Rogue gene networks gone awry in Alzheimer's disease

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The human brain consists of billions of cells encompassing hundreds of distinct cell-types, each with unique functions and properties. Identification of the molecular architecture of the brain has been revolutionized by next-generation sequencing (NGS), as evident by recent transcriptomic and genetic/epigenetic studies. NGS paved the way to perform large-scale, genome-wide sequencing studies on human postmortem brain tissue, and this offered an unprecedented opportunity to elucidate the genetic bases of polygenic neurological disorders, like Alzheimer's disease (AD).

AD is a highly prevalent neurodegenerative disorder that manifests as significant memory loss and other cognitive deficits, and AD research has primarily focused on amyloid beta (Aβ) and tau, the proteins that make up the disease's hallmark pathological aggregates (amyloid plaques and neurofibrillary tangles). Despite remarkable advancements in our understanding of Aβ and tau, we still lack effective therapeutics for AD. Over the years, we have continued to see disappointing results with several Aβ-targeting treatments, discontinued in clinical trials. Therefore, AD research has begun to look for targets other than Aβ and tau. Before genome-wide association studies (GWAS), we had only identified rare causal genetic mutations (APP, PS1/2), which are not present in most afflicted individuals. GWAS initially discovered only APOE as an AD genetic risk factor for non-familial (sporadic) AD cases, but quickly more and more AD genetic risk variants were revealed. A recent AD GWAS has identified more than twenty AD-associated risk loci (TREM2, BIN1, CLU, for example) across almost 100,000 individuals (Kunkle et al., 2019), while another recent study has revealed sex-dependency of AD GWAS loci (Fan et al., 2020).

However, many AD genetic risk variants are in non-coding regions, and currently, biological interpretation of these AD risk loci remains a large hurdle, impeding the advancement of novel therapeutics. Thus, it has become clear that functional genomic approaches are necessary to further our understanding of AD. Functional genomics aims to unravel the biological functions of genes and their encoded proteins through high-throughput sequencing techniques, and we examine genes at the network level, rather than in isolation, to better recapitulate the biological processes of the brain and to identify biologically relevant gene targets.

To this end, there have been multiple transcriptomic (microarray and RNA-sequencing, RNA-seq) studies of human AD, identifying AD dysregulated genes and microRNAs. However, there is great, inherent variability between human samples, requiring large sample numbers to detect robust disease-specific changes. Three large-scale RNA-seq datasets on postmortem human brain tissue, consisting of multiple brain regions, were generated by the National Institute on Aging's Accelerating Medicines Partnership-Alzheimer's Disease (AMP-AD) Target Discovery and Preclinical Validation Project (Allen et al., 2018; Mostafavi et al., 2018). Individual studies of these datasets identified AD-associated gene networks and found shared molecular pathways between AD and another tauopathy, progressive supranuclear palsy. In addition, mRNA splicing was found altered in AD (Raj et al., 2018). Most transcriptomic studies, including the aforementioned though, have limited their analyses to data from a single brain tissue repository, which can be a confounding variable.

While the AMP-AD consortium has propelled the generation of large-scale genomic and transcriptomic datasets, it has also led to the development of new analytical tools and thinking by making these datasets accessible for other researchers. More recent studies have begun to take a meta-analytical approach to discover AD-specific changes across several cohorts, significantly increasing the number of samples. One example is our group's recent work in which we applied consensus weighted gene co-expression network analysis (cWGCNA) to the three AMP-AD cohorts (Mayo Clinic, Mount Sinai, ROSMAP), identifying AD-specific gene co-expression modules conserved across the 1268 samples from the respective brain tissue repositories (Morabito et al., 2020). Unlike traditional WGCNA, cWGCNA constructs only co-expression modules found in all datasets, ignoring any solely in one dataset. Additionally, many transcriptomic studies have concentrated on only one brain region, but we examined data from multiple brain regions. While we found brain region specificity in the degree of gene co-expression changes in AD, we effectively defined the disease-associated transcriptional alterations shared across brain regions. The AD gene co-expression modules were also highly preserved in human AD microarray datasets, demonstrating the reproducibility of our work.

In addition, RNA-seq now can be performed at the single-cell level (single-cell RNA-seq, scRNA-seq; single-nucleus RNA-seq, snRNA-seq), and already scRNA-seq studies have revealed the brain's cellular heterogeneity is far more complex than previously thought (Tasic et al., 2018). It also has been established that cell-type proportions drastically change in the course of AD progression, skewing RNA-seq data obtained only at the tissue-level. There is significant neuronal loss, while glial cells increase. Thus, it is no surprise that AD downregulated genes are related to neuron function or that upregulated genes are related to glial immune processes. Therefore, a few snRNA-seq studies have been performed on human AD samples, detecting cell-type specific gene expression changes, and importantly, these cell-type specific changes were often incongruent to that at the tissue-level (Grubman et al., 2019; Mathys et al., 2019; Zhou et al., 2020). In addition, Mathys et al. (2019) identified cell-type specific disease transcriptional changes that were sex-specific. However, the sample numbers of these snRNA-seq studies have been limited due to high costs. In order to combat this limitation, our group integrated the large-scale bulk tissue RNA-seq data with a smaller snRNA-seq dataset, allowing us to define the cellular context of the gene co-expression changes occurring in AD (Morabito et al., 2020). This provided us greater insight into the AD dysregulated transcriptome, as we were able to discern downregulated genes related to mitochondria function were neuron-specific and upregulated genes involved in transcriptional regulation were oligodendrocyte-specific. Although snRNA-seq costs may decrease over time, we foresee that the integration of bulk tissue RNA-seq and snRNA-seq will continue to be valuable due to the inherent sparsity of single-cell data and the high computing power required for increasing cell numbers.

Further, the integration of multi-scale datasets is critical for a thorough dissection of the AD transcriptome and to yield novel insights into disease biology. As previously mentioned, AD genetic risk variants have been identified by GWAS, and by intersecting AD GWAS genes with transcriptomic data, we and others have highlighted the role of microglia in AD pathophysiology. We found specific enrichment of AD GWAS genes in a microglial module, and by also looking at genes identified in GWAS of other tauopathies, like progressive supranuclear palsy, we found that this enrichment is unique to AD, suggesting that AD microglial changes are associated with the presence of both Aβ and tau pathology (Morabito et al., 2020). Additionally, we examined GWAS of multiple other neurological disorders and unrelated traits, and we found enrichment of multiple sclerosis GWAS hits in the same microglial module, indicating similarities in the disease biology of AD and multiple sclerosis.

We also investigated the enrichment of transcription factor binding sites in order to identify potential regulators of AD gene co-expression changes. The nuclear factor kappa B (NFκB) pathway has been previously

implicated in AD, and we established RELA (NFκB p65 subunit) as a modulator of both neuronal and non-neuronal AD transcriptional changes (Morabito et al., 2020). This was further supported by the association of non-neuronal modules with NFKB 1 (p50), which interacts with RELA in canonical NFκB signaling. We additionally found RELB (RELB Proto-Oncogene, NFKB subunit) as a regulator of non-neuronal modules, implicating the non-canonical NFκB pathway as well. Further, we utilized the NIH's Library of Integrated Network-Based Cellular Signatures database and found Withaferin A, a drug that inhibits NFκB signaling, as a modulator of one neuronal and two non-neuronal modules.

Moreover, integrating epigenetic datasets can help to further clarify the gene regulatory programs mediating AD-associated transcriptional changes. Previous studies have identified AD-associated DNA methylation, as well as H3K9ac marks associated with Aβ or tau pathology (De Jager et al., 2014; Klein et al., 2019). The PsychENCODE consortium also has generated large-scale human brain epigenetic datasets (H3K27ac ChIP-seq, Hi-C). In our meta-analysis, we examined the enrichment of AD-associated methylation and H3K9ac marks in our co-expression modules and observed a striking lack of enrichment in the same microglial module that was enriched in AD GWAS hits (Morabito et al., 2020). This was even more noticeable since all other co-expression modules were enriched in at least one of the epigenetic annotations. Previous studies though have indicated that histone modifications regulate microglia activation, which would seem to contradict our findings. However, currently there have been no studies on human AD brain samples examining cell-type specific epigenetic changes, representing a large gap in knowledge. While single-cell transcriptomics has been able to identify transcriptionally distinct disease-associated cell states, it is imperative to understand the gene regulatory programs controlling these cell states to develop therapeutics targeting diseased cell states.

It is important to note, though, that there are several limitations when studying postmortem human brain tissue, such as postmortem artifacts and disease comorbidity. Single-cell sequencing is fairly new technology, and researchers are still exploring how differences in tissue processing can affect results. Additionally, since AD is an aging disorder, many AD brains are also diagnosed with disorders like Lewy body dementia and hippocampal sclerosis, making it difficult to determine changes specific to AD. Another major concern we would like to point out is that postmortem human brain tissue cannot concretely provide us information on disease progression. Mouse models, however, can allow us to study disease progression but have been criticized for their inability to fully recapitulate human AD. The development of new mouse models, informed by data from diseased human samples, is critical to advancing our understanding of AD, in addition to discovering new therapeutics. The Model Organism Development and Evaluation for Late-Onset Alzheimer's Disease (MODEL-AD) consortium is currently developing and extensively characterizing novel AD mouse models and promises to bring robust model systems of neurodegeneration and AD biology.

As technology continues to advance, we will continue to see the generation of new and exciting data, but we emphasize that the integration of multiple model systems, in addition to multiple data modalities, is key to advancing our knowledge of disease biology.

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References

Allen M, Wang X, Burgess JD, Watzlawik J, Serie DJ, Younkin CS, Nguyen T, Malphrus KG, Lincoln S, Carrasquillo MM, Ho C, Chakrabarty P, Strickland S, Murray ME, Swarup V, Geschwind DH, Seyfried NT, Dammer EB, Lah JJ, Levey AI, et al. (2018) Conserved brain myelination networks are altered in Alzheimer's and other neurodegenerative diseases. Alzheimers Dement 14:352-366.

De Jager PL, Srivastava G, Lunnon K, Burgess J, Schalkwyk LC, Yu L, Eaton ML, Keenan BT, Ernst J, McCabe C, Tang A, Raj T, Replogle J, Brodeur W, Gabriel S, Chai HS, Younkin C, Younkin SG, Zou F, Szyf M, et al. (2014) Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. Nat Neurosci 17:1156-1163.

Fan CC, Banks SJ, Thompson WK, Chen CH, McEvoy LK, Tan CH, Kukull W, Bennett DA, Farrer LA, Mayeux R, Schellenberg GD, Andreassen OA, Desikan R, Dale AM (2020) Sex-dependent autosomal effects on clinical progression of Alzheimer's disease. Brain 143:2272-2280.

Grubman A, Chew G, Ouyang JF, Sun G, Choo XY, McLean C, Simmons RK, Buckberry S, Vargas-Landin DB, Poppe D, Pflueger J, Lister R, Rackham OJL, Petretto E, Polo JM (2019) A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. Nat Neurosci 22:2087-2097.

Klein HU, McCabe C, Gjoneska E, Sullivan SE, Kaskow BJ, Tang A, Smith RV, Xu J, Pfenning AR, Bernstein BE, Meissner A, Schneider JA, Mostafavi S, Tsai LH, Young-Pearse TL, Bennett DA, De Jager PL (2019) Epigenome-wide study uncovers large-scale changes in histone acetylation driven by tau pathology in aging and Alzheimer's human brains. Nat Neurosci 22:37-46.

Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, Boland A, Vronskaya M, van der Lee SJ, Amlie-Wolf A, Bellenguez C, Frizatti A, Chouraki V, Martin ER, Sleegers K, Badarinarayan N, Jakobsdottir J, Hamilton-Nelson KL, Moreno-Grau S, Olaso R, et al. (2019) Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. Nat Genet 51:414-430.

Mathys H, Davila-Velderrain J, Peng Z, Gao F, Mohammadi S, Young JZ, Menon M, He L, Abdurrob F, Jiang X, Martorell AJ, Ransohoff RM, Hafler BP, Bennett DA, Kellis M, Tsai LH (2019) Single-cell transcriptomic analysis of Alzheimer's disease. Nature 570:332-337.

Morabito S, Miyoshi E, Michael N, Swarup V (2020) Integrative genomics approach identifies conserved transcriptomic networks in Alzheimer's disease. Hum Mol Genet 29:2899-2919.

Mostafavi S, Gaiteri C, Sullivan SE, White CC, Tasaki S, Xu J, Taga M, Klein HU, Patrick E, Komashko V, McCabe C, Smith R, Bradshaw EM, Root DE, Regev A, Yu L, Chibnik LB, Schneider JA, Young-Pearse TL, Bennett DA, et al. (2018) A molecular network of the aging human brain provides insights into the pathology and cognitive decline of Alzheimer's disease. Nat Neurosci 21:811-819.

Raj T, Li YI, Wong G, Humphrey J, Wang M, Ramdhani S, Wang YC, Ng B, Gupta I, Haroutunian V, Schadt EE, Young-Pearse T, Mostafavi S, Zhang B, Sklar P, Bennett DA, De Jager PL (2018) Integrative transcriptome analyses of the aging brain implicate altered splicing in Alzheimer's disease susceptibility. Nat Genet 50:1584-1592.

Tasic B, Yao Z, Graybuck LT, Smith KA, Nguyen TN, Bertagnolli D, Goldy J, Garren E, Economo MN, Viswanathan S, Penn O, Bakken T, Menon V, Miller J, Fong O, Hirokawa KE, Lathia K, Rimorin C, Tieu M, Larsen R, et al. (2018) Shared and distinct transcriptomic cell types across neocortical areas. Nature 563:72-78.

Zhou Y, Song WM, Andhey PS, Swain A, Levy T, Miller KR, Poliani PL, Cominelli M, Grover S, Gilfillan S, Cella M, Ulland TK, Zaitsev K, Miyashita A, Ikeuchi T, Sainouchi M, Kakita A, Bennett DA, Schneider JA, Nichols MR, et al. (2020) Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. Nat Med 26:131-142.

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