

1           **The Landscape of Sex- and APOE Genotype-Specific Transcriptional Changes in**  
2           **Alzheimer's Disease at the Single Cell Level**

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30      **Abstract**  
31  
32      Alzheimer's disease (AD) is the most common form of dementia, with approximately two-thirds  
33      of AD patients are females. Basic and clinical research studies show evidence supporting sex-  
34      specific differences contributing to the complexity of AD. There is also strong evidence supporting  
35      sex-specific interaction between the primary genetic risk factor of AD, *APOE4* and AD-associated  
36      neurodegenerative processes. Recent studies by us and others have identified sex and/or *APOE4*  
37      specific differentially expressed genes in AD based on the bulk tissue RNA-sequencing data of  
38      postmortem human brain samples in AD. However, there lacks a comprehensive investigation of  
39      the interplay between sex and *APOE* genotypes at the single cell level. In the current study, we  
40      systematically explore sex and *APOE* genotype differences in single cell transcriptomics in AD.  
41      Our work provides a comprehensive overview of sex and *APOE* genotype-specific transcriptomic  
42      changes across 54 high-resolution cell types in AD and highlights individual genes and brain cell  
43      types that show significant differences between sexes and *APOE* genotypes. This study lays the  
44      groundwork for exploring the complex molecular mechanisms of AD and will inform the  
45      development of effective sex- and *APOE*-stratified interventions for AD.

46

47 **INTRODUCTION**

48  
49 Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder of aging <sup>1</sup>, with  
50 approximately two-thirds of AD patients are females <sup>2</sup>. There are conflicting literature reports  
51 regarding AD incidents among males and females with some suggesting a higher AD incidence in  
52 females than males and others suggesting no differences <sup>3-7</sup>. Evidence from basic and clinical  
53 research studies supports sex-specific differences contributing to the complexity of AD. For  
54 example, sex-biased changes in brain structures and connectome have been demonstrated in AD  
55 subjects using various neuroimaging modalities. It was found that the rates of brain atrophy in  
56 females were 1-1.5% faster than those in males, most prominently seen in the MCI group with a  
57 1% increase in atrophic rates and 2% in ventricular expansion <sup>8</sup>. Sex-specific brain region atrophy  
58 have also been reported <sup>9</sup>. Moreover, a significantly higher reduction of hippocampal integrity in  
59 female AD subjects was reported when compared to male subjects with Magnetic Resonance  
60 Imaging studies of the Minimal Interval Resonance Imaging in AD database <sup>10</sup>.

61 There is strong evidence supporting sex-specific interaction between the primary genetic  
62 risk factor of AD, *APOE4* <sup>11</sup> and AD-associated neurodegenerative processes <sup>12, 13</sup>. The sex-  
63 specific association between *APOE4* and tau has been reported <sup>14, 15</sup>. Furthermore, studies suggest  
64 a stronger association of *APOE4* with cortical thinning, volume loss, brain connectivity and hypo-  
65 metabolism in AD females than males <sup>16-18</sup>. Moreover, stronger associations of *APOE4* with  
66 cognitive impairment, memory decline, and neuropsychiatric symptoms of AD in females were  
67 reported <sup>19, 20</sup>. On the other hand, male-specific associations with cerebral amyloid angiopathy  
68 (CAA) and microbleed in AD have been reported <sup>21</sup>. Even so, little has been done to understand  
69 the interplay between *APOE* and sex in ageing and AD.

70 Recently, we systematically identified sex and *APOE4* specific differentially expressed  
71 genes in AD<sup>22</sup> based on the bulk tissue RNA-sequencing data of postmortem human brain samples  
72 from the Mount Sinai Brain Bank (MSBB) cohort <sup>23</sup> and the Religious Orders Study and Rush  
73 Memory and Aging Project (ROSMAP) cohort <sup>24</sup>. We further developed sex-specific gene  
74 network models of AD to predict sex specific driver genes of AD and subsequently validated  
75 *LRP10* (lipoprotein receptor related protein 10) as a female-specific driver of AD pathogenesis<sup>22</sup>.

76 In the current study, we further investigate the interplay between sex and *APOE* in AD at  
77 single cell level through differential gene expression analysis of single-nucleus RNA sequencing  
78 data. The findings not only provide a comprehensive overview of sex and *APOE* genotype-specific  
79 transcriptomic changes across 54 high-resolution cell types in AD but also highlight individual  
80 genes and brain cell types that show significant differences between sexes and *APOE* genotypes.  
81 This study lays the groundwork for exploring the complex molecular mechanisms of AD and will  
82 inform the development of effective sex- and *APOE*-stratified interventions for AD.

83  
84 **RESULTS**

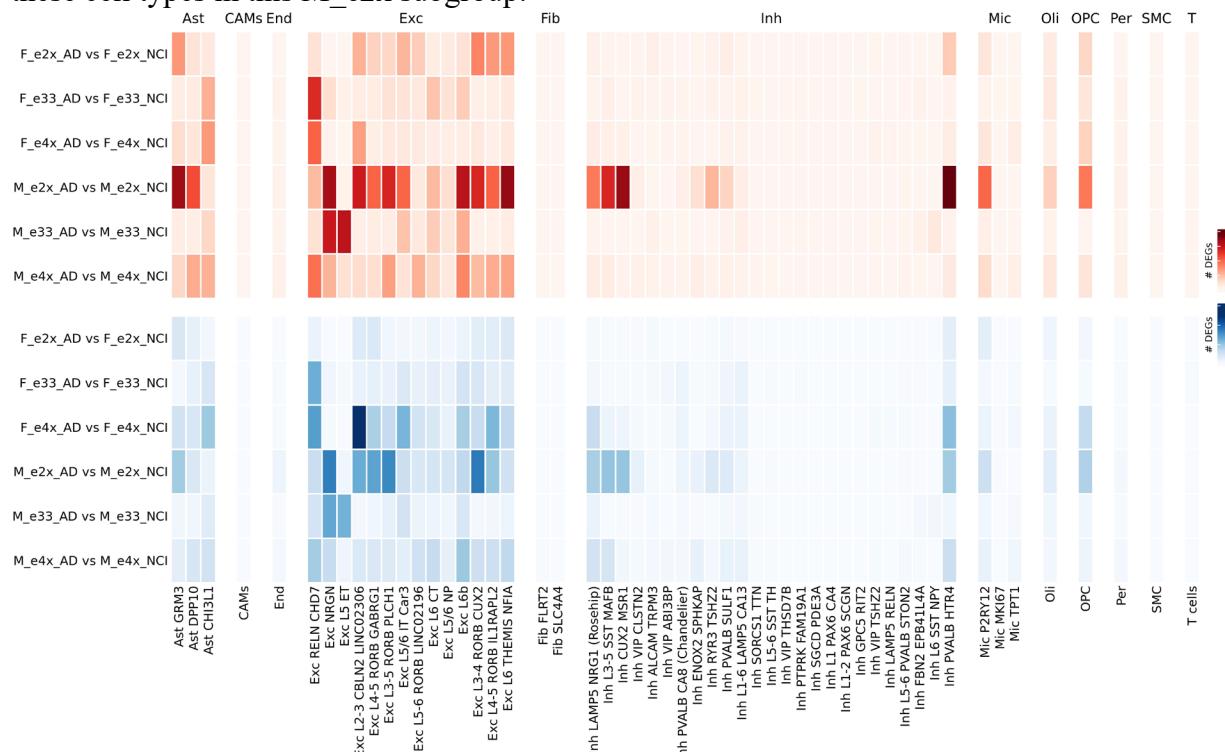
85  
86 We leveraged a single-nucleus RNA sequencing (snRNA-seq) dataset which was generated from  
87 postmortem brain tissues of subjects with or without dementia by Mathys et al.<sup>25</sup>. The dataset  
88 profiled 2,359,994 nuclei from 427 prefrontal cortex brain samples, including 215 female and 212  
89 male donors. Disease status was categorized using the final consensus cognitive diagnosis score  
90 (cogdx), where 1 = no cognitive impairment (NCI), 2 = mild cognitive impairment (MCI), 4 =  
91 Alzheimer's disease (AD), and 3, 5, 6 were categorized as other dementia. For this study, we  
92 focused solely on NCI (n = 146) and AD (n = 144) samples. To investigate the impact of *APOE*

genotypes, we grouped participants as follows: *APOE* e22 and e23 were categorized as *APOE* e2 carriers (e2x; n = 58), e33 remained as e33 (n = 252), and e34 and e44 were classified as *APOE* e4 carriers (e4x; n=105). *APOE* e24 carriers were excluded from the analysis due to the controversial nature of their association with disease risk.

We utilized the 54 cell clusters identified in the original study<sup>25</sup>: 14 excitatory neuron clusters, 25 inhibitory neuron clusters, 1 oligodendrocyte cluster, 1 oligodendrocyte precursor cell cluster, 3 astrocyte cell clusters, 3 microglia cell clusters, 1 central nervous system (CNS)-associated macrophage (CAM) cluster, 1 T cell cluster, 1 endothelial cells (End) cluster, 1 smooth muscle cell (SMC) cluster, 2 fibroblasts (Fib) clusters, and 1 pericytes (Per) cell cluster.

### Cell type specific gene expression changes in AD in each sex-*APOE* subgroup

To investigate the transcriptional changes associated with AD in each *APOE* genotype (e2x, e33, and e4x) or sex group (female (F) and male (M)), we performed cell-cluster-specific differential gene expression (DEG) analysis across six stratified comparisons. For each sex-*APOE* group (F\_e2x, F\_e33, F\_e4x, M\_e2x, M\_e33, and M\_e4x), we compared gene expression profiles of the individuals with AD with those with no cognitive impairment (NCI). This stratification allowed us to capture genotype- and sex-specific transcriptional alterations in AD in each cell cluster, offering a detailed view of how these factors shape the molecular landscape in AD. The numbers of up-regulated and down-regulated DEGs identified in these comparisons are shown in **Figure 1**. The distribution of DEGs across cell clusters revealed distinct patterns. Excitatory neurons exhibited a substantial number of DEGs in almost all sex-*APOE* groups. Notably, the AD males with *APOE* e2 carriers (M\_e2x) had the highest number of DEGs compared to other sex-*APOE* groups, particularly in the excitatory neuron (Exc), inhibitory neuron (Inh), OPC, and microglia (Mic) clusters. This suggests a potentially heightened transcriptional response to AD in these cell types in this M\_e2x subgroup.



119 **Figure 1. Heatmap showing the number of differentially expressed genes (DEGs) between**  
120 **AD samples and NCI samples within each sex-APOE group (F\_e2x, F\_e33, F\_e4x, M\_e2x,**  
121 **M\_e33, M\_e4x) across all the 54 cell clusters.** The upper heatmap represents the number of  
122 upregulated DEGs (red), while the lower heatmap depicts the number of downregulated DEGs  
123 (blue). Each row corresponds to a specific comparison, and each column represents a distinct cell  
124 cluster.

125

## 126 **Comparative study of transcriptional signatures between different sex-APOE subgroups**

127 To further explore transcriptional changes in AD, we conducted a series of pairwise  
128 comparisons to identify the overlap and divergence in differentially expressed genes (DEGs)  
129 across APOE genotypes and between sexes. These analyses aimed to identify both shared and  
130 unique transcriptional responses among subgroups, offering insights into how genotype and sex  
131 interact to influence molecular changes in AD in each brain cell type.

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## 133 **Comparison of transcriptional signatures across *APOE* genotypes in females and males**

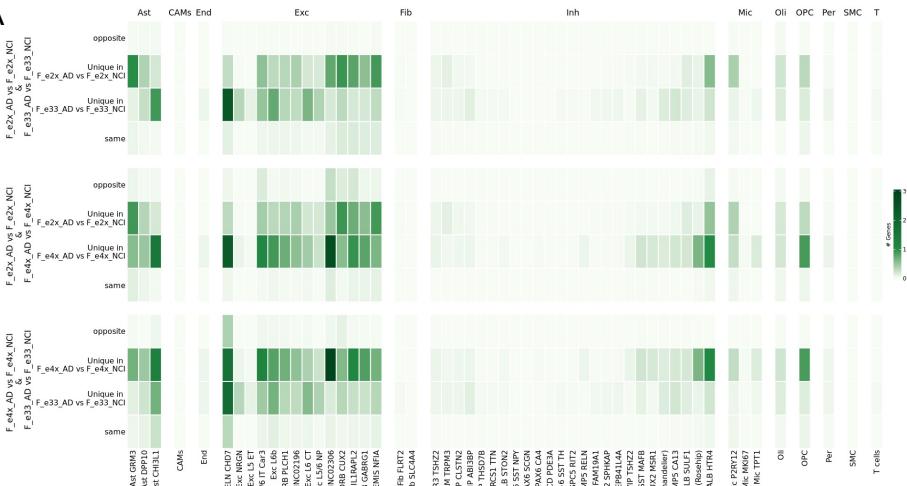
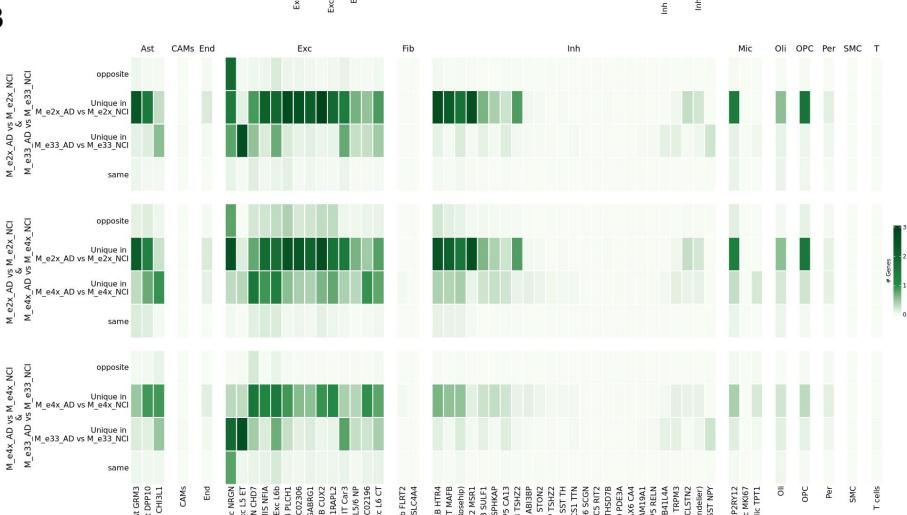
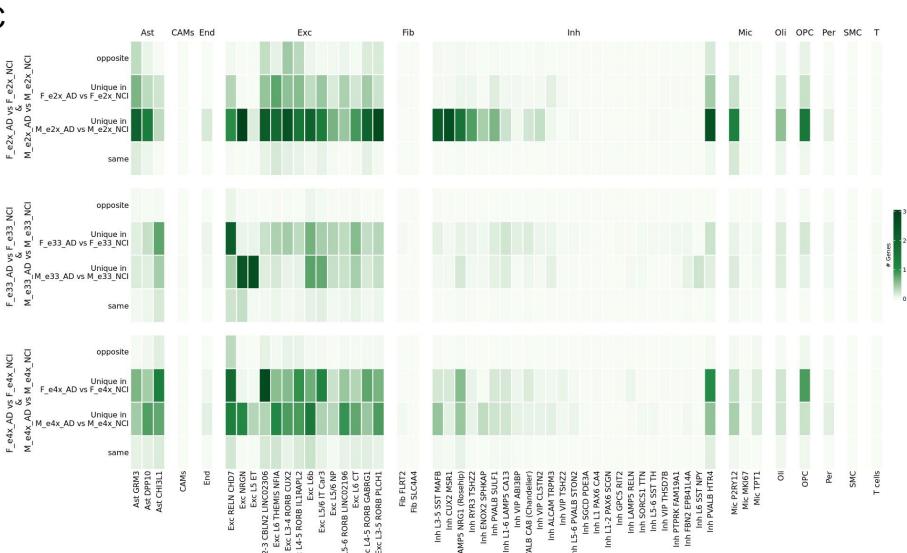
134 We first compared the DEG signatures between females across *APOE* genotypes (F\_e2x,  
135 F\_e33, and F\_e4x) to explore *APOE* genotype-specific transcriptional patterns in female (**Fig. 2A**).  
136 Comparisons included 1). F\_e2x\_AD vs. F\_e2x\_NCI and F\_e33\_AD vs. F\_e33\_NCI, 2).  
137 F\_e2x\_AD vs. F\_e2x\_NCI and F\_e4x\_AD vs. F\_e4x\_NCI, and 3). F\_e4x\_AD vs. F\_e4x\_NCI and  
138 F\_e33\_AD vs. F\_e33\_NCI. Notably, *APOE* e4 carriers showed more distinct transcriptional  
139 changes than the other genotypes, highlighting the unique impact of the e4 genotype on AD  
140 females.

141 The same analysis was applied to the male *APOE* groups (M\_e2x, M\_e33, and M\_e4x),  
142 including 1) M\_e2x\_AD vs. M\_e2x\_NCI and M\_e33\_AD vs. M\_e33\_NCI, 2) M\_e2x\_AD vs.  
143 M\_e2x\_NCI and M\_e4x\_AD vs. M\_e4x\_NCI, and 3) M\_e4x\_AD vs. M\_e4x\_NCI and  
144 M\_e33\_AD vs. M\_e33\_NCI. As shown in **Fig. 2B**, *APOE* e2 carriers exhibited a more pronounced  
145 transcriptional divergence from other genotypes, particularly within excitatory neurons, inhibitory  
146 neurons and astrocytes. This pattern suggests that *APOE* e2 carriers in males may activate certain  
147 distinct transcriptional programs in AD, with greater cell-type specificity than observed in females.

148

## 149 **Comparison Across Sexes Within Each *APOE* Genotype**

150 Lastly, we examined the overlap and difference in DEGs between sexes for each *APOE*  
151 genotype (**Fig. 2C**). Comparisons included 1) F\_e2x\_AD vs. F\_e2x\_NCI and M\_e2x\_AD vs.  
152 M\_e2x\_NCI, 2) F\_e33\_AD vs. F\_e33\_NCI and M\_e33\_AD vs. M\_e33\_NCI, and 3) F\_e4x\_AD  
153 vs. F\_e4x\_NCI and M\_e4x\_AD vs. M\_e4x\_NCI. The results, visualized in **Figure 2C**,  
154 demonstrate significant sex-specific transcriptional differences in *APOE* e2 and *APOE* e4  
155 genotypes. *APOE* e33 groups (F\_e33\_AD vs. F\_e33\_NCI and M\_e33\_AD vs. M\_e33\_NCI) had  
156 relatively fewer transcriptional differences between sexes across most cell clusters compared to  
157 e2x or e4x carriers. This observation aligns with the known biological role of the e33 genotype,  
158 which is generally considered neutral in terms of AD risk, in contrast to e2, which is protective,  
159 and e4, which is associated with heightened risk. The limited sex-specific divergence in e33  
160 carriers suggests a more stable transcriptional landscape, potentially reflecting the absence of  
161 strong protective or risk-associated pressures on gene expression within this genotype.

**A****B****C**

163 **Figure 2. Comparative study of transcriptional signatures between different sex-APOE**  
164 **subgroups.** A) Heatmap illustrating the overlap, unique, and opposite differentially expressed  
165 genes (DEGs) between female APOE groups (F\_e2x, F\_e33, and F\_e4x) across cell clusters. Each  
166 row corresponds to a specific comparison, including: 1). F\_e2x\_AD vs. F\_e2x\_NCI and  
167 F\_e33\_AD vs. F\_e33\_NCI, 2). F\_e2x\_AD vs. F\_e2x\_NCI and F\_e4x\_AD vs. F\_e4x\_NCI, and 3).  
168 F\_e4x\_AD vs. F\_e4x\_NCI and F\_e33\_AD vs. F\_e33\_NCI. B) Heatmap illustrating the overlap,  
169 unique, and opposite differentially expressed genes (DEGs) between male APOE groups (M\_e2x,  
170 M\_e33, and M\_e4x) across cell clusters. Each row corresponds to a specific comparison, including:  
171 1). M\_e2x\_AD vs. M\_e2x\_NCI and M\_e33\_AD vs. M\_e33\_NCI, 2). M\_e2x\_AD vs. M\_e2x\_NCI  
172 and M\_e4x\_AD vs. M\_e4x\_NCI, and 3). M\_e4x\_AD vs. M\_e4x\_NCI and M\_e33\_AD vs.  
173 M\_e33\_NCI. C) Heatmap illustrating the overlap, unique, and opposite differentially expressed  
174 genes (DEGs) between males and females within each APOE genotype (e2x, e33, and e4x) across  
175 cell clusters. Each row corresponds to a specific comparison, including: 1). F\_e2x\_AD vs.  
176 F\_e2x\_NCI and M\_e2x\_AD vs. M\_e2x\_NCI, 2). F\_e33\_AD vs. F\_e33\_NCI and M\_e33\_AD vs.  
177 M\_e33\_NCI, and 3). F\_e4x\_AD vs. F\_e4x\_NCI and M\_e4x\_AD vs. M\_e4x\_NCI.

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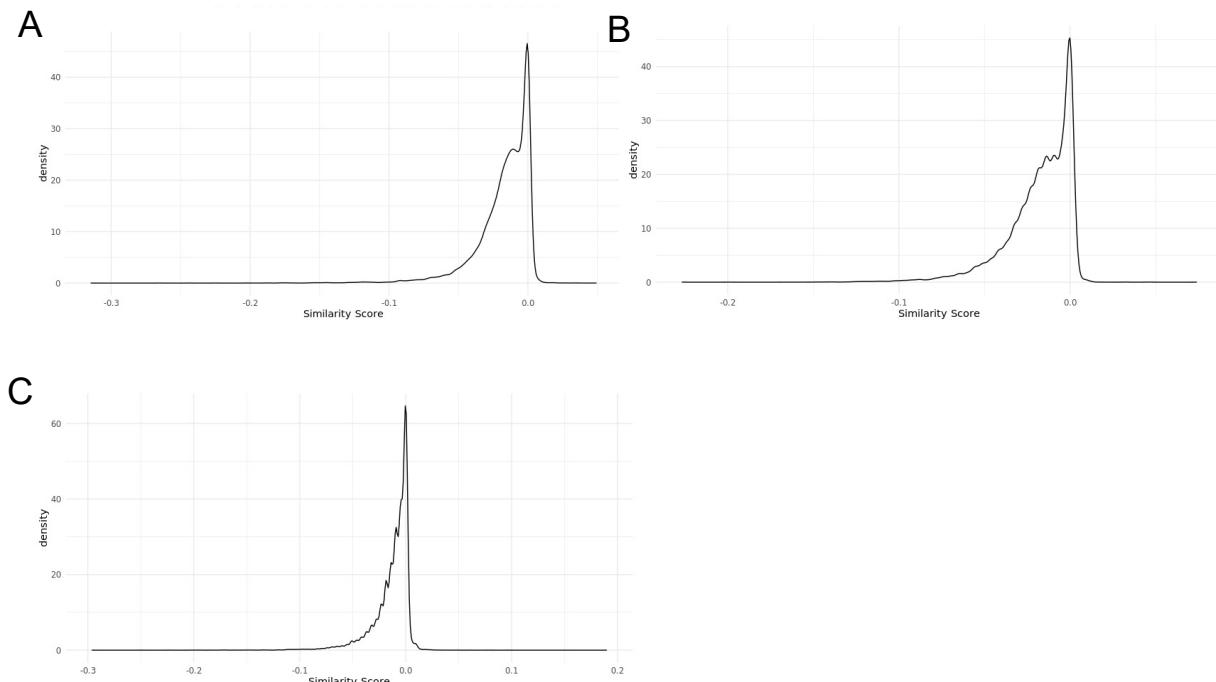
180 **Similarity measures reveal shared and divergent transcriptional changes across sexes and**  
181 **APOE genotypes in AD**

182 To quantitatively evaluate the similarity of differential expression patterns across sexes and  
183 APOE genotypes, we developed a new similarity measure for ternary vectors, termed as Zhang-  
184 Yu similarity measure (see **the Materials and Methods section** for the details). Each element in  
185 a ternary vector represents the differential expression status of a gene from a specific comparison  
186 with -1 standing for significant down-regulation, 0 for no significant change, and +1 for significant  
187 upregulation. The Zhang-Yu similarity measure quantitatively assesses the overlap, divergence,  
188 and directional consistency of gene expression changes across comparisons. Higher similarity  
189 scores indicate greater similarity while lower scores reflect divergence.

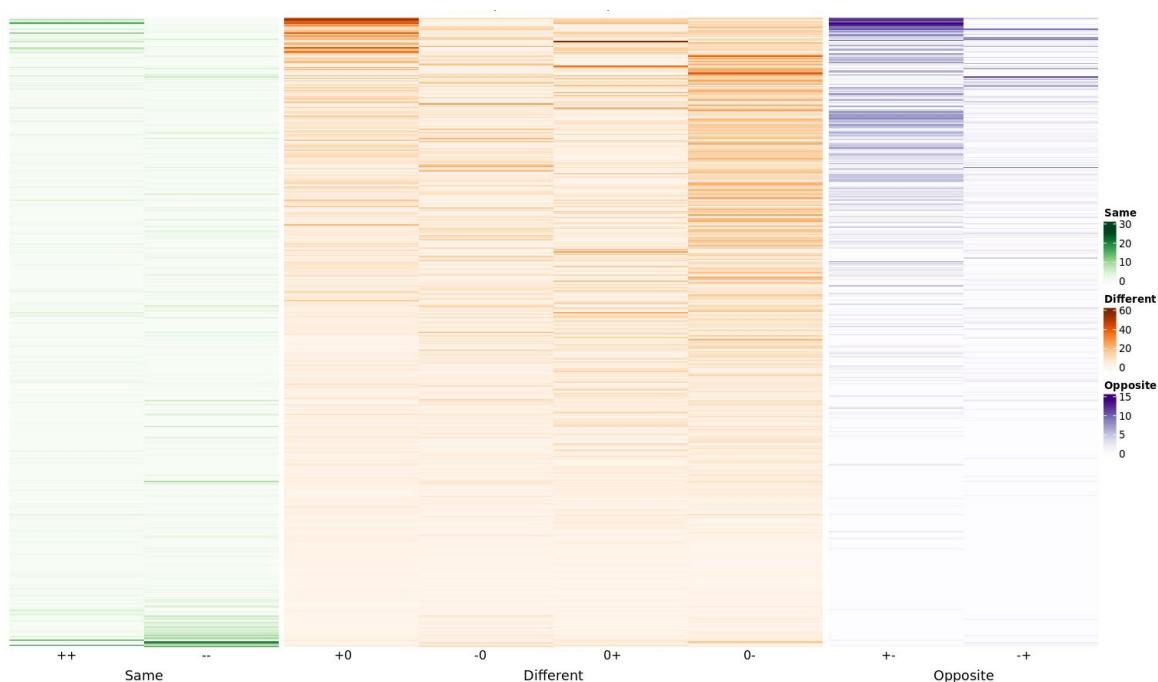
190 We employed this similarity measure to compare transcriptional patterns between females  
191 and males, incorporating results across all APOE genotypes (e2x, e33, and e4x) and all cell  
192 subclusters (**Figure 3A**). This allowed us to assess the consistency of sex-specific transcriptional  
193 responses across AD and NCI samples. Next, we extended the analysis to genotype-based  
194 comparisons, examining the similarity of transcriptional responses between APOE e2x and e33  
195 carriers, as well as between e4x and e33 carriers (**Figure 3B, 3C**). For these analyses, we combined  
196 results across sexes and cell subclusters to identify genotype-specific patterns of differential  
197 expression.

198 To evaluate the effectiveness of the similarity metric, we plotted the expression patterns of  
199 approximately 500 genes, sampled evenly across the range of similarity scores, incorporating  
200 transcriptional changes associated with AD versus NCI across all APOE genotypes (e2x, e33, and  
201 e4x) and cell clusters for both females and males (**Figure 4**). For each gene, we analyzed the  
202 frequency of all possible combinations of expression patterns between females and males, such as  
203 consistent upregulation, consistent downregulation, or divergent patterns. As shown in the plot,  
204 genes with lower similarity scores are predominantly characterized by "opposite" and "different"  
205 cases, indicating divergent transcriptional responses between sexes. In contrast, genes with higher  
206 similarity scores tend to show more "same" direction cases, reflecting consistent transcriptional  
207 changes between females and males. This analysis demonstrates that the similarity metric

208 effectively distinguishes shared versus divergent transcriptional patterns, supporting its utility in  
209 identifying sex- and genotype-specific transcriptional responses in Alzheimer's disease.  
210



211  
212 **Figure 3. Distribution of similarity scores for different comparisons.** A) The distribution of  
213 similarity scores for the comparison of females and males across all APOE genotypes (e2x, e33,  
214 and e4x). B) The distribution of similarity scores for the comparison of APOE e2x and e33  
215 genotypes across both sexes. C) The distribution of similarity scores for the comparison of APOE  
216 e4x and e33 genotypes across both sexes.  
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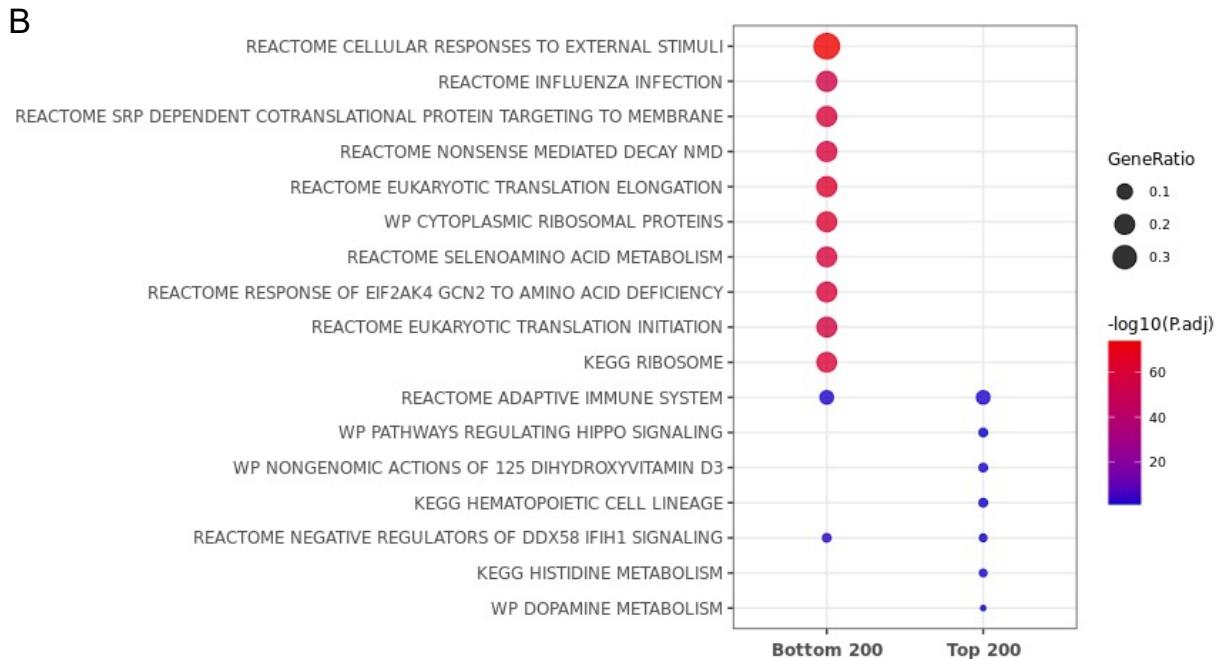
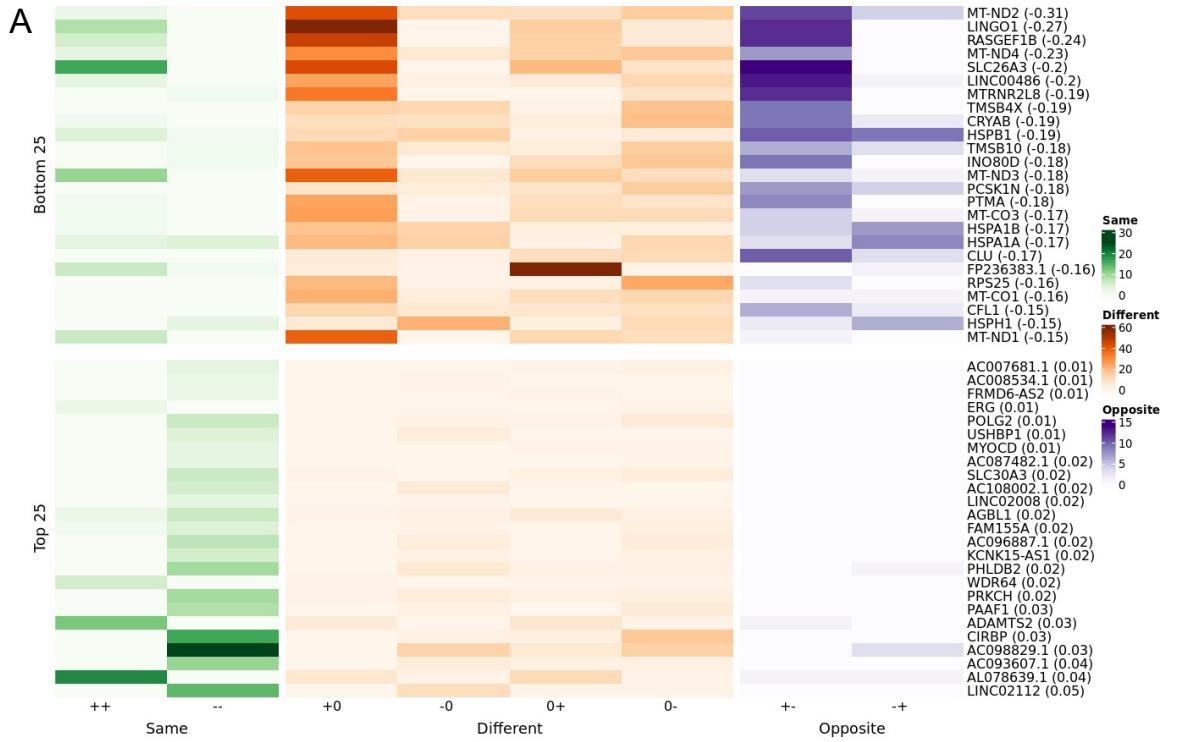
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219 **Figure 4.** Heatmap illustrating the a set of approximately 500 genes that are evenly  
220 distributed across the full range of similarity scores for the comparison of differential  
221 expression patterns between females and males across all APOE genotypes. The heatmap  
222 categorizes gene expression changes as "Same" (++, --), "Different" (+0, 0+, -0, 0-), or "Opposite"  
223 (+-, -+), with color intensities indicating the number of occurrences across all cell subclusters.  
224

225 Using the similarity score, we identified the top 25 (with the highest similarity) and bottom  
226 25 (with the lowest similarity) genes for each of the three comparisons of our interest: sex  
227 differences, *APOE* e2x vs e33, and *APOE* e4x vs e33. For sex differences, the bottom 25 genes  
228 included *CLU* (Clusterin), a gene associated with AD risk and neuroinflammation, and *HSPB1*  
229 (Heat Shock Protein Beta-1) which is associated with stress responses in neurodegeneration. Both  
230 genes show divergent expression patterns between sexes (**Figure 5A**). Pathway enrichment  
231 analysis of the top 200 and bottom 200 genes further revealed distinct biological processes  
232 associated with shared and divergent transcriptional responses (**Figure 5B**). The top 200 genes,  
233 enriched in pathways such as "dopamine metabolism" and "histidine metabolism" suggest  
234 conserved roles in cellular metabolism, and hormone signaling across sexes. In contrast, the  
235 bottom 200 genes, representing divergent transcriptional responses, were enriched in immune-  
236 related and stress response pathways, including "cellular responses to external stimuli" "adaptive  
237 immune system" and "nonsense-mediated decay". These enriched pathways point to sex-specific  
238 differences in immune and stress-response mechanisms in AD, highlighting potential molecular  
239 targets for understanding sex-related variability in disease progression.

240 For the comparison between *APOE* e2x and *APOE* e33 genotypes, the top 25 genes with  
241 the highest similarity scores and the bottom 25 genes with the lowest similarity scores are shown  
242 in **Figure 6A**. Pathway enrichment analysis revealed that the top 200 genes were enriched in  
243 processes such as "fatty acid metabolism" and "SHC1 events in ERBB4 signaling," emphasizing  
244 conserved roles in lipid metabolism and cellular signaling across these genotypes (**Figure 6B**).  
245 Conversely, the bottom 200 genes were enriched in pathways associated with translational  
246 regulation, such as "cytoplasmic ribosomal proteins" and "eukaryotic translation elongation," as  
247 well as stress-response pathways like "cellular responses to external stimuli." These enrichments  
248 highlight genotype-specific responses that may contribute to the differential impact of *APOE* e2  
249 and *APOE* e33 in AD pathology. For the comparison between *APOE* e4x and *APOE* e33 genotypes,  
250 the top 25 genes with the highest similarity scores and the bottom 25 genes with the lowest  
251 similarity scores are shown in **Figure 7A**. Genes with the lowest similarity scores were enriched  
252 in mitochondrial function and energy metabolism pathways, including "oxidative  
253 phosphorylation" "electron transport chain" and "HSP90 chaperone cycle for steroid hormone  
254 receptors" (**Figure 7B**). These results suggest that mitochondrial processes and stress response  
255 pathways diverge significantly between *APOE* e4x and *APOE* e33 carriers.  
256  
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260 **Figure 5. The top 25 genes displaying the greatest and least differences between sexes across**  
 261 **APOE genotypes and cell types. A)** Heatmap illustrating the top 25 and bottom 25 genes based  
 262 on similarity scores for the comparison of differential expression patterns between females and  
 263 males across all APOE genotypes and cell types. The heatmap categorizes gene expression  
 264 changes as "Same" (++, --), "Different" (+0, 0+, -0, 0-), or "Opposite" (+-, -+), with color  
 265 intensities indicating the number of occurrences across all cell subclusters. **B)** Pathway enrichment

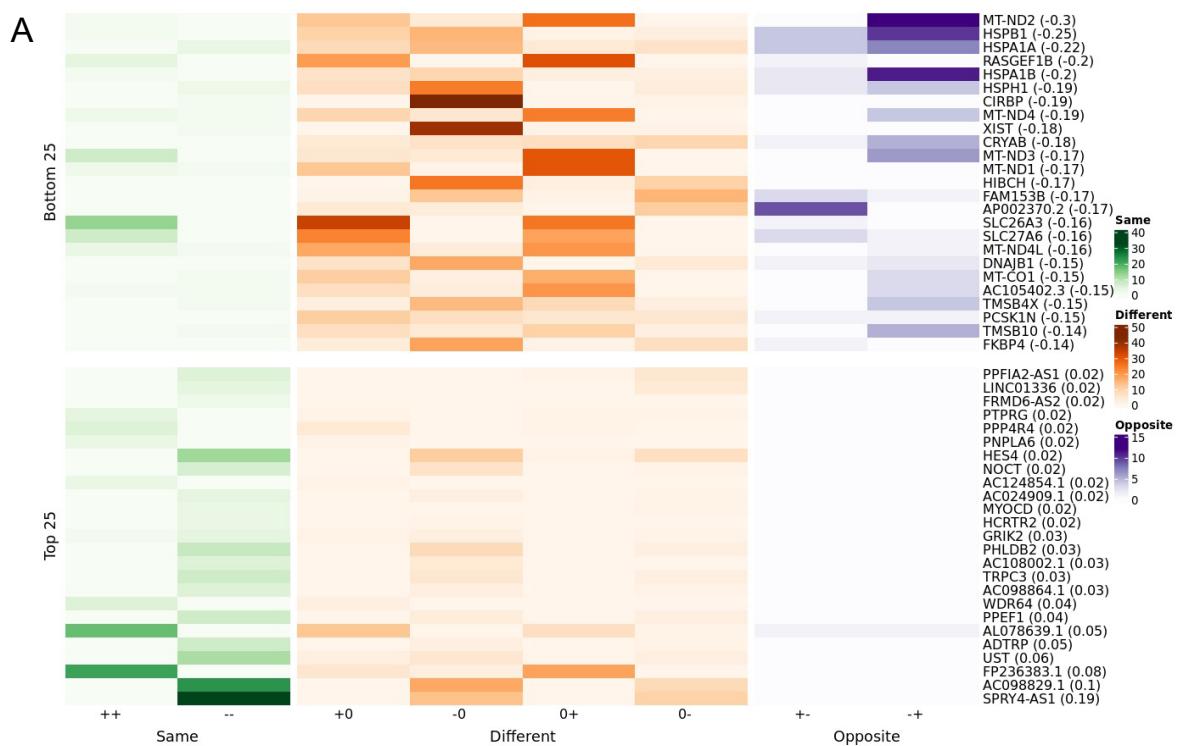
266 analysis for the top 200 and bottom 200 genes based on similarity scores in the comparison  
 267 between females and males.



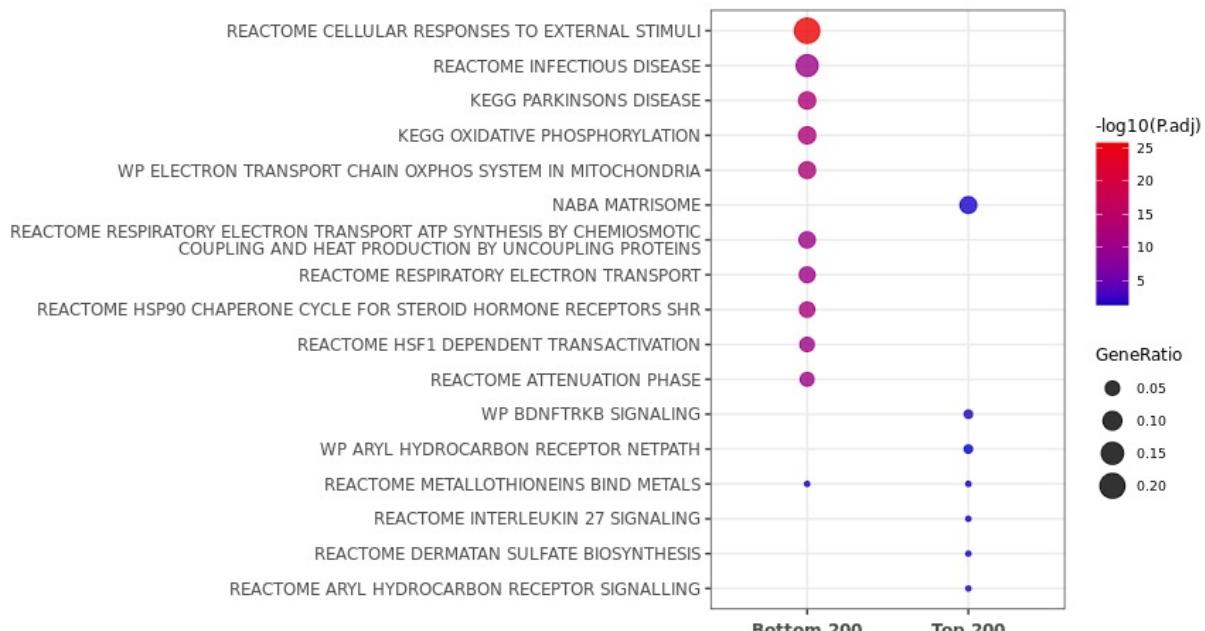
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269 **Figure 6. The top 25 genes displaying the greatest and least differences between APOE e2x  
 270 and e33 carriers across sexes and cell types. A) Heatmap illustrating the top 25 and bottom 25  
 271 genes based on similarity scores for the comparison of differential expression patterns between**

272 APOE e2x and e33 carriers across sexes and cell types. The heatmap categorizes gene expression  
 273 changes as "Same" (++, --), "Different" (+0, 0+, -0, 0-), or "Opposite" (+-, -+), with color  
 274 intensities indicating the number of occurrences across all cell subclusters. **B)** Pathway enrichment  
 275 analysis for the top 200 and bottom 200 genes based on similarity scores in the comparison  
 276 between APOE e2x and e33 carriers.



**B**



278 **Figure 7. The top 25 genes displaying the greatest and least differences between APOE e4x**  
279 **and e33 carriers across sexes and cell types. A)** Heatmap illustrating the top 25 and bottom 25  
280 genes based on similarity scores for the comparison of differential expression patterns between  
281 APOE e4x and e33 carriers across sexes and cell types. The heatmap categorizes gene expression  
282 changes as "Same" (++, --), "Different" (+0, 0+, -0, 0-), or "Opposite" (+-, -+), with color  
283 intensities indicating the number of occurrences across all cell subclusters. **B)** Pathway enrichment  
284 analysis for the top 200 and bottom 200 genes based on similarity scores in the comparison  
285 between APOE e4x and e33 carriers.

## 286 DISCUSSION

287 This study provides a comprehensive analysis of sex- and *APOE* genotype-specific transcriptional  
288 changes in AD using a snRNA-seq dataset from the ROSMAP cohort. By performing differential  
289 gene expression analysis across six stratified sex-*APOE* groups and applying a novel similarity  
290 metric, Zhang-Yule similarity measure, for ternary vectors, we uncovered shared and divergent  
291 transcriptional patterns across sexes and *APOE* genotypes. These findings highlight the interplay  
292 between genetic risk, sex, and cell-type-specific transcriptional responses in AD pathophysiology.

293 Male *APOE* e2 carriers exhibited the highest number of DEGs when comparing AD to NCI  
294 samples, suggesting a heightened transcriptional response in this subgroup. A majority of AD  
295 signatures were identified in excitatory neurons, inhibitory neurons, astrocytes, oligodendrocyte  
296 precursor cells, and one specific microglia cluster, highlighting the importance of these cell types  
297 in transcriptional responses to AD across all sex-*APOE* groups. Conversely, several cell clusters,  
298 including endothelial cells, CNS-associated macrophages (CAMs), pericytes, smooth muscle cells  
299 (SMCs), and T cells, showed no significant transcriptional changes, indicating a limited or  
300 negligible response to AD-related pathology in these populations.

301 In the comparison of transcriptional signatures across *APOE* genotypes in females, F\_e4x  
302 AD signatures were notably divergent from those of F\_e2x and F\_e33. This divergence was  
303 particularly evident in comparisons such as F\_e2x\_AD vs. F\_e2x\_NCI and F\_e4x\_AD vs.  
304 F\_e4x\_NCI, and F\_e4x\_AD vs. F\_e4x\_NCI and F\_e33\_AD vs. F\_e33\_NCI, which displayed a  
305 higher number of DEGs that were unique to one condition. These unique DEGs suggest distinct  
306 transcriptional responses in F\_e4x carriers, aligning with the heightened AD risk associated with  
307 *APOE* e4. Similarly, in males, M\_e2x AD signatures were significantly divergent from M\_e4x  
308 and M\_e33 AD signatures. These findings highlight the distinct transcriptional responses  
309 associated with *APOE* e4 in females and *APOE* e2 in males, reflecting genotype-specific roles in  
310 AD pathology.

311 The use of the Zhang-Yule similarity measure allowed us to quantify shared and divergent  
312 transcriptional patterns across groups. Genes with high similarity scores represented conserved  
313 transcriptional responses, reflecting core molecular processes shared across sexes and genotypes.  
314 Conversely, genes with low similarity scores highlighted subgroup-specific pathways, particularly  
315 in immune responses and stress-related mechanisms. These patterns reinforce the complexity of  
316 AD pathology and emphasize the importance of examining both shared and divergent molecular  
317 changes in understanding the disease.

318 Overall, this study highlights the intricate interplay of sex and *APOE* genotype in shaping  
319 transcriptional responses in AD. By integrating cell-cluster-specific analyses with a similarity  
320 scoring approach, we provide a detailed view of the molecular heterogeneity underlying AD. These  
321 findings pave the way for future research to further elucidate the functional implications of

322 identified pathways and develop precision medicine strategies that address the diverse molecular  
323 profiles of AD patients.

324

## 325 MATERIALS AND METHODS

326 To investigate the genes, pathways, and cell types underlying sex and *APOE* isoform differences  
327 in AD pathology, we leveraged one of the most comprehensive single-nucleus RNA sequencing  
328 (snRNA-seq) datasets in AD research by Mathys et al<sup>1</sup>. This dataset includes 2.3 million nuclei  
329 isolated from the prefrontal cortex of 427 participants in the ROSMAP cohort, spanning a wide  
330 range of AD progression.

331 We used preprocessed read count data from the original study, which identified 54 high-  
332 resolution cell types grouped into 12 major categories. These include 14 excitatory neuron  
333 subtypes (Exc), 25 inhibitory neuron subtypes (Inh), oligodendrocytes (Oli), oligodendrocyte  
334 precursor cells (OPCs), 3 astrocyte subtypes (Ast), and 5 immune cell types (microglia (Mic),  
335 CNS-associated macrophages (CAMs), and T cells). The dataset also encompasses several  
336 vascular cell types, including endothelial cells (End), smooth muscle cells (SMCs), fibroblasts  
337 (Fib), and pericytes (Per).

### 338 Cell cluster specific differential expression analysis

339 Differential expression analysis was performed for each sex-*APOE* group, including F\_e2x,  
340 F\_e33, F\_e4x, M\_e2x, M\_e33, and M\_e4x, comparing individuals with Alzheimer's disease (AD)  
341 to control samples with no cognitive impairment (NCI). This analysis was conducted at the cell-  
342 cluster level to account for cell-type-specific transcriptional changes. We utilized the FindMarkers  
343 function in Seurat version 5 for cluster-specific DEG identification. Genes were included in the  
344 analysis if they were expressed in at least 10% of the cells in either group (min.pct = 0.1). To adjust  
345 for potential confounding factors, we incorporated covariates including nCount\_RNA (total RNA  
346 counts), post-mortem interval (PMI), and age at death. The Benjamini-Hochberg (BH) method was  
347 used to adjust p-values for multiple testing. Significant differentially expressed genes (DEGs) were  
348 defined as those meeting the following thresholds: adjusted p-value (adj.p) < 0.05 and absolute  
349 fold change (|FC|) > 1.3. Filtered genes that passed these criteria were included in downstream  
350 analyses. Enrichment analysis was performed by using R package GTest (V1.0.9).

351

### 352 Similarity measure for evaluating similarity of transcriptional patterns between sex and 353 *APOE* genotype subgroups

354 To quantitatively evaluate the similarity of differential expression patterns across sexes and  
355 *APOE* genotypes, we developed and applied a novel similarity measure termed Zhang-Yu  
356 similarity measure. This similarity measure calculates the similarity between two N-dimensional  
357 ternary vectors, i.e., *X* and *Y*, representing differential expression statuses (AD versus NCI) of the  
358 genes in two different groups. In a ternary vector where each element takes three discrete values  
359 including -1, 0 and 1, each dimension represents the differential expression status of a gene as  
360 either up-regulation (+1), down-regulation (-1), or no significant change (0). The similarity score  
361 for two ternary vectors is defined as:

362

363 
$$Score = \frac{(S_{11} + S_{(-1)(-1)}) - 0.5 * (S_{10} + S_{(-1)0} + S_{01} + S_{0(-1)}) - (S_{1(-1)} + S_{(-1)1})}{N}$$

364 where,  $S_{ij}$  represents the number of occurrences of  $X(k) = i$  and  $Y(k) = j$ , where  $k=1, \dots, N$ .  
365 Therefore  $S_{11}$  represents for the number of the up-regulation matches in both groups,  $S_{(-1)(-1)}$  for the  
366 number of the down-regulation matches in both groups, and  $S_{10}$  for the number of mismatches with  
367 the upregulation in  $X$  and no change in  $Y$ , and so on so forth. N is the number of genes evaluated  
368 in the differential expression analysis.

369 We applied this similarity measure to three key comparisons:

370 1. **Female vs. Male (F vs. M):**

- 371     ○ **X:** Differential expression results ( $\{-1,0,1\}$ ) for females across all cell clusters  
372       and *APOE* genotypes: F\_e2x\_AD vs. F\_e2x\_NCI, F\_e33\_AD vs. F\_e33\_NCI,  
373       and F\_e4x\_AD vs. F\_e4x\_NCI.
- 374     ○ **Y:** Differential expression results ( $\{-1,0,1\}$ ) for males across all cell clusters and  
375       *APOE* genotypes: M\_e2x\_AD vs. M\_e2x\_NCI, M\_e33\_AD vs. M\_e33\_NCI, and  
376       M\_e4x\_AD vs. M\_e4x\_NCI.
- 377     ○ **N:** Total number of cell clusters (54) multiplied by 3 comparisons = 162.

378 2. ***APOE* e2x vs. e33:**

- 379     ○ **X:** Differential expression results ( $\{-1,0,1\}$ ) across all cell clusters for *APOE* e2x:  
380       F\_e2x\_AD vs. F\_e2x\_NCI and M\_e2x\_AD vs. M\_e2x\_NCI.
- 381     ○ **Y:** Differential expression results ( $\{-1,0,1\}$ ) across all cell clusters for *APOE* e33:  
382       F\_e33\_AD vs. F\_e33\_NCI and M\_e33\_AD vs. M\_e33\_NCI.
- 383     ○ **N:** Total number of cell clusters (54) multiplied by 2 comparisons = 108.

384 3. ***APOE* e4x vs. e33:**

- 385     ○ **X:** Differential expression results ( $\{-1,0,1\}$ ) across all cell clusters for *APOE* e4x:  
386       F\_e4x\_AD vs. F\_e4x\_NCI and M\_e4x\_AD vs. M\_e4x\_NCI.
- 387     ○ **Y:** Differential expression results ( $\{-1,0,1\}$ ) across all cell clusters for *APOE* e33:  
388       F\_e33\_AD vs. F\_e33\_NCI and M\_e33\_AD vs. M\_e33\_NCI.
- 389     ○ **N:** Total number of cell clusters (54) multiplied by 2 comparisons = 108.

390

## 391 **CONFLICT OF INTEREST STATEMENT**

392 All authors declared no conflict of interest.

393

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