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Abstract

Alzheimer's disease (AD) is an age-related disease pathologically defined by the deposition of amyloid plaques and neurofibrillary tangles in the brain parenchyma. Single cell profiling reveals Alzheimer's dementia involves the complex interplay of virtually every major brain cell type. Here, we highlight cell-type specific molecular perturbations in AD. We discuss how genomic information from single cells expand existing paradigms of AD pathogenesis and highlight new opportunities for therapeutic interventions.

Introduction

Single cell genomics have defined the complex molecular regulation of major cell types in the mouse[1–4] and human brain[5,6]. Profiling genetic information from single cells from individuals with various stages of AD pathology (**Table 1**) uncovers detailed cell-type specific molecular programs in AD (**Figure 1**). Keeping in mind challenges related to interpreting genomic studies from single cells, such as the common necessity to profile single nuclei instead of single cells from archived brain tissue (**Box 1**), we argue molecular disturbances across major cell types converge on common signaling pathways such as lipid handling, immune response, and metabolic reprogramming (**Figure 2**). Further defining and manipulating core signaling nodes may generate new opportunities for therapeutic intervention.

Excitatory neurons. Synaptic alterations and neuronal loss are well-established in AD, and single cell profiling has revealed molecular programs regulating neuronal dysfunction (**Figure 3**). Several single nucleus RNA-sequencing (snRNA-seq) studies reveal excitatory neurons from AD patients alter genes regulating neurotransmitter release, synaptic vesicle recycling, and glutamate metabolism[7–9]. Histological findings indicate post-synaptic terminals are lost in AD, and several differentially expressed genes relate to

post-synaptic scaffolding molecules, glutamate receptor trafficking, and calmodulin signaling[8,9]. Importantly, in situ hybridization confirms some of these findings, such as the reduced number of excitatory neurons[8] and the downregulation of *NTNG1*, a gene involved in the regulation of neurite outgrowth[8]. Inhibitory synapses, which are highly plastic in the adult brain and are thought to enable flexible modulation of stable excitatory connections[10], are also reduced in number in AD[11]. These findings are partly reflected in altered expression of genes critical for inhibitory synapses, such as modulation of some integrin genes[7,8]. The integrity of myelinated axons is critical for long-range projections, and genes related to neuronal-oligodendrocyte interactions are modulated in AD neurons, such as upregulation of *LINGO1*[7–9], a negative regulator of myelination (a finding repeated across several transcriptomic studies[7–9] and by in situ hybridization[8]). These transcriptional changes suggest synaptic elements are not simply structurally lost in AD, but the very molecular machinery governing their integrity are dysregulated.

Several studies highlight how AD neurons modulate stress related genes[8,12], particularly genes related to chaperone mediated protein folding[7–9,13,14] (e.g., *DNAJA1*). Altered genes in AD neurons also relate to mitochondrial translocase, glucose sensing, and glycolysis (e.g., *SLC2A3*, which encodes a glucose transporter enriched at synaptic terminals)[8,9]. Synaptic mitochondria are critical for sustained synaptic efficacy[15,16], and transcriptional profiles provide insight into metabolic programs disrupted in AD neurons.

Defining genetic signatures of neurons selectively vulnerable to dysfunction may reveal the molecular logic governing AD degeneration (**Box 2**). Tau aggregates form neurofibrillary tangles within some neurons, a canonical pathological hallmark of AD closely associated with neuronal loss. Fluorescence activated cell sorting (FACS) based on neurons with neurofibrillary tangles reveal synaptic genes are dysregulated in tangle-bearing neurons compared to non-tangle-bearing neurons from AD individuals[12]. A separate snRNA-seq study revealed *BAG3*, a master regulator for proteotoxicity-induced signaling, regulates tau homeostasis[17]. Reducing *BAG3* in primary cortical neurons led to tau accumulation, and *BAG3* overexpression attenuated tau pathology[17], suggesting selective vulnerability related to tau metabolism may be governed in part by *BAG3*. Similarly, the transcription factor RORB is thought to mark a population of tangle-burdened neurons that modulate genes related to synaptic proteins and neurotransmitter

receptors[18]. Thus, RORB may be a marker for selectively vulnerable neurons[18]. Conversely, some neurons may be preferentially resilient to AD: a subset of individuals harbor extensive amyloid and tau pathology but do not exhibit dementia[19] and therefore offer a tractable opportunity to interrogate the molecular basis of resilience to cognitive decline. *MEF2C* is upregulated in excitatory neurons in individuals with high AD pathology and normal cognition compared to age-matched individuals with high pathology and low cognition[19]. Impairing *Mef2* in mice causes neuronal hyperexcitability, and *Mef2* overexpression in Tau P301S mice rescued tauopathy-induced hyerpexcitability[19]. These findings suggest properties related to neuronal firing may explain individual neuronal susceptibility or resilience to degeneration.

Risk variants from genome wide-association studies (GWAS) further highlight differential risk to AD (**Box 3**). The ε4 allele of the apolipoprotein E gene (*APOE*) is considered the most highly validated genetic risk factor for sporadic late-onset AD. While *APOE* is predominantly expressed by astrocytes and microglia[8], variable expression of *APOE* among individual neurons suggests some neurons express relatively higher levels of *APOE*[20]. Increased *APOE* levels were associated with cellular stress and death[20], suggesting neuronal *APOE* and *MHC-I* signaling might be an important factor driving differential vulnerability to AD neurodegeneration. Apolipoproteins are critical for lipid transport, and other lipid related genes modulated in AD neurons include those related to cholesterol transport (e.g., *NPC1*[8,9], which encodes a lysosomal cholesterol transporter whose loss of function is associated with neuronal death[21]) and lipid signaling (e.g., *LAMTOR5,* related to endosomal/lysosomal transport[7–9,14]). The findings highlight complex lipid-related signaling networks disrupted in AD neurons.

Neuronal DNA damage. DNA damage is well known to occur in AD neurons[22], and snRNA-seq reveals AD neurons modulate genes related to DNA repair enzymes[7–9,14] (e.g., *XRCC6*, which is involved in DNA repair initiation, is downregulated in AD neurons[8]). Moreover, single cell whole genome amplification sequencing suggests DNA damage in neurons is elevated in neurodegeneration[23]. High DNA damage in human neurons is enriched in differentially expressed genes of AD individuals[24], potentially suggesting dysregulated gene expression in AD neurons may be related to impaired DNA repair. DNA damage may be part of a switch in cellular states associated with metabolic stress (e.g., upregulation of the DNA damage

inducible transcript *DDIT3*[8,9] may be associated with broader program in cellular stress). Neurons burned with DNA damage also active inflammatory signaling in neurodegeneration[25]. DNA damage is known to occur at neuronal enhancers and promoters[26], particularly during learning[27], and is required for learning-related immediate early gene expression[27]. Disentangling learning-related DNA damage and aging-related DNA damage in AD may lead to new insights into the progression of neural circuit disruption in AD.

Neurogenesis. Single cell genomics have generated molecular insight into the progression of new neurons in the adult brain, including the molecular definition of stem cells and their terminal fates[28–33], although common markers for neural progenitors may complicate efforts to define human neurogenesis[34]. Mutations in *PSEN1* may alter the stem cell niche[35] and some neural stem cells may be particularly affected by amyloid toxicity[36], so AD risk genes could potentially alter neurogenesis in the context of AD.

Interneurons. Interneurons critically sculpt synchronized patterns of neural activity, and single cell transcriptomics provide molecular definitions of interneuron subtypes from mouse[3,37,38] and human[39,40] brain, which reflect their functional repertoire[41]. Interneurons share transcriptional changes with excitatory neurons in AD, including genes related to metabolic stress, ion transport, DNA damage, perineuronal net assembly, and ErbB signaling[7,8,13]. Despite the well-documented functional perturbations of interneuron-dependent neuronal rhythms in amyloid mouse models[42] and mice with targeted replacement of mouse *Apoe* with human *APOE4*[43], transcriptional alterations within distinct interneuron subtypes have evaded comprehensive characterization in AD[7–9,13,14]. Downregulated genes in AD interneurons include marker genes for canonical interneuron subtypes, including *SST, PVALB,* and *VIP*[7–9]. Interneuron neuropeptides are thought to regulate inhibitory circuits[44,45] and neurovascular coupling[46], so impaired interneuron peptide signaling in AD may broadly impair neuronal signaling in dementia. The loss of canonical markers might also suggest interneurons lose core aspects of their transcriptional identity, which may reflect global loss of cellular function (**Box 2**).

Microglia. GWAS findings highlight AD-associated variants in immune related genes (e.g., *TREM2, CD33, HLA-DR*)[47]; accordingly, microglia have received a great deal of attention by single cell profiling[48] (**Figure 4**).

Plaque-associated microglia. One of the first single cell transcriptomic studies profiling microglia revealed a distinct subtype in 5XFAD mice, a popular amyloid model harboring five mutations associated with familial AD. Microglia from the hippocampi of 5XFAD mice were dubbed "disease-associated microglia" (DAM)[49]. Microglia harboring DAM transcriptional signatures were revealed to localize to amyloid plaques. One study elegantly sorted microglia from 5XFAD mice according to their levels of plaque labeled with methoxy-X04, a fluorescent probe that binds to fibrillar β-sheets of amyloid[50]. Interestingly, methoxy-labeled microglia increased expression of hypoxia-inducible factor *Hif1a*[50], which may be associated with a broad switch in metabolic programs in human AD microglia associated with *HIF1* signaling[51]. Studies in 5XFAD mice indicate plaque-associated microglia upregulate genes related to cell surface receptors (*Trem2, Tyrobp, Clec7a*), integrins (*Itgax*), and immune-related pathways (*Csf1, Ccl6*)[9,49,50,52]. This transcriptional signature is partly recapitulated in CKp25[52], APP-PS1[53], APP$^{NL\text{-}G\text{-}F}$ [54], Tau P301S, and P301L mice[53] (although genetic background may drive substantial microglial diversity[55]). While additional studies are needed to clarify the extent to which mice recapitulate human AD microglia, several studies indicate human AD microglia modulate genes related to cell migration and phagocytosis[7–9,14]. Collectively, these findings highlight the transcriptional basis for microglia to cluster around plaques and phagocytose debris[7–9,14].

Lipid metabolism in AD microglia. TREM2 acts in microglia as a sensor for a wide array of lipids, and the R47H variant of *TREM2* is associated with increased risk of AD and enhanced Akt signaling in microglia[56]. In adipose tissue of non-AD individuals, *TREM2* drives a gene expression program involved in phagocytosis, lipid catabolism, and energy metabolism[57], suggesting *TREM2* may broadly regulate lipid-related metabolic programs in macrophages. In accord with experimental studies highlighting the importance of lipid sensing and metabolism in microglia, microglial lipid metabolism pathways are broadly disrupted in AD[7–9]. Interestingly, the DAM phenotype in 5XFAD mice is dependent on *Trem2*[9,49]. Several findings in mice suggest manipulating microglial lipid pathways regulate pathology. For example, knocking

down mouse *Apoe* conferred neuroprotection in APP/PS1 mice[58], and overexpressing low-density lipoprotein receptor, which mediates lipid clearance, alleviates pathology when overexpressed in Tau P301S mice and shifts microglia transcriptional signatures to a homeostatic state[59]. Lipid metabolism is critical for microglia to rapidly remodel plasma membrane for local brain surveillance and regulate neural activity, and these findings suggest targeting microglial lipid-related pathways may alleviate AD pathology.

Microglial perturbations in neuronal support. It is increasingly relevant how microglia regulate neuronal circuits[60,61]. Microglia regulate neural computations in part by complement-mediated synapse elimination[62], fractalkine signaling[63], and purine sensing[60], and, accordingly, several studies indicate AD microglia modulate genes related to complement (*C1Q*[7,8]), fractalkine receptors (e.g., *CX3CR1*[8,14]), and purine receptors (e.g., *P2Y12R*[7]). Thus, neuronal dysfunction in AD may arise in part due in part to alterations in microglial capacity to sense and control neuronal activity. Perturbations in microglial metabolic state, related in part to cellular stress and glycolytic shifts, may converge on signaling pathways that impair microglial capacity to regulate neuronal activity (for example, a model using CRISPR-edited induced pluripotent stem cells found lipid accumulation induced by APOE4 impairs microglial surveillance of neuronal network activity[64]). Microglia state may also be associated with distinct GABAergic circuits[65] and pyramidal neuron subtypes[66], so future work defining microglial-neuronal crosstalk may reveal the molecular logic of microglia governing neuronal dysfunction in AD.

Vascular function of microglia and macrophages. Capillary-associated microglia are thought to regulate blood flow via purinergic signaling[67,68]. Microglia and perivascular macrophages are thought to harbor distinct ontogeny[69], and subpopulation of cells marked by high *Mrc1*, a marker of peripheral macrophages[70], may represent a transcriptionally distinct population of vascular-associated macrophages[71,72]. Signaling between vascular-associated microglia/macrophages and vascular cells may influence neurovascular function in AD. For example, secreted factors from AD microglial might regulate the integrity of endothelial tight junctions. Chemokines secreted by microglia may influence the inflammatory state of endothelial walls (which in turn can lead to neutrophil adhesions and capillary stall-induced blood flow reductions[73]). Future

studies may further define how vascular-associated macrophages influence vascular permeability and neurovascular coupling in AD.

White matter associated microglia. Microglia in white matter have a distinct transcriptional state compared to grey matter microglia[74] and share genes associated with disease-associated microglia (e.g., upregulation of *APOE*, complement, and lipid metabolism related genes, and downregulation of homeostatic markers)[75]. Similar transcriptional signatures of white matter microglia are present in 5XFAD and $APP^{NL\text{-}G\text{-}F}$ mice, and *TREM2* knockout reduces the presence of white matter microglia[75]. The dysfunction of microglia in white matter may relate to repairing and phagocytosing myelin[76]. Further defining white matter associated microglia may reveal interventions to promote the health of myelinated axons.

State transitions of microglia inflammation. Morabito et al. performed snATAC-seq and snRNA-seq to define AD-associated gene-regulatory programs at the epigenomic and transcriptomic levels[77]. Some microglia in AD had more open binding sites for *SPI1*, which encodes PU.1, a master regulator of myeloid cell differentiation. PU.1 may act as a transcriptional repressor in late-stage AD microglia[77], and a complex network of transcription factors in AD microglia (e.g., *ELF5, ETS1, ETV5, SPIC)*[77] may be involved in microglia state transitions in AD[77,78]. Indeed, PU.1 expression levels[79] and PU.1-dependent transcriptional control[53,80] are thought to critical regulate microglial function, such as microglial clearance of amyloid. Single cell transcriptomics and CRISPRi/a screens might further reveal gene programs modulating microglial state[81].

Astrocytes. Astrocytes are involved in neuronal trophic support, extracellular ion homeostasis, and brain fluid balance. Single cell profiling reveals the molecular heterogeneity of astrocytes[82–85] and astrocytic perturbations in AD (**Figure 5**).

Astrocyte metabolism. Astrocytes are a central driver of energy homeostasis in the brain. Several snRNA-seq from human AD cortex reveal AD astrocytes alter genes related to cellular stress and metabolic reprogramming genes related to cell stress (e.g., *CIRBP*, *CABLES*, *CSRP1*)[7–9], as well as many genes

related to the structural remodeling of the astrocytic cytoskeleton (e.g., *GFAP*)[7–9] and extracellular matrix (e.g., the versican gene *VCAN* and integrin genes *ITGB8* and *ITGB4*)[7–9]. Upregulation of *GFAP* in AD astrocytes is also observed cross several snRNA-seq datasets from AD patients[7–9] and 5XFAD mouse hippocampus[86], which may contribute to clinically relevant biomarkers (**Box 3**). Given that astrocytes critically respond to and regulate the inflammatory state of the brain following injury and neurodegeneration, potentially in a region-specific manner[84], these findings highlight transcriptional underpinnings of astrocytic metabolic reprogramming in AD.

Many dysregulated pathways in AD astrocytes converge on lipid signaling (e.g., *PLCE1*, which encodes a phospholipase, and apolipoprotein family genes)[7–9]. Genes related to lipid synthesis and transport (e.g., *LDLR*)[8] are perturbed in AD astrocytes[7–9]. Metabolic reprogramming in AD astrocytes may contribute to impaired capacity to regulate neuronal circuits. One study showed that astrocytes break down cytotoxic fatty acids secreted by hyperactive neurons[87]. Lipid associated pathways are dysregulated in disease-associated astrocytes from 5XFAD mouse hippocampus, including those in cholesterol pathways[86], recapitulating aspects of AD astrocyte dysfunction in humans related to transport and storage in lipid droplets, and detoxification of reactive oxygen species (e.g., *SOD2*)[9]. Removing astrocytic *APOE4* in mice decreases disease-associated transcriptional signatures across multiple cell types and protects against tau-mediated neurodegeneration[88]. These findings suggest lipid-related signaling networks in astrocytes may represent a core perturbation in AD.

Dysfunctional synaptic communication in AD astrocytes. Astrocytes facilitate neurotransmitter shuttling and synaptic trophic support. Several snRNA-seq from human patients reveal AD astrocytes alter genes related to glutamate receptor subunits (e.g., *GRIA2*, *GRM3, GRID2*)[7,8,13,86]. Gap junctions and potassium handling in astrocytes critically support neural function, and several snRNA-seq studies from human AD patients reveal genes related to gap junctions (e.g., upregulation of *GJA1,* which encodes the major astrocytic gap junction component connexin 43)[8] and ion transporters (e.g., downregulation of *KCNIP4*)[7–9] are dysregulated in AD astrocytes. Collectively, these transcriptional changes highlight molecular pathways dysregulated in astrocytes governing extracellular ion homeostasis and neuronal function.

To gain insight into the molecular basis driving transitions between astrocytic cell states, several groups have analyzed transcription factor expression profiles. The transcription factor AEBP1, a coactivator of the master immune signaling regulator NFκB, may regulate AD-related state transitions in astrocytes[7]. The master lysosomal regulator *TFEB* was upregulated in astrocytes in AD patients[7,8], and given the importance of TFEB to lysosomal pathways[89], dysfunction in intracellular lipid related processes may represent a key driver of AD related dysfunction[7]. Several genes overlap between AD microglia and astrocytes (e.g., *CTSB, CTSD,* and *APOE*)[90], potentially suggesting a common glial inflammatory milieu may emerge in AD including weakened metabolic coordination with neurons[9]. The complex, bidirectional inflammatory milieu generated by astrocytes and microglia (and shared in part likely is shared by oligodendrocyte related cells), collectively may regulate neuronal function. Dysfunction in secreted cytokines and lipid related trafficking may represent a functionally redundant glial response to AD pathology. For example, the metabolic shift in microglia highlighted above may be shared in part by AD astrocytes, which modulate genes involved in cell growth and signal transduction. These changes collectively highlight common lipid- and immune-related signaling pathways shared across major cell types in AD.

Oligodendrocytes. Single cell transcriptomics from mouse[91] and human brain[92,93] reflect the highly heterogeneous nature of oligodendrocytes. Molecular programs perturbed in AD oligodendrocytes reflect alterations in the diverse functional repertoire of these cells, including myelination, sensing neural activity, and immune function[7–9,13,14,18] (**Figure 6**).

Deficits in white matter volume and myelination rates are well appreciated in AD[94]. Many myelin related genes are perturbed in AD oligodendrocytes, such as upregulation of *LINGO1*[7,8] (a negative regulator of myelination, which is also upregulated in AD excitatory neurons) and downregulation of *CNTN2* and *OPALIN* (genes thought to regulate axon homeostasis)[14]. Genes involved in myelin production are altered in oligodendrocytes early in AD, including genes for enzymes related to fundamental cellular building blocks (e.g., *CERS2*[8], *CARNS1*[7–9], and *QDPR*[7–9], which encode genes important for the processing of molecules important for white matter integrity) and myelination itself (such as *PLP1*, *OLIG1*, and *MBP*)[8]. Several

studies from human brain also highlight alterations in genes for lipid transport, including genes related to apolipoproteins and lipid receptors (e.g., *ABCA6*, *LRP1, LDLRAD4*)[7–9], lysosome function (e.g., *LAMP1*)[8], and solute carriers (e.g., *SLC38A2, SLC5A11*)[7–9]. These findings highlight cellular pathways related to biosynthetic processes and lipid handling converge on myelin synthesis, and may explain in part how oligodendrocyte dysfunction contributes to deficits in the structure and plasticity of myelination in AD.

Myelination is a highly regulated and dynamic process that may be specific for anatomically distinct neural circuits[95,96]. The plasticity of myelin may relate to the capacity of oligodendrocytes to sense neural activity, such as the sensory experience-dependent myelination remodeling on parvalbumin inhibitory interneurons but not on excitatory callosal projection neurons[97]. snRNA-seq suggests AD oligodendrocytes modulate genes related to ion channels (e.g., *KCNH8*)[7–9] and glutamate receptor subunits (e.g., *GRM3, GRID2)*[7,8,13,14]. These findings suggest AD oligodendrocytes may exhibit impaired capacity to sense and regulate neural activity. Memory preservation is thought to require new myelin formation[98], so impaired oligodendrocyte capacity to adaptively monitor neural activity and facilitate myelin remodeling may govern cognitive decline in AD.

Oligodendroglial immune and vascular functions. Studies in mice reveal antigen processing and presentation capabilities of oligodendrocytes[92,99], and AD oligodendrocytes modulate genes related to MHC-I and MHC-II[8]. Many other immune pathways are perturbed in AD, including interferon response and inflammation (e.g., *CD63* and *IRF2*, which are involved in the activation of immune cells)[13]. Oligodendrocytes display major transcriptomic alterations in 5XFAD mice[100]. Reactive oligodendrocytes marked by *Serpina3n+ C4b+* in plaque bearing regions of 5XFAD mice are thought to emerge in a TREM2-dependent manner[9]. Oligodendrocytes may also respond to[101] and participate in[102] blood-brain barrier integrity by secreting growth factors that regulate vascular cell function (e.g., PDGF signaling, which is thought to regulate vascular function, is dysregulated AD oligodendrocytes[8]). Oligodendrocytes also support extracellular matrix remodeling, a critical factor in remyelination, and AD oligodendrocytes modulate genes related to collagens, laminins, and chondrotins[8]. Some of these changes may reflect injury

related to white matter injury, a common clinical finding in AD. Collectively, these findings highlight heterogeneous molecular programs adopted by AD oligodendrocytes.

Oligodendrocyte precursor cells (OPCs). OPCs are distributed throughout grey and white matter. snRNA-seq reveals heterogeneous subtypes of OPCs in mouse[91,103,104] and in the human cortex[105,106]. OPCs regulate neural activity and harbor immune and vascular related function, and snRNA-seq reflects alterations in OPC state perturbations in these processes. For example, several snRNA-seq studies from human subjects indicate AD OPCs downregulate genes for ion channels (e.g., *KCNIP1, CACNA1D*)[7,8], and may alter genes encoding neurotransmitter receptors (e.g., *GALR1*)[7,8], glutamate receptors (e.g., *GRIA2*)[9], and synaptic genes (e.g., *SNAP25*)[8]. Like oligodendrocytes, OPCs are thought to dynamically sense and modulate neural activity[107]. Transcriptional findings highlighting OPC modulation of genes related to voltage gated ion channels suggest OPCs may harbor altered capacity to sense neural networks, which may explain in part dysfunction in adaptive myelination and neuronal integrity in AD. OPCs modulate immune related genes (e.g., *IFIT1*)[13], highlighting a potential role for inflammation and immune mechanisms in OPC mediated dysfunction in AD. Oligodendrocyte differentiation is partly dependent on *OLIG1*, which is upregulated in AD OPCs[8], supporting a role for alterations in the dynamic reprogramming of OPC fate that may be responsible for oligodendrocyte alterations in AD. OPCs with DNA damage may alter their differentiation programs, and, notably, amyloid itself is thought to induce senescence in OPCs, which can be reversed by senolytic treatment[108].

Vascular cells. Alterations in vascular function critical contribute to brain homeostasis, and reduced blood flow may emerge early in AD[109]. snRNA-seq has provided molecular definitions of functionally distinct vascular cells in mouse[71,110–113] and human brain[114–117]. These studies suggest vascular cells in AD have altered immune signaling, neurovascular coupling, and permeability[14,116].

Brain endothelial cells control the movement of ions, molecules, and cells between the blood and the parenchyma, a constellation of properties collectively referred to as the blood brain barrier (BBB)[118]. snRNA-seq from AD patients reveal transcriptional perturbations in many BBB processes, including

alterations in tight junctions (e.g., *CLDN5*), solute transporters (e.g., *SLC2A1*), and adhesion molecules[14,116]. In endothelial cells of APP/PS1 mice, mNat1, a regulator of insulin sensitivity, was found to govern endothelial cell necroptosis[119].

Pericytes reside in the basement membrane and wrap around capillaries. Pericytes express genes involved in actomyosin contraction[71], consistent with functional evidence suggesting pericyte contractility controls vascular dynamics (e.g., by controlling blood flow at capillary junctions[120] and regulating basal capillary diameters[121]), which may contribute to AD related hypoperfusion[122]. Human hippocampal pericytes from *APOE4* carriers elevate expression of *NFAT*, and modulating *NFAT* through calcineurin signaling reduces *APOE* expression and ameliorates amyloid deposition *NFAT*[123]. Mice with targeted expression of APOE4 in vascular mural cells modulate the transcriptome of many cells, particularly astrocytes[124]. Collectively, these studies provide transcriptomic evidence for observations related to pericyte dysfunction in vascular dysfunction in AD related to neurovascular coupling and BBB integrity.

Future Directions

Single cell profiling reveals cell-type specific alterations in AD, and highlights core signaling pathways that are dysfunctional across cell types. Combining genetic information with other metrics of cellular functions will enhance our understanding of AD alterations in distinct cell types (**Figure 7**). For example, preserving anatomical information using spatial transcriptomics may generate insight into the anatomical progression of AD and define distinct neuronal projections susceptible to AD dysfunction. Expanding patient samples to resolve contributions from sex, race/ethnicity[125,126], genetic risk variants, and factors such as education, sleep, and exercise habits, will further enhance our understanding of the cellular phenotypes driving memory decline in AD patients. Existing datasets must be integrated and made easily shareable to expand the heterogeneity of AD response and to define concordant gene expression changes across studies. Further defining how human molecular signatures are recapitulated in mouse models and cell culture preparations will lead to new experimental opportunities to dissect disease mechanisms.

Further characterizing distinct neuronal microcircuits and cell types that become dysfunctional in AD—and defining which cell states contribute to CSF and plasma biomarkers—may lead to new frameworks to define cellular substrates of AD progression. By identifying vulnerable cell types and the molecular programs that give rise to them, therapeutic interventions might reverse aberrant cellular trajectories. While many transcriptional alterations cell type specific, these changes ultimately might converge on shared signaling pathways across cell types that might represent targets for new therapeutic strategies.

Conclusion

Single cell profiling facilitates a nuanced portrait of the diverse cellular processes perturbed in the AD brain. These varied molecular programs help explain the divergence between healthy aging and cognitive decline, and highlight cell-type specific molecular programs involved in AD. Core signaling modules are disrupted across multiple cell types, and manipulating disrupted cellular states will pave the way for new therapeutic opportunities.

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All figures were created with BioRender.

Author contributions

M.H.M. and L-H.T. conceived the original idea and wrote the paper.

Competing interests

The authors declare no competing interests related to this project.

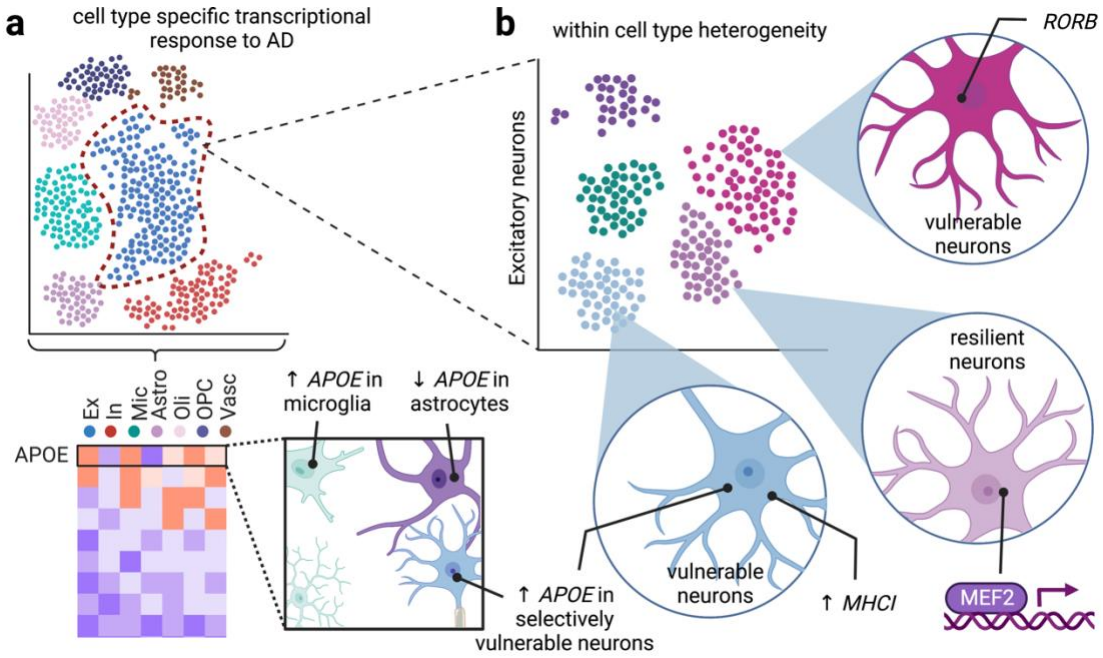

Figure 1: Overview of central advantages of single cell approaches for the study of AD. Single cell approaches highlight cell type and cell subtype specific vulnerability to disease. **(a)** *Cell type specific responses to disease*. Bulk quantifications of gene expression report population averages, which belie changes from specific cell populations that may drive distinct pathological responses. For example, snRNA-seq revealed that APOE is downregulated in AD astrocytes but upregulated in microglia[7,8] and some neurons[20]. **(b)** C*ell subtype responses to disease*. Bulk profiling based on cell type markers might mask within-cell type heterogeneity, such as layer-specific neurons, non-myelinating oligodendrocytes. In contrast, single cell profiling unmasks differential vulnerabilities to AD within distinct subsets of major cell types. For example, neurons selectively vulnerable to AD neurodegeneration are marked by *RORB*[18] and elevated APOE/MHC-I signaling[20], and neurons resilient to AD pathology are enriched in *MEF2*[19].

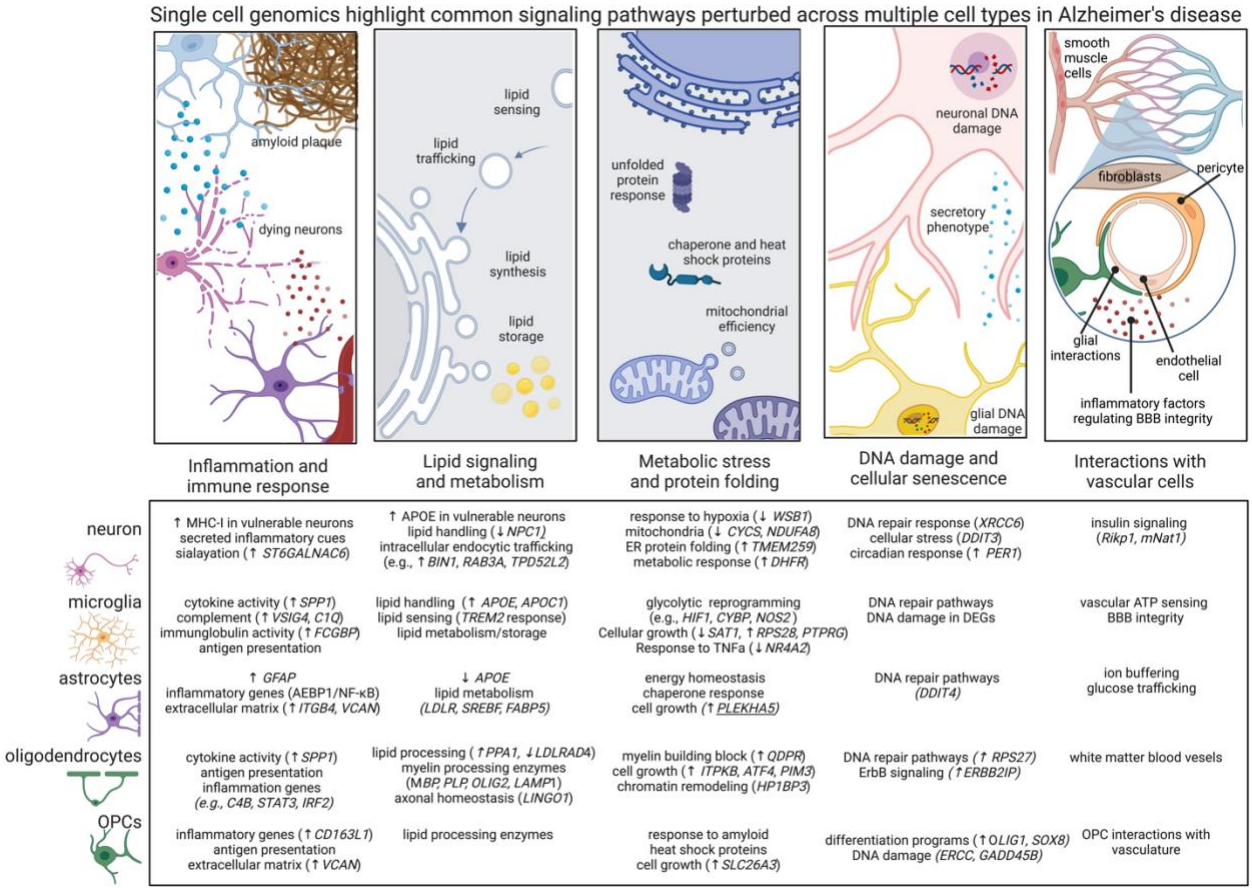

Figure 2. Shared cellular pathways disturbed in AD as revealed by single cell genomics. Differentially expressed genes across cell types are related to shared signaling motifs. Identifying common disrupted pathways may uncover core nodes of perturbation and lead to new therapeutic interventions related to multiple cells. Below we highlight common cellular pathways that are disrupted across multiple cell types in AD. Arrows denote transcriptional directions from prefrontal cortex[8] (up arrow means up in AD compared to non-AD) and with a focus on genes showing concordant expression changes from other datasets and brain regions as highlighted in the text.

•*Immune signaling*. Nearly every cell type generates immune responses in AD, including transcriptional responses related to cytokine, chemokine, and MHC signaling. MHC signaling may related to synaptic plasticity and the unfolded protein response. The low-grade AD-related inflammation in every cell type associated with AD may be associated with metabolic reprogramming.

•*Lipid handling.* Lipid signaling is crucial for many cell functions, such as sensing and shuttling lipid species and to accommodate the dynamic remodeling of plasma membrane required for the structural plasticity of dendritic spines, microglial processes, astrocytic endfeet, and nodes of Ranvier. Perturbed lipid signaling in many brain cell types in AD, underscore the importance of lipid signaling and metabolism.

•*Unfolded protein response*. Nearly every major cell type modulates protein misfolding pathways and integrated stress responses, and, related, mitochondrial function, highlighting energetic disruptions in AD cells. These findings suggest the milieu of the AD brain affects unfolded protein response and cellular stress even in cells not directly burdened by pathology.

•*DNA damage and cellular senescence*. DNA damage in neurons is associated with aging and is elevated in neurodegeneration[23], DNA damage is essential for the expression of learning related immediate early gene expression[27]. Many cells in AD have impaired DNA repair enzyme pathways, potentially suggesting senescent state and loss of core cellular functions.

•*Vascular interactions*. Recent studies are beginning to profile the complex network of vascular cells in AD. Existing datasets highlight signaling pathways perturbed across multiple brain cell types relating to neurovascular coupling and BBB dysfunction in AD, including the cell-type specific secretion of inflammatory molecules known to regulate vascular cells.

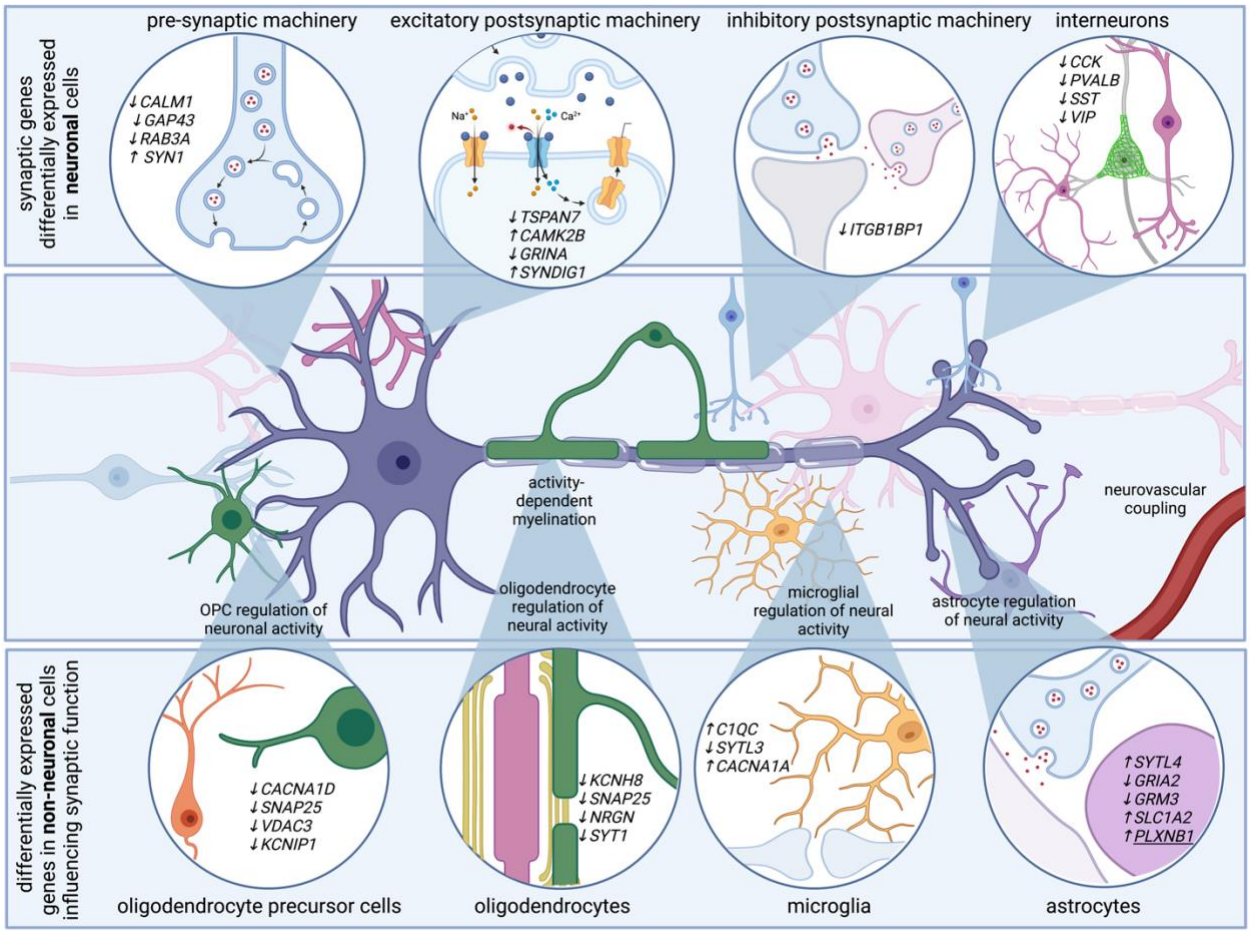

Figure 3. Single cell genomics reveal cell type specific perturbations in sensing and regulating neural activity in AD. Neurons account for the vast majority of differentially expressed genes in AD. Genes related to pre-synaptic, post-synaptic, and inhibitory synaptic machinery emerge in single transcriptomes of AD neurons. For example, AD neurons upregulate *SYN1*, a gene that encodes synapsin 1, critical for synaptic vesicle function, and downregulate *TSPAN7*, which encodes a tetraspanin thought to regulate post-synaptic dendritic spine structure. Transcriptional programs associated with altered electrical properties may be associated with neuronal vulnerability to AD. Additionally, non-neuronal cells modulate genes that are involved in synaptic function. For example, genes related to synaptotagmin related genes are differentially expressed in astrocytes, oligodendrocytes, oligodendrocyte precursor cells, and microglia. Several differentially expressed genes in non-neuronal cells converge on pathways that ultimately influence neuronal function, such as genes related to synaptic pruning and activity-dependent ion channels. For example, voltage gated ion channels, which might help non-neuronal cells sense neuronal activity, are also modulated in multiple cell types. These highlight how many brain cell types are involved in sensing and regulating neural activity, and suggest neural circuit dysfunction in AD is likely the consequence of multi-cellular signaling cascades.

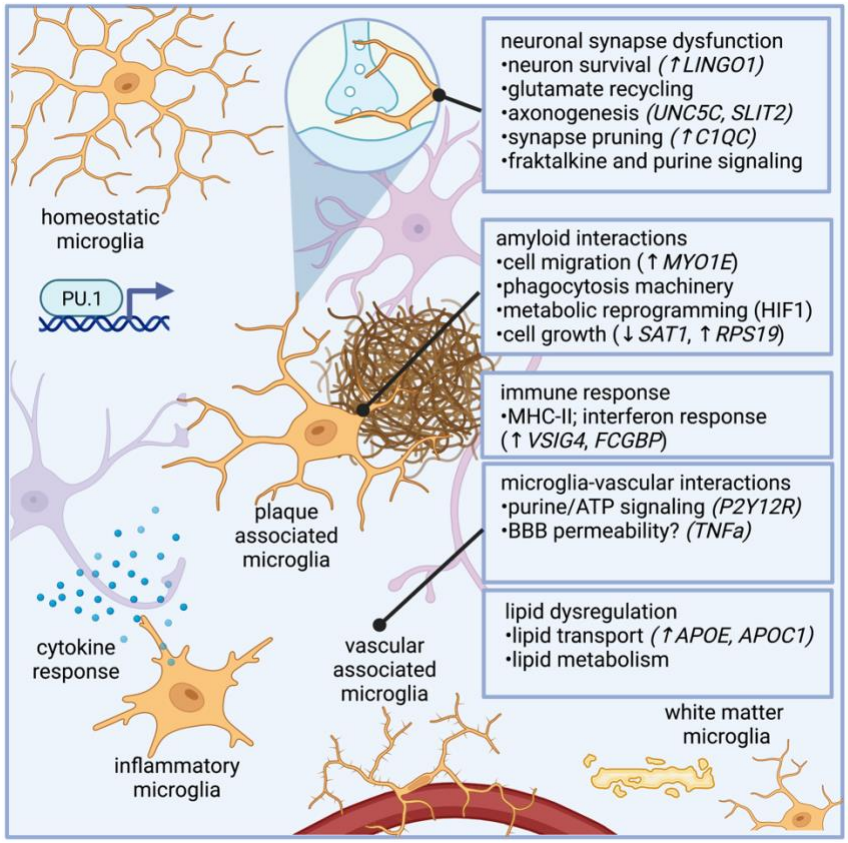

Figure 4: Molecular programs adopted in AD microglia revealed by single cell genomics. Microglia dysfunction in AD modulate genes related to synapse function, phagocytosis, and immune response. Microglia regulate genes involved in myelination, such as *LINGO1*, a negative regulator of myelination, as well as genes involved in axonogenesis (e.g., *UNC5C* and *SLTI2*). AD microglia also modulate complement related genes, such as *C1QC*, which regulate synaptic pruning. Microglial harbor properties associated with phagocytosis, and microglial response to amyloid has been well characterized, and genes in AD microglia potentially related to amyloid response include those related to microglial plaque clustering phenotypes, such as cell migration (e.g., *MYO1E*, which encodes a gene related to myosin), as well as genes involved in metabolic reprogramming and cell growth (e.g., *SAT1*, which encodes an acetyltransferase, and *RPS19*, a ribosomal subunit). As the innate immune cells of the brain, microglia are intimately involved in immune response, and several differentially expressed genes in AD microglia are involved in immune response, such as *VSIG4* and *FCGBP*, genes involved in immunoglobulin response. TREM2 is a lipid receptor that is thought to govern microglia transitions to disease-associated states[9,49]. Subtypes of microglia that regulate plaques are marked by *Hif1a* in 5XFAD mice, which is associated with metabolic reprogramming in human AD microglia.

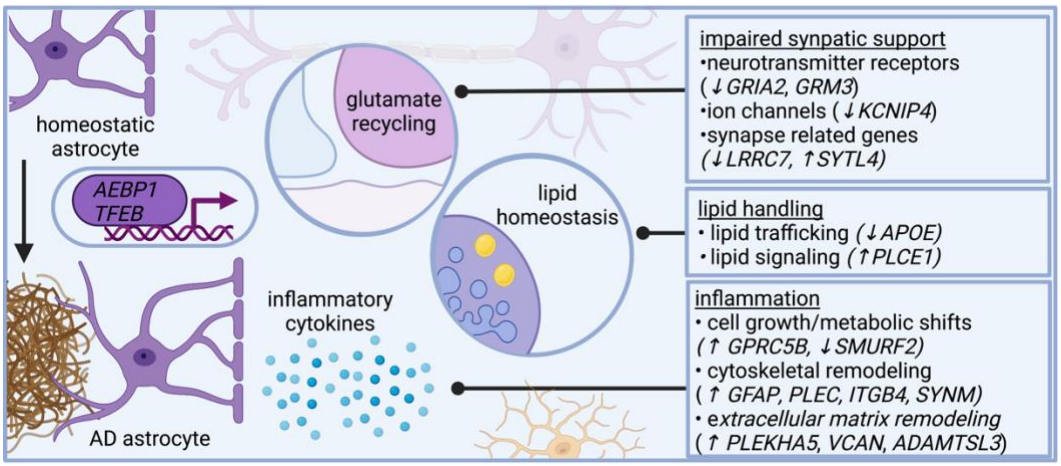

Figure 5. Molecular programs adopted in AD astrocytes revealed by single cell genomics. Several lines of evidence suggest astrocytes in AD become inflammatory and impair neural circuit function, including plaque-associated barriers, and modulating lipid-related signaling networks. Single cell genomics shed additional insight on these pathways and reveals astrocytes in AD modulate genes related to neurotransmitter recycling, inflammatory response, and lipid metabolism. AD dysregulates astrocytic genes involved in neurotransmitter receptors (such as *GRIA2* and *GRM3*, which encode subunits of glutamate receptors), ion channels (*such as KCNIP4*, which encodes a protein that interacts with voltage-gated potassium channels), and even genes involved in synapses (such as *LRRC7*, which encodes a component of the post synaptic density of excitatory synapses, and *SYTL4*, which encodes a synaptotagmin). AD astrocytes also modulate genes involved in lipid metabolism, including *APOE* and *PLCE1*, which encodes a phospholipase. Several astrocytic genes differentially expressed in AD relate to cytoskeletal remodeling, including *GFAP* (which encodes an intermediate filament), *PLEC* (which encodes plectin, a protein that interacts with intermediate filaments), *SYNM* (which encodes another intermediate filament), and *ITGB4* (which encodes an integrin). AD astrocytes modify genes involved in cell growth, such as *SMURF2* (a member of the SMAD family important for cell growth). Collectively, these transcriptional changes highlight signaling pathways altered in AD astrocytes.

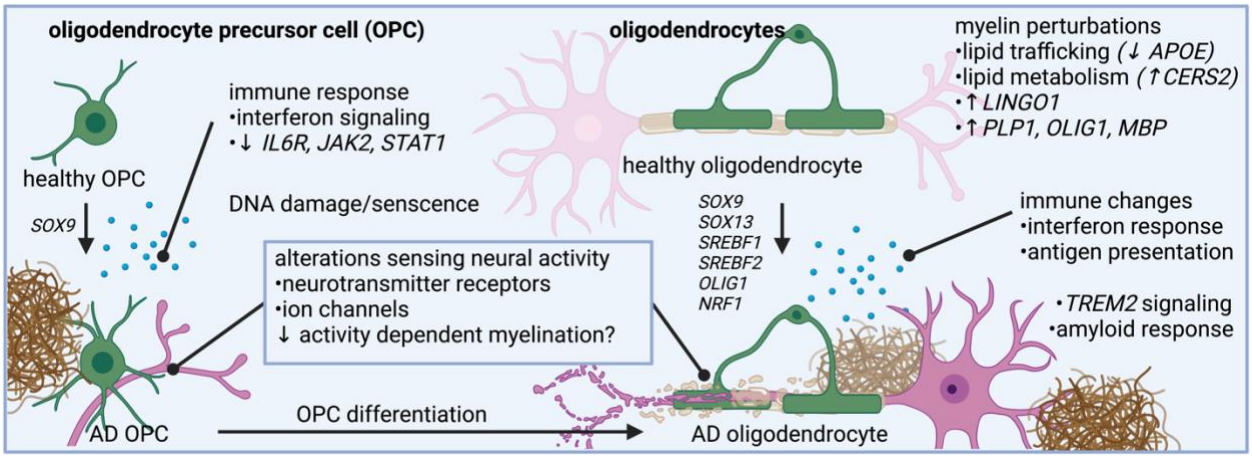

Figure 6. Molecular programs adopted in AD oligodendroglia revealed by single cell genomics. Oligodendrocytes in AD have altered pathways related to myelin synthesis, lipid trafficking, lipid metabolism, and immune related changes. Oligodendrocyte precursor cells also changes expression of genes related to neurotransmitter sensing and immune response.

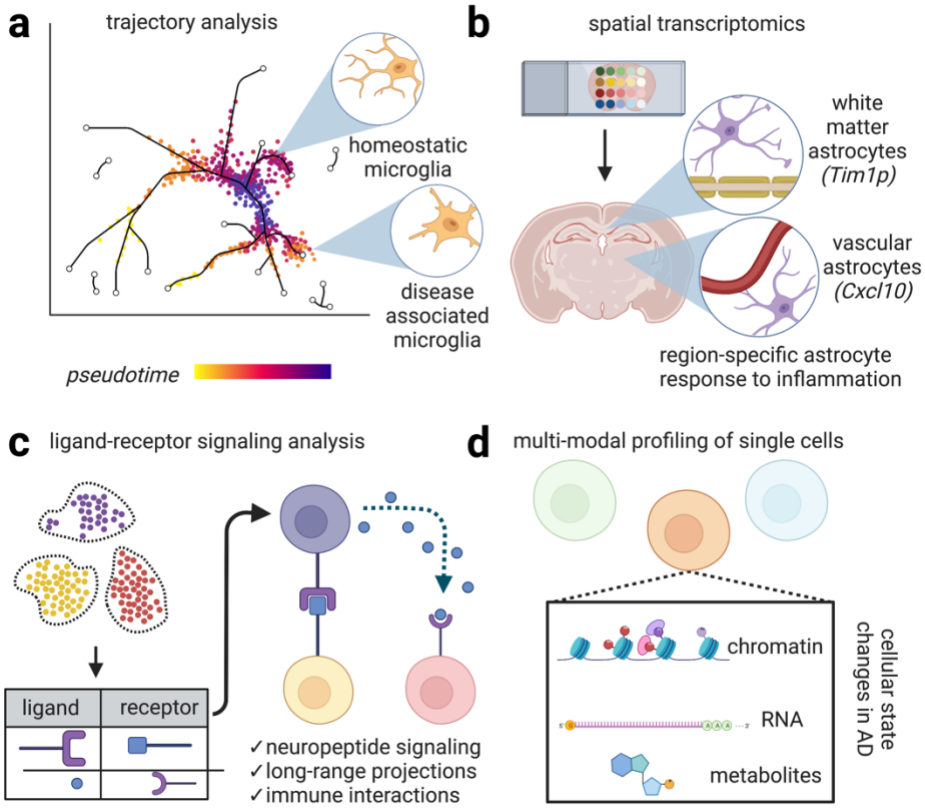

Figure 7. Emerging methods to interrogate single cell profiles in AD.
Emerging methods in single cell profiling will enhance our understanding of the distinct cellular signaling networks perturbed in AD. **(a)** Genetic analysis of single cells potentially enables the construction of dynamical cellular models according to "pseudotime," a quantitative measure of biological progression through a cellular process. Applying these models to AD potentially enables the trajectory of distinct cell types adopting new transcriptional states relating to disease progression, progression of microglia from homeostatic states to disease-associated states[52]. **(b)** Spatial transcriptomics is an umbrella term referring to techniques that combine mRNA readouts with spatial information. For example, one study revealed reactive subsets of astrocytes occupy distinct anatomical locations, such one astrocyte population lining white matter tracts that express the matrix metalloprotease inhibitor *Timp1*, which has been shown to be involved in amyloid response and has been shown to drive oligodendrocyte production and myelination and another astrocyte population marked by the cytokine *Cxcl10*+ adjacent to blood vessels[84]. Preserving spatial information while performing single cell profiling will likely further enhance our understanding of the molecular mechanisms driving AD. **(c)** Ligand-receptor signaling involves predicting signaling interactions based on ligand/receptor databases. This analysis has revealed, for example, dense peptidergic intracortical signaling networks[44]. Expanding our understanding of cellular physical and signaling interactions between brain cells with enhanced methods of examining these interaction networks will undoubtedly yield important insight into the progression of signaling networks in AD. **(d)** Multi-modal profiling of single cells to simultaneously chromatin, RNA, and potentially metabolites are emerging methods. These studies have revealed additional levels of regulation in distinct cell types in AD[77]. Combined with enhanced sequencing depth, these methods will generate a richer portrait of cellular function in neurodegeneration.

Table 1. Single-cell transcriptomic and epigenetic datasets from post-mortem AD tissue.
♦signifies particularly noteworthy study

Study	Data ID	Patient cohort	Brain region	Sequencing strategy	Total nuclei
♦Mathys[8]	syn18485175	48	PFC (BA10)	snRNA-seq	80,660
Davila[13]	N/A	112	Hippocampus	snRNA-seq	489,558
			Entorhinal cortex		
♦Grubman[7]	GSE138852	12	Entorhinal cortex	snRNA-seq	13,214
♦Leng[18]	GSE147528	10	Caudal entorhinal cortex	snRNA-seq	42,528
			Superior frontal gyrus	snRNA-seq	63,608
Zhou[9]	syn21125841	32	Dorsolateral prefrontal cortex	snRNA-seq	66,311
Lau[14]	GSE157827	21	PFC (BA6, BA8, and BA9)	snRNA-seq	169,496
Otero-Garcia[12]	GSE129308	8	Prefrontal cortex (BA9)	AT8 and MAP2 FACS	63,110
Alsema[127]	GSE146639	27	superior parietal lobe superior frontal gyrus	CD11/CD45 FACS; bc-Smart-seq2	
Marinaro[128]	N/A	12	PFC (BA9)	FACS neurons and glia; snRNA-seq	89,325
♦Yang[116]	GSE163577	17	hippocampus	Vascular enriched fraction then snRNA-seq	143,793
		8	superior frontal cortex		
Gerrits[129]	GSE148822	18	occipitotemporal cortex and fusiform gyrus	$NEUN^-/OLIG2^-$ FACS, then snRNA-seq	482,472 nuclei
Del-Aguila[130]	http://ngi.pub/snuclRNA-seq/	3	Parietal lobe	snRNA-seq	26,331
Olah[131]		14	dorsolateral prefrontal cortex	CD11b+/CD45+, snRNA-seq	16,242
		3	TNC		
♦Morabito[77]	syn3219045	20	PFC	snATAC-seq and snRNA-seq	191,890
Xu[132]	GSE181279	5	PBMCs	CD45 selection, then TCR-seq	36,849
Gate[133]	GSE134578	18	peripheral CD8+ TEMRA; CSF cells	TCR-seq	21,267
Smith[134]	GSE160936	12	entorhinal and somatosensory cortex	NEUN-/SOX10-	52,706 astrocytes and 27,592 microglia

Box 1. Considerations related to interpreting single cell data for the study of Alzheimer's disease.

AD classification. Assigning AD status is nontrivial because some individuals bearing AD pathology are cognitively normal, while some individuals clinically diagnosed with AD are found to not harbor AD pathology[19]. Variants in genetic risk factors such as APOE or TREM2[9] generate pleiotropic molecular effects. These factors underscore the importance of considering AD classification in profiling patient samples and interpreting single cell data.

Patient selection. Sex is a critical consideration in patient selection, as sex-specific AD associations have emerged in single cell profiling[8]. Additionally, racial and ethnic factors may be associated with differential AD risk[125,126], but these potential differences driving AD susceptibility are poorly understood at the single cell level. Many other confounds, including education, diet, sleep patterns, and exercise habits are known to generate epidemiological effects on AD risk and therefore may affect conclusions from single cell profiling—and may confer a tractable opportunity to define underpinnings of AD vulnerability.

Brain region. The anatomical routes AD pathology progresses through the brain are incompletely understood. Single cell studies are beginning to unravel this complexity by examining multiple brain regions, and spatial transcriptomics is facilitating insight into brain-wide transcriptional modules associated with AD dysfunction.

Single cell preparations. Archived brain tissue often suffers from compromised structural integrity, complicating efforts to purify whole cells, so most studies of human tissue rely on nuclei purification[135]. However, single nuclei preparations of microglia may not capture many AD-associated microglial genes that are observed in single soma preparations[136], and cytoplasmic mRNA may be lost in many other cell types. Additionally, debris related to single cell preparations following nuclei purification can complicate downstream analyses; while sorting nuclei by FACS prior to loading cells may remove some debris, stress-associated artifacts from cell sorting may confound some analyses.

Tissue preparation. Enzymatic dissociation induces stress-related transcriptional changes in microglia, and a cocktail of transcription/translation inhibitors (actinomycin D, ansiomycin, and triptolide) are thought to prevent dissociation related transcriptional responses[137]. Differences in single cell preparations between experimenters may further bias the enrichment of certain cell types, which may explain differences in proportions in brain cell types across datasets. Furthermore, some cell types evade certain preparations. For example, many vascular cells resist single cell dissociation protocols, which may account for their low yields in most datasets, and strainer-based methods to capture intact blood vessels helps enrich vascular segments for single cell analysis[116].

Computational analysis. Quality filtering, such as metrics of cell health (e.g., number of mitochondrial genes in nuclei datasets) and feature selection (e.g., number of genes detected, which can be a proxy for doublets), can affect downstream conclusions. Additionally, clustering resolution to define cell types can be highly dependent on individual experiments, and individual clustering algorithms can require user-defined parameters. Differential gene expression analysis is also highly variable, reinforcing the importance of biological validation. As sample sizes increase, batch correction and data integration across multiple datasets presents several statistical challenges. Thus, harmonizing single cell datasets may be essential to generate biological insight across analytical methods and tissue preparation protocols.

Genomic programs and biological function. AD-specific transcriptional differences may not reflect biological differences, partly because gene transcripts may not generate differences in protein levels owing to multiple levels of regulation. Furthermore, non-genomic programs may account for many aspects of AD dysfunction, including post-translational modifications and mRNA regulation, such as regulation of local protein synthesis in distinct cellular compartments and cell types, which may not be captured by single cell profiling.

Box 2. Cellular identity, cell states, and disease-associated molecular programs

Classifying cell types requires multiple levels of characterization to correlate transcriptional and epigenomic profiles of cellular identity with functional and developmental state[138]. In the study of AD, single cell genomics have provided a great deal of insight into transcriptional alterations in major brain cell types, but how these transcriptional profiles correlate with functional state in AD progression are incompletely understood. Sub-clustering analysis within major cell types often reveals transcriptionally distinct subtypes of cells within the AD brain that may be associated with AD pathology and cognitive dysfunction. Given the functional plasticity of cells, these sub-clusters may represent functionally distinct cell states that emerge in disease. Nevertheless, even neurons not burdened by tau pathology[18] and microglia not directly phagocytosing amyloid plaque[50] modulate genes related to cellular stress and protein folding in AD. These findings highlight the heterogeneity of disease-related molecular programs across and within cell types.

Disease-related molecular signatures may reflect cells sampling a distinct space of their cell identity, and the plasticity of these cell states may reflect the capacity for disease-modifying treatments to return cells to a homeostatic equilibrium. For example, the transcription factor PU.1 is thought to govern aspects of microglial state in AD[77,79], and modulating PU.1 can alter microglial gene expression[139] in a potentially therapeutically useful manner. Similarly, genes related to neuronal hyperactivity may relate to vulnerability or resilience to degeneration, and targeting neuronal hyperactivity using levetiracetam, an anti-epileptic agent which reduces hyperactivity and improves memory in APP/PS1 mice[140], may provide benefit for some AD patients[141]. Other efforts have identified molecular regulators of disease-associated states in astrocytes[77,86] and oligodendrocytes[77]. Further defining the molecular regulators of cellular states—and interrogating how therapeutic intervention might target state-related pathways—may advance the treatment of AD.

Single cell transcriptomics reveal downregulation of cell-type specific marker genes for some cell types. For example, microglia, interneurons, and endothelial cells downregulate canonical markers in AD. Impairment of signaling pathways directly encoded by these marker genes may influence neuronal circuits (such as reduced fractalkine and purine signaling in microglia[67,68], or reduced neuropeptide signaling in interneurons[46]). More broadly, loss of marker genes may indicate the very transcriptional programs governing the identity of distinct cell types is lost in AD, potentially leading to cellular senescence. Accordingly, senolytic treatment, which ameliorates pathology and cognitive impairment in APP/PS1 mice[108] may represent a therapeutic strategy for the treatment of AD[142].

Box 3. Single cell genetics, GWAS risk variants, and AD biomarkers.

Genome-wide association studies (GWAS) have highlighted genetic variants associated with sporadic, late-onset Alzheimer's disease[47,143,144]. Genomic information from single cells facilitates biological insight captured from GWAS studies in several ways:

•Single cell approaches help define which cell types highly express AD risk genes. For example, compared to other brain cell types, microglia are thought to express relatively higher levels of the risk variants *TREM2* and *CD33*[58,145]*.* The observation that expression of AD risk genes are enriched in immune cells has contributed to the hypothesis that immune mechanisms may play causal roles in AD[47].

•Distinct cell types differentially modulate the expression of AD risk genes[144]. For example, the risk gene *CNTNAP2*, a neuroxin family gene involved in cell adhesion, is upregulated in late-pathology AD neurons[8] but downregulated in astrocytes[7,8]; *BIN1* is upregulated only in AD excitatory neurons[7,8]; *APOE* is upregulated in microglia[7–9] and vulnerable populations of neurons[20] but downregulated in astrocytes[7–9]. The gene products of these risk variants likely affect many cellular processes in AD. Manipulations of risk variants within certain cells types, such as selective removal of neuronal APOE4[146] or astrocytic APOE4[88] in mice, highlight how cell type specific expression of risk gene variants governs signaling alterations in AD.

•Mutations in one gene can modify the expression and function of many other genes across many cell types. For example, individuals harboring *APOE4*[123] and *TREM2 R47H*[9] mutations carry differential expression for many genes across many cell types. Single genetic variants, such as 5XFAD mice lacking *Ccr7*[147] or *Trem2*[9]*,* and *APOE4*-knock in mice[88,148], generate widespread transcriptional variations in many cell types. Collectively, these findings underscore how single genetic perturbations vastly alter many cell types. Recent *in vitro* studies enable the perturbation of AD risk factors in distinct cell types, such as *APOE* genotype in distinct vascular[123] and glial[149] cell types, which further illustrate how risk variants affect distinct cell types. Single cell studies also suggest highlight expression of genes upstream of GWAS genes. For example, AD astrocytes upregulate of *TFEB*, which is upstream of ten GWAS loci, and which might control astrocytic disease-state transition[7].

Single cell genomics also generate insight into cell-type specific contributions to AD biomarkers. For example, *CHI3L1*, which encodes chitinase-like protein, a candidate cerebrospinal fluid (CSF) biomarker for preclinical AD, is upregulated in AD microglia[9]. Similarly, AD microglia upregulate *SORL1*[9] and *A2M*[9]*,* which encode CSF biomarkers, and downregulate *FTH1*[9], a serum marker. These findings highlight the potentially causal roles of immune mechanisms in AD. AD neurons downregulate *NEFL* and *BDNF*[7,8], which encode plasma biomarkers. Glial fibrillary acidic protein (GFAP), another biomarker, is upregulated in astrocytes from AD patients[7,8] and 5XFAD mice[50]. These findings showcase how single cell approaches define cell states potentially responsible for AD biomarkers, which may facilitate efforts to define cellular substrates driving distinct clinically distinct subtypes and pathological stages of AD.

Collectively, the overlap between risk variants and single cell genomics provide insight into cell types and signaling pathways that may govern AD progression. Further defining how genetic risk interface with non-genetic factors my further reveal signaling nodes governing AD pathogenesis, potentially informing new therapeutic approaches.

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