Ancestry-related differences in chromatin accessibility and gene expression of *APOE4* are associated with Alzheimer disease risk

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Author contributions

K.C, J.M.V, J.I.Y and A.J.G overall study design. K.C performed experiments. M.D.M.M and A.J.G performed bioinformatics analysis. F.R and K.H performed ancestry analysis. P.W. and C.L.D performed genotyping arrays and whole genome sequencing. M.F, S.W, C.G, M.G, D.A.B, T.S and M.A.P provided brain samples and advice. D.M.D, K.N, L.W and F.J provided insight for the functional analysis. K.C, J.M.V, J.I.Y, A.J.G and M.D.M.M wrote the manuscript with input from all authors. All authors read and approved the final manuscript.

Consent Statement

Individuals included in this study provided informed autopsy consent to the Institutional Review Board from their participating center.

Consent for publication

All authors consent for publication.

Conflicts of Interest

K.C, M.D.M.M, F.R, P.W, K.H, D.M.D, K.N, L.W, M.F, S.W, C.G, M.G, C.L.D, F.J, D.A.B, T.S, M.A.P, A.J.G, J.I.Y and J.M.V have nothing to disclose. The authors declare that they have no competing interests.

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Abstract

INTRODUCTION: European local ancestry (ELA) surrounding *APOE4* confers higher risk for Alzheimer Disease (AD) compared to African local ancestry (ALA). We demonstrated significantly higher *APOE4* expression in ELA vs ALA in AD brains from *APOE4/4* carriers. Chromatin accessibility differences could contribute to these expression changes.

METHODS: We performed single nuclei Assays for Transposase Accessible Chromatin sequencing from frontal cortex of six ALA and six ELA AD brains, homozygous for local ancestry and *APOE4*.

RESULTS: Our results showed an increased chromatin accessibility at the *APOE4* promoter area in ELA vs ALA astrocytes. This increased accessibility in ELA astrocytes extended genome wide. Genes with increased accessibility in ELA in astrocytes were enriched for synapsis, cholesterol processing and astrocyte reactivity.

DISCUSSION: Our results suggest that increased chromatin accessibility of *APOE4* in ELA astrocyte contributes to the observed elevated *APOE4* expression, corresponding to the increased AD risk in ELA vs ALA *APOE4/4* carriers.

Graphical Abstract
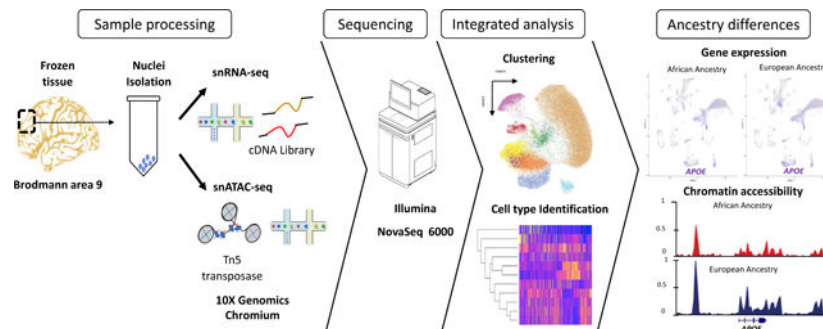
Keywords

Alzheimer disease; ancestry; *APOE4* ; chromatin accessibility; gene expression; snATAC-seq; snRNA-seq; European; African

BACKGROUND:

Alzheimer disease (AD) is the most common neurodegenerative disorder and one of the leading causes of disability worldwide for individuals aged 75 and older[1]. The strongest genetic risk factor for late-onset AD is the apolipoprotein E4 (*APOE4*) allele located on chromosome 19[2,3]. However, the risk associated with *APOE4* differs dramatically between individuals of European vs African ancestries[4–7] with *APOE4* carriers of European ancestry having substantially increased risk for AD compared to individuals with African ancestry.

This observed difference in risk has recently been shown in three independent studies to be due to differences in the genetic local ancestry (LA) region surrounding *APOE* rather than overall global European or African ancestry[8–10]. Using single nucleus RNA sequencing (snRNA-seq) of frontal cortex we have previously demonstrated that *APOE4/4* homozygous carriers with European local ancestry (ELA) expressed significantly higher *APOE* compared to those with African local ancestry (ALA), especially in astrocytes[11], suggesting a potential mechanism underlying the differential AD risk seen between ancestries.

Control of gene expression is orchestrated by the integration of cis-regulatory modules, such as enhancer and promoter elements, along with transcription factors[12–14]. The cis-regulatory modules of actively transcribed genes are generally in 'accessible' euchromatin with low nucleosome occupancy and few high-order structures[15–17], while 'closed' chromatin (heterochromatin) generally associates with transcriptional silencing[16,18,19]. Accordingly, ancestry-specific changes in accessibility of cis-regulatory elements that modulate the binding of transcription factors is a potential mechanism responsible for the difference in *APOE4* expression seen between ancestries.

The single nuclei Assay for Transposase Accessible Chromatin followed by sequencing (snATAC-seq), facilitates the creation of chromatin accessibility maps with single cellular resolution. For example, Corces et al[20] demonstrated that open chromatin regions in promoter or enhancer areas are associated with increased gene expression through snATAC-seq. Investigation of chromatin accessibility profiles specifically in AD have mostly focused on pathological hallmarks[21]. More recently, Morabito et al[22] conducted an integrated analysis using snATAC-seq and snRNA-seq from brains of AD and control individuals and reported that cell-type-specific chromatin accessibility changes in regulatory elements may be key to gene expression changes in AD. However, to date, the role of chromatin accessibility in AD between different ancestries, specifically at the *APOE4* locus, has not been investigated.

Therefore, we performed snATAC-seq combined with snRNA-seq to investigate the cell specific patterns of chromatin accessibility in ELA and ALA *APOE4/4* brains. We observed increased accessibility in *APOE* ELA relative to ALA in astrocytes. The differentially accessible peaks at the *APOE* promoter are predicted to bind a subset of transcription factors that exhibit differential gene expression in ELA astrocyte samples. These data support the hypothesis that European ancestry at the *APOE* promoter and in the area around *APOE* have a more open chromatin conformation than African ancestry. We speculate that this difference is permissive for transcription factor binding and transcriptional activation of *APOE*. Increased chromatin accessibility in ELA samples was also observed genome-wide in this astrocytic cell type. Thus, these findings provide novel insights into the molecular mechanisms of ancestry specific differences in AD risk.

METHODS:

Brain Samples

Brain autopsy samples were obtained as part of a multi-center collaboration from four Alzheimer Disease Research Centers (ADRCs; Emory University, Northwestern University,

Rush University Medical Center, and the University of Pennsylvania) and from the John P. Hussman Institute for Human Genomics (HIHG) at the University of Miami. All ADRC samples were initially selected from the National Alzheimer Coordinating Center (NACC) and compliant with site-specific approved institutional review board protocols as previously described[11].

Local ancestry determination

Genotyping arrays were processed to assess both global and local genetic ancestry (LA) in the *APOE* region. To determine the LA, we phased independently our datasets with the SHAPEIT tool (version 2) using 1000 Genomes Phase 3 as the reference panel[8]. The *APOE* region was defined within 1Mb on either side of *APOE,* using chr19:44–46Mb as the LA boundaries for ancestry assignments, broad enough to include potential enhancers and topological associated domains. Individuals homozygous for the ELA or ALA haplotype in this region were Whole Genome Sequenced (WGS) at either the Center for Genome Technology (CGT) at the HIHG or The American Genome Center at Uniformed Services University of the Health Sciences (USUHS).

snATAC-seq and snRNA-seq data generation and analysis

Isolation of Nuclei: Nuclei were isolated from frozen frontal cortex brain tissue (Brodmann area 9) as described previously[23,24]. A suspension of 65,000 nuclei was prepped according to the 10X Genomics Chromium Next GEM Single Cell ATAC 1.1 protocol. Libraries were sequenced on an Illumina NovaSeq 6000 targeting 75,000 reads per nuclei in paired end 50bp sequencing reactions. FASTQ files were processed using the CellRanger ATAC software package v1.2 (10X Genomics). snRNA-seq was performed as previously described[11].

Integrated analysis of the snATAC-seq and snRNA-seq data: This was performed using the ArchR software package v1.0.1[25]. Clustering of cells was performed with five iterations of Iterative Latent Semantic Indexing (LSI)[26,27] followed by batch correction with Harmony[28]. ATAC clusters were identified using FindClusters in Seurat v4.0 software package[29] and refined the cell type definitions by integration with snRNA-seq from the same samples using Seurat's FindTransferAnchors.

SnATAC-seq peak calling and differential accessibility

We created pseudo-bulk replicates combining all ELA and all ALA samples separately and called peaks for each ancestry within each cluster using the addReproduciblePeakSet function in ArchR utilizing the default parameters of the MACS2 callpeak command v2.2.7.1[30]. Differential accessibility for each peak within each cluster was calculated using ArchR getMarkerFeatures.

ATAC peak annotations

HOMER[31] (version 4.11[32]), was used to annotate peaks closest to transcription start sites (TSS) and to indicate exon, intron, intergenic, or promoter-TSS regions. A peak was assigned to a gene promoter-TSS when the peak location was ±2kb from the TSS. GREAT[33]

(version 4.0.4, http://great.stanford.edu/public/html/) was used to refine annotations where the peak was near multiple genes or intergenic. Distal enhancers were identified with the ELITE GeneHancer database from UCSC[34] of enhancers and promoters. We considered as distal enhancer peaks those annotated by GREAT or HOMER farther than ±2kb from a TSS and with coordinates overlapping one of these ELITE enhancers.

Transcription Factor Analysis

Bedtools getfasta[35] was used to extract the sequence of each peak (refdata-cellranger-atac-GRCh38–1.2.0 version). The algorithm FIMO[36] within MEME Suite version 5.4.1[37] was used to identify known transcription factor (TF) binding motifs in the two *APOE* promoter differentially accessible peaks (DAP). We surveyed defined transcription factor binding site databases JASPAR CORE 2022 (vertebrates non redundant)[38], Jolma 2013 (human and mouse)[39], and Swiss regulon (human and mouse)[40], and selected those with false discovery rate (FDR) adjusted *p*-value <0.05. In addition, we used all the human specific JASPAR 2022 defined TFs of the UCSC HG38 table browser[41] filtering by a JASPAR score 300.

Functional Enrichment Analysis

Functional analysis was performed using enrichR[42]. using as reference the human specific gene-set libraries KEGG 2021[43], the Gene Ontology Biological Process 2021[44], Reactome 2021[45] and ENCODE Histone post-translational modifications[46]. Enrichment for cell type specific markers was performed using the cell type gene-set atlas lists from Azimuth 2021[47] and PanglaoDB 2021[48]. Disease gene enrichment analysis used GeneSigDb[49], HDSigDb[50] and MsigDB[51]. Enrichment in chromosomal location was performed using a Fischer exact test.

RESULTS:

Brain Sample Characteristics

Twelve brain samples were used for the study (Table 1). Six ELA samples (European global ancestry >96%) were obtained from the HIHG Brain Bank (3), Rush University Medical Center (2) and Emory University (1). Six ALA samples (African global ancestry 68% to 88%) were obtained from Emory University (2), Northwestern University (1) and Rush University Medical Center (3). Samples included seven females (four ALA and three ELA) and five males (two ALA and three ELA). All samples had BRAAK staging scores ranging from IV to VI and had a mean age-of-death of 79 years. Whole genome sequencing confirmed the homozygous *APOE4/4* genotype and absence of mutations in known Mendelian genes for AD (*PSEN1, PSEN2, APP, MAPT*) as well as in known rare AD risk variants in *ABCA7, TREM2* and *SORL1*.

Cell type identification

We obtained data from a total of 60,306 nuclei from snATAC-seq and 94,411 nuclei from snRNA-seq. The snATAC-seq libraries had an average of ~13,000 fragments per nucleus and the snRNA-seq libraries had a median depth of ~116,000 reads per nucleus with on average ~1,900 genes/nucleus (Supplementary Table 1). The clusters with more than 500 nuclei in ELA and ALA showed no statistical differences in the number of nuclei per

cluster between the ancestry groups (*p*-value= 0.497). Integration of the snATAC-seq and snRNA-seq resulted in the identification of 11 cell type clusters (Figure 1A) with no sample specific bias (Supplementary Figure 1A and 1B, Supplementary Table 2). The cell type identity of each cluster was determined using top 50 snATAC-seq predicted gene scores for marker genes of known cell types in the frontal cortex (Figure 1B). We used *SLC17A7* for excitatory neurons; *SLC32A1* for inhibitory neurons; *MAG* for oligodendrocytes; *AIF1* for microglia; *GFAP* for astrocytes, *CSPG4* for oligodendrocyte precursor cell (OPCs) and *COL1A2* for vascular leptomeningeal cell (VLMC) (Supplementary Figure 2). We identified one oligodendrocyte cluster (55.6% of total cells), four excitatory neuron clusters (20.8% of the total cells), one inhibitory neuron cluster (8.3% of total cells), one microglia cluster (7.4% of total cells), one OPC cluster (3.9% of total cells), two astrocyte clusters (2.5% of total cells), and one vascular leptomeningeal cell (VLMC) cluster (1.5% of total cells) (Supplementary Table 2).

To further characterize the two astrocyte cell clusters, we determined the genes that identified astrocyte cluster 1 and astrocyte cluster 2 and investigated their expression in the single cell atlas of the Entorhinal Cortex in Human Alzheimer's Disease (ECHAD)[52] and astrocyte transcriptomic data from frontal cortex[53]. We observed that the genes characterizing astrocyte cluster 1 were primarily expressed in astrocyte subclusters a4 to a8, while the genes from our astrocyte cluster 2 were primarily expressed in astrocyte subclusters a2 and a3 from ECHAD, which correspond to reactive astrocytes subpopulations (Supplementary Figure 3)[53].

Accessibility analysis at the APOE locus and LA

We first investigated whether the ancestry-related differential expression of *APOE4* previously described[11] was recapitulated when additional samples from Rush University Medical Center were included in our analyses. Indeed, *APOE4* was differentially expressed when considering expression over all cell types between ancestries with greater expression in ELA (FC= 1.31; *p*-value=$9.66E^{-219}$), except one excitatory neurons cluster with higher expression in ALA (FC= 1.28; *p*-value=$5.87E^{6}$). This pattern of increased expression in ELA samples was true for four cell types (excitatory and inhibitory neurons, astrocytes and microglia) (Supplementary Table 3). Astrocytes had the highest fold change difference in ELA (FC = 1.56) and most significant *p*-value (*p*-value=$1.24E^{-129}$).

We next determined whether the *APOE* expression difference in astrocytes is associated with differences in chromatin accessibility. The snATAC-seq and snRNA-seq integrated UMAP showed that *APOE* expression as well as accessibility was highest in the astrocyte cluster 1 (Figure 2A). Comparison of accessibility between ancestries revealed two peaks with significantly increased accessibility in ELA at the *APOE* promoter in astrocyte cluster 1 (Figure 2B). These peaks were located at −19bp and −1990bp upstream of the *APOE* transcription start site (TSS) (FC: 2.57 and 4.52; FDR: 0.001 and 0.02, respectively). Notably, although *APOE* showed significantly increased expression in several cell types, significant differences in accessibility were only detected in astrocyte cluster 1 (Supplementary Figure 4).

To gain insight into the molecular mechanisms involved in the increased accessibility and increased expression of *APOE*, we determined which known transcription factor binding sites were present in the differentially accessible peaks and analyzed the accessibility and gene expression of those transcription factors. The two differentially accessible peaks at the *APOE* promoter overlap predicted binding sites for a set of 15 transcription factors (*GLI2, NPAS2, KLF15, HIF1A, LHX2, RXRA, MXI1, FOS, JUNB, KLF2, PURA, SREBF1, TEAD1, KLF6, ZBTB7C*) that are also differentially expressed and have differentially accessible genomic region between ancestries (Supplemental Table 4). From these 15 putative *APOE* binding transcription factors, *PURA* and *SREBF1* have increased accessibility and expression in ELA samples, *KLF6* has increased accessibility and expression in ALA samples and the other 12 transcription factors have increased accessibility in ELA and increased expression in ALA.

We identified 32 additional differentially accessible peaks with greater accessibility in ELA than ALA astrocytes in gene promoters (within 2kb from the TSS) in the LA region surrounding *APOE* (chr19:44–46MB), which corresponds to 19 additional genes, including the *APOE* proximal genes *TOMM40* and *APOC1* (Table 2). No genes in the defined LA region were more accessible in the ALA. In addition, by overlapping the differentially accessible peaks in the *APOE* LA region with previously classified enhancers (ELITE GeneHancer from USCS) we identified 32 additional peaks among 23 LA genes, all with increased accessibility in ELA brains (Supplementary Table 5).

Genome-wide differential accessibility

Since the global ancestry of the ELA samples were predominantly European and the European local ancestry blocks are uniformly distributed among ALA samples (Supplementary Figure 5), we performed genome-wide differential accessibility analysis. In total we identified 5,154 significant (FDR 0.05 and FC 2) differentially accessible peaks between the ancestries in three cell types (astrocytes, excitatory neurons and microglia), representing less than 1% of all called ATAC peaks. 99% of these differentially accessible peaks were seen in the astrocyte cluster 1, with an overall increased chromatin accessibility in the European ancestry blocks compared to African ancestry blocks (more accessible in ELA: 4,546 peaks; more accessible in ALA: 107 peaks) (Figure 3A). This astrocyte specific accessibility difference was widely spread across all chromosomes (Figure 3B). Among all differentially accessible peaks, 25.8% (2,248 peaks) are in promoters and TSS of genes, 33.5% (2,920 peaks) are intragenic, 34.1% (2,970 peaks) are intergenic and 6.6% (574 peaks) are in distal ELITE enhancers (Figure 3C), altogether corresponding to 6,067 genes.

Sixteen percent of the differentially accessible peaks were found on chromosome 19, a significant enrichment as compared to a random chromosomal distribution of peaks (Fischer exact test adjusted *p*-value=2.501E^{-31}) with 36.4% of these differentially accessible peaks in the promoter regions of chromosome 19 genes (Supplementary Figure 6).

Pathway enrichment of differentially accessible and expressed genes

To gain mechanistic insights from the integrative snRNA-seq and snATAC-seq approach, we determined the genome-wide overlap between DEG in the astrocyte clusters identified by

snRNA-seq and genes associated with differentially accessible peaks identified by snATAC-seq (DAG). From the 6,067 genes with differentially accessible peaks, 239 genes were both differentially expressed and accessible (DEG-DAG) between the ancestries (Supplementary table 6). However only 55 genes had concordant differences in expression and accessibility: 48 with increased accessibility and expression in ELA (including *APOE*) and 7 with reduced expression and accessibility in ELA. This set of 55 DEG-DAG in astrocytes were used to compute a functional enrichment analysis.

KEGG pathways (KEGG) and Gene Ontology (GO) analysis of gene set enrichment consider different category sets. KEGG pathway analyses identify over-representation of genes participating in the same biological process, while GO analyses aim to reduce complexity and focus on the common functional properties of the gene products. Thus, both analyses were performed, as it is expected that they will highlight different aspects of the data. Analysis of KEGG Molecular functional pathways showed enrichment in signaling pathways including calcium signaling and MAPK signaling pathway. The GO gene-sets highlighted alterations in the biological process of cholesterol metabolism, including regulation of cholesterol metabolic process and biosynthetic process, pathways linked to synapses and axonal transport, spine development and pathways linked to glial cell and astrocyte projection (Supplementary Table 7).

Using Human MSigDB database of disease associated genes from published transcriptomic studies revealed the highest overlap with lipopolysaccharide (LPS) treated astrocytes to induce reactivity (adjusted *p*-value =8.16E^{-10}, GSE75246)[54] and with genes showing altered expression in brains (adjusted *p*-value =2.26E^{-19}, GSE79666)[55] and astrocytes (adjusted *p*-value =2.92E^{-19})[56] of Huntington Disease (HD) individuals. The overlapping genes between the ancestry DEG-DAG and genes up-regulated in astrocytes of HD patients include a molecular signature of reactive astrocyte markers also observed in the HD striatal astroglia data, but also extends to pathways related to glutamatergic synapse and signaling. In addition, the ENCODE database of brain histone post-translational modifications identified an enrichment of DEG-DAG in those genes having H3K27me3 signals in astrocytes (H3K27me3 astrocytes Hg19, adjusted *p*-value = 0.02) (Supplementary Table 7).

Chromatin accessibility in AD candidate genes

We also assessed chromatin accessibility differences between the ancestries in AD associated genes, including Mendelian genes (*APP, PS1, PS2*, and *MAPT*) and genes suggested by GWAS and rare variant association studies across ancestries[57,58]. 32 differentially accessible peaks between the ancestries were identified in seven out of 76 AD-associated loci as defined by the ADSP Gene Verification Committee (https://adsp.niagads.org/gvc-top-hits-list/)[58]. The correlation between accessibility and expression of these seven genes with differential accessibility is shown in Table 3. Aside from *APOE*, nine differentially accessible peaks were observed to fall in the promoter areas of three genes, *SORL1, VRK3* and *ABCA7*, all with increased accessibility in ELA regions. Ten differentially accessible peaks fall in intergenic regions close to five AD genes, all with increase accessibility in ELA. In addition, twelve peaks are found in the gene body of four AD-associated genes (*CLU, PTK2B, SORL1, SPHK1*), also with increased accessibility in

ELA. Nonetheless, none of these genes containing differentially accessible peaks between local ancestries showed significant differential expression between ancestries (Table 3).

DISCUSSION:

To understand the complex mechanisms by which *APOE4* confers differential risk for AD in the context of genetic ancestry, we evaluated both gene expression and chromatin accessibility profile differences between homozygous *APOE4/4* ELA and ALA Alzheimer disease brains at single cell resolution. We confirmed our previous finding[11] that *APOE* is significantly more expressed in astrocytes of ELA compared to ALA. In fact, *APOE* is the most differentially expressed gene in astrocytes in the 2Mb local ancestry region surrounding *APOE*. Beyond being the most differentially expressed, the promoter of *APOE* in astrocytes showed a significant increase in accessibility in ELA compared to ALA. These results suggest that the increased *APOE4* expression previously reported in ELA *APOE4* carriers[11] may be partly due to differences in chromatin remodeling at the promoter of *APOE4* between ancestries. We have also shown using Capture Chromatin Conformation analysis and massively parallel reporter assays that specific DNA sequence differences in areas physically interacting with the *APOE* promoter have functional effects in microglia and astrocytes, with greater expression occurring in ELA sequence variants than ALA[59]. Thus, multiple mechanisms affecting *APOE4* gene expression in astrocytes may be activated at different times in life or by stress and could affect the differences in AD risk seen between ancestral homozygous *APOE4* carriers. This emphasizes that *APOE* loci is under a complex regulatory environment that involves cell type-specific processes and strengthens the primary functional role of *APOE* in astrocytes[60].

Though our principal research question focused on the underlying mechanisms leading to the higher expression of *APOE* in ELA brains using snATAC-seq, this study also provides a global view on the chromatin landscape of AD *APOE4/4* carriers from different genetic ancestries. One challenge is the relative scarcity of available autopsy material for African Americans. This is made further challenging as we required homozygous local ancestry for African local ancestry and homozygosity for the *APOE4* genotype. These requirements limited the available number for final analysis. It highlights the need for an effort to understand the concerns of the African American and Hispanic/Latino populations on participating in autopsy studies and work with these populations to increase the number of autopsies in AD family members. Non-Hispanic Europeans are not admixed and thus almost exclusively European local ancestry, so that the only additional limiting criteria is *APOE4* homozygosity.

We observed in astrocyte cluster 1 a significantly higher chromatin accessibility in ELA brains genome wide. A recent study using mesenchymal progenitor cells has reported that *APOE* accumulation in the nucleus can disrupt and destabilize heterochromatin, which would increase chromatin accessibility, similar to what we observe here in the ELA with higher *APOE* expression[61]. As astrocytes are the primary cells expressing *APOE*, this could explain the finding of increased global accessibility primarily in this cell type. Notably, only a small percent of significant peaks of higher chromatin accessibility overlap with the significant differentially expressed genes identified by snRNA-seq. Conversely, only 40%

of the significant differentially expressed genes showed significant changes in accessibility between the ancestries. This is in accordance with the growing understanding that gene expression depends on multiple regulatory elements[62], many located far from the gene locus itself[22]. Some of the DEG-DAG show a positive correlation between expression and accessibility, suggesting a functional relationship. However, we also observed an anti-correlation in accessibility-expression pairs. This anti-correlation could represent binding by repressors leading to a decrease in expression as previously described[63,64]. In addition, highly accessible sites not associated with enhanced expression could represent poised enhancers/promoter with no active transcription occurring[65]. Besides this known biological partial correlation of accessibility and expression, in this study the snATAC and snRNA were derived from separate, but adjacent, brain tissue. Differences in tissue composition could compound the discordance between these two outcomes. Future studies with paired snATAC-seq and snRNA-seq from the same nuclei such as the 10X Genomics Single Cell Multiome will minimize tissue composition effects if present. In fact, while we do identify differential expression of *APOE* between ancestries in several cell types (e.g., microglia), we only see significant differential accessibility of its promoter and immediate region in astrocytes.

Astrocytes have well established roles in neuronal metabolic support and neuroprotection, synapse formation and function, ion signaling homeostasis, integrity of the blood brain barrier, tissue repair and complex brain functions such as memory and sleep[66–70]. Thus, it is not surprising that a relevant role for astrocytes in AD pathology is suggested[71]. Our data suggest that astrocytes are involved in the modulation of *APOE4*-afforded AD risk and pinpoint this cell type as one of the most sensitive to differences in genetic ancestry. The significant enrichment of astrocytic ancestry-associated DEG-DAG in genes upregulated in HD astrocytes suggest that the differences associated with diverse ancestry entail functional properties that are potentially common to other neurodegenerative disorders. In addition to this astrocyte-ancestry association, we also evidence a difference in DEG-DAG between our astrocyte clusters 1 and 2. We observed that our astrocyte cluster 1 was transcriptionally most similar to astrocytes subclusters a4 and a8 described by Grubman et al[52], clusters where *APOE* was upregulated in AD[52]; while our astrocyte subcluster 2 was transcriptionally comparable to astrocytes subcluster a2 where *APOE* was downregulated.

Interestingly, there was a significant enrichment of differential chromatin accessibility and expression on chromosome 19 relative to the rest of the genome (Supplemental Figure 5) in astrocyte cluster 1. These results correlate with the observation of a comparatively high number of ATAC-seq peaks in chromosome 19[72], potentially due to the high gene density, GC content and DNA binding proteins in chromosome 19 compared to other chromosomes[73–75]. It is possible the high gene density and the structural nature of chromosome 19 could modulate the differential binding of chromatin modifying enzymes, explaining the enrichment in differentially accessible chromatin and gene expression in this specific chromosome observed between ancestries.

Our analysis of transcription factors with binding sites in the differentially accessible peaks at the 5'end of *APOE* that were both differentially expressed and accessible between ancestries suggests transcriptional modulators of *APOE* expression differences. Of the 15

transcription factors we identified, only *SREBF1* and *PURA* showed increased accessibility (intergenic and intronic) and expression in ELA samples and *KLF6* showed increased accessibility (distal enhancer) and expression in ALA samples. A polymorphism in *SREBF1* has been shown to influence the risk of AD specifically in *APOE4* carriers[76], while *PURA* has been shown to regulate expression of AD and Aβ clearance related genes[77]. On the other hand, *KLF6* is found to be differentially expressed in AD brains and implicated in Aβ-induced oxidative stress[78]. Their role with *APOE* activation has not been previously described, although *SREBF1* is a major regulator of lipid metabolism.

Although many genetic risk factors for disease are expected to be shared among populations, genomic diversity among ancestries can provide new opportunities for discoveries regarding disease susceptibility. In AD, understanding why the *APOE4* allele confers a lower risk to African ancestry carriers versus European ancestry carriers is instrumental for the identification of potential AD therapeutics. Here we provide chromatin accessibility and gene expression data from AD *APOE4* carriers of African and European ancestries at single cell resolution. One of the main challenges in comparing local ancestry effects is the current limitation of available samples from diverse ancestries. The need for both specific genotype and local ancestry criteria further restricted the number of samples available. This highlights the importance of increasing autopsy material from diverse populations. Expansion of this study into other *APOE* genotypes and more diverse ancestries could provide further insight into the importance of brain astrocytes and chromatin landscape of *APOE,* as well as genome-wide changes, implicating specific molecular pathways and regulatory proteins in this risk difference. Finally, these findings support the concept of reducing *APOE4* expression as a potential therapeutic pathway for AD.

CONCLUSIONS:

Our results provide novel compelling insights to understand the AD risk difference known to exist between European and African *APOE4* local ancestry carriers. Here we demonstrate that differences in chromatin accessibility between the African and European *APOE* locus could explain the expression differences in *APOE* expression in astrocytes and supports the concept that this contributes to the risk difference between African American and European populations for AD in *APOE4* carriers. We found that this increase in accessibility in the region surrounding *APOE4* in European astrocytes extended genome-wide, suggesting a wider mechanism of regulatory dysfunction is occurring in these cells. Not only is it important to include diverse ancestries to ensure all individuals are represented in research, but also we have shown here and in previous work that including diversity in research can provide additional windows of opportunity to elucidate disease and biological mechanisms. Understanding regulatory dysfunction in AD has not been well studied and should be and area of future research in AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Availability of data and material

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/datasets/ng00067/

LIST OF ABBREVIATIONS:

AD	Alzheimer Disease
ABCA7	ATP Binding Cassette Subfamily A Member 7
ACER3	Alkaline Ceramidase 3
ADRC	Alzheimer Disease Research Centers
AIF1	Allograft inflammatory factor 1
ALA	African Local Ancestry
APOC1	Apolipoprotein C1
APOE	Apolipoprotein E
APOE4	Apolipoprotein E allele 4
APOE4/4	Homozygous for Apolipoprotein E allele 4
APP	Amyloid Beta Precursor Protein
ATAC	Assay for Transposase-Accessible Chromatin
Azimth	App for reference-based single-cell analysis
Aβ	Amyloid beta
BRAAK	Braak staging (pathology score for Alzheimer Disease)
CGT	Center for Genome Technology
CLU	Clusterin

COL1A2	Collagen Type I Alpha 2 Chain
CSPG4	Chondroitin Sulfate Proteoglycan 4
DAG	Differentially Accessible Genes
DAP	Differentially Accessible Peaks
DEG	Differentially Expressed Genes
DEG-DAG	Differentially Expressed and Accessible Genes
ECHAD	Entorhinal Cortex in Human Alzheimer's Disease
ELA	European Local Ancestry
ELITE	enhancer gene with both a high likelihood enhancer definition and a strong enhancer–gene association
ENCODE	Encyclopedia of DNA Elements
FC	Fold Change
FDR	false discovery rate
FOS	Fos Proto-Oncogene, AP-1 Transcription Factor Subunit
GC	Guanine-Cytosine content
GeneSigDb	Gene Signature Data Base
GFAP	Glial Fibrillary Acidic Protein
GLI2	GLI Family Zinc Finger 2
GO	Gene Ontology
GRCh38	Genome Reference Consortium Human Build 38
GREAT	Genomic Regions Enrichment of Annotations Tool
H3K27me3	tri-methylation of lysine 27 on histone H3 protein
HD	Huntington Disease
HDSigDb	Huntigton Disease Signature Database
HIF1A	Hypoxia Inducible Factor 1 Subunit Alpha
HIHG	John P. Hussman Institute for Human Genomics
HOMER	Hypergeometric Optimization of Motif EnRichment
JASPAR	transcription factor binding profile database
JOLMA	Human specific transcription factor binding

JUNB	JunB Proto-Oncogene, AP-1 Transcription Factor Subunit
KEGG	Kyoto Encyclopedia of Genes and Genomes
KLF15	Kruppel Like Factor 15
KLF2	Kruppel Like Factor 2
KLF6	Kruppel Like Factor 6
LA	Local Ancestry
LHX2	LIM Homeobox 2
LPS	lipopolysaccharide
LSI	Iterative Latent Semantic Indexing
MAG	Myelin Associated Glycoprotein
MAPT	microtubule-associated protein tau
MB	Million Base pair
Mb	Mega base
MsigDB	Molecular Signatures Database
MXI1	MAX interactor-1
NACC	National Alzheimer Coordinating Center
NPAS2	Neuronal PAS Domain Protein 2
OPCs	oligodendrocyte precursor cells
PanglaoDB	Single-cell sequencing mouse and human database
PCR	Polymerase chain reaction
PSEN1	Presenillin-1
PSEN2	Presenillin-2
PURA	Purine Rich Element Binding Protein A
Reactome	pathway database
RSB	Resuspension Buffer
RXRA	Retinoid X Receptor Alpha
SLC17A7	Solute Carrier Family 17 Member 7
SLC32A1	Solute Carrier Family 32 Member 1

snATAC-seq	single nuclei Assay for Transposase-Accessible Chromatin sequencing
snRNA-seq	single nuclei RNA sequencing
SORL1	Sortilin Related Receptor 1
SPHK1	Sphingosine Kinase 1
SREBF1	Sterol Regulatory Element Binding Transcription Factor 1
TEAD1	TEA Domain Transcription Factor 1
TF	Transcription Factor
TOMM40	Translocase Of Outer Mitochondrial Membrane 40
TREM2	Triggering Receptor Expressed On Myeloid Cells 2
TSS	Transcription Starting Site
TTS	transcription terminating site
UCSC	University of California Santa Cruz
USUHS	Uniformed Services University of the Health Sciences
VLMC	vascular leptomeningeal cell
VRK3	VRK Serine/Threonine Kinase 3
ZBTB7C	Zinc Finger And BTB Domain Containing 7C
WGS	Whole Genome Sequencing

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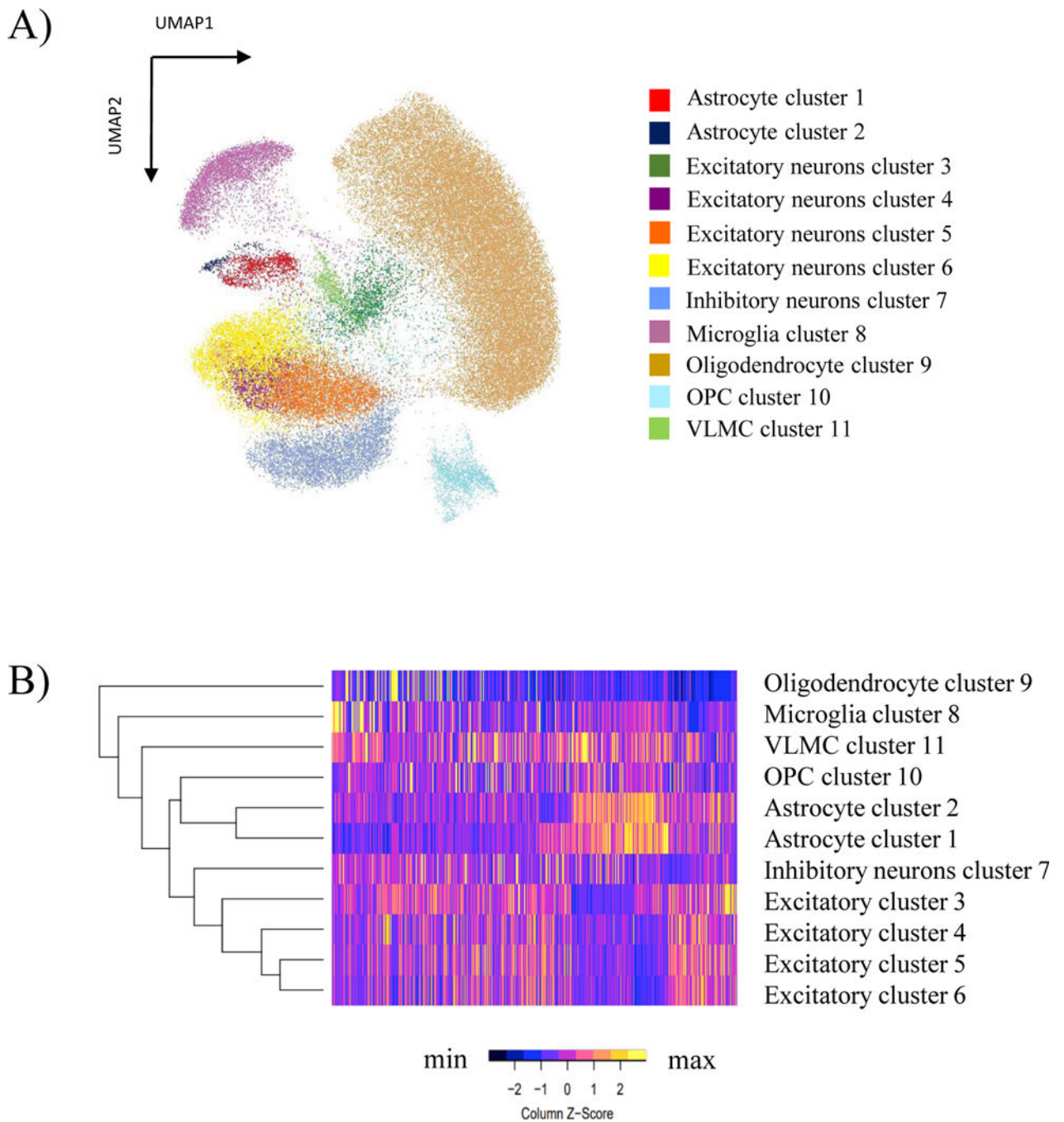
Figure 1. Visualization of the integrated single nuclei ATAC and RNA sequencing clusters and cell identification across the samples.

A) UMAP reduction plot using resolution of 0.4 resulting in 11 cell type clusters from the integration of snATAC-seq and snRNA-seq data. B) Heatmap showing cell cluster identification by chromatin accessibility patterns using the top 50 snATAC-Seq predicted gene scores for markers genes of the known cortex cell types.
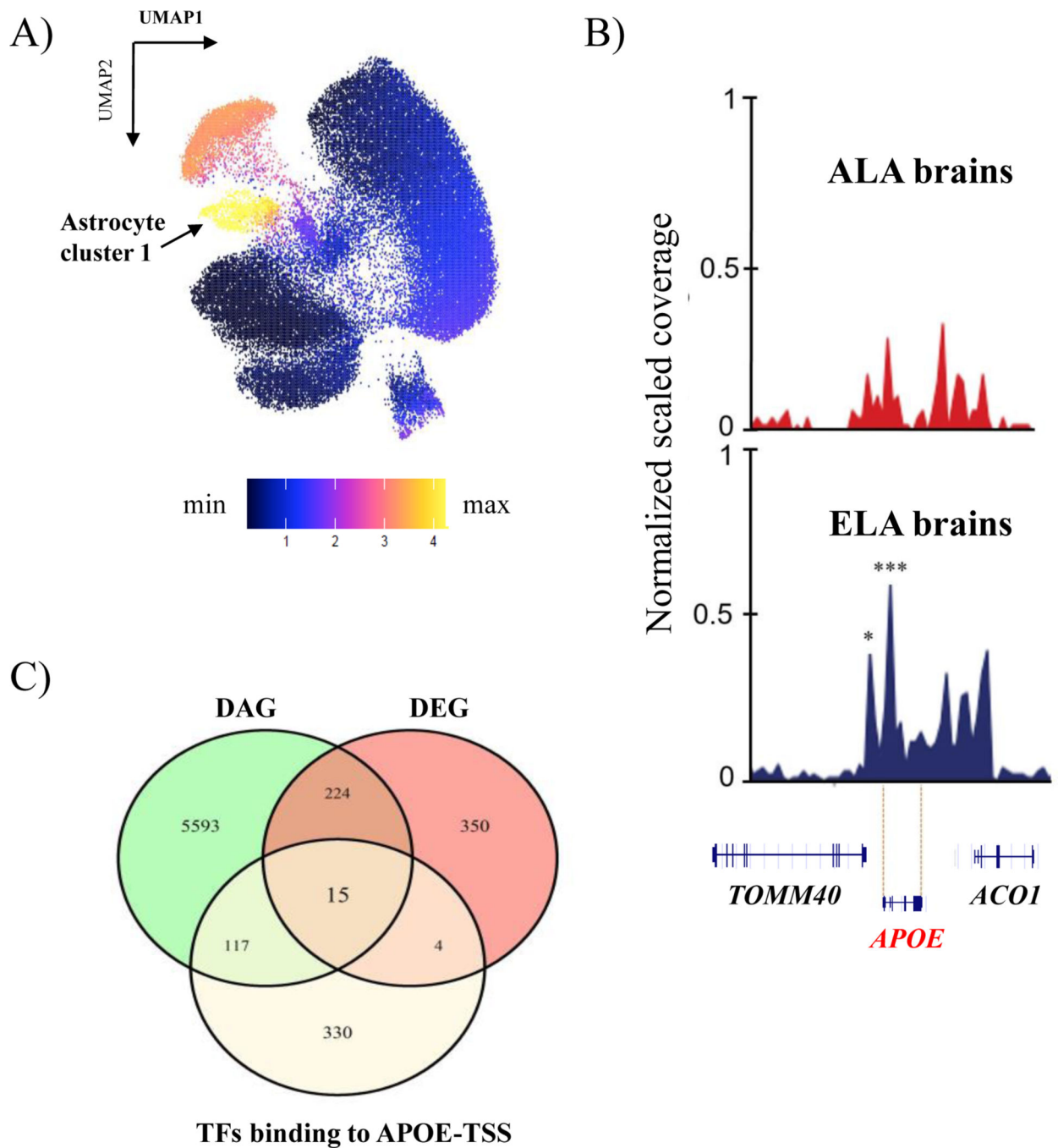
Figure 2. Differential chromatin accessibility and expression of *APOE* in Astrocyte cluster 1.
A) *APOE* expression represented in clusters generated by the integrated snATAC-seq and snRNA-seq data; B) Visualization of chromatin differential accessible peaks in the *APOE* locus between ancestries from Astrocyte cluster 1. *Represents significantly differentially accessible peaks between ancestries (*FDR=0.02; ***FDR=0.001). C) Venn diagram showing transcription factors biding to *APOE* that are differentially expressed (DEG) and differentially accessible (DAG).
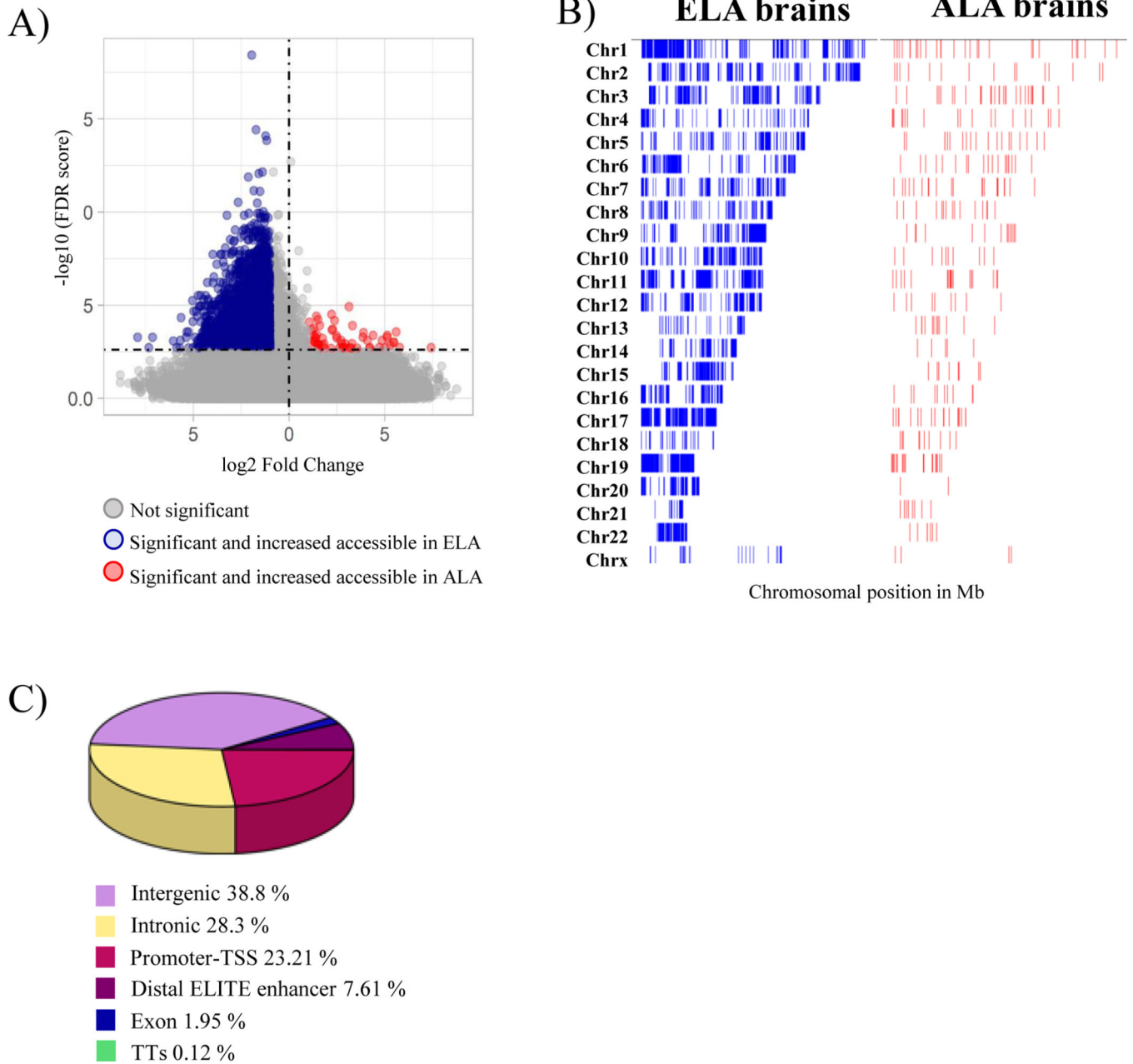
Figure 3. Chromatin accessibility differences between ancestries genome-wide in Astrocyte cluster 1.

A) Volcano plot representation of global chromatin accessibility difference between ancestries in Astrocyte cluster 1; B) Chromatin accessibility differences across the genome displayed by chromosomes. Blue is increased accessibility in Europeans, Red is increased accessibility in African ancestry; C) Pie chart showing the differentially accessible peak region distribution in Astrocyte cluster 1.

Table 1.

Demographic characteristics of the samples

Sample	Center	Sex	AOD	*APOE* genotype	Local Ancestry (Chr19: 44–46Mb)	% Of Global Ancestry	BRAAK staging score
1	Emory	Female	82	4,4	AF/AF	87% AF	V
2	NW	Female	85	4,4	AF/AF	86% AF	V
3	Emory	Female	80	4,4	AF/AF	88% AF	VI
4	Rush	Male	93	4,4	AF/AF	68% AF	V
5	Rush	Male	83	4,4	AF/AF	79% AF	VI
6	Rush	Female	77	4,4	AF/AF	85% AF	VI
7	UM/Duke	Male	75	4,4	EU/EU	95% EU	IV
8	Rush	Male	71	4,4	EU/EU	98% EU	VI
9	UM/Duke	Female	70	4,4	EU/EU	95% EU	VI
10	Rush	Male	76	4,4	EU/EU	97% EU	IV
11	UM/Duke	Female	76	4,4	EU/EU	96% EU	V
12	Rush	Female	86	4,4	EU/EU	98% EU	VI

AOD: Age of Death; UM: University of Miami; NW: Northwestern University; RUSH: RUSH University Medical Center; AF: African; EU: European

Table 2.

Differentially accessible peaks in promoter regions of Local Ancestry genes in Astrocyte cluster 1.

Gene	Chromosome	Peak location	Distance to TSS	FC	FDR	Region
CTB-171A8.1	chr19	44718456	−21	3.66	0.003	promoter-TSS
BCL3	chr19	44748309	12	2.77	0.007	promoter-TSS
BCAM	chr19	44808802	−7	5.11	0.043	promoter-TSS
BCAM	chr19	44808802	−51	5.11	0.043	promoter-TSS
CTB-129P6.4	chr19	44890205	421	2.70	0.002	promoter-TSS
TOMM40	chr19	44890205	−781	2.70	0.002	promoter-TSS
APOE*	chr19	44903514	−1990	4.52	0.022	promoter-TSS
APOE*	chr19	44905485	−19	2.57	0.001	promoter-TSS
APOC1	chr19	44914897	278	2.05	0.000	promoter-TSS
APOC1	chr19	44914897	900	2.05	0.000	promoter-TSS
APOC1P1	chr19	44926992	266	2.23	0.025	promoter-TSS
CLPTM1	chr19	44955080	84	3.61	0.007	promoter-TSS
CLPTM1	chr19	44955080	84	3.61	0.007	promoter-TSS
CTB-179K24.3	chr19	45091377	−1236	2.19	0.027	promoter-TSS
PPP1R37	chr19	45091377	−1335	2.19	0.027	promoter-TSS
MARK4*	chr19	45294609	504	3.65	0.011	promoter-TSS
ERCC2	chr19	45370425	−88	2.40	0.002	promoter-TSS
ERCC2	chr19	45370425	−57	2.40	0.002	promoter-TSS
PPP1R13L	chr19	45405279	−491	3.68	0.004	promoter-TSS
CD3EAP	chr19	45405279	−1162	3.68	0.004	promoter-TSS
PPP1R13L	chr19	45405279	820	3.68	0.004	promoter-TSS
FOSB	chr19	45467194	−551	2.61	0.024	promoter-TSS
VASP	chr19	45506348	−818	3.58	0.020	promoter-TSS
OPA3	chr19	45584758	−166	2.16	0.000	promoter-TSS
EML2*	chr19	45644874	505	7.63	0.032	promoter-TSS
EML2*	chr19	45645399	−20	3.28	0.002	promoter-TSS
FBXO46	chr19	45730087	567	4.28	0.025	promoter-TSS
FBXO46	chr19	45730692	−38	2.01	0.028	promoter-TSS
FBXO46	chr19	45730692	−38	2.01	0.028	promoter-TSS
RSPH6A	chr19	45815742	−673	3.10	0.033	promoter-TSS
IRF2BP1	chr19	45885449	471	4.72	0.025	promoter-TSS
NOVA2	chr19	45973825	−529	5.11	0.004	promoter-TSS
CCDC61	chr19	45995053	−158	2.38	0.000	promoter-TSS
CCDC61	chr19	45995053	−164	2.38	0.000	promoter-TSS

FC: Fold change; TSS: Transcription Starting Site; Negative value in Distance to TSS reflects peaks falling upstream of TSS

Table 3.

Differentially accessible peaks in Astrocyte cluster 1 in Alzheimer disease GWAS candidate genes.

Gene	Chr.	Peak location	Cell cluster	Distance to TSS	SnATAC FC	SnATAC FDR	SnRNA FC	SnRNA FDR	Region
PTK2B	chr8	27340471	Astrocyte 1	29239	2.327	0.026	−1.05	4.67E-13	intron
CLU	chr8	27596089	Astrocyte 1	18692	4.235	0.019	−1.15	3.39E-73	intergenic
CLU	chr8	27597084	Astrocyte 1	17697	2.556	0.035	−1.15	3.39E-73	exon
CLU	chr8	27597084	Astrocyte 1	17697	2.556	0.035	−1.15	3.39E-73	intron
CLU	chr8	27597917	Astrocyte 1	16864	18.113	0.008	−1.15	3.39E-73	exon
CLU	chr8	27597917	Astrocyte 1	16864	18.113	0.008	−1.15	3.39E-73	intron
CLU	chr8	27607577	Astrocyte 1	7204	6.695	0.032	−1.15	3.39E-73	intron
ACER3	chr11	76799174	Astrocyte 1	−61449	2.41	0.008	1.04	1.12E-30	intergenic
SORL1	chr11	121566449	Astrocyte 1	114496	3.538	0.044	−1.05	4.51E-53	exon
SORL1	chr11	121566449	Astrocyte 1	114496	3.538	0.044	−1.05	4.51E-53	intron
SORL1	chr11	121566449	Astrocyte 1	−26	3.538	0.044	−1.05	4.51E-53	promoter-TSS
SORL1	chr11	121591362	Astrocyte 1	139409	7.332	0.011	−1.05	4.51E-53	intron
SORL1	chr11	121591362	Astrocyte 1	1193	7.332	0.011	−1.05	4.51E-53	promoter-TSS
SORL1	chr11	121596337	Astrocyte 1	6168	6.485	0.046	−1.05	4.51E-53	intron
SORL1	chr11	121596337	Astrocyte 1	144384	6.485	0.046	−1.05	4.51E-53	intron
SORL1	chr11	121695992	Astrocyte 1	105823	3.063	0.039	−1.05	4.51E-53	intergenic
SORL1	chr11	121695992	Astrocyte 1	244039	3.063	0.039	−1.05	4.51E-53	intergenic
SORL1	chr11	121795198	Astrocyte 1	205029	3.099	0.027	−1.05	4.51E-53	intergenic
SORL1	chr11	121795198	Astrocyte 1	343245	3.099	0.027	−1.05	4.51E-53	intergenic
SORL1	chr11	121929505	Astrocyte 1	477552	3.494	0.004	−1.05	4.51E-53	intergenic
SORL1	chr11	121932043	Astrocyte 1	480090	11.926	0.031	−1.05	4.51E-53	intergenic
SPHK1	chr17	76397939	Astrocyte 1	13539	4.176	0.041	−1.01	9.98E-43	intergenic
SPHK1	chr17	76397939	Astrocyte 1	12897	4.176	0.041	−1.01	9.98E-43	intron
SPHK1	chr17	76414406	Astrocyte 1	30006	15.903	0	−1.01	9.98E-43	intergenic
ABCA7	chr19	1039766	Astrocyte 1	−87	2.429	0.001	1.09	2.95E-21	promoter-TSS
ABCA7	chr19	1040877	Astrocyte 1	−119	4.091	0.002	1.09	2.95E-21	promoter-TSS
ABCA7	chr19	1040877	Astrocyte 1	1024	4.091	0.002	1.09	2.95E-21	promoter-TSS
VRK3	chr19	50024717	Astrocyte 1	360	5.864	0.043	1.09	4.88E-24	promoter-TSS
VRK3	chr19	50024717	Astrocyte 1	979	5.864	0.043	1.09	4.88E-24	promoter-TSS
VRK3	chr19	50025268	Astrocyte 1	−116	3.408	0.002	1.09	4.88E-24	promoter-TSS
VRK3	chr19	50025268	Astrocyte 1	428	3.408	0.002	1.09	4.88E-24	promoter-TSS

Gene	Chr.	Peak location	Cell cluster	Distance to TSS	SnATAC FC	SnATAC FDR	SnRNA FC	SnRNA FDR	Region
VRK3	chr19	50050285	Astrocyte 1	−24589	5.267	0.002	1.09	4.88E-24	intergenic

FC: Chr.: Chromosome; FC:Fold change; TSS: Transcription Starting Site; Negative value in Distance to TSS reflects peaks falling upstream of TSS