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Increased *APOEε4* expression is associated with the difference in Alzheimer Disease risk from diverse ancestral backgrounds

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Abstract

INTRODUCTION: *APOEε4* confers less risk for Alzheimer disease (AD) in carriers with African local genomic ancestry (ALA) than *APOEε4* carriers with European local ancestry (ELA). Cell type specific transcriptional variation between the two local ancestries (LAs) could contribute to this disease risk differences.

METHODS: Single-nucleus RNA sequencing was performed on frozen frontal cortex of homozygous *APOEε4* AD patients: seven with ELA, four with ALA.

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RESULTS: 60,908 nuclei were sequenced. Within the LA region (chr19:44–46Mb), *APOEε4* was the gene most differentially expressed, with ELA carriers having significantly more expression (overall $p < 1.8E^{-317}$) in 24 of 32 cell clusters. The transcriptome of one astrocyte cluster, with high *APOEε4* expression and specific to ELA, is suggestive of A1 reactive astrocytes.

DISCUSSION: AD patients with ELA expressed significantly greater levels of *APOE* than ALA *APOEε4* carriers. These differences in *APOEε4* expression could contribute to the reduced risk for AD seen in African *APOEε4* carriers.

Keywords

APOE ; Single-nucleus RNA-seq; Local Ancestry; Alzheimer Disease

1. Introduction

Alzheimer disease (AD) is the most common form of dementia among older adults, affecting an estimated fifty million individuals worldwide [1]. The *APOEε4* allele is the strongest common genetic risk factor for AD [2]. However, the risk for AD conveyed by the *APOEε4* allele varies with ancestral background. The homozygous *APOEε4* genotype presents a stronger AD risk for Non-Hispanic Whites (NHW) (Odds Ratio (OR) ~15) [3–6] than for African Americans (AA) (OR~8) and Africans (OR~3) [3–9]. The lower AD risk in African and AA *APOEε4* carriers is not due to different environments between the populations or different genetic structure across the AA and NHW genomes. Rather, the protective factor lowering the risk for AD from *APOEε4* is associated with a genetic difference in the local genomic ancestry region (LA) surrounding *APOEε4* [10,11]. That is, if you inherited your *APOEε4* allele and its African local ancestry (ALA) from your African ancestor, you have the African *APOEε4* risk for AD. If you inherited your *APOEε4* allele and its surrounding European local ancestry (ELA) from your European ancestor, you have the European risk for AD associated with *APOEε4*.

There are no consistent differences observed in the *APOEε4* amino acid coding sequence between AA and NHW. Thus, the most likely source of the relative protective effect for *APOEε4* found in the ALA are non-coding variant(s). We hypothesized that these regulatory elements could affect the expression of genes mediating the risk difference. Thus, unlike previous gene expression studies comparing expression differences between unaffected individuals and those affected with AD, in this study we are specifically focused on identifying expression differences between the ALA and ELA surrounding the *APOEε4* region. To do this we obtained brain tissue from both AA and NHW Alzheimer patients, all homozygous carriers of the *APOEε4* genotype but differing in the ancestry of their surrounding LA (African or European).

The cellular heterogeneity of the human brain makes bulk transcriptome studies difficult to interpret for any specific cell, gene or genotype. Single-nucleus RNA-sequencing (snRNA-seq) provides a method to dissect this tissue complexity, allowing unbiased characterization and quantitative expression profiles from tens of thousands of individual cells from archived frozen brain. The large number of cells that can be examined increases the statistical power of the analyses.

We demonstrate here that in the dorsal lateral frontal cortex (Brodmann area 9), AA homozygous *APOEε4* carriers with surrounding ALA expressed significantly less *APOE* than NHW homozygous *APOEε4* carriers with ELA. Identifying the underlying mechanism of the *APOEε4* risk differences between NHW and AA could provide insight into potential therapeutic interventions to reduce the risk posed by *APOEε4* for AD.

2. Material and Methods

2.1 Sample Source

All patients presented clinically with a progressive dementia consistent with AD, had a confirmed diagnosis of AD upon neuropathological examination and were *APOEε4* homozygotes. To identify African ancestry patients, the National Alzheimer Coordinating Center (NACC) was screened for patients who self-identified as AA and were *APOEε4* carriers. Autopsy material for the NACC-selected AA samples was obtained from the Alzheimer Disease Research Centers (ADRC) at Emory University, Northwestern University, and the John P. Hussman Institute for Human Genomics (HIHG). *APOEε4* expression was not measured prior to sample selection and thus was not a criterion in selecting tissue. All samples were acquired with informed consent for research use and approved by the institutional review board of each center.

2.2 Sample Genomic Characterization

Global and *APOE* local ancestry (LA) were assessed using genome-wide genotyping data as previously described [10]. Briefly, we phased the genotyping data with the SHAPEIT tool ver. 2 [12] using 1000 Genomes Phase 3 reference panel [13]with default settings. We then used the RFMix algorithm [14] using the Human Genome Diversity Project (HGDP) European and African data as a reference panel to label each admixture block using the RFMix estimates. We defined the primary LA region of interest as that within 1Mb on either side of *APOE* (chr19:44–46Mb), broad enough to include potential enhancers, primary topological associated domains, and other regulatory factors, while narrow enough to ensure contiguous LA blocks [10].

We selected individuals homozygous for ELA and ALA haplotypes. Whole Genome Sequencing (WGS) was performed at either the Center for Genome Technology at the John P. Hussman Institute for Human Genomics or The American Genome Center at Uniformed Services University of the Health Sciences (USUHS) using standard Illumina protocols and GATK Best Practices analysis recommendations [15,16]. All individuals used in the study were confirmed homozygous for the *APOEε4* allele by Sanger and WGS and screened for pathogenic variants in known AD genes.

2.3 Nuclei isolation

Nuclei were isolated from ~100mg of frozen tissue from Brodmann area 9 at the HIHG using the Nuclei Isolation Kit: Nuclei EZ Prep (Sigma, #NUC101). All tissues were homogenized in ice-cold EZ Lysis buffer. Pelleted nuclei (500× g, 5 min and 4 °C) were washed in ice-cold EZ Lysis buffer, and Nuclei Suspension Buffer (NSB; consisting of 1X PBS, 1% BSA and 0.2 U/μl RNase inhibitor (NxGEN #97065–224). Isolated nuclei

were resuspended in NSB, filtered through a 70μm and 40μm cell strainer and the pellet re-suspended in 2% BSA in PBS. Homogenates (2 mL) were layered onto a 1.8M sucrose cushion and ultra-centrifuged at 24,400 rpm at 4°C for 2 hours (Beckman Coulter Optimal centrifuge #L90K). The nuclear pellet was re-suspended in 2% BSA in 1X PBS. After resuspension, nuclei were re-filtered through a 40μm cell strainer and an aliquot was trypan-blue stained for visual quality assessment and counted using the Countess Automated Cell Counter (Thermo Fisher).

2.4 Single nucleus RNA sequencing

Single nucleus sequencing was performed in the Center for Genome Technology at the HIHG. Nuclei at a concentration of 1200 nuclei/μl were loaded on the 10X Genomics Chromium platform to isolate ~7,000 nuclei per sample and create individually barcoded Gel bead-in-Emulsions (GEMs) which were processed using the Chromium Single Cell 3' Reagent Version 3 Kit. Sequencing libraries were evaluated for quality on the Agilent Tape Station (Agilent Technologies, Palo Alto, CA, USA), and quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and qPCR before sequencing on the Illumina NovaSeq 6000 targeting 100,000 reads per cell with sequencing parameters: Read1, 28 cycles; Index1, 8 cycles; Read2, 98 cycles.

2.5 Single nucleus RNA Quality Control and Analysis

Reads were processed and analyzed as described in detail in the Supplementary Methods. Briefly, 10X Genomics CellRanger v3.0.2 software was used for primary bioinformatics with alignment to a customized GRCh38 reference genome that includes both intronic and exonic regions during gene quantification and further processing with the Seurat 3.1 pipeline [17]. We removed poor quality nuclei and potential doublets by excluding nuclei outside the 5th and 95th percentile of the number of genes and the number of unique molecular identifiers (UMIs) and nuclei with greater than 10% mitochondrial reads.

The first 100 principal components were calculated for each sample and plotted in an elbow plot (Supplementary Figure 1) to demonstrate that data integration considering 50 principal components captured most of the variability across the samples. Data integration was performed to identify shared cell states across different samples following a recently published Seurat protocol [18]. Similar nuclei were clustered using a final resolution of 0.4 in Seurat resulting in 32 distinct clusters. Differentially expressed genes (DEG) between ancestral groups within each cluster were identified using the MAST test which employs a generalized linear model framework using cell detection rate within replicates and across groups as a co-variate [19]. The MAST test has low error and false discovery rates in comparison with other single nuclei differential expression methods [20]. This was followed by pathway analysis performed using the gene enrichment analysis tools METASCAPE [21] and Enrichr [22,23].

2.5 Data Availability

Raw sequencing data (FASTQ) and CellRanger pipeline outputs (barcode, feature, and matrix files) are available for each sample by email request to the corresponding author.

3. Results

3.1 Brain Samples

Forty-five AA samples were identified in the NACC database, but only 14 AA samples were available at the time of the study, from the Alzheimer Disease Research Centers (ADRC) of Northwestern (four) and Emory University (ten). LA was assessed and identified three AA samples homozygous for ELA and three heterozygous for ELA and ALA, excluding them for the study. Two additional samples were eliminated due to the presence of other identified neurologic abnormalities (e.g. global head injury, glioblastoma) that could affect the analysis. This left four samples (three females, one male) that were homozygous for *APOEε4* and ALA. The African global ancestry of these four samples ranged from 85 to 92%, with the remaining admixture European.

Initial RNA single nuclei seq analysis used four NHW AD samples (three females, one male) obtained from the HIHG Brain Bank, with samples collected prior to 2007 at Duke University. In order to eliminate the possibility of differences in processing between centers affecting *APOEε4* expression, three additional homozygous *APOEε4*, homozygous ELA samples were obtained from the Emory ADC, added into the total analysis, and separately compared only with the ALA samples from that same site. Samples were chosen based on tissue availability, sex match, neuropathology (had no other identified neurologic abnormalities that would affect expression), homozygous *APOEε4* genotype and ELA. Global ancestry for the NHW samples was >96% European. Comparison of the overall linkage disequilibrium structure between ALA and ELA was similar, with slightly smaller and more numerous LD blocks in ALA as would be expected (Supplementary Figure 2).

All donors included in the study were clinically diagnosed with AD using standard cognitive testing (Clinical Dementia Rating (CDR) or Mini-Mental State Exam (MMSE)), and met the neuropathological criteria of the National Institute on Aging-Alzheimer's Association for the diagnosis of AD. We assessed brain pH levels (see Supplementary Methods) and an overview of the results is given on Table 1, with no statistically significant difference between the groups ($p = 0.44$). Description of the samples is shown in Table 1 and in Supplementary Table 1. Whole genome sequencing revealed absence of mutations in any known Mendelian genes for AD (*PSEN1, PSEN2, APP,* and *MAPT*) as well as absence of known risk rare variants in *ABCA7*, *TREM2* and *SORL1* in all samples.

3.2 Single nucleus RNA-seq clusters

After quality control, we obtained data from a total of 60,908 total nuclei (2,805 – 7,774 nuclei per sample), sequenced at a median depth of ~126,000 reads per cell with on average ~1,900 genes/nucleus (Table 2). There was no significant difference between ELA and ALA in terms of percentage of aligned and unaligned reads (86 ± 8% vs 90% ± 4% of reads mapped to the hg38 genome in ALA and ELA, respectively). A UMAP plot for the integrated eleven samples was performed at a resolution of 0.4, resulting in 32 distinct clusters (Figure 1A). We identified thirteen excitatory neuron clusters (comprising ~33% of the total cells), eight inhibitory neuron clusters (~13% of total cells), two oligodendrocyte clusters (~29% of total cells), three astrocyte (~11% of total cells), two microglia clusters

(~6% of total cells), one oligodendrocyte precursor cell (OPC) cluster (~5% of total cells), two endothelial cell cluster (~2% of total cells), and one vascular leptomeningeal cell (VLMC) cluster (~1% of total cells). (Figure 1B and Supplementary Table 2). A heatmap of the top ten marker genes defining each of the 32 clusters is shown in Supplementary Figure 3.

3.3 Comparison of clusters between ancestries

The cell count and percentage of the total cells found in each cluster, separated by ancestries, is shown in Figure 2 and Supplementary Table 2. The proportion of cells per cluster between the four cases with ALA and the seven cases with ELA was similar for all clusters with greater than 500 cells, with the exception of clusters 3, 20 and 21. Cluster 21 (excitatory neurons) was more than 2-fold greater in the ALA cluster and excitatory neuronal cluster 3 was more than 2-fold greater in ELA. Cluster 20's proportion of the total cell number contributed by ELA was 13 times greater than the proportion of the total cells represented by cluster 20 from the ALA samples (Figure 2), but was derived from primarily two ELA samples (5 and 6).

3.4 APOE expression

We compared *APOEε4* expression between ALA and ELA clusters, as shown in Figure 3. *APOEε4* expression was significantly increased overall in ELA versus ALA samples (adjusted p-value < $1.8E^{-317}$, FC = 1.56). *APOEε4* demonstrated significant differential expression (DE) in 24 of the 32 clusters with all fold changes in the same direction (Table 3). The biggest fold change in *APOEε4* expression was observed in a microglia cluster (cluster 4), in which ALA cells express 2.2-fold less *APOEε4* than ELA cells.

To ensure that the differential expression for *APOEε4* between the ALA and ELA was not secondary to differences in processing between ADRCs, we performed a second analysis using three ELA samples and three ALA samples, all from the Emory ADRC. Using the same methods as for the combined analysis, we identified a similar cluster pattern when integrating just these six samples (Supplementary Figure 4). Importantly, in these samples from the same ADRC, the expression of *APOEε4* was still significantly higher in the ELA (p < $3.5E^{-318}$, FC = 1.42) (Supplementary Figure 5). Thus, the difference in *APOEε4* expression is not due differences in processing of tissue between sites.

3.5 Characterization of astrocyte cluster 20

Cluster 20 was unusual in that the cluster had the highest proportion of cells expressing *APOEε4* (74.9%) and *APOEε4* expression was significantly higher in cluster 20 when compared to all other clusters (p = <$1.39E^{-319}$, FC = 2.76). Examination of this cell cluster revealed that the vast majority of these cells (78%) originate from two of the Duke samples (5 and 6), and not the other ELA samples.

Examination of the transcriptional signature of cluster 20 revealed a strong enrichment for known astrocyte-specific markers (Supplementary File 1). To further characterize cluster 20, we compared the transcriptome of this cluster with the other astrocyte clusters (2 and 10). Analysis of the identified DEG indicates that cluster 20 preferentially expressed markers of

reactive astrocytes [24–26], with significantly higher levels of *GFAP* (p= 2.88E-192, FC= 2.11), *VIM* (p= 9.33E-267, FC = 2.09), and *HSPB1* (p= 2.39E-173, FC= 1.90) compared to other astrocyte clusters. Interestingly, compared to the other astrocyte clusters, cluster 20 also overexpressed *IFITM3* (p = 1.08E-215, FC = 1.74), *OLFM1* (p= 8.17E-204, FC= 1.90), *B2M* (p= 3.01E-160, FC= 1.56), *TAPBP* (p= 2.04E-168, FC= 1.35), *CHI3L1* (p= 2.51E-43, FC= 1.19) which are specifically upregulated by neuroinflammation in A1-type reactive astrocytes in mice [24].

3.5 Other Local Ancestry and Known AD genes

We identified 116 genes (GENCODE version 32) that lie within the LA region. Of those 116, only three had significantly DE in any cluster: *APOE, ERCC1,* and *MARK4*. If we expand the analyzed region to two Mb on either side of the *APOE* locus, *CALM3, PNMA8A, PLAUR, ,* and *HIF3A* were differentially expressed genes (Table 4).

We also evaluated DE of AD candidate genes, including Mendelian AD genes (*APP*, *PS1*, *PS2,* and *MAPT*), genes suggested by GWAS studies in both ethnic groups [27–29], and genes with rare variants recently reported to be associated with AD in the AA population. We observed DE between NHW and AA in five reported AD genes including*, APOE, CLU, BIN1, SORL1,* and *SPI1*. Interestingly, we also found significant DE in three recently suggested novel African American AD genes [29]: *RBFOX1*, *ALCAM*, and *GPC6* (Table 5).

3.6 AA versus NHW transcriptome

While this study was designed to evaluate the differences in ALA versus ELA, it also provides one of the first looks at comparing snRNA-seq data between AA and NHW AD patients. The top ten significant DEG between AA and NHW across all clusters are shown in Supplementary Table 3. The DEG of the full set of clusters is shown in Supplementary File 2.

3.7 Cell type specific pathway analysis

To better understand ancestry-specific changes in gene expression, we performed an unbiased analysis of enriched pathways. We pooled the DEG from all clusters of a given cell-type to achieve a more complete cell-type transcriptome representation (Supplementary File 3). The DEG in the astrocytic lineages were enriched in GO and KEGG pathways containing the term “stress”, suggestive of differential cellular stress responses, and ontologies like “synaptic signaling” and “cellular responses to external stimuli”. Further, enriched analysis for terms “Alzheimer Disease”, “Cognition” and “Aging” were statistically significant. Pathways enriched in DEG in neurons (both, in inhibitory and excitatory) include neuronal development, synaptic transmission and response to toxicity. DEG in oligodendrocytes are enriched in pathways related to synaptic transmission and apoptosis. Microglia DEG were enriched in immune-related pathways. DEG in endothelial cells are enriched in pathways related to responses to stress and angiogenesis.

4. Discussion

We hypothesized that the DE of genes lying in the LA region between ALA and ELA could provide insight to the locus or gene(s) contributing to the risk difference between African and European LA carriers of *APOEε4*. We found that the gene with the greatest DE in the LA region was *APOE*. No other gene in the LA region displayed the amount or extent of DE that was seen for *APOE*. Carriers of the *APOEε4* allele on the ELA surrounding the locus produced significantly more *APOEε4* than did carriers of the *APOEε4* allele on the ALA. Importantly, the expression of *APOEε4* was low in all four ALA tested and higher in all seven ELA, supporting the biological importance of this DE in AD risk between the two groups. Further, the DE of *APOE* was found to be in the same direction in all the clusters in which it is differentially expressed.

Rajabli et al [10] used a region of 1Mb on either side of *APOE* as the area of LA. This should include any regulatory factors interacting with *APOE* and the strongest reported topologically associated domains [30], while providing the most contiguous LA. In this region, two other genes, *ERCC1* and *MARK4* demonstrated DE, but not to the extent seen in *APOEε4* (Table 4). The gene ERCC excision Repair 1, Endonuclease Non-Catalytic Subunit (*ERCC1)* was the next most extensive DE gene in the LA area, with increased expression in the ALA relative to the ELA in the four clusters of inhibitory neurons. *ERCC1* functions in DNA repair and interestingly has been associated with human longevity along with *APOE* [31]. DNA repair has been proposed to be a contributing pathway to AD pathogenesis as well [32]. As the LA region is not a fixed region in individuals, we also extended our analysis to genes lying within 2Mb upstream or downstream of *APOE*. Only *CALM3*, a subunit of calmodulin, had significant DE in multiple clusters, again with less expression in the ALA than ELA. Calmodulin and calcium homeostasis have been implicated in AD [33–35], though *CALM3* itself was not. *CALM1*, on chromosome 10, is also DE and lower in AA, suggesting the DE of *CALM3* is not likely due to local ancestry effects.

Our comparison of UMAP clusters between ALA and ELA revealed an obvious difference in the cell proportion in cluster 20, which was identified as potentially an A1 reactive astrocyte cluster. Reactive astrocytes have been categorized into two primary groups, A1 and A2. A1 reactive astrocytes, activated by neuroinflammatory conditions, are proposed to be “toxic” astrocytes, resulting in destructive actions towards neuronal synapses [36]. A2 astrocytes are activated by hypoxic or ischemic conditions and are postulated to help protect neuronal cells [24]. While there is heterogeneity in gene expression reported for these reactive astrocytes [26], elevated expression of *GFAP, IFITM3,B2M* and *OLFM1* tags them as A1 astrocytes [24], all of which were significantly overexpressed in the ELA cluster 20 relative to the other astrocyte clusters. A1 reactive astrocytes have been previously reported to be increased in AD versus control brains [37] and are known to increase in the brain with increasing age [26]. However, mouse models have shown that the high expression of *APOEε4* can stimulate the formation of A1 reactive astrocytes [38] and cluster 20 cells expressed a high proportion and expression level of *APOEε4*. Thus, whether the finding that two ELA and no ALA contributed to cluster 20 just represents the biological heterogeneity of AD or is secondary to the higher *APOEε4* expression in ELA cells is not clear and requires further studies.

The mechanism leading to this difference in *APOEε4* expression is currently not known. Sequence differences between the two LA leading to differences in methylation or enhancer/repressor activity seem most likely [39,40]. Whether these differences apply to *APOEε*3 homozygotes with different local ancestries is not known. We have recently shown that *APOEε*3 homozygotes with the Very Long repeat length of the TOMM40 523' polyT repeat on the ELA background have less risk for AD than those *APOEε*3 homozygotes with smaller repeats [41]. This repeat is likely tagging a sequence difference in its haplotype, as the same effect is not seen in ALA homozygous *APOEε*3 carriers. Therefore, while we would expect an expression difference between *APOEε*3 carriers on different LAs, this may not be the case.

There is also a difference in age-of-onset between the two groups, though both are well within the normal age-of-onset for AD. As *APOEε*4 in NHW drives and earlier age-of-onset, this is perhaps not unexpected. This seems unlikely to have contributed to any differences between the two groups. First, age-of-onset for AD is at best an estimation, usually in retrospect. Second, it is now well accepted that AD starts pathologically years before its clinical manifestation, with amyloid deposition in *APOEε*4 homozygotes seen to rise in their 30s [42]. Thus, comparison of the clinical onset of the disease is not necessarily reflective of pathological differences, nor the more important duration of the course of pathological disease. Pathologically there was no significant difference in BRAAK staging or pH of the sample tissues between groups.

Recently, a SNP in the promoter of *APOE* has been suggested to be related to the risk difference for AD between NHW and Koreans (rs405509) [9]. However, rs405509 is not significantly different in allele frequency between AA and NHW LAs on the *APOEε4* haplotype (Rajabli, personal communication). Thus, it does not appear to explain the risk difference between AA and NHW. Little is also known about the similarity of open reading frames or topologically associated domains between the two ancestries in the brain, which could affect expression differences as well.

We chose to compare clinically affected individuals in this study to minimize neuropathic heterogeneity of the disease and because the number of control African-American *APOEε*4 carriers with autopsies is very few, if any. There is no reason to expect that expression differences we observe here are not present preclinically in homozygous *APOEε*4 carriers, but confirmation of this will await the inclusion of many more autopsy samples from diverse ancestries.

One of the challenges in this study was identifying AA brains that were homozygous for *APOEε4* and ALA The number of AA brain samples currently available for study is very limited relative to NHW samples. Further, as an admixed population, many AA samples are heterozygous for ELA and ALA or even homozygous for ELA ancestry surrounding *APOEε4*. In general, there is a great need for increased genomic and autopsy studies in diverse ancestries outside of NHW. Indeed, this is a primary research foci of the Alzheimer Disease Research Sequencing Project (ADSP) of the National Institutes of Aging (https://www.niagads.org/adsp/content/home).

Finally, there has been discussion by many authors debating whether increasing or decreasing *APOEε4* production would be a therapeutic approach [43]. The data presented here, along with antisense oligonucleotides studies in mouse models [44], would support the premise that decreasing *APOEε4* production could lead to a positive therapeutic effect, reducing the overall risk for AD in NHW *APOEε4* carriers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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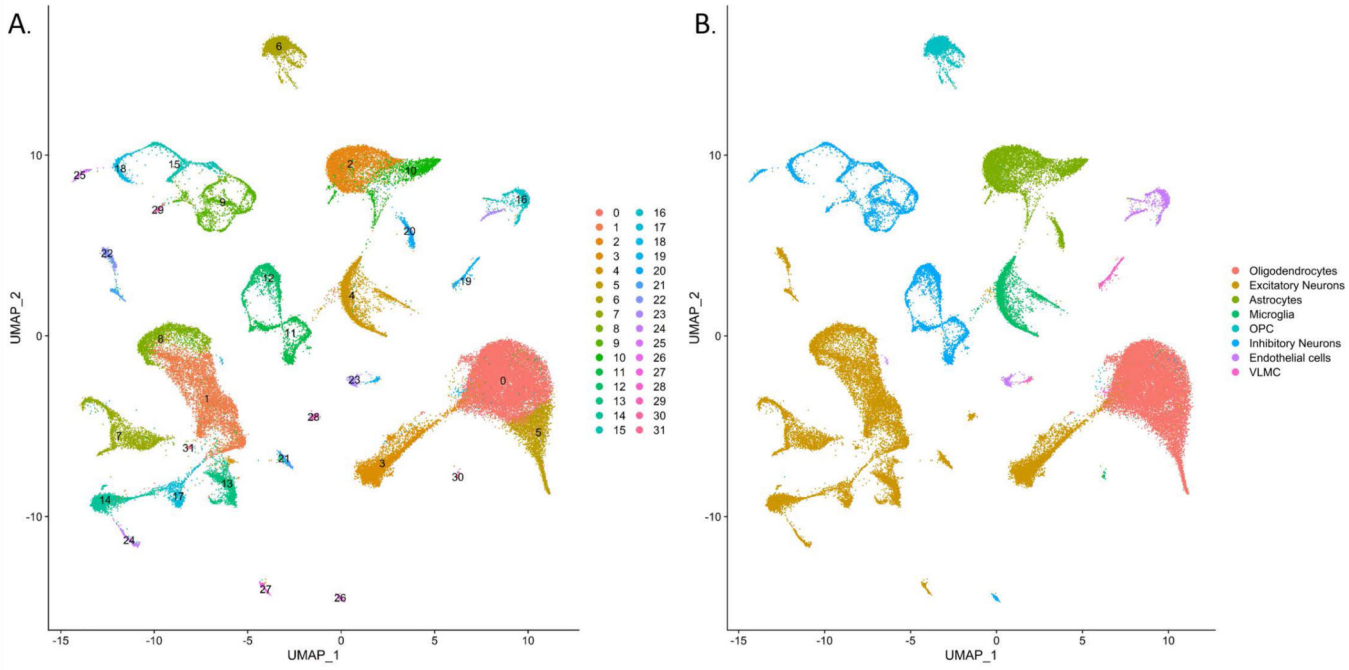

Figure 1.

Visualization of single nuclei clusters from 60,908 nuclei integrated across 11 samples. A) UMAP dimensionality reduction plot of using a resolution of 0.4 resulting in 32 unique clusters. B) UMAP dimensionality reduction plot with clusters colored by cell type.

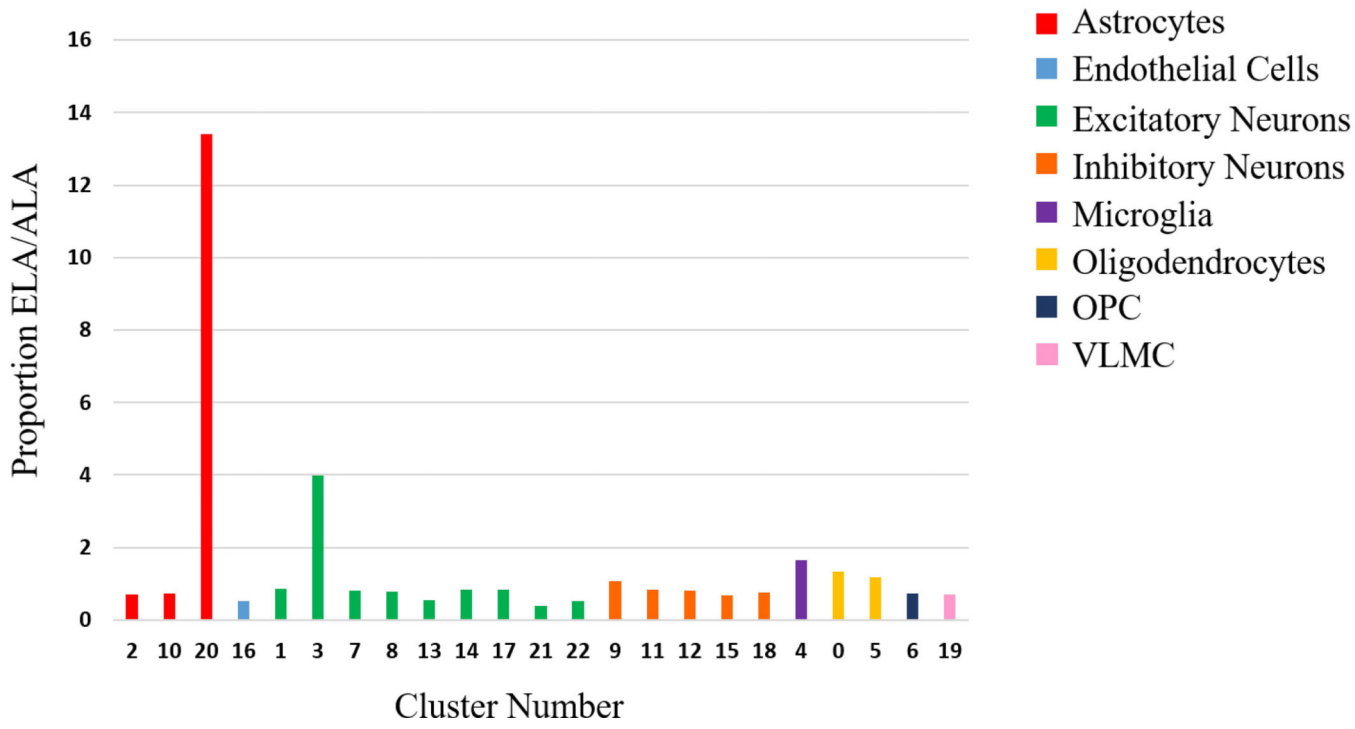

Figure 2.

ELA/ALA cell proportions by clusters. The proportion of cells in ELA / proportion of cells in ALA plotted in a bar graph. Bars are grouped and colored by cell type of the cluster.

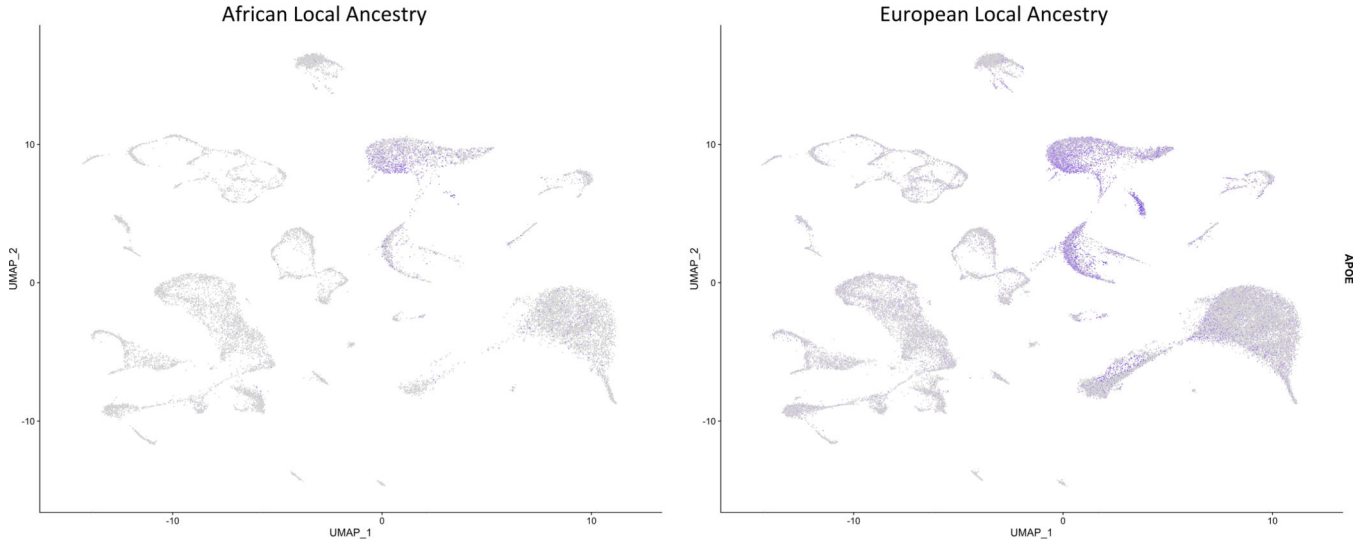

Figure 3.

Visualization of *APOE* expression in nuclei from AA local ancestry and EU local ancestry. Cells are overlaid with gene expression information with expression depicted from gray (low) to purple (high).

Table 1.

Demographic characteristics of the samples.

Sample	Center	Sex	AOD	*APOE* genotype	Local Ancestry	CDR Score	Freezer time (years)	PMI (hours)	BRAAK Score	ABC Score	pH
1	Emory	Male	86	4,4	AF/AF	3	3	7	IV	A2B2C3	6.1
2	Emory	Female	82	4,4	AF/AF	3	10	NA	V	A3B3C3	6.3
3	NW	Female	85	4,4	AF/AF	3	5	27	V	A3B3C3	6.8
4	Emory	Female	80	4,4	AF/AF	2	5	14	VI	A3B3C3	6.3
5	Duke/UM	Male	75	4,4	EU/EU	3	15	2	IV	A2B2C3	6.5
6	Duke/UM	Female	76	4,4	EU/EU	3	15	6	V	A3B3C3	6.4
7	Duke/UM	Female	72	4,4	EU/EU	3	15	12	IV	A2B2C3	6.1
8	Duke/UM	Female	70	4,4	EU/EU	3	15	4	VI	A3B3C3	6.4
9	Emory	Female	72	4,4	EU/EU	3	1	6	VI	A3B3C3	6.3
10	Emory	Female	83	4,4	EU/EU	NA	19	12	VI	A3B3C3	6.2
11	Emory	Female	76	4,4	EU/EU	3	12	3.5	VI	A3B3C3	6.3

AOD: Age of Death, PMI: Post-Mortem Interval, UM: University of Miami, NW: Northwestern University, NA: Not Available

Table 2.

Summary of Single Nuclei RNA sequencing results.

Sample	Raw # of cells	# of cells post-QC	Median # of reads/nucleus	Median # genes/nucleus
1	10,160	3,587	169,439	2,593
2	6,660	5,592	126,054	2,677
3	5,984	4,993	180,736	1,331
4	3,233	2,805	322,773	1,388
5	8,853	7,774	86,528	1,253
6	10,492	7,765	96,055	3,332
7	5,151	4,353	204,838	1,735
8	7,100	6,001	126,487	1,759
9	12,472	6,752	78,945	1,820
10	11,566	5,618	86,167	1,900
11	18,596	5,668	47,890	1,117

QC: quality control

Table 3.

APOE differential expression analysis.

Cluster	Cell Type	Fold Change	adj-p-value
2	Astrocytes	−1.49	6.76E-93
16	Endothelial cells	−1.64	1.22E-11
23		−1.53	5.31E-07
1	Excitatory Neurons	−1.23	1.70E-162
13		−1.22	5.33E-23
14		−1.22	3.21E-33
17		−1.21	1.92E-19
21		−1.21	2.67E-19
22		−1.21	8.54E-12
3		−1.79	1.95E-13
7		−1.21	2.53E-59
8		−1.26	2.50E-74
11	Inhibitory Neurons	−1.36	2.14E-39
12		−1.30	3.69E-27
15		−1.47	1.10E-25
18		−1.32	2.40E-17
26		−1.20	0.00050
29		−1.36	0.02576
9		−1.34	5.73E-39
4	Microglia	−2.16	2.53E-76
0	Oligodendrocytes	−1.30	1.18E-128
5		−1.31	7.31E-29
6	OPC	−1.25	4.98E-25
19	VLMC	−1.28	0.03288

Table 4.

Differentially expressed genes in the 2Mb local ancestry region.

Cluster	Cell Type	Gene	Fold Change	adj-p-value
2	Astrocytes	*CALM3*	−1.30	1.97E-108
2		*APOE*	−1.50	6.76E-93
2		*MARK4*	1.22	2.94E-66
2		*HIF3A*	−1.20	3.50E-62
16	Endothelial cells	*APOE*	−1.64	1.22E-11
23		*APOE*	−1.54	5.31E-07
1	Excitatory Neurons	*APOE*	−1.24	1.70E-162
1		*CALM3*	−1.27	4.66E-134
3		*APOE*	−1.80	1.95E-13
7		*APOE*	−1.22	2.53E-59
7		*CALM3*	−1.31	1.70E-45
8		*CALM3*	−1.34	1.70E-94
8		*APOE*	−1.27	2.50E-74
13		*APOE*	−1.23	5.33E-23
13		*CALM3*	−1.32	1.85E-12
14		*CALM3*	−1.51	8.38E-61
14		*APOE*	−1.23	3.21E-33
17		*PNMA8A*	−1.25	1.30E-19
17		*APOE*	−1.22	1.92E-19
17		*CALM3*	−1.38	1.80E-18
21		*APOE*	−1.21	2.67E-19
21		*CALM3*	−1.20	0.00683
22		*APOE*	−1.21	8.54E-12
22		*CALM3*	−1.22	5.85E-05
24		*CALM3*	−1.38	6.90E-11
27		*CALM3*	−1.27	0.00283
9	Inhibitory Neurons	*APOE*	−1.34	5.73E-39
9		*CALM3*	−1.26	4.06E-38
11		*APOE*	−1.36	2.14E-39
11		*CALM3*	−1.24	1.45E-16
11		*ERCC1*	1.25	4.00E-11
12		*APOE*	−1.31	3.69E-27
12		*ERCC1*	1.35	7.34E-19
12		*CALM3*	−1.20	5.33E-15
15		*CALM3*	−1.37	3.40E-27
15		*APOE*	−1.48	1.10E-25
15		*PNMA8A*	−1.19	3.88E-19

Cluster	Cell Type	Gene	Fold Change	adj-p-value
18		*APOE*	−1.33	2.40E-17
18		*ERCC1*	1.31	2.78E-09
26		*MARK4*	1.21	6.38E-05
26		*CALM3*	−1.26	0.00018
26		*APOE*	−1.20	0.00049
29		*CALM3*	−1.38	0.01493
29		*APOE*	−1.37	0.02575
4	Microglia	*APOE*	−2.16	2.53E-76
4		*CALM3*	−1.22	2.48E-17
4		*PLAUR*	1.24	6.99E-13
0	Oligodendrocytes	*APOE*	−1.31	1.18E-128
0		*CALM3*	−1.30	3.33E-105
5		*APOE*	−1.31	7.31E-29
5		*CALM3*	−1.29	2.71E-19
6	OPC	*HIF3A*	−1.25	4.31E-36
6		*CALM3*	−1.25	3.38E-33
6		*APOE*	−1.26	4.98E-25
19	VLMC	*CALM3*	−1.45	0.00652
19		*APOE*	−1.28	0.03287

Table 5.

Alzheimer disease candidate genes differentially expressed. Negative fold changes = less expression in ALA.

Gene	# of Clusters with DE	Average fold change	Average adjusted p-value
Genome-wide-significant loci in Non-Hispanic Whites [27]			
CLU	28	−1.67	6.55E-05
APOE	24	−1.37	2.46E-03
SORL1	3	−1.27	1.98E-04
BIN1	2	−1.22	5.94E-06
SPI1	1	1.26	2.36E-15
Novel loci in African American Meta-Analysis [29]			
RBFOX1	7	1.28	2.21E-04
ALCAM	5	−1.26	7.30E-03
GPC6	7	1.44	5.41E-04