transdisciplinary Approach to Alzheimer's Disease).

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Collaborators of the EADB, GR@ACE, DEGESCO, DemGene, GERAD, and EADI Groups: Collaborators of the EADB, GR@ACE, DEGESCO, DemGene, GERAD, and EADI Groups are listed in Supplement 2.

Additional Contributions: The European Alzheimer's disease DNA Biobank Group thanks the study participants, researchers, and staff for collecting and contributing to the data, the High-Performance Computing Service at the University of Lille, and the staff at the Atomic Energy Commission of the National Center of Human Genomics Research for their help with sample preparation and genotyping, and technical assistance.

Additional Information: Data used in the preparation of this article were obtained from the EADB, GR@ACE, DEGESCO, DemGene, EADI, and GERAD groups. As such, the investigators within these consortia contributed to the design and implementation of their respective consortium and/or provided data. Additional funders of individual investigators and institutions who contributed to data collection and genotyping are provided in the eAppendix in Supplement 1.

REFERENCES

1. Bellenguez C, Küçükali F, Jansen I, et al. New insights on the genetic etiology of Alzheimer's and related dementia. *medRxiv*. Preprint posted online December 14, 2020. doi:10.1101/2020.10.01.20200659

2. de Rojas I, Moreno-Grau S, Tesi N, et al; EADB contributors; GR@ACE study group; DEGESCO consortium; IGAP (ADGC, CHARGE, EADI, GERAD); PGC-ALZ consortia. Common variants in Alzheimer's disease and risk stratification by polygenic risk scores. *Nat Commun*. 2021;12(1):3417. doi:10.1038/s41467-021-22491-8

3. Corder EH, Saunders AM, Risch NJ, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet*. 1994;7(2):180-184. doi:10.1038/ng0694-180

4. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993;261(5123):921-923. doi:10.1126/science.8346443

5. Ridge PG, Hoyt KB, Boehme K, et al; Alzheimer's Disease Genetics Consortium (ADGC). Assessment of the genetic variance of late-onset Alzheimer's disease. *Neurobiol Aging*. 2016;41:200.e13-200.e20. doi:10.1016/j.neurobiolaging.2016.02.024

6. Morris JC, Roe CM, Xiong C, et al. *APOE* predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Ann Neurol*. 2010;67(1):122-131. doi:10.1002/ana.21843

7. Castellano JM, Kim J, Stewart FR, et al. Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. *Sci Transl Med*. 2011;3(89):89ra57. doi:10.1126/scitranslmed.3002156

8. Belloy ME, Napolioni V, Greicius MD. A quarter century of *APOE* and Alzheimer's disease: progress to date and the path forward. *Neuron*. 2019;101(5):820-838. doi:10.1016/j.neuron.2019.01.056

9. Mak ACY, Pullinger CR, Tang LF, et al. Effects of the absence of apolipoprotein e on lipoproteins, neurocognitive function, and retinal function. *JAMA Neurol*. 2014;71(10):1228-1236. doi:10.1001/jamaneurol.2014.2011

10. Le Guen Y, Belloy ME, Eger SJ, et al. *APOE* missense variant R145C is associated with increased Alzheimer's disease risk in African ancestry individuals with the *APOE* E3/E4 genotype. *medRxiv*. Preprint posted online October 26, 2021. doi:10.1101/2021.10.20.21265141

11. Arboleda-Velasquez JF, Lopera F, O'Hare M, et al. Resistance to autosomal dominant Alzheimer's disease in an *APOE3* Christchurch homozygote: a case report. *Nat Med*. 2019;25(11):1680-1683. doi:10.1038/s41591-019-0611-3

12. Liu CC, Murray ME, Li X, et al. *APOE3*-Jacksonville (V236E) variant reduces self-aggregation and risk of dementia. *Sci Transl Med*. 2021;13(613):eabc9375. doi:10.1126/scitranslmed.abc9375

13. Medway CW, Abdul-Hay S, Mims T, et al. ApoE variant p.V236E is associated with markedly reduced risk of Alzheimer's disease. *Mol Neurodegener*. 2014;9(1):11. doi:10.1186/1750-1326-9-11

14. Taliun D, Harris DN, Kessler MD, et al; NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium. Sequencing of 53,831 diverse genomes from the NHLBI TOPMed program. *Nature*. 2021;590(7845):290-299. doi:10.1038/s41586-021-03205-y

15. Rasmussen KL, Tybjaerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. *APOE* and dementia—resequencing and genotyping in 105,597 individuals. *Alzheimers Dement*. 2020;16(12):1624-1637. doi:10.1002/alz.12165

16. Beecham GW, Bis JC, Martin ER, et al. The Alzheimer's Disease Sequencing Project: study design and sample selection. *Neurol Genet*. 2017;3(5):e194. doi:10.1212/NXG.0000000000000194

17. Weiner MW, Aisen PS, Jack CR Jr, et al; Alzheimer's Disease Neuroimaging Initiative. The Alzheimer's Disease Neuroimaging Initiative: progress report and future plans. *Alzheimers Dement*. 2010;6(3):202-11.e7. doi:10.1016/j.jalz.2010.03.007

18. Bennett DA, Schneider JA, Buchman AS, Barnes LL, Boyle PA, Wilson RS. Overview and findings from the Rush Memory and Aging Project. *Curr Alzheimer Res*. 2012;9(6):646-663. doi:10.2174/156720512801322663

19. Kunkle BW, Grenier-Boley B, Sims R, et al; Alzheimer Disease Genetics Consortium (ADGC); European Alzheimer's Disease Initiative (EADI); Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (CHARGE); Genetic and Environmental Risk in AD/Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease Consortium (GERAD/PERADES). Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat Genet*. 2019;51(3):414-430. doi:10.1038/s41588-019-0358-2

20. Kunkle BW, Schmidt M, Klein HU, et al; Writing Group for the Alzheimer's Disease Genetics Consortium (ADGC). Novel Alzheimer disease risk loci and pathways in African American individuals using the African Genome Resources Panel: a meta-analysis. *JAMA Neurol*. 2021;78(1):102-113. doi:10.1001/jamaneurol.2020.3536

21. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 2015;4(1):7. doi:10.1186/s13742-015-0047-8

22. Karczewski KJ, Francioli LC, Tiao G, et al; Genome Aggregation Database Consortium. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434-443. doi:10.1038/s41586-020-2308-7

23. Le Guen Y, Belloy ME, Napolioni V, et al; Alzheimer's Disease Neuroimaging Initiative. A novel age-informed approach for genetic association analysis in Alzheimer's disease. *Alzheimers Res Ther*. 2021;13(1):72. doi:10.1186/s13195-021-00808-5

24. Le Guen Y, Napolioni V, Belloy ME, et al. Common X-chromosome variants are associated with Parkinson disease risk. *Ann Neurol*. 2021;90(1):22-34. doi:10.1002/ana.26051

25. Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. Robust relationship inference in genome-wide association studies. *Bioinformatics*. 2010;26(22):2867-2873. doi:10.1093/bioinformatics/btq559

26. Chen CY, Pollack S, Hunter DJ, Hirschhorn JN, Kraft P, Price AL. Improved ancestry inference using weights from external reference panels. *Bioinformatics*. 2013;29(11):1399-1406. doi:10.1093/bioinformatics/btt144

27. Auton A, Brooks LD, Durbin RM, et al; 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature*. 2015;526(7571):68-74. doi:10.1038/nature15393

28. Bis JC, Jian X, Kunkle BW, et al; Alzheimer's Disease Sequencing Project. Whole exome sequencing study identifies novel rare and common Alzheimer's-associated variants involved in immune response and transcriptional regulation. *Mol Psychiatry*. 2020;25(8):1859-1875. doi:10.1038/s41380-018-0112-7

29. Gogarten SM, Sofer T, Chen H, et al. Genetic association testing using the GENESIS R/Bioconductor package. *Bioinformatics*. 2019;35(24):5346-5348. doi:10.1093/bioinformatics/btz567

30. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94(446):496-509. doi:10.1080/01621459.1999.10474144

31. Conomos MP, Miller MB, Thornton TA. Robust inference of population structure for ancestry prediction and correction of stratification in the presence of relatedness. *Genet Epidemiol*. 2015;39(4):276-293. doi:10.1002/gepi.21896

32. Conomos MP, Laurie CA, Stilp AM, et al. Genetic diversity and association studies in US Hispanic/Latino populations: applications in the Hispanic Community Health Study/Study of Latinos. *Am J Hum Genet*. 2016;98(1):165-184. doi:10.1016/j.ajhg.2015.12.001

33. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw*. 2010;36(3):1-48. doi:10.18637/jss.v036.i03

34. Huang Y, Weisgraber KH, Mucke L, Mahley RW. Apolipoprotein E: diversity of cellular origins, structural and biophysical properties, and effects in Alzheimer's disease. *J Mol Neurosci*. 2004;23(3):189-204. doi:10.1385/JMN:23:3:189

35. Huang Y, Mahley RW. Apolipoprotein E: structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. *Neurobiol Dis*. 2014;72(pt A):3-12. doi:10.1016/j.nbd.2014.08.025

36. Harris FM, Brecht WJ, Xu Q, et al. Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice. *Proc Natl Acad Sci U S A*. 2003;100(19):10966-10971. doi:10.1073/pnas.1434398100

37. Bien-Ly N, Andrews-Zwilling Y, Xu Q, Bernardo A, Wang C, Huang Y. C-terminal-truncated apolipoprotein (apo) E4 inefficiently clears amyloid-β (Abeta) and acts in concert with Abeta to elicit neuronal and behavioral deficits in mice. *Proc Natl Acad Sci U S A*. 2011;108(10):4236-4241. doi:10.1073/pnas.1018381108

38. Huang YA, Zhou B, Wernig M, Südhof TC. ApoE2, ApoE3, and ApoE4 differentially stimulate APP transcription and Aβ secretion. *Cell*. 2017;168(3):427-441.e21. doi:10.1016/j.cell.2016.12.044

39. Choy N, Raussens V, Narayanaswami V. Inter-molecular coiled-coil formation in human apolipoprotein E C-terminal domain. *J Mol Biol*. 2003;334(3):527-539. doi:10.1016/j.jmb.2003.09.059

40. Westerlund JA, Weisgraber KH. Discrete carboxyl-terminal segments of apolipoprotein E mediate lipoprotein association and protein oligomerization. *J Biol Chem*. 1993;268(21):15745-15750. doi:10.1016/S0021-9258(18)82318-3

41. Flowers SA, Rebeck GW. *APOE* in the normal brain. *Neurobiol Dis*. 2020;136:104724. doi:10.1016/j.nbd.2019.104724

42. Dyer CA, Cistola DP, Parry GC, Curtiss LK. Structural features of synthetic peptides of apolipoprotein E that bind the LDL receptor. *J Lipid Res*. 1995;36(1):80-88. doi:10.1016/S0022-2275(20)39756-X

43. Weisgraber KH, Shinto LH. Identification of the disulfide-linked homodimer of apolipoprotein E3 in plasma. impact on receptor binding activity. *J Biol Chem*. 1991;266(18):12029-12034. doi:10.1016/S0021-9258(18)99060-5

44. Minami SS, Cordova A, Cirrito JR, et al. ApoE mimetic peptide decreases Abeta production in vitro and in vivo. *Mol Neurodegener*. 2010;5:16. doi:10.1186/1750-1326-5-16

45. Minagawa H, Gong JS, Jung CG, et al. Mechanism underlying apolipoprotein E (ApoE) isoform-dependent lipid efflux from neural cells in culture. *J Neurosci Res*. 2009;87(11):2498-2508. doi:10.1002/jnr.22073

46. Zhao N, Liu CC, Qiao W, Bu G. Apolipoprotein E, receptors, and modulation of Alzheimer's disease. *Biol Psychiatry*. 2018;83(4):347-357. doi:10.1016/j.biopsych.2017.03.003

47. Williams T, Borchelt DR, Chakrabarty P. Therapeutic approaches targeting apolipoprotein E function in Alzheimer's disease. *Mol Neurodegener*. 2020;15(1):8. doi:10.1186/s13024-020-0358-9