Ancestral origin of \textit{ApoE $\varepsilon 4$} Alzheimer disease risk in Puerto Rican and African American populations

Farid Rajabi, Briseida E. Feliciano, Katrina Celis, Kara L. Hamilton-Nelson, Patrice L. Whitehead, Larry D. Adams, Parker L. Bussies, Clara P. Manrique, Alejandro Rodriguez, Vanessa Rodriguez, Takiyah Starks, Grace E. Byfield, Carolina B. Sierra Lopez, Jacob L. McCauley, Heriberto Acosta, Angel China, Brian W. Kunkle, Christiane Reitz, Lindsay A. Farrer, Gerard D. Schellenberg, Badri N. Vardarajan, Jeffery M. Vance, Michael L. Cuccaro, Eden R. Martin, Jonathan L. Haines, Goldie S. Byrd, Gary W. Beecham, Margaret A. Pericak-Vance

1 John P. Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, Florida, United States of America, 2 Universidad Central del Caribe, Bayamón, Puerto Rico, United States of America, 3 Center for Outreach in Alzheimer’s, Aging and Community Health at North Carolina A&T State University, Greensboro, North Carolina, United States of America, 4 Clinica de la Memoria, San Juan, Puerto Rico, United States of America, 5 Gertrude H. Sergievsky Center, Taub Institute for Research on the Aging Brain, Departments of Neurology, Psychiatry, and Epidemiology, College of Physicians and Surgeons, Columbia University, New York, New York, United States of America, 6 Departments of Medicine (Biomedical Genetics), Neurology, Ophthalmology, Epidemiology, and Biostatistics, Boston University Schools of Medicine and Public Health, Boston, Massachusetts, United States of America, 7 Penn Neurodegeneration Genomics Center, Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States of America, 8 Dr. John T. MacDonald Foundation Department of Human Genetics, Miller School of Medicine, University of Miami, Miami, Florida, United States of America, 9 Department of Population & Quantitative Health Sciences, Institute for Computational Biology, Case Western Reserve University School of Medicine, Cleveland, Ohio, United States of America

* These authors contributed equally to this work.

Citation: Rajabi F, Feliciano BE, Celis K, Hamilton-Nelson KL, Whitehead PL, Adams LD, et al. (2018) Ancestral origin of \textit{ApoE $\varepsilon 4$} Alzheimer disease risk in Puerto Rican and African American populations. PLoS Genet 14(12): e1007791. https://doi.org/10.1371/journal.pgen.1007791

Received: May 18, 2018

Accepted: October 25, 2018

Published: December 5, 2018

Copyright: © 2018 Rajabi et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: Data are available through the National Institute on Aging Genetics of Alzheimer’s Disease Data Storage Site (NIAGADS) Data Sharing Service (DSS): https://dss.niagads.org (accession number NG00039).

Funding: This work was supported by National Institute of Health grants AG054074 (MAPV, GWB), AG032984 (GDS) and AG052410 (MAPV, GWB, GSB, RPM). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

The \textit{ApoE $\varepsilon 4$} allele is the most significant genetic risk factor for late-onset Alzheimer disease. The risk conferred by $\varepsilon 4$, however, differs across populations, with populations of African ancestry showing lower $\varepsilon 4$ risk compared to those of European or Asian ancestry. The cause of this heterogeneity in risk effect is currently unknown; it may be due to environmental or cultural factors correlated with ancestry, or it may be due to genetic variation local to the \textit{ApoE} region that differs among populations. Exploring these hypotheses may lead to novel, population-specific therapeutics and risk predictions. To test these hypotheses, we analyzed \textit{ApoE} genotypes and genome-wide array data in individuals from African American and Puerto Rican populations. A total of 1,766 African American and 220 Puerto Rican individuals with late-onset Alzheimer disease, and 3,730 African American and 169 Puerto Rican cognitively healthy individuals (> 65 years) participated in the study. We first assessed average ancestry across the genome (“global” ancestry) and then tested it for interaction with \textit{ApoE} genotypes. Next, we assessed the ancestral background of \textit{ApoE} alleles (“local” ancestry) and tested if ancestry local to \textit{ApoE} influenced Alzheimer disease risk while controlling for global ancestry. Measures of global ancestry showed no interaction with \textit{ApoE}...
risk (Puerto Rican: p-value = 0.49; African American: p-value = 0.65). Conversely, ancestry local to the ApoE region showed an interaction with the ApoE ε4 allele in both populations (Puerto Rican: p-value = 0.019; African American: p-value = 0.005). ApoE ε4 alleles on an African background conferred a lower risk than those with a European ancestral background, regardless of population (Puerto Rican: OR = 1.26 on African background, OR = 4.49 on European; African American: OR = 2.34 on African background, OR = 3.05 on European background). Factors contributing to the lower risk effect in the ApoE gene ε4 allele are likely due to ancestry-specific genetic factors near ApoE rather than non-genetic ethnic, cultural, and environmental factors.

Author summary

The strongest risk gene identified for late-onset Alzheimer disease is ApoE. However, the risk for Alzheimer disease due to ApoE is not consistent across populations. For example, individuals with African ancestry experience less risk from ApoE ε4 than individuals of European or Asian ancestry. The cause of the difference in risk effect is currently unknown. This has led us to ask: What is/are the factor(s) contributing to the risk effect variation of ApoE across the populations? We hypothesized two possibilities for the variability of ApoE risk: 1) ethnic-related environmental factors that vary across populations, such as diet and lifestyle activities, or 2) a population-specific genetic difference in the ApoE gene, or in its surrounding region. We tested our hypothesis using populations with more than one genetic ancestral background, specifically African Americans and Puerto Ricans. Our study showed that the risk of Alzheimer disease is lower for individuals who inherited the genomic region surrounding the ApoE gene from an African ancestor than it is for risk allele carriers who inherited the region from a European ancestor. These findings suggest that protective genetic variant(s) most likely lie(s) within the genetic region surrounding the ApoE gene on the African ancestral background.

Introduction

Late-onset Alzheimer disease (LOAD) is a progressive neurodegenerative disorder characterized by loss of memory and other cognitive functions. It is the most common form of dementia worldwide [1], with prevalence increasing with age (e.g., ~30–40% by 85–89 years) [2]. The etiology of AD is multifactorial with genetic, and environmental factors all influencing risk.

The most significant genetic risk factor for LOAD is the ApoE gene [3,4]. Three common ApoE alleles have been identified (ε2, ε3, and ε4). The ε3 allele is the most frequent and is typically considered “neutral” regarding AD risk. The ApoE ε4 allele both increases the risk and decreases the age-of-onset of developing AD [4]. Conversely, the ε2 allele is protective against AD [4,5]. Although ApoE is an AD risk factor in nearly all populations, the risk of AD for ε4 carriers differs among racial/ethnic groups [6]. The strongest reported risk for ε4 allele is in East-Asian populations (ε3/ε4 odds ratio OR: 3.1–5.6; ε4/ε4 OR: 11.8–33.1) [6,7] followed by non-Hispanic Whites (NHW) (ε3/ε4 odds ratio [OR]: 3.2; ε4/ε4 OR: 14.9) [6,8–10] with a considerably lower risk to develop AD for an ε4 carrier in African-Ancestry populations, such as African Americans (AA) and Caribbean Hispanics (CHI). Studies in African-ancestry cohorts consistently reported significant association between ApoE ε4 homozygosity and AD, but showed inconsistent results for ε4 heterozygote allele individuals (ε3/ε4 OR:1.1–2.2; ε4/ε4
OR: 2.2–5.7) [6,8–13]. The reason for this heterogeneous risk effect of ApoE is currently unknown. This disparity in risk may be due to ethnic-related environmental factors that vary across populations, such as diet and lifestyle activities, or the difference may be due to population-specific genetic factors. Exceptions include studies among the Wadi Ara and American Indian populations, but these studies may suffer from low power due to small sample sizes [14–16].

Ancestral methods examining both global (GA) and local (LA) ancestry can be used to explore these different hypotheses. GA refers to an individual’s average ancestry across his/her entire genome while LA refers to the ancestral background of a particular (i.e., “local”) chromosomal region within an individual genome (Fig 1). GA is predominantly correlated with ethnic, cultural, and environmental factors that are related to broader definitions of race and ethnicity [17–20]. Conversely, LA is often correlated with ancestry-specific genetic factors that are located in or near the genomic region in question [21,22]. As such, an understanding of LA around the ApoE region may help inform how we interpret the race/ethnicity differences observed in ε4 risk. Specifically, if cultural and environmental effects play a major role in ApoE heterogeneity, we would expect GA to interact with ε4 to influence AD risk. There will also be GA and allele interaction if there is epistasis with alleles on other chromosomes that have different frequencies between ancestral populations. However, if genetic modifiers or protective factors local to the ApoE region (e.g., cis-acting enhancers, eQTL, etc.) play a major role in ApoE ε4 heterogeneity, we would expect LA to interact with ε4 to influence AD risk.

Admixed populations, due to their ancestral heterogeneity, often show complex patterns of GA and LA, enabling us to test these hypotheses. As such, we utilized two admixed populations (CHI from Puerto Rico (PR), and AA) to assess the relationship between ApoE ε4 risk and patterns of GA and LA. PR individuals commonly have European (EU), African (AF) and Amerindian (AI) ancestors, while AA individuals often have both EU and AF ancestors. To test the hypothesis that the population-specific risk is due to ethnic-related environmental factors that vary across populations, we compared those ApoE ε4 carriers who inherited most of their chromosomes from AF ancestors to those who inherited most of their chromosomes from their EU ancestors by using GA. If there are additional genomic loci outside of the ApoE gene contributing to the population risk difference, then individuals with the highest GA load of EU (or AF) ancestry would match the EU (or AF) population risk. Alternatively, to test the hypothesis that the disparity in risk may be due to genetic modifiers or protective factors local to ApoE, we compared the LAs in the admixed populations with those of the corresponding ancestral population (e.g., if one inherited his/her ApoE LA from the EU ancestors, his/her risk for AD would be similar to the EU population risk).

Our results strongly suggest that an ancestry-specific region surrounding the ApoE gene is contributing to the lower risk of AD in AA and PR ε4 carriers, supporting the hypothesis that the “protective” effect is due to the ancestry-specific genetic factors around the ApoE genomic region.

**Results**

First, we performed two genotype-based regression tests to assess global ancestry and local ancestry interaction with ApoE genotype (see Methods for details). Results showed that the LA by ApoE interaction term (dose of AF ancestry by dose of ε4 allele; LAxApoE) was significantly different from 0 in both PR and AA populations (PR: likelihood ratio test (LRT), p-value = 0.019; AA: LRT, p-value = 0.005). The effect size of the interaction term was negatively correlated with AD (PR: OR = 0.2 (CI: 0.05–0.76); AA: OR = 0.75 (CI: 0.61–0.91)). This was in
contrast to the GA by ApoE interaction term (GAxApoE), which was not significant in either PR or AA (PR: LRT, p = 0.49; AA: LRT, p-value = 0.65).

Since we identified a significant interaction, we performed a haplotype-based regression test to assess the effect size of ancestry-specific alleles (see Methods for details). We found that the effect size of the ε4 risk allele was significant across the ancestral haplotypes, even while accounting for correlations with GA (Table 1). In the PR dataset, the ε4 alleles on an EU ancestral background were significantly associated with AD (p-value = 3.7e-05; OR = 4.49)

![Image of chromosome 19 from Puerto Rican, African American, and European ancestry individuals. (A) The colored chromosomal segments represent the admixture blocks "local" to each genomic region, with each ancestry coded by a different color (red: African (AF), blue: European (EU), green: American Indian (AI)). (B) The global ancestry estimated by the average ancestry across the whole genome. The Puerto Rican individual has one African block and one European block around ApoE (represented by the dashed line); that is, the local ancestry around ApoE is African/European for this individual. The African American individual, though mostly African genome-wide, also has African/European local ancestry at the ApoE gene.](https://doi.org/10.1371/journal.pgen.1007791.g001)
compared to ε3 alleles from an EU ancestral background. However, ε4 vs ε3 showed no significant effect on the AF LA background (p-value = 0.67; OR = 1.26). Similarly, in the AA dataset, the ε4 haplotypes of EU ancestry showed a stronger risk effect (OR = 3.05; p-value = 4.9e-17) than those in the AA dataset of AF ancestry (OR = 2.34; p-value = 9.2e-45). We tested the difference between the effect sizes of ancestral backgrounds by using t-test for means. Test results showed that effect sizes between the ancestral backgrounds are different with nominal significance in both populations (PR: p-value = 0.059; AA: p-value = 0.068). It is of note that these models all include GA as covariates, indicating that the effects seen are independent of the GA.

In the subgroup of individuals with homozygote ε4 and ε3 alleles, results showed that ε4 haplotypes of EU ancestry have a stronger risk effect (OR = 18.44 (CI: 9.6–35.6); p-value = 3.5e-18) than those with AF ancestry (OR = 6.48 (CI: 3.4–12.5); p-value = 4.3e-63). The t-test of means showed that effect sizes of EU and AF backgrounds are significantly different (p-value = 0.003).

Since we observed that AF ancestral background surrounding the ApoE gene is contributing to the lower risk of AD, we examined the genetic region surrounding ApoE by using 1000 Genome sequence data from three populations of the Utah Residents with Northern and Western European Ancestry (CEU), Japanese in Tokyo (JPT), and Yoruba in Ibadan (YRI). We identified 43 variants using Pearson’s chi-square test between the CEU vs. YRI and JPT vs. YRI populations, which were significant following the Bonferroni correction for multiple comparisons. Table 2 shows the list of 15 most significant variants with the Bonferroni corrected

| Marker ID | Base Position | Reference Allele | Alternative Allele | Bonferroni corrected p-values |
|-----------|---------------|------------------|-------------------|------------------------------|
|           |               |                  |                   | CEU vs. YRI | JPT vs. YRI |
| rs6857    | 45392254      | C                | T                 | 8.44E-12 | 5.88E-06 |
| rs157590  | 45398716      | A                | C                 | 1.31E-09 | 5.85E-07 |
| rs157588  | 45398264      | C                | T                 | 1.31E-09 | 5.85E-07 |
| rs157585  | 45397512      | A                | C                 | 1.31E-09 | 5.85E-07 |
| rs769449  | 45410002      | G                | A                 | 1.31E-09 | 5.85E-07 |
| rs157584  | 45396899      | T                | C                 | 6.45E-09 | 5.85E-07 |
| rs111789331 | 45427125 | T               | A                 | 6.45E-09 | 4.75E-09 |
| rs12721046 | 45421254 | G               | A                 | 6.45E-09 | 3.71E-10 |
| rs142042446 | 45386467 | G               | GTAA             | 3.02E-08 | 5.85E-07 |
| rs71352238 | 45394336 | T               | C                 | 3.02E-08 | 5.85E-07 |
| rs12972156 | 45387459 | C               | G                 | 3.02E-08 | 5.85E-07 |
| rs12972970 | 45387596 | G               | A                 | 3.02E-08 | 5.85E-07 |
| rs34342646 | 45388130 | G               | A                 | 3.02E-08 | 5.85E-07 |
| rs66626994 | 45428234 | G               | A                 | 4.37E-08 | 4.97E-08 |

Table 2. Top list of the potential protective variants at the local ancestry blocks surrounding the ApoE gene.
p-values less than $1 \times 10^{-5}$. The whole list of variants is shown in the S2 Table. Fig 2 demonstrates Bonferroni corrected p-values for the pairwise comparisons between CEU and YRI, and JPT and YRI populations. The primary CEU and JPT peaks align, and lie within the strongest Topologically Associated Domain (TAD) containing the \textit{ApoE} gene. None of the significantly different variants were in the protein-coding DNA in the defined region around the \textit{ApoE} gene. It is noteworthy that just 6 variants in sequence data comparison showed significant difference (with the lowest p-value = 0.0052) between the CEU and JPT and all of them were found out of the TAD region containing the \textit{ApoE}.

**Discussion**

These findings strongly support our hypothesis that genetic modifiers local to the \textit{ApoE} region influence the risk of the \( \varepsilon 4 \) allele, showing a weaker risk effect on the AF ancestral background.
and stronger effect on the EU ancestral background. There was no evidence that overall ancestry (GA) has an effect on the heterogeneity of ApoE ε4 risk within the populations, which we used as a surrogate for non-genetic cultural/ethnic differences. Additionally, we observed a stronger risk effect on the EU ε4 haplotypes (or conversely, a protective effect on AF ε4 haplotypes). This effect was especially pronounced in an analysis of ε4 homozygotes against ε3 homozygotes, a result consistent with previous reports on ApoE risk across populations [6,8–13].

The overlapping of the subTAD (~50kb) region and the peaks of the allele frequency differences between the CEU, JPT and YRI support the hypothesis that the variant(s) modifying ε4 risk are most likely to lie in this region. The significant differences found in non-protein-coding DNA, suggests the protective effect is due to a regulatory difference between the local ancestries. This would also suggest that possible a modifier(s) would affect ApoE expression itself and supports the hypothesis that the genomic region surrounding ApoE with AF background reduces the risk for ε4 carriers and is evidence that genetic factors may be underlying the discrepancy in ε4 allele risk effect across populations.

It should be noted that this study was not well-powered to test AI background influence on ε4 risk allele. Further research is needed to study populations with higher AI ancestral background, such as Peruvian, Mexican, and Central American populations, to understand the correlation between the AI ancestry and ApoE. Similarly, limitations in sample size prevented us from assessing effects in ε2 carriers.

Our findings suggest that the ApoE region from AF populations may contain protective factors that help mitigate the effect of the ε4 allele. In particular, comprehensive analysis of the ApoE region and testing for protective loci may reveal previously unappreciated biological pathways and provide translational opportunities. Research that focuses on locating protective variants represents a complementary approach to accelerating the identification of more effective targets for drug development. This, in turn, will lead to better treatments, and help reduce health disparities.

**Materials and methods**

**Sources of participants**

All AA cases and controls selected for genotyping were obtained from the John P. Hussman Institute for Human Genomics (HIHG) at the University of Miami Miller School of Medicine (Miami, FL), North Carolina A&T State University (Greensboro, NC), Case Western Reserve University (Cleveland, OH), and the Alzheimer’s Disease Genetic Consortium (ADGC). Samples were collected as described previously [23,24]. The AA dataset contained 1,766 AD cases (69.8% female, mean age at onset (AAO) 77.6 years [SD 8.2]) and 3,730 cognitively healthy controls (72.0% female, mean age-of-examination (AOE) 76.5 years [SD (8.3)]).

PR individuals were ascertained as a part of the Puerto Rico Alzheimer Disease and Related Disorders Initiative study. Ascertainment was focused in metropolitan areas of New York, North Carolina, Miami, and Puerto Rico. Participants were recruited and enrolled after they (or a proxy) provided written informed consent and with approval by the relevant institutional review boards. For the PR cohort, 220 unrelated cases (69.6% female, mean AAO 75.1 years [SD 9.7]) and 169 unrelated cognitively intact controls (66.4% female, mean AOE 73.6 years [SD 7.1]) were ascertained.

For both AA and PR datasets, cases were defined as individuals with AD with AAO >65 years of age; controls were defined as individuals with no evidence of cognitive problems and AOE >65 years of age. All participants were evaluated to determine case or control status based on the National Institute of Neurological and Communicative Disorders and Stroke—
Alzheimer’s Disease and Related Disorders Association, criteria [25,26]. Individuals with known or suspected dementia were evaluated using the LOAD study reference [27]. Individuals who were deemed to be cognitively normal were screened with the Mini-Mental State Examination [28] or the Modified Mini-Mental State [29]. The participants were classified as AA and PR based on self-report, and the GWAS analysis confirmed these data.

**Genotyping and quality control procedures**

Genome-wide single-nucleotide polymorphism (SNP) genotyping was processed on three different platforms: Expanded Multi-Ethnic Genotyping Array, Illumina 1Mduo (v3) and the Global Screening Array (Illumina, San Diego, CA, USA). ApoE genotyping was performed as in Saunders et al. [30]. Quality control analyses were performed using the PLINK software, v.2 [31]. The samples with a call rate less than 90% and with excess or insufficient heterozygosity (+/- 3 standard deviations) were excluded. Sex concordance was checked using X chromosome data. To eliminate duplicate and related samples, relatedness among the samples was estimated by using identity by descent (IBD). SNPs with minor allele frequencies less than 0.01 and SNPs available in samples with the call rate less than 97%, or those not in Hardy-Weinberg equilibrium (p<1.e-5), were eliminated from further analysis [32]. Further details of the QC analysis can be found in the Supplement (S1 Table).

To explore the reasons for the differences in ε4 allele risk between the populations we first assessed the genetic ancestry (LA and GA), and then tested the effect of LA and GA on the ε4 allele by building three logistic regression models.

**Assessment of genetic ancestry**

To assess the LA, we phased our datasets independently applying the SHAPEIT tool ver. 2 [33] using 1000 Genomes Phase 3 reference panel [34] with default settings. We defined a region around the ApoE that was broad enough (chr19: 44,000,000–46,000,000) to include potential enhancers, topological associated domains, etc. while narrow enough to ensure contiguous LA blocks for most individuals in the study. After selecting the ApoE region, we used RFMix [35], discriminative modeling approach, to infer LA at loci across the genome. We ran RFMix with the TrioPhased option and a minimum node size of 5. We used Human Genome Diversity Project (HGDP) data as the reference panel; two for AA (EU, and AF), and three for PR (EU, AF, and AI). Then, we eliminated samples with ancestral break points across the 2Mb window (N = 892) and labeled each admixture block using the RFMix estimates. As a result, we obtained haplotype data with three LA states (AF, EU, AI) in PRs and two (AF, EU) in AAs. Afterwards, we defined haplotypes according to LA states and ApoE variants. S1 Fig illustrates the defining of LA at the ApoE gene and S2 Table shows the number of e3 and e4 alleles along AF and EU local ancestry in each population for cases and controls.

Next, we assessed GA by performing principal components analysis (PCA) using the Eigenstrat program [36]. The AA and PR datasets were combined with reference panels (using HGDP reference panels) representing diverse ancestries: EU and AF for AA, and EU, AF and AI for PR.

**Statistical analyses**

To assess the effects of GA and LA on ε4 risk we used three logistic regression-based models. The first model utilized a genotype-based test to assess GA interaction with ApoE genotype. This model evaluated the role of GA and factors strongly correlated with GA (e.g., ethnic-related environmental factors) on ApoE risk variation among populations. The second model utilized a genotype-based approach to assess LA interaction with ApoE genotype. In this
model, we examined the role of genetic modifiers or protective factors local to \textit{ApoE} in risk variation. The third model utilized a haplotype-based approach to assess the effect sizes of ancestry-specific alleles (e.g., e4 and e3 alleles on the AF background) while accounting for correlations with GA. Statistical analyses were performed using the “GLM2” \cite{37} and “GEE” \cite{38} packages available in R computing environment.

**Global ancestry by \textit{ApoE} interaction.** We tested the significance of the GA by \textit{ApoE} genotype interaction (GAX\textit{ApoE}) using the LRT. To assess the influence of the GAX\textit{ApoE} on AD we used an age- and sex-adjusted logistic regression model. A "full model" was built that included homozygote (\(e4/e4\)) and heterozygote (\(e3/e4\)) genotypes (with \(e3/e3\) being the referent) as well as measures of GA (PC1, PC2, and PC3), and GAX\textit{ApoE} (Eq 1). This full model was tested (by the LRT) against a reduced model without the interaction terms.

\[
AD \sim Age + Sex + GA + ApoE + GA \times ApoE
\]  

(1)

**Local ancestry by \textit{ApoE} interaction.** LA interaction was tested in a similar fashion; individuals were assigned LA “types” (for AA individuals: AF/AF, AF/EU, EU/EU; for PR individuals AF/AF, AF/EU, AF/AI, EU/EU, EU/AI, AI/AI). LA by \textit{ApoE} interaction was tested by comparing a full model to a reduced model. The full model (Eq 2) included homozygote (\(e4/e4\)) and heterozygote (\(e3/e4\)) genotypes (with \(e3/e3\) being the referent), measures of GA, LA, and LA\textit{ApoE} interaction term. The reduced model lacked the LA\textit{ApoE} interaction term.

\[
AD \sim Age + Sex + LA + GA + ApoE + LA \times ApoE
\]  

(2)

These datasets had few \(e2/\) genotypes and AI/ ancestral backgrounds, so individuals with these genotypes and ancestral backgrounds were excluded from the comparisons.

**Assessment of effect sizes: Haplotype approach.** A haplotype model was also tested to assess \(e4\) risk in an allele-specific manner. This approach tests \(e4\) of a particular LA background against \(e3\) alleles of the same background, rather than genotypes tested in the context of a LA “dose” across both parental haplotypes. To perform the analysis, \(e3\) and \(e4\) alleles were grouped by their LA (AF in one and EU in the other; the sample size of AI was too small to test adequately) and tested for association. AA and PR datasets were analyzed separately. Within each group, the effect of the \(e4\) allele was assessed via logistic regression using the generalized estimating equation (GEE), with principal components 1, 2, and 3 used as covariates and the individual as the grouping variable. We chose the GEE to account for the individual haplotypes correlation (since each allele is counted individually). This effectively tests the association of AF (or EU) \(e4\) alleles against \(e3\) while controlling for the effects of global ancestry, and allows us to estimate effect sizes of ancestry-specific haplotypes. In addition, we tested a haplotype-based approach among the individuals with homozygote \(e4\) and \(e3\) alleles to assess the effect size of ancestry-specific alleles on those with \(e4/e4\) genotype (it was not applicable to the PR dataset since only 12 samples had homozygote \(e4\) alleles.). Finally, we tested the significance of difference between the effect sizes of ancestral backgrounds using t-test for means.

**Defining potential protective variants at the LA blocks around the \textit{ApoE}**

To define the potential genetic factors modifying the \textit{ApoE} effect size we assessed the sequence differences between the ancestral backgrounds among the \(e4\) haplotypes. First, using the 1000 genomes database, we obtained genomic DNA sequence data from three populations of the CEU, JPT, and YRI. Secondly, we extracted the \(e4\) haplotypes across the defined LA block of 2 mB. In addition to EU, we tested Japanese haplotypes because \(e4\) allele in East Asian populations has a high-risk effect as well \cite{6,7}. Then, we performed Pearson’s chi-square test using
allele frequencies at the region of interest among the populations (CEU vs. YRI and JPT vs. YRI) to identify the list of significantly different variants that likely contain the protective variant(s). We assessed the allele frequency difference on \(\varepsilon_3\) and \(\varepsilon_4\) haplotypes separately. To make a list of \(\varepsilon_4\) haplotype-specific alleles with the significantly different frequencies we removed those that showed significant difference also among the \(\varepsilon_3\) haplotypes. Finally, we performed the Bonferroni correction [39] for the multiple comparisons.

**Supporting information**

S1 Table. Number of individuals and SNPs excluded after QC analysis.

(DOCX)

S2 Table. Counts of \(\varepsilon_3\) and \(\varepsilon_4\) alleles along African and European local ancestries in African Americans and Puerto Ricans for cases and controls.

(DOCX)

S3 Table. The complete list of the potential protective variants at the local ancestry blocks surrounding the ApoE gene.

(DOCX)

S1 Fig. This figure illustrates how local ancestry is estimated at ApoE. (A) Phasing, selecting the ApoE region, and classifying the haplotypes into three groups: \(\varepsilon_2\), \(\varepsilon_3\), \(\varepsilon_4\) haplotypes. (B) Building reference panels and inferring the local ancestry by using RFMix. Haplotypes classified as Reference ancestries \(\varepsilon_2\), \(\varepsilon_3\), \(\varepsilon_4\) haplotypes and admixed \(\varepsilon_2\), \(\varepsilon_3\), \(\varepsilon_4\) haplotypes. (C) Building statistical model to test the association of ancestry-aware \(\varepsilon_4\) alleles against \(\varepsilon_3\) within AF and EU subgroups, and admixed haplotypes within ApoE were excluded from analysis.

(TIF)

**Acknowledgments**

We thank the families and community members who graciously agreed to participate in the study and made this research possible.

**Author Contributions**

**Conceptualization:** Farid Rajabli, Jeffery M. Vance, Gary W. Beecham, Margaret A. Pericak-Vance.

**Data curation:** Kara L. Hamilton-Nelson, Brian W. Kunkle, Lindsay A. Farrer, Gerard D. Schellenberg, Jonathan L. Haines, Goldie S. Byrd, Gary W. Beecham, Margaret A. Pericak-Vance.

**Formal analysis:** Farid Rajabli, Kara L. Hamilton-Nelson.

**Funding acquisition:** Gerard D. Schellenberg, Goldie S. Byrd, Gary W. Beecham, Margaret A. Pericak-Vance.

**Investigation:** Farid Rajabli, Jeffery M. Vance, Gary W. Beecham, Margaret A. Pericak-Vance.

**Methodology:** Farid Rajabli, Jeffery M. Vance, Eden R. Martin, Gary W. Beecham, Margaret A. Pericak-Vance.

**Project administration:** Margaret A. Pericak-Vance.

**Resources:** Briseida E. Feliciano, Katrina Celis, Patrice L. Whitehead, Larry D. Adams, Parker L. Bussies, Clara P. Manrique, Alejandra Rodriguez, Vanessa Rodriguez, Takiyah Starks,
Grace E. Byfield, Carolina B. Sierra Lopez, Jacob L. McCauley, Heriberto Acosta, Angel Chinea, Christiane Reitz, Lindsay A. Farrer, Michael L. Cuccaro, Jonathan L. Haines.

**Supervision:** Gary W. Beecham, Margaret A. Pericak-Vance.

**Validation:** Farid Rajabli, Gary W. Beecham.

**Visualization:** Farid Rajabli, Gary W. Beecham.

**Writing – original draft:** Farid Rajabli, Michael L. Cuccaro, Gary W. Beecham, Margaret A. Pericak-Vance.

**Writing – review & editing:** Briseida E. Feliciano, Jacob L. McCauley, Christiane Reitz, Lindsay A. Farrer, Gerard D. Schellenberg, Badri N. Vardarajan, Jeffery M. Vance, Eden R. Martin, Jonathan L. Haines.

**References**

1. Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (2010–2050) estimated using the 2010 Census. Neurology. 2013; 80:1778–83. https://doi.org/10.1212/WNL.0b013e31828726f5 PMID: 23390181

2. Borenstein AR, Mortimer JA. Alzheimer Disease Life Course Perspectives on Risk Reduction. San Diego, CA: Elsevier; 2016.

3. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. Science. 1993; 261:921–923. PMID: 8346443

4. Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC Jr, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet. 1994; 7(2):180–4. https://doi.org/10.1038/ng0694-180 PMID: 7920638

5. Conejero-Goldberg C, Gomar JJ, Bobes-Bascaran T, Hyde TM, Kleinman JE, Herman MM, et al. APOE2 enhances neuroprotection against Alzheimer’s disease through multiple molecular mechanisms. Mol Psychiatry. 2014; 19:1243–1250. https://doi.org/10.1038/mp.2013.194 PMID: 24492349

6. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: A meta-analysis. JAMA. 1997; 278(16):1349–1356. PMID: 9343467

7. Liu M, Bian C, Zhang J, Wen F. Apolipoprotein E gene polymorphism and Alzheimer’s disease in Chinese population: a meta-analysis. Sci Rep. 2014; 4:4383. https://doi.org/10.1038/srep04383 PMID: 24632849

8. Tang MX, Stern Y, Marder K, Bell K, Gurland B, Lantigua R, et al. The APOE-epsilon4 allele and the risk of Alzheimer disease among African Americans, whites, and Hispanics. JAMA. 1998; 279:751–755. PMID: 9508150

9. Tang MX, Cross P, Andrews H, Jacobs DM, Small S, Bell K, et al. Incidence of AD in African-Americans, Caribbean Hispanics, and Caucasians in northern Manhattan. Neurology. 2001; 56(1):49–56. PMID: 11148235

10. Tang MX, Maestre G, Tsai WY, Liu XH, Feng L, Chung WY, et al. Relative risk of Alzheimer disease and age-at-onset distributions, based on APOE genotypes among elderly African Americans, Caucasians, and Hispanics in New York City. Am J Hum Genet. 1996; 58(3):574–584. PMID: 8644717

11. Sahota A, Yang M, Gao S, Hui SL, Baiyewu O, Gureje O, et al. Apolipoprotein E-associated risk for Alzheimer’s disease in the African-American population is genotype dependent. Ann Neurol. 1997; 42:659–661. https://doi.org/10.1002/ana.410420418 PMID: 9382479

12. Hendrie HC, Murrell J, Baiyewu O, Lane KA, Purnell C, Ogunniyi A, et al. APOE ε4 and the risk for Alzheimer disease and cognitive decline in African Americans and Yoruba. Int Psychogeriatr. 2014; 26(9):977–985

13. Graff-Radford NR, Green RC, Go RC, Hutton ML, Edeki T, Bachman D, et al. Association Between Apolipoprotein E Genotype and Alzheimer Disease in African American Subjects. Arch Neurol. 2002; 59(4):594–600. PMID: 11939894

14. Farrer LA, Friedland RP, Bowirrat A, Waraska K, Korczyn A, Baldwin CT. Genetic and environmental epidemiology of Alzheimer’s disease in arabs residing in Israel. J Mol Neurosci. 2003; 20(3):207–12. https://doi.org/10.1385/JMN:20:3:207 PMID: 14500999
15. Henderson JN, Crook R, Crook J, Hardy J, Onstead L, Carson-Henderson L, et al. Apolipoprotein E4 and tau allele frequencies among Choctaw Indians. Neurosci Lett. 2002; 324(1):77–9. PMID: 11983299

16. Bowirrat A, Friedland RP, Chapman J, Korczyn AD. The very high prevalence of AD in an Arab population is not explained by APOE epsilon4 allele frequency. Neurology. 2000; 55(5):731.

17. Mersha TB, Abebe T. Self-reported race/ethnicity in the age of genomic research: its potential impact on understanding health disparities. Hum Genomics. 2015;7: 9-1.

18. Gravlee CC, Non AL, Mulligan CJ. Genetic ancestry, social classification, and racial inequalities in blood pressure in Southeastern Puerto Rico. PLoS One. 2009; 4(9):e6821. https://doi.org/10.1371/journal.pone.0006821 PMID: 19742303

19. Aldrich MC, Selvin S, Wrensch MR, Sison JD, Hansen HM, Quesenberry CP Jr, et al. Socioeconomic status and lung cancer: unraveling the contribution of genetic admixture. Am J Public Health. 2013; 103(10):e73–80. https://doi.org/10.2105/AJPH.2013.301370 PMID: 23948011

20. Fenesi B, Fang H, Kovacevic A, Oremus M, Raina P, Heisz JJ. Physical Exercise Moderates the Relationship of Apolipoprotein E (APOE) Genotype and Dementia Risk: A Population-Based Study. J Alzheimers Dis. 2017; 56(1):297–303. https://doi.org/10.3233/JAD-160424 PMID: 27911292

21. Martin ER, Tunc I, Liu Z, Siller SH, Beecham A, Beecham G. Properties of global- and local-ancestry adjustments in genetic association tests in admixed populations. Genet Epidemiol. 2018; 42(2):214–229. https://doi.org/10.1002/gepi.22103 PMID: 29288582

22. Zhu X, Wang H. The Analysis of Ethnic Mixtures. Methods Mol Biol. 2017; 1666:505–525. https://doi.org/10.1007/978-1-4939-7274-6_25 PMID: 28980262

23. Reitz C, Jun G, Naj A, Vardarajan BN, Wang LS, Valladares O, et al. Variants in the ATP-binding cassette transporter (ABCA7), apolipoprotein E 4, and the risk of late-onset Alzheimer disease in African Americans. JAMA. 2013; 309:1483–1492. https://doi.org/10.1001/jama.2013.2973 PMID: 23571587

24. Cukier HN, Kunkle BW, Vardarajan BN, Rolati S, Hamilton-Nelson KL, Kohli MA, et al. ABCA7 frameshift deletion with Alzheimer disease in African Americans. Neurol Genet.2016; 2(3):e79. https://doi.org/10.1212/NXG.0000000000000079 PMID: 27231719

25. McKhann G, Drachman D, Folstein M, Katzman R. Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. Neurology.1984; 34(7): 939–44. PMID: 6610841

26. Albert MS, Dekosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer’s disease: Recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement.2011; 7:270–9. https://doi.org/10.1016/j.jalz.2011.03.008 PMID: 21514249

27. Wijsman EM, Pankratz ND, Choi Y, Rothstein JH, Faber KM, Cheng R, et al. Genome-wide association of familial late-onset Alzheimer’s disease replicates BIN1 and CLU and nominates CUGBP2 in interaction with APOE. PLoS Genet. 2011; 7:e1001308 https://doi.org/10.1371/journal.pgen.1001308 PMID: 21379329

28. Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res.1975; 12(3):189–98. PMID: 1202204

29. Teng EL, Chui HC. The Modified Mini-Mental State (3MS) examination. J Clin Psychiatry.1987; 48(8):314–8. PMID: 3611032

30. Saunders AM, Hulette C, Welsh-Bohmer KA, Schmechel DE, Crain B, Burke JR, et al. Specificity, sensitivity, and predictive value of apolipoprotein-E genotyping for sporadic Alzheimer’s disease. Lancet.1996; 348(9020):90–93. PMID: 8676723

31. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a toolset for whole-genome association and population-based linkage analysis. Am J Hum Genet.2007; 81(3):559–75 https://doi.org/10.1086/519795 PMID: 17701901

32. Anderson AC, Petterson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. Nat Protoc. 2010; 5(9):1564–1573. https://doi.org/10.1038/nprot.2010.116 PMID: 21085122

33. Delaneau O, Marchini J. The 1000 Genomes Project Consortium. Integrating sequence and array data to create an improved 1000 Genomes Project haplotype reference panel. Nature Communications.2014; 5:3934. https://doi.org/10.1038/ncomms4934 PMID: 25653097

34. The 1000 Genomes Project Consortium. A global reference for human genetic variation. Nature.2015; 526: 68–74 https://doi.org/10.1038/nature15393 PMID: 26432245

35. Maples BK, Gravel S, Kenny EE, Bustamante CD. RFMix: a discriminative modeling approach for rapid and robust local-ancestry inference. Am. J. Hum. Genet.2013; 93:278–288 https://doi.org/10.1016/j.ajhg.2013.06.020 PMID: 23910464
36. Price AL, Patterson N, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006;904–909. https://doi.org/10.1038/ng1847 PMID: 16862161

37. Marschner I. glm2: Fitting Generalized Linear Models. R package version 1.1.2.2014; https://CRAN.R-project.org/package=glm2

38. Vincent JC. gee: Generalized Estimation Equation Solver. R package version 4.13–19.2015; https://CRAN.R-project.org/package=gee

39. Dunn OJ. Multiple Comparisons Among Means. Journal of the American Statistical Association. 1961; 56 (293): 52–64.