Integrative multi-omics analyses identify cell-type disease genes and regulatory networks across schizophrenia and Alzheimer's disease

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Abstract

Strong phenotype-genotype associations have been reported across brain diseases. However, understanding underlying gene regulatory mechanisms remains challenging, especially at the cellular level. To address this, we integrated the multi-omics data at the cellular resolution of the human brain: cell-type chromatin interactions, epigenomics and single cell transcriptomics, and predicted cell-type gene regulatory networks linking transcription factors, distal regulatory elements and target genes (e.g., excitatory and inhibitory neurons, microglia, oligodendrocyte). Using these cell-type networks and disease risk variants, we further identified the cell-type disease genes and regulatory networks for schizophrenia and Alzheimer's disease. The cell-type regulatory elements (e.g., enhancers) in the networks were also found to be potential pleiotropic regulatory loci for a variety of diseases. Further enrichment analyses including gene ontology and KEGG pathways revealed potential novel cross-disease and disease-specific molecular functions, advancing knowledge on the interplays among genetic, transcriptional and epigenetic risks at the cellular resolution between neurodegenerative and neuropsychiatric diseases. Finally, we summarized our computational analyses as a general-purpose pipeline for predicting gene regulatory networks via multi-omics data.

Introduction

Recent Genome Wide Association Studies (GWAS) studies have reported strong phenotype-genotype associations in brain diseases. For example, 97% of Alzheimer's disease patients, for instance, develop neuropsychiatric symptoms over the course of the disease [1]. In addition, a number of genetic risk variants have recently been found to be associated with multiple disorders. For example, 109 pleiotropic loci were found to significantly associate with at least two brain disorders [2], and many cross-disease common genetic risk factors have revealed many shared functional consequences in clinical presentations [3]. In addition, recent studies have revealed shared symptoms at both psychiatric and physical levels between neurodegenerative and neuropsychiatric diseases [3,4], and additional insights into the progression and causes of each have further demonstrated the highly interlinked nature of both disease types [5]. Building upon this, the progression of each disease rarely occurs within a neurological vacuum, and as such, there potentially exists a high degree of interplay between regulatory elements including various signaling mechanisms, immune response, and hallmark pathway development. The primary actors that sense, generate, and respond to changes are various cell types that simultaneously work in tandem to create complex networks of effect. Often, when looking at specific diseases, it is possible to discern cell type specific response factors at various stages of disease progression as well as between diseases. Thus, examining the genetic variability of multiple cell types with regard to expression and regulation presents an interesting insight into the network of potential interactions.

In neurodegenerative diseases, Alzheimer’s Disease (AD) is broadly characterized by the accumulation of Amyloid Beta plaques and Tau protein tangles within the brain. The pathology is complex, and a wide array of factors and pathways have been associated with it. Broadly speaking, the general progression contains both neuroimmune response elements and general interruptions in normal neuronal functioning. Brain atrophy is a hallmark feature of AD, primarily due to the Amyloid plaques and Neurofibrillary tangles that cause slow, but irreversible cell death over time. Primarily, the plaques that are normally cleared away and broken down remain in between neurons of patients with AD; this is further exacerbated by irregular microtubule formations that eventually collapse within the neurons, starving them of their transport mechanisms and leading to cell death. In tandem with considerations of brain atrophy as recognized through brain imaging, this leads to the three prominent clinically relevant “stages” of the disease: cognitively normal (CN), mild cognitive impairment (MCI), and Alzheimer’s Disease (AD). Neuroimaging assists this in two key ways: first, it helps with tracking initial brain state; second, changes in brain matter over time often inform disease progression rates. Specifically, Magnetic Resonance Imaging (MRI) utilize the gold standard of Brain Boundary Shift Integrals (BBSI), Hippocampus Boundary Shift Integrals (HBSI), and Ventricular Boundary Shift Integrals (VBSI) to quantify and track these changes over time. Computed Tomography (CT) scans help to rule out alternative causes of similar symptoms such as internal hemorrhages, brain tumors, and strokes. Lastly, Proton Emission Tomography scans are often used with radioactively labeled tracers to map glucose metabolism, but new techniques have recently been proposed to track other substances such as the characteristic amyloid plaques. Methods like these are useful in tracking the decline over time, but largely fail at performing preventative measures and are often used in late stage care. Some studies have suggested links between decreased gray matter within patients with Down Syndrome and AD as well as higher levels of glucose uptake, which makes PET an ideal method for studying this connection. Lastly, the diagnosis of AD is only confirmed in post-mortem analysis, making the need for a mechanistic understanding even more crucial as it can help shed light on early-stage risk factors. Much of the conversation surrounding AD research today concerns forms of the apolipoprotein E (APOE). Certain variants like APOE2 are thought to have neuroprotective effects due to their ability to properly clear formed molecules [6–8]. At the same loci, the APOE4 gene has the opposite effect, drastically increasing the likelihood of developing AD due to the formation of a salt bridge between the standard sites of clearance. This then results in accumulation in the brain and eventual neuron death due to the inflammatory response and reactive oxygen species-based impacts that amyloid beta depositions develop [9–14]. Eventually, neurodegeneration at both the cellular and cognitive levels develop, leading to standard AD pathology.

Schizophrenia (SCZ) is a neuropsychiatric disease characterized molecularly through disruptions in dopamine, glutamate, and GABA-based receptor pathways and presenting clinically through both positive symptoms such as altered perceptions of reality as well as negative symptoms including anti-social behaviors [15,16]. SCZ often leads to the need for lifelong continual treatment in the form of antipsychotic medications, which is similar in nature to chronic AD treatment. However, this creates the potential for increased tolerance and decreased sensitization to specific effects of target therapies (general antipsychotic drug resistance with regard to mechanism of action remains unclear, but the consensus remains that resistance is rarely the entire functional pharmacology of a specific drug and rather a specific behavioral element or effect) [17]. In light of this fact, a new wave of “atypical” antipsychotic medications has emerged that fundamentally operates with new mechanisms of action compared to the original first and second line treatments [18]. On the front of pathology, patients diagnosed with SCZ exhibit decreased grey matter volume - a key player in both muscle control and sensory perceptions - that forms due to decreased neuron size [19,20]. A juxtaposition between AD and SCZ, where the former leads to characteristic neurodegeneration and degradation over the course of the disease whereas the latter affects neuron size and density but not count, can be drawn. To this end, exploring the connection between AD and SCZ at the level of shared phenotype-based interactions presents an interesting opportunity to explore the interplay between each of these diseases. The increasing relative prevalence of AD in the ever aging population presents a growing need for better understanding both of disease specific and potentially cross disease elements [21]. At the level of clinical presentation, shared symptoms (primarily psychiatric effects) create a key point of intersection to be explored. General psychosis, for instance, is found in up to 60% of AD patients, including hallucination events as well as other effects mirroring those of the positive symptoms found in SCZ patients [22–24]. Genome-based analysis of AD patient cohorts has revealed multiple conserved genetic loci that could encode shared risk factors between the two diseases [22]. Patients who exhibit symptoms of psychosis in addition to standard AD pathology and symptomology experience significantly worse clinical outcomes than those who do not [24]. The combination of each of these shared genetic and clinical factors presents an opportunity to explore regulation of multiple factors at a cellular resolution between common neurodegenerative and neuropsychiatric diseases - in this case, AD and SCZ respectively.

Recent analyses have also revealed that brain disease risk variants are located in non-coding regulatory elements (e.g., enhancers) and that the risk genes likely have cell-type specific effects including neuronal and non-neuronal types [25,26]. In addition, recent single-cell studies suggest changes to cell-type specific gene expression in brain diseases [27,28]. The gene expression is fundamentally driven by a variety of gene regulatory factors like transcriptional factors (TFs) and regulatory elements. These factors work cooperatively as gene regulatory networks (GRNs) to carry out cellular and molecular functions. However, our understanding of the gene regulatory networks driving cell-type and disease-specific gene expression, especially across diseases remains elusive. To address this, a number of computational methods have been proposed to predict cell-type GRNs [29], such as PIDC [30], GENIE3 [31] and GRNBoost2 [32]. However, the methods typically use single omics only (e.g., transcriptomics) and predict networks based on statistical associations (e.g., co-expression), providing insufficient mechanistic insights into gene regulation at the cellular resolution. Thus, it is essential to integrate emerging multi-omics data for a deeper understanding of cell-type gene regulation, especially involving non-coding regulatory elements. Recent studies have shown that integrating multi-omics data can not only reduce the impact of noise from a single omics data, but can also achieve better prediction accuracy [33].

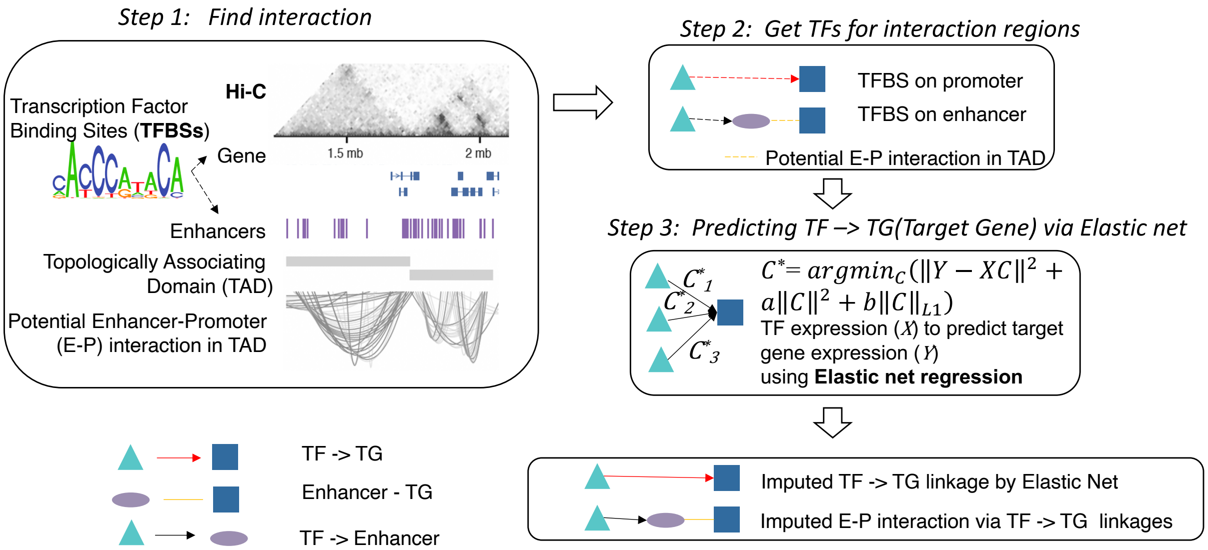
Step 1: Find interaction
Hi-C
Transcription Factor Binding Sites (TFBSs)
Gene
1.5 mb
2 mb
Enhancers
Topologically Associating Domain (TAD)
Potential Enhancer-Promoter (E-P) interaction in TAD
Step 2: Get TFs for interaction regions
TFBS on promoter
TFBS on enhancer
Potential E-P interaction in TAD
Step 3: Predicting TF –> TG(Target Gene) via Elastic net
C^*_1
C^*_2
C^*_3
$C^* = argmin_C(\|Y - XC\|^2 + a\|C\|^2 + b\|C\|_{L1})$
TF expression (X) to predict target gene expression (Y) using Elastic net regression
TF -> TG
Enhancer - TG
TF -> Enhancer
Imputed TF -> TG linkage by Elastic Net
Imputed E-P interaction via TF -> TG linkages

Figure 1. The computational pipeline for predicting the gene regulatory network via multi-omics data. The scGRN pipeline inputs the chromatin interactions (e.g., from Hi-C) of regulatory elements (e.g., enhancer-promoter), identifies the transcription factor binding sites (TFBSs) on the regulatory elements, predicts TF-target gene expression relationships (e.g., high coefficients from Elastic net regression), and finally outputs a gene regulatory network linking TFs (cyan), regulatory elements (purple) to target genes (blue).

To explore these ideas, this paper presents a computational pipeline, *scGRN* by integrating multi-omics data to predict cell-type gene regulatory networks (GRNs) linking TFs, regulatory elements (e.g., enhancers and promoters) and target genes (**Figure 1**, Methods). In particular, we applied the pipeline to the multi-omics data at the cellular resolution such as chromatin interactions, epigenomics and single-cell transcriptomics of major cell types in the human brain including excitatory and inhibitory neurons, microglia, and oligodendrocyte. Our predictions provide additional information on cell-type gene regulation such as linking regulatory elements to genes, and they are found to be consistent with the state-of-the-art methods [29]. A recent study has benchmarked a number of methods of predicting cell-type GRNs and found the ones with high prediction accuracy [29] (Methods). We also found that the enhancers in our cell-type GRNs (e.g., microglia) are enriched with GWAS SNPs in human diseases including mental disorders and Alzheimer's disease. Thus, we further linked GWAS SNPs to cell-type disease genes and regulatory networks for schizophrenia (SCZ) and Alzheimer's disease (AD), two majorly represented neuropsychiatric and neurodegenerative diseases as described above and found cross-disease and disease-specific genomic functions (**Figure 2**).

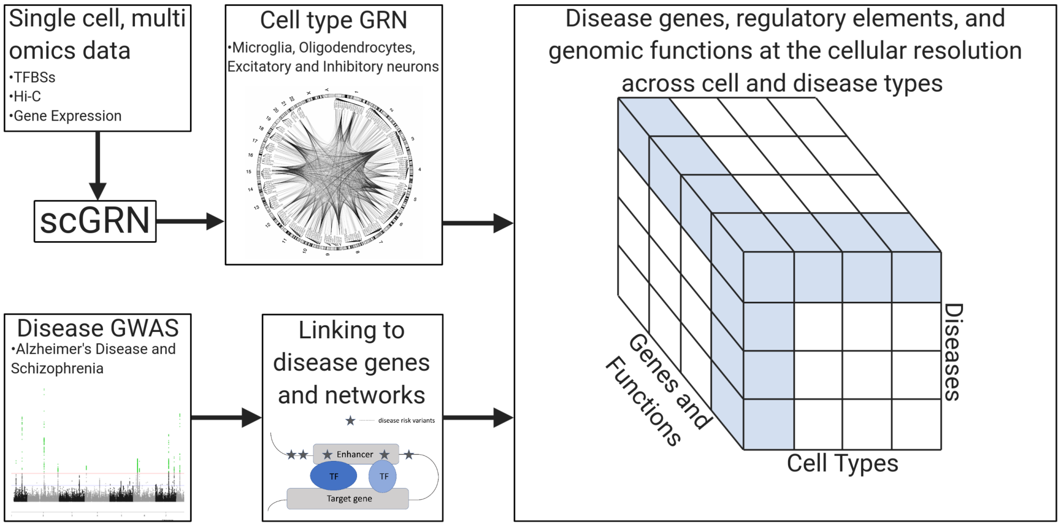
Single cell, multi omics data
•TFBSs
•Hi-C
•Gene Expression
scGRN
Cell type GRN
•Microglia, Oligodendrocytes, Excitatory and Inhibitory neurons
Disease GWAS
•Alzheimer's Disease and Schizophrenia
Linking to disease genes and networks
disease risk variants
Enhancer
TF
TF
Target gene
Disease genes, regulatory elements, and genomic functions at the cellular resolution across cell and disease types
Genes and Functions
Cell Types
Diseases

Figure 2. Identification of cell-type disease genes, regulatory elements and functions. We applied our pipeline to the multi-omics data of the human brain at the cellular resolution and predicted the cell-type gene regulatory networks for four major cell types in the human brain: excitatory and inhibitory neurons, microglia and oligodendrocytes. We further linked GWAS SNPs to cell-type disease genes, regulatory elements and functions using these cell-type gene regulatory networks. Our analyses enable revealing the interplay among variants, genes, cell types and disease types in a functional genomic resource at the cellular resolution for the human diseases (e.g., Alzheimer’s Disease and Schizophrenia as demonstrated in the paper).

Results

Predicting cell-type specific gene regulatory networks in the human brain

We applied our pipeline, scGRN to the multi-omics data for the human brain including cell-type chromatin interactions [25], transcription factor binding sites [34], and single-cell transcriptomics [28]. As shown on **Figure 3A** (and Supplemental file 1), we predicted cell-type GRNs for major cell types: excitatory neuron (254 TFs, 5126 TGs, 45258 TF-TG links with 30066 enhancers), inhibitory neuron (192 TFs, 4739 TGs, 37231 TF-TG links with 29210 enhancers), microglia (68 TFs, 1768 TGs, 5249 TF-TG links with 7090 enhancers), and oligodendrocyte (210 TFs, 3496 TGs, 29556 TF-TG links with 15544 enhancers).

In addition, we compared our predicted microglial gene regulatory network with existing state-of-the-art methods for predicting cell-type gene regulatory networks, particularly, those that are consistent and highly accurate PIDC, GENIE3 and GRNBoost2 benchmarked by BEELINE [29]

(Methods). Out of 14135 TF-TG links in our microglial GRN, 10222 links (approximately 72%) were also shared in the predicted networks by these methods, suggesting a high consistency between scGRN and these methods. However, these methods predicted TF-TG links without providing information on involved regulatory elements like enhancers. Thus, we looked further at the enhancers from shared and unique links in our microglial GRN and found that they are significantly enriched with disease risk SNPs ($p<0.05$), suggesting potential pleiotropic roles in gene regulation for a number of diseases (Methods). In particular, as shown on **Figure 3B**, we found that a number of diseases - including mental disorders, nervous system diseases and immune system diseases - are significantly enriched in shared microglial enhancers. Additionally, Inflammatory Bowel Diseases (IBD), digestive system diseases and AD are significantly enriched with our unique microglial enhancers. Also according to our observations, the enhancers from other cell-type GRNs are enriched with a number of disease-associated SNPs including neuropsychiatric and neurodegenerative diseases (Supplemental file 2).

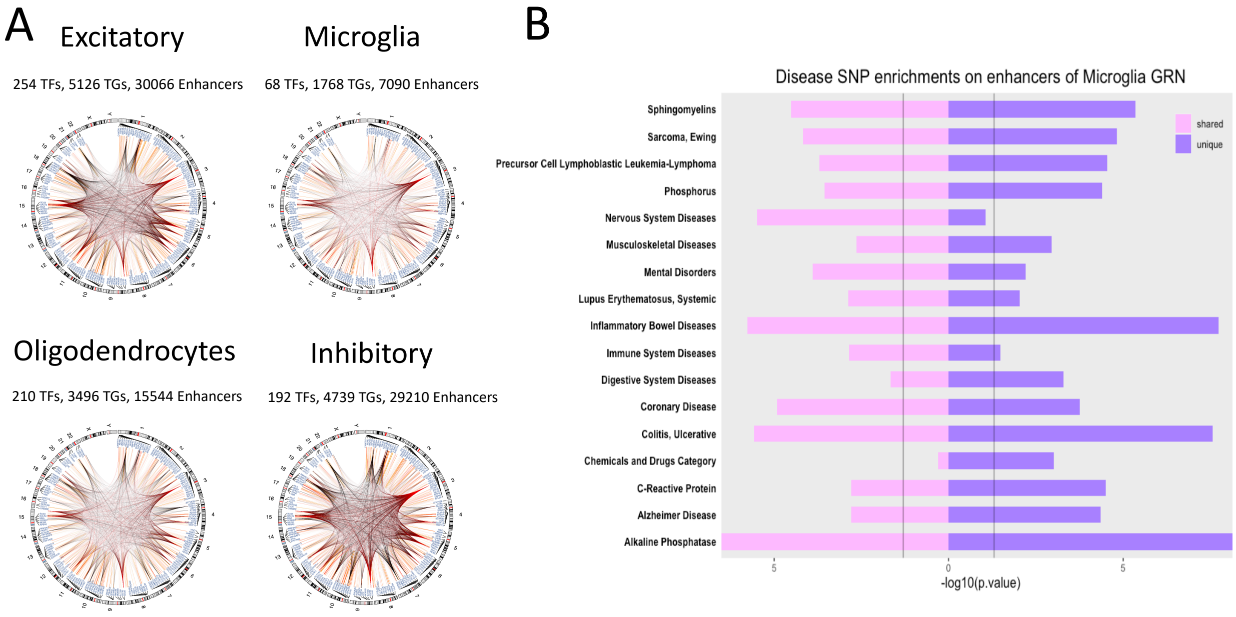

Figure 3. Cell-type gene regulatory networks (GRNs) for four major cell-types, excitatory and inhibitory neurons, microglia and oligodendrocytes in the human brain. (**A**) Cell-type GRNs. The black, yellow and red links represent the interactions of TF to enhancer, enhancer to target gene, and TF to target gene promoter, respectively. (**B**) The enrichments of SNPs associated with various diseases and traits (bar) on the enhancers of Microglia GRN. Bar height is -log10(p value) of the enrichment. Pink represents the enhancers predicted by both scGRN and other state-of-the-art methods. Purple represents the enhancers uniquely predicted by scGRN only.

Identifying cell-type disease genes in AD and SCZ for both neuronal and non-neuronal types

We further used these cell-type GRNs to link GWAS SNPs to disease risk genes for each type, advancing knowledge on cross-disease and disease-specific interplays among genetic, transcriptional and epigenetic risks at cellular resolution. In particular, we chose schizophrenia

(SCZ) and Alzheimer's disease (AD), two majorly represented neuropsychiatric and neurodegenerative diseases with potential convergent underlying mechanisms [1], and we linked a number of cell-type disease genes (Methods, Supplemental file 3). We then performed the enrichment analyses for these cell-type disease genes to understand cross cell-type and specific functions (**Figure 4**, Supplemental file 4). The majority of the microglia-based functionality revolves around the standard operations of the cell primarily concerning neuroimmune response elements of disease pathology. For example, in AD, we observed known key pathways surrounding the functional consequences of Amyloid Beta formation ($p<3.21e-6$) [35] including MAPK1/MAPK3 signaling ($p<3.92e-8$) [36] and neurite growth (axonogenesis) ($p<2.23e-14$) [37]. In a similar path, the oligodendrocyte analysis reveals Tau protein binding ($p< 1.72e-5$) [38] and tubulin binding ($p<2.29e-4$) [39] are heavily associated with the enrichment. This is vital to the understanding of a multitude of diseases that commonly demonstrate atrophy of cortical tissue as a hallmark feature. In SCZ, then, at a neuroimmune response level, we found that multiple key hallmark pathways were implicated, including dopaminergic synapse p($<7.78e-9$) [40,41], and signaling by both Receptor Tyrosine Kinase ($p<1.86e-10$) [42,43] and cGMP-PKG ($p<2.94e-7$) [44]. From an oligodendrocyte-based context, we observed that multiple forms of synapse function and regulation were enriched - specifically, the regulation of trans-synaptic signaling ($p<1.95e-8$) [45], modulation of chemical synaptic transmission ($p<1.80e-8$) [46], and neuron-to-neuron synapses ($p<2.57e-11$) [47]. The overarching theme of these observations concerns furthered impacts on signaling and transmission between neurons which Schizophrenia pathology widely believes to be implicated [45,46,48,49]. This is in line with the physical effects of SCZ, both positive and negative, as overactive dopamine channels have been suggested to create hallucinations (visual and auditory) [50], which is a hallmark feature of SCZ diagnoses.

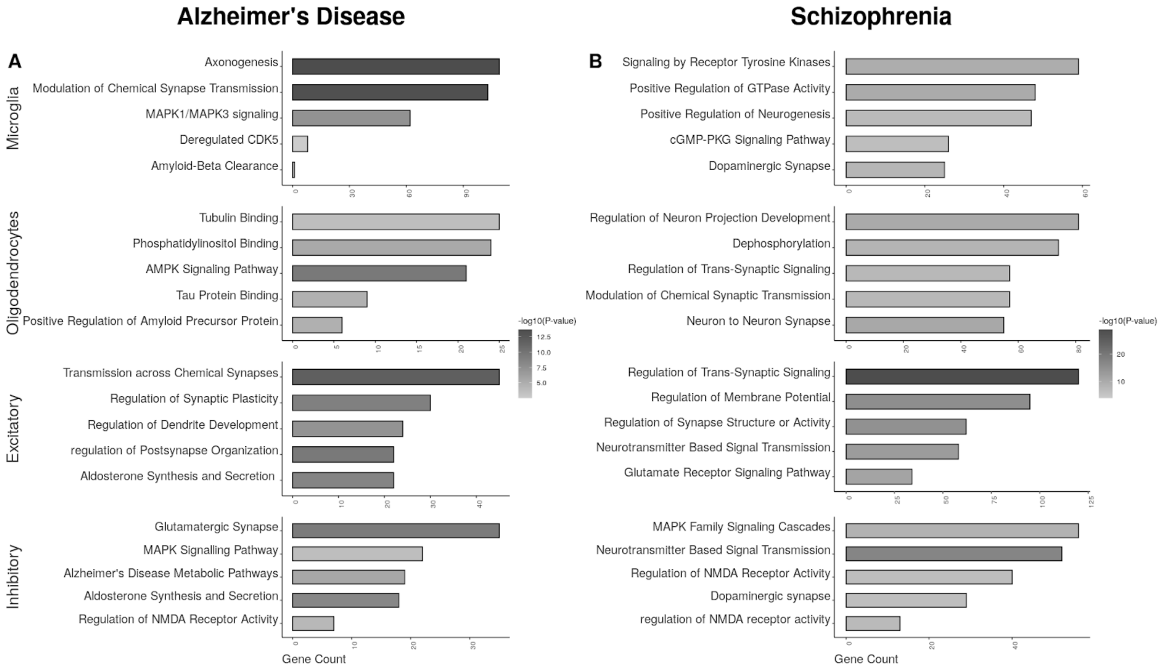

Figure 4. Cell-type specific pathways and functions enriched in the disease genes. (**A**) Alzheimer’s Disease. (**B**) Schizophrenia. Darkness is proportional to -log10(p value) of the enrichment. Bar height represents the gene count.

In neuronal types, excitatory and inhibitory neuronal functions generally revolve around cell signaling pathways within the brain. In particular, our analysis identified both ion channel specific and broad signaling mechanisms as affected. In AD, multiple forms of chemical transmission and neuronal cell regulation were found to contribute to the neurodegenerative nature of the disease: transmission across chemical synapses (p<1.04e-12) [51,52] as well regulation of synaptic plasticity (p<1.12e-9) [52,53], dendrite development (p<1.01e-8) [53,54], and postsynapse organization (p<1.12e-9) [55]. Similarly, when examining inhibitory neurons within the context of AD progression we noted additional forms of signaling and regulatory pathways as enriched: glutamatergic synapses (p<4.22e-10) [55], MAPK signaling (p<2.38e-4) [36], and NMDA receptor activity regulation (p<7.19e-5) [29]. Lastly, SCZ enrichment for both excitatory and inhibitory neurons were similar to those of AD, possessing unique, disease-specific interactions. For excitatory neurons, we observed neurotransmitter-based signal transmission (p<1.11e-13) [56] as well as regulation of trans-synaptic signaling (p<1.46e-29) [45], synapse structure and activity (p<1.02e-15) [49], and glutamate receptor signaling (p<3.90e-12) [41]. From the inhibitory neuron standpoint, SCZ enrichment shared multiple enrichment terms with AD, suggesting abstraction from standard disease-specific pathology and broader atypical neuronal signaling and activity. Specifically, we observed MAPK family signaling (p<1.63e-9) [42], regulation of NMDA receptor activity (p<5.09e-8) [56], dopaminergic synapses (p<2.07e-7) [40,41], and neurotransmitter-based signal transmission (p<3.71e-18) [56] to be enriched. The general consensus is that similar pathways are observed to be enriched between diseases and cell types, but there are disease and cell type specific implications for clinical presentation.

Revealing disease related functions involving multiple cell-types in AD and SCZ

In addition to cell-type specific pathways in these diseases, we also identified those involving multiple cell types in each disease (**Figure 5**), which implies that potential cell-type interactions are driving the disease pathology. Specifically, in AD, multiple pathways implicated in neuro-degradation and intercellular signaling were found to be significantly enriched across multiple cell types. For instance, AMPK signaling (p_excitatory<9.65e-7, p_oligodendrocytes<2.98e-10) [57,58], asymmetric and dopaminergic synapses (p_microglia<2.27e-21, p_oligodencrocytes<2.26e-5, p_excitatory<5.82e-17, p_inhibitory<3.29e-15) [59] and (p_microglia<7.94e-8, p_oligodencrocytes<2.78e-7, p_excitatory<8.07e-7, p_inhibitory<1.37e-7) [60], respectively, as well as postsynaptic specialization (p_microglia<5.88e-21, p_excitatory<1.73e-17, p_inhibitory<1.37e-15) [61] were all found to be significantly enriched in more than two cell types. The AMPK signaling is involved as a master kinase regulation element in ATP synthesis production and in the context of Alzheimer's Disease, has aided both amyloid production and tau phosphorylation [30]. In terms of synapse-based analysis, we found that both asymmetric synapses (AS) and dopaminergic synapses (DS) are enriched. The asymmetric synapses are characteristic of excitatory neurons compared to symmetric synapses which are more relevant in the context of inhibitory neurons. Previous work has shown AS deficits may be linked to cognitive decline in AD patients [62], but recent work suggests that no such disparity exists [63]. There is, however, evidence that synapse projection targets change in

AD patients as the disease progresses [64]. Additionally, there is a consistent and growing body of work in the study of dopaminergic synapses that supports the role of both dopamine and associated receptors, synapses, and regulatory mechanisms within the context of AD pathology [60], suggesting its vital role in memory and cognition - especially within the hippocampus. Clinically, imaging biomarkers like the peripheral hippocampal brain boundary shift integral (BBSI) [65] are commonly used to track the neurodegeneration of neural and hippocampal volumes over time in order to measure dementia progress in living patients. The combination of both hippocampal atrophy and altered chemical signaling suggests that dopamine-based memory pathways enrichment across all four cell types for AD analysis has vital functional consequences for AD in clinical presentation. Postsynaptic specialization was additionally highly enriched across microglia as well as across excitatory and inhibitory neurons. It has been shown to be a preclinical biomarker for neurodegeneration [66]; however, such methods suffer from the need to retrieve cerebral spinal fluid (CSF) based samples which are difficult to obtain from humans and lead to poor compliance in clinical trials. Finally, at a higher level, protein-protein interaction (PPI) level analysis through Metascape [61] revealed additional elements to be enriched through our analysis for intersecting loci of interest (**Figure 5C**). Here, the plasma membrane cell projection morphogenesis (p<3.98e-17) was significantly enriched. Biologically, this presents remarkable implications for the communication between cell types in the process of amyloid plaque formation [67].

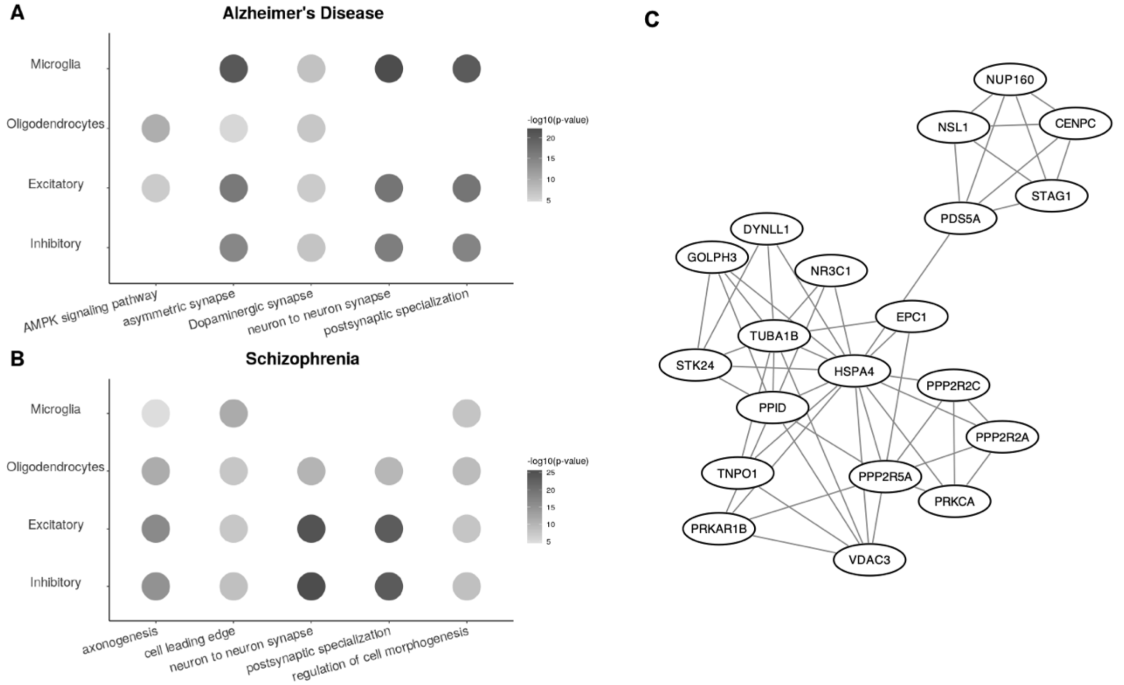

Figure 5. Cross-cell-type conserved pathways and functions in the diseases. Darkness is proportional to -log10(p value) of the enrichment. Terms are selected with p values < 1e-5. (**A**) Alzheimer’s Disease. (**B**) Schizophrenia. (**C**) A protein-protein interaction (PPI) network of shared disease genes across at least three cell types in Alzheimer's Disease (AD), implying cellular conserved PPIs in the disease.

The primary elements of SCZ include changes in cell shape and type rather than strict degradation, as demonstrated in AD-based enrichment. Clinically, this is consistent with the general consensus that SCZ is strictly neuropsychiatric as opposed to degenerative. In this same vein, axonogenesis (p_microglia<2.91e-5, p_oligodencrocytes<4.56e-12, p_excitatory<5.27e-17, p_inhibitory<7.91e-16) [68], regulation of cell morphogenesis (p_microglia<1.01e-8, p_oligodencrocytes<9.11e-10, p_excitatory<1.56e-8, p_inhibitory<2.65e-9) [69], neuronal cell synapses (p_oligodencrocytes<7.26e-11, p_excitatory<2.28e-25, p_inhibitory<2.17e-26) [49] were all enriched. Multiple axon guidance pathways associated with general axon growth in axonogenesis have been heavily implicated in the broad mechanism of SCZ pathology; elucidating this mechanism has remained difficult in part due to the polygenic nature of the disease. Broadly, it is thought that missteps in guidance cues can lead to eventual presentation and disease onset, but the underlying pathway remains unclear [68]. On the front of cell morphogenesis, early life neurodevelopmental genetic markers may suggest causal links with alterations in hippocampal cell differentiation points leading to cascades of downstream effects [69]. This has primarily been studied and modeled within the scope of iPSC-based analyses, which make correlations and connections to the clinical presentation more difficult due to the additional abstraction from standard pathology-based analysis. Finally, neuron-to-neuron synapses were enriched in SCZ analysis across all four cell types. This is characteristic of standard SCZ progression and onset and has been widely supported through a growing body of evidence. In particular, multiple studies have been performed within the hippocampus that measure key levels of synapse proteins significantly declining [49]. While there are some forms of decline in neuronal cell content, SCZ enrichment generally focused on molecular cues that led to eventual pathology as opposed to specific hallmark molecules as observed in AD pathology. The lack of these molecules makes mechanistic understandings more complex as it often associates multiple genetic loci as well as metabolic, neurodevelopmental, and homeostasis with maintaining based pathways.

Comparative analyses reveal the interplays between genomic functions, cell types, and diseases

We found that a variety of cross-disease conserved functions are involved in one or multiple cell types, revealing potential novel functional interplays across cell types and diseases (**Figure 6A**). For example, active regulators of proline residue protein folding sites in the family of peptidyl-prolyl isomerases (PPIases) have been found to relate to AD hallmark molecules. This includes, for example. the microtubule tau tangles, due to their proline-rich nature of repeat regions within tau binding sites [70]. Additional study also suggests that these phosphorylation sites lead to hallmark oligomerization and aggregation of the tau proteins within standard AD progression [71]. Our analysis observed fairly uniform enrichments of PPIases across all four types in both AD and SCZ; however, within the context of SCZ, Microglia are more enriched than others, suggesting potential microglia-specific mechanisms of PPIases across AD and SCZ. In AD, it has been noted that microglial activation precedes standard tau pathology within P301S tau gene mutant mice models [72]. In SCZ, however, little work has been done to quantify and study the effects of potential alterations in PPIases, especially in the Microglia. A broad proteomic analysis of gray matter in the anterior cingulate cortex (ACC) has revealed differential PPIase levels within SCZ patients, suggesting the ACC as a potentially novel therapeutic target area.

Although conserved between AD and SCZ, we also found that a number of functions involve different cell types across diseases. For example, we found that the neuron to neuron synapses have conserved high enrichments for Excitatory and Inhibitory neurons between two diseases but different enrichments for Microglia and Oligodendrocytes. The functional role of signaling consequences for both Microglia and Oligodendrocytes in each disease has been well characterized. AD patients usually demonstrate progressive and irreversible synapse loss and dysfunction as well as alterations in synaptic transmission. SCZ patients are thought to have a relative imbalance in the signaling between these cell types that leads to neuropsychiatric symptoms [52,73]. Thus, the relative functional impact manifests in disease-specific ways but with a shared origin. More interestingly, when exploring the interactions between cell types that change between diseases, the disease-specific pathologies enter into the picture to help explain the cause of discrepancies. In particular, for AD, it is shown that phagocytic Microglia are activated during early stages of synaptic decline which leads to eventual neuroinflammation and programmed cell death [74]. For SCZ, Oligodendrocyte analysis reveals similar intercellular mechanisms between Excitatory and Inhibitory Neurons, specifically those regarding disruption of interneuron synaptic signaling, providing potential direction for future exploration and validation of the communication role of Oligodendrocytes [75].

Finally, we also found a set of cross-disease conserved functions enriched with unique cell types in AD and SCZ. For instance, the cGMP dependent Protein Kinase G (cGMP-PKG) signaling shows higher enrichment within Microglia for AD and shifted into higher enrichment in both Excitatory and Inhibitory neurons for SCZ. First, cGMP-PKG signaling is involved in numerous pathways throughout the central nervous system (CNS) [76], and the functional consequence between diseases surrounds decreased neuronal plasticity [77]. In AD, Microglia are likely involved at a neuroinflammatory level [78]; similarly in SCZ, Excitatory and Inhibitory neurons are likely involved with alterations in synaptic plasticity through perturbed signaling between regulatory elements [44]. Overall, we reveal the intrinsic difference between the disparities at the clinical presentation level but also potential similarities at the cellular level for shared dysfunction.

Gene regulatory networks associated with functional interplays across cell types and diseases reveal additional disease risk regulatory elements and network dynamics in the development of disease functions

To further understand gene regulatory mechanisms in functional interplays among cell types and diseases, we looked at the enhancers targeting the genes of the cross-disease enriched terms in **Figure 6A** in the cell-type GRNs. We found that these enhancers (+/- 1kb) are significantly enriched with GWAS SNPs for a number of additional diseases (**Figure 6B**) - for example, Parkinson disease for peptide-threonine phosphorylation and modification, Nervous system disease for exocytic vesicle, and Amyotrophic lateral sclerosis for the neuron-to-neuron synapse. This suggests that these distant regulatory elements are likely potential novel disease risk regions across multiple diseases and that they have cell-type specific regulatory networks targeting the genes of cross-disease functions.

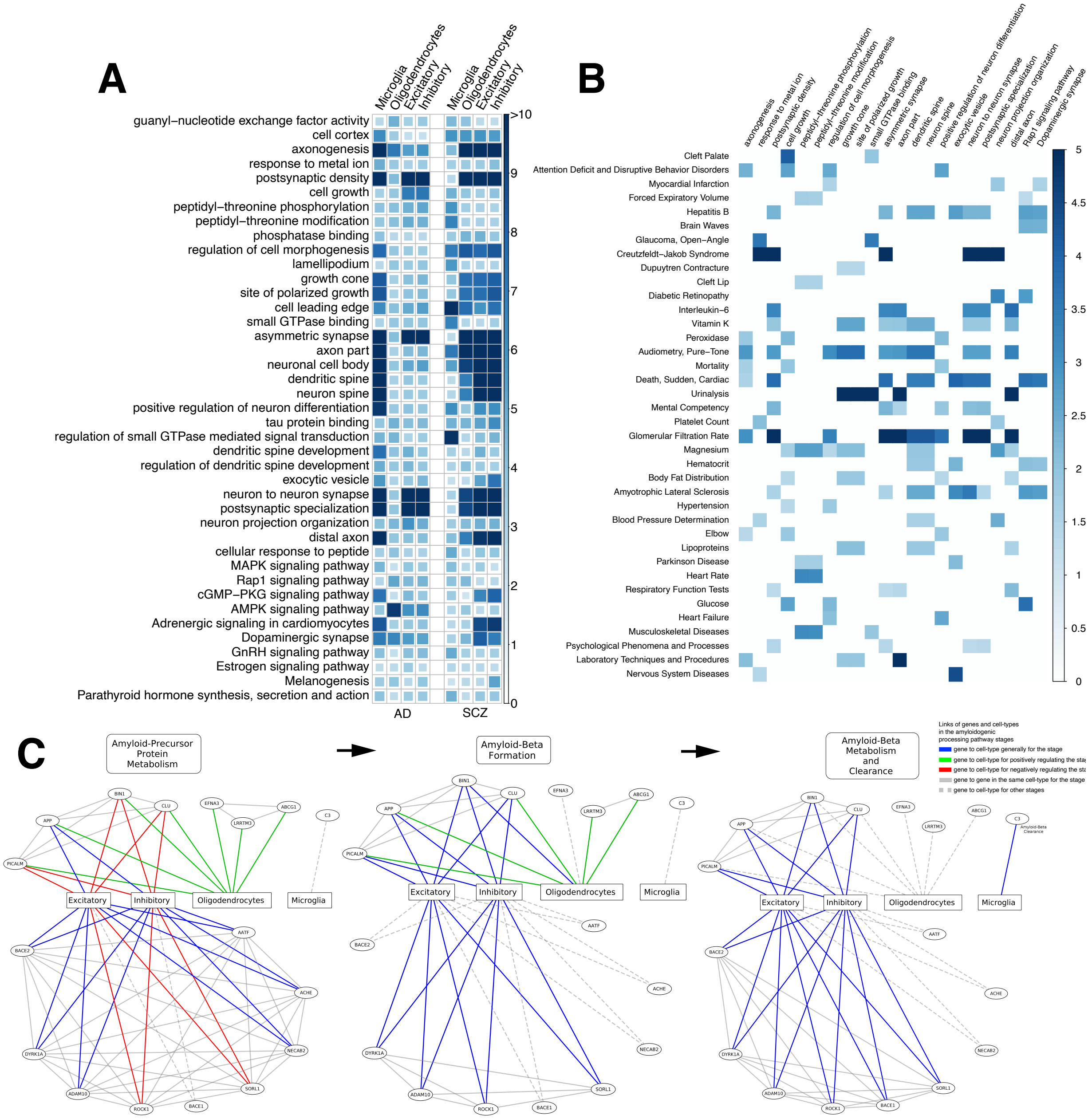

Figure 6. Comparative analyses revealed the interplay of genes, cell types, pathways/functions and diseases. (**A**) Cross-disease conserved cell-type pathways and functions between Alzheimer's Disease and Schizophrenia. Darkness is proportional to -log10(p value) of the enrichment. (**B**) The enrichments of SNPs associated with various diseases and traits on the enhancers regulating disease genes of cross-disease conserved pathways and functions in (**A**). Darkness is proportional to -log10(p value) of the enrichment. (**C**) Dynamic networks linking disease genes and cell types across the amyloidogenic processing pathway stages (shown on top of each network) in Alzheimer's Disease. Blue: links of genes and cell types for the stage. Green/Red: links of genes and cell types for positively/negatively regulation of the stage. Grey: links of genes in the same cell types for the stage. Dashed: links of genes and the cell types that are associated with other stages.

We also observed that the cell-type gene regulatory networks in these disease risk enhancers change in the development of disease functions, revealing dynamic regulatory mechanisms at the system level. For example, a complex multi-cell-type mechanism for amyloid beta formation and processing was established in AD. Our gene regulatory networks show the changes to amyloid precursor protein (APP) interactions in amyloid beta formation, clearance, and metabolism (**Figure 6C**). In particular, the APP gene was found to be enriched across multiple cell types, following the central mechanism of enzymatic cleavage of APP to form the hallmark amyloid beta protein through the amyloidogenic processing pathway. Here, the non-amyloidogenic processing pathway typically generates the P3 terminal fragments that do not proceed to oligomerize and form plaques within the brain [79–81]. Additionally, their role in clearance is vital because amyloid beta is typically cleared from the brain and processed in healthy aging [82,83] which creates a commonly explored site for therapeutic efforts [84]. Unlike the APP gene involved in multiple cell types, our network analysis observed the cell-type specific regulation changes in the AD amyloid for all four cell types. For example, we found that the C3 gene is specific in Microglia for amyloid beta clearance, which was also reported by previous studies; C3 knockout mice surround engulfment of synapses by microglia fulfilling a standard neuroimmune function in healthy controls [85] that declines in the absence of C3 [86,87].

We also identified multiple genes that were both unique and conserved between multiple stages of the amyloidogenic processing pathway (**Figure 6C**). For example, BIN1 and CLU were both found to be enriched among Oligodendrocytes, Excitatory and Inhibitory neurons in Amyloid Precursor Protein metabolism, and Amyloid Beta formation and metabolism. BIN1 was found to be the second most predominant loci involved in AD progression (following APOE) [88] and was not involved in later steps of amyloidogenic processing from later stage AD progression, falling in line with previously identified involved steps [89]. In addition, BIN1 has been found to link to microglia [25] and be commonly implicated in Tauopathy, further supporting the status of BIN1 as a high risk loci for AD [90]. Similarly, CLU has been shown to be enriched and heavily implicated in multigene pathways, particularly APOE, to lead to eventual AD progression. Like BIN1, it is a key marker gene as well, and it is screened for AD patients to determine the progression of the disease clinically [88]. Mechanistically, the function of CLU remains unclear and conflicting prevailing theories as to the exact method of progression remain unanswered [91]; at a broader level, however, the interaction between APOE and CLU is certain. Instances of functionally conserved genes were documented as well. Here, BACE2 was found to be enriched across Excitatory and Inhibitory neurons at both metabolism stages of the process. Beginning first with Amyloid-Precursor Protein and Amyloid-Beta Metabolism, the role of BACE2 is well-documented and understood to be heavily involved in the proteolytic cleavage of APP and heavily colocalizes with APP gene expression within targeted cells [92,93]. With this in mind, looking to the cell type specificity reveals interesting potential mechanisms for how the functional role of BACE2 could interact with other genes to lead to the eventual process of Amyloid-Precursor Protein Metabolism. The cell-cell interactions have not been thoroughly studied until this point, and the interaction between excitatory and inhibitory neurons through other gene-based pathways suggests a high degree of involvement throughout the Amyloid-Precursor Protein metabolism pathway. Better understanding of the cellular level effects of multiple genes within specific steps of the Amyloidogenic Processing mechanism elucidates the broader path of action through which AD pathology ensues. Lastly, when looking to potentially novel loci and pathways in Oligodendrocytes, we observed EFNA3 as specifically enriched within APP metabolism; EFNA3 encodes the Ephrin-A3 receptor protein tyrosine-kinase, which is part of the larger family of Ephrin based molecules commonly involved in developmental pathways within the nervous system. Ephrin-A3 specifically has been implicated widely as a diagnostic marker in oncological diagnosis primarily due to accumulation over time [94], and the broad functional significance of signaling oligodendrocyte precursor elements has been widely documented [95]. However, the interaction between EFNA3 and Ephrin-A3 with Oligodendrocytes in the AD progression is previously unreported, presenting a potentially novel mechanism in Oligodendrocytes.

Materials and Methods

The scGRN pipeline for predicting gene regulatory networks from multi-omics data

scGRN is a computational as an R package to integrate multi-omics datasets for predicting gene regulatory networks linking transcription factors, noncoding regulatory elements and target genes. To achieve this, scGRN has three steps (Figure 1), each of which is available as an R function:

Step1: Finding chromatin interactions. The function, *scGRN_interaction* inputs the chromatin interaction data (e.g., Hi-C) and predicts all possible interactions between enhancers and promoters in the data or the user-provided list - for example, those from Topologically Associating Domains (TADs) in Hi-C data. In addition, the function uses an R package, *GenomicInteractions* [96], to annotate interacting regions and link them to genes;

Step 2: Inferring the transcription factor binding sites on interacting regions. The function, *scGRN_getTF* infers the transcription factor binding sites (TFBS) based on consensus binding site sequences in the enhancers and promoters that potentially interact from the previous step, *scGRN_interaction*. It outputs a reference gene regulatory network linking these TF, enhancers and/or promoters of genes. In particular, this function uses TFBSTools [34] to obtain the position weight matrices of the TFBS motifs from the JASPAR database [97] and predicts the TFBS locations on the enhancers and promoters via mapping TF motifs. It further links TFs with binding sites on all possible interacting enhancers and promoters, and outputs the reference regulatory network. Furthermore, this function can run on a parallel computing version via an R package, *motifmatchr* [98] for computational speed-up;

Step 3: Predicting the gene regulatory network. The function, scGRN_getNt predicts the final gene regulatory network based on the TF-target gene expression relationships in the reference network. The reference gene regulatory network from the previous step provides all possible regulatory relationships (wires) between TF, enhancers, and target genes. However, changes in gene expression may trigger different regulatory wires. To refine our maps and determine the activity status of regulatory wires, this function applies elastic net regression, a machine learning method that has successfully modelled gene regulatory networks in our previous work [28]. Given a gene expression dataset and a reference network from *scGRN_getTF*, the function uses the TF expression to predict each target gene expression and finds the TF with high regression coefficients. This indicates an active regulatory influence on the target gene's expression in the gene expression data. The final gene regulatory network consists of the TF with high elastic net coefficients, target genes and the linked enhancers from their reference network links if any. The single cell gene expression data (UMI) for each cell type was normalized by *Seurat 3.0* [99].

Multi-omics datasets at the cellular resolution in the human brain

We applied our computational pipeline, scGRN to the multi-omics datasets at the cellular resolution in the human brain and predicted cell-type specific gene regulatory networks for major cell types: excitatory and inhibitory neurons, microglia and oligodendrocyte. We first input recently published cell-type chromatin interactome data in the human brain [25] to *scGRN_interaction* in order to reveal all possible interactions from enhancers to gene promoters in the neuronal, microglia and oligodendrocyte types. The genome annotation was from TxDb.Hsapiens.UCSC.hg19.knownGene [100]. We then predicted a reference regulatory network for each of these cell types using *scGRN_getTF*. When given a cell type, we input its normalized single cell gene expression data for the human brain, summarized by a recent functional genomic resource for the human brain [28] and the cell-type reference networks from *scGRN_getNt*, and we predicted the cell-type specific gene regulatory network consisting of the top 10% of TFs with highest Elastic net coefficients for each target gene. In particular, we randomly split the dataset into training and testing sets with a ratio of seven to three and then selected the best Elastic net model that minimized the mean square error. In addition, after normalizing gene expression and filtering out lowly expressed genes and cells by *Seurat 3.0* [99], we included 302 microglial cells with 4022 genes, 2653 oligodendrocyte cells with 12755 genes, 6034 inhibitory neuronal cells with 12100 genes, and 13703 excitatory neuronal cells with 15017 genes.

Comparison with state-of-the-art methods

We chose our predicted gene regulatory network, consisting of 68 TFs, 1768 target genes (TGs) and 46217 TF-TG links to compare with existing state-of-the-art methods for predicting cell-type gene regulatory networks. In particular, we input the single-cell gene expression data for microglia to a recently published benchmark framework, BEELINE [29], and predicted three microglial regulatory networks using three of the most consistent and highly accurate methods, PIDC, GENIE3 and GRNBoost2. These methods input gene expression data only, so they predict all possible TF-TG regulatory links based on their expression relationships. To make these networks comparable, we restricted TGs present in our microglial network and selected top 30% weighted TFs for each TG using each method. scGRN, PIDC, GENIE3 and GRNBoost2 predicted 14135, 36951, 37080, 29251 TF-TG links, respectively. Now with a target gene, we further checked whether its TFs were also present in the networks predicted by three methods.

Enrichment analyses of disease risk SNPs on the enhancers in the cell-type gene regulatory networks in the human brain

Genome Wide Association Studies (GWAS) have identified a variety of genetic risk variants including single nucleotide polymorphisms (SNPs) that are significantly associated with diseases and phenotypes (i.e., the traits). We used an R package, *traseR* [101] to calculate the enrichments of trait-associated SNPs for various diseases and phenotypes on the enhancers of cell-type gene regulatory networks. while calculating the enrichments, we used the binomial tests and the trait-associated SNPs in linkage disequilibrium (LD).

Identification and enrichment analysis of cell-type disease genes

First, we obtained the disease risk SNPs associated with Alzheimer's Disease (AD, 2357 credible SNPs) and Schizophrenia (SCZ, 6105 credible SNPs) from recent GWAS studies [102,103]. Second, given a cell-type, we used an R package, *GenomicRanges* [104], to overlap these SNPs with the enhancers and promoters of its gene regulatory network, and then linked to the cell-type disease genes from the overlapped enhancers or promoters. Finally, we used an R package, *clusterProfiler* [105], to find the enriched pathways and functions of cell-type disease genes, and the web app, *Metascape* [61] to find the enriched protein-protein interactions.

Conclusions

We developed a computational pipeline, scGRN for integrating multi-omics data and predicting gene regulatory networks (GRNs) which link TFs, non-coding regulatory regions (e.g., enhancers) and target genes. With applications to the data from single-cell multi-omics of the human brain, we predicted cell-type specific GRNs for both neuronal (e.g., excitatory, inhibitory) and non-neuronal types (e.g., microglia, oligodendrocyte), and used them to further link disease genes at the cell-type level for brain diseases like Alzheimer's and schizophrenia. These disease genes revealed conserved and specific genomic functions across neuropsychiatric and neurodegenerative diseases, providing potential novel disease mechanistic insights at the cellular resolution. Although this paper focuses on Alzheimer's and schizophrenia, our pipeline is general-purpose for understanding functional genomics across other diseases.

Our pipeline and analyses serve as a baseline and general framework for future research, although we have demonstrated a few basic use cases for multiple disease types including neurodegenerative and neuropsychological cases. As presented, the networks were able to identify multiple genes that have been well documented within the process of amyloidogenic processing, a hallmark pathway within Alzheimer's Disease progression. Future studies utilizing the scGRN pipeline would be able to take advantage of the ever growing number of GWAS for an extensive variety of diseases. Existing tools such as FUMA [106] have linked GWAS loci to genes by integrating information from multiple resources, providing functional insights from genotype to phenotype in human diseases. Thus, incorporating GWAS data from various brain regions exposes key areas of observed phenotypes. Previous studies in the same field have demonstrated the caution that must be exercised when attempting to correlate GWAS data with clinical phenotypes, and methods such as scGRN-based analysis mitigate these effects [26]. A similar methodology as outlined could be used where common loci within each set of summary statistics are incorporated and established prior to integration into the cell type GRNs, thus linking neuronal spatial information with known mutation sites in disease case patients along with potentially cell type specific functionality. Expanding past the four cell types examined here into additional forms of multicellular analyses is also possible given expanded interactome data. In particular, this would allow for further analysis of complex neuropsychological diseases as well as cases where the line between different clinical classifications becomes blurred and leads to additional complications with regard to clinically relevant genetic therapies. One such example includes Autism Spectrum Disorder (ASD), where clinical presentations can vary in multiple axes of severity which creates a broad spectrum of phenotypes. In such cases, being able to potentially link specific symptoms or aspects of a particular subset of ASD to certain brain regions and cell types allows for a better-informed picture of functional consequences associated with genetic mutation sites. Such connections could aid in determining genetic risk factors associated with variations in edge case patients; they also create the opportunity to take advantage of modern day Induced Pluripotent Stem Cell (iPSC) technology using genetic engineering technologies to create point mutations matching computationally identified genes.

Machine learning has also been widely used to analyze multi-omics such as multiview learning and deep learning [28,107]. In particular, multiview learning has great potential for understanding functional multi-omics and for revealing nonlinear interactions across omics. Therefore, integrating such emerging machine learning approaches will potentially enable the identification of additional cross-omic patterns - especially for increasing single cell multi-omics data and providing more comprehensive mechanistic insights in cell-type gene regulation and in linking to disease genes. This means, for example, adding more omics such as methylation data that reflect epigenetic changes which may occur due to wide variations of inherited and environmental factors [108]. At a deeper functional level, variations in methylation have been attributed to alterations in splicing activity, ultimately impacting the regulation and expression of key genes [109]. Additionally, integrating proteomic data at a single-cell level enhances the broader picture formed through additional data sources even further [110]. Lastly, expanding past simple methylation and proteomics allows for the ability to include all forms of data incorporated through the single-cell cytometry [111].

Supplementary information

Supplemental file 1 - cell-type gene regulatory networks (TF, enhancer, target gene)
Supplemental file 2 - enrichments of SNPs associated with diseases and traits on the enhancers of cell-type GRNs
Supplemental file 3 - cell-type disease genes for AD and SCZ
Supplemental file 4 - functional enrichments of cell-type diseases genes

Data availability

Our pipeline for predicting gene regulatory networks via multi-omics is open-source available as well as a tutorial at https://github.com/daifengwanglab/scGRN. All data supporting this study are included in this paper, the supplemental files, and/or available from the corresponding author on request.

Author contributions

D.W. conceived and designed the study. M.Y. implemented the software. P.R., M.Y. and D.W. analyzed the data. P.R., M.Y. , Pa.R. and D.W wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

None declared.

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