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Postmortem human brain genomics in neuropsychiatric disorders — how far can we go?

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Large-scale collection of postmortem human brain tissue and subsequent genomic data generation has become a useful approach for better identifying etiological factors contributing to neuropsychiatric disorders. In particular, studying genetic risk variants in non-psychiatric controls can identify biological mechanisms of risk free from confounding factors related to epiphenomena of illness. While the field has begun moving towards cell type-specific analyses, homogenate brain tissue with accompanying cellular profiles, can still identify useful hypotheses for more focused experiments, particularly when the dysregulated cell types are unknown. Technological advances, larger sample sizes, and focused research questions can continue to further leverage postmortem human brain research to better identify and understand the molecular etiology of neuropsychiatric disorders.

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Introduction

Schizophrenia (SCZD), bipolar disorder (BPD), major depression (MDD) and autism (ASD) are prevalent neuropsychiatric disorders that are diverse in their clinical presentations and symptomatology. While the underlying etiology in the majority of patients with these disorders is largely unknown, the strongest recent etiological clues have come from genome-wide association studies (GWAS), and while all four disorders share varying degrees of overlap in common genetic risk variants, they all feature extensive genetic heterogeneity [1••]. Postmortem human brain tissue has long been an essential substrate for understanding the molecular pathology of brain disorders and their risk [2], and the last several years have featured a renewed push for generating and interrogating genomic, transcriptomic and epigenomic data using new technologies in large samples to better understand how genetic risk variation contributes to etiology of illness. Here I review recent literature for generating and utilizing human postmortem brain genomics data — including analyses to interrogate individual genes as well as their networks and approaches that move from homogenate tissue towards cell type-specific analysis — that can be used to better understand the etiology of neuropsychiatric disorders.

Characterizing genomic signatures in the human brain using postmortem tissue

Large-scale and multi-site efforts in postmortem human brain collections combined with advances in genomic technologies such as sequencing now permit the ability to fully characterize and analyze the genome, epigenome and transcriptome in hundreds of neuropsychiatric disorder donors as well as non-psychiatric control subjects across many brain regions. There have been recent large public RNA sequencing (RNA-seq) datasets in homogenate brain tissue focused both on understanding the normal functionality of the human brain, as well as characterizing dysregulation associated with serious mental illness. Two popular resources focused on studying non-psychiatric control subjects free from the epiphenomena of illness include the GTEX project, which aims to integrate genetic and expression data across many different brain regions in the same subjects [3••] and the BrainSpan project, which aims to better characterize developmental regulation of genes and transcript expression levels across multiple brain regions [4]. Other recent projects have sought to identify transcriptional and epigenetic correlates of disease risk as well as differences between neuropsychiatric disorder patients and controls, such as the psychENCODE project [5] and BrainSeq Consortium [6].

These postmortem brain resources can be especially informative given the overall lack of etiological insight in many neuropsychiatric disorders. The strongest clues for the etiological underpinnings of schizophrenia come from recent genetic studies which have identified hundreds of common loci that each contribute to small effects of risk via genome-wide association studies (GWAS) [1••],

but the mechanisms guiding any individual risk locus remain largely unknown [7,8]. GWAS for other disorders have been much less successful, although there is also substantial overlap between the clinical risk variants identified in schizophrenia and those marginally significant in bipolar disorder, major depression and autism [1••,9], all of which are hypothesized to have neurodevelopmental components [10–12]. While common variants associated with these disorders are thought to converge on distinct pathogenic gene pathways and networks, including genes related to histone methylation, the immune system and neuronal signaling pathways [13] including more specific synaptic pathways involving plasticity [14], there has been substantial variability in the pathways associated with the illness from the GWAS results. Furthermore, many of associated loci identified contain multiple genes, creating uncertainty around identifying the likely causal gene(s) to enter into pathway or network analyses. Many groups have therefore utilized postmortem human brain tissue to better understand the molecular correlates of the both genetic [15–18] and non-genetic [19,20], aspects of schizophrenia and related disorders, as gene expression and other molecular data levels and transcript variants may better illuminate mechanisms of risk [2].

Functional genomics in the human brain

Identifying molecular targets of neuropsychiatric disorders in postmortem tissue can utilize both gene- and/or network based approaches. In studies comparing gene expression of patients with schizophrenia and related disorders to unaffected control samples using PCR and oligonucleotide microarrays, hundreds of candidate genes and disrupted gene networks have been reported in the past decade [21–25]. These early postmortem studies identified a wide range of biological processes dysregulated in schizophrenia patients compared to control subjects — some array-based expression profile studies identified differences in myelination-related as well as neurotransmitter-related genes [21] supporting potential roles of both oligodendrocyte dysfunction [26] as well as synaptic dysfunction [22]. Technical advances in sequencing have further permitted the largely unbiased characterization of the transcriptome using RNA sequencing (RNA-seq), furthering the ability beyond microarray and PCR-based technologies to identify expression-based genes and their networks implicated in neuropsychiatric disorders [27,28•]. Several recent reports using RNA-seq have identified changes in immune/inflammation-related genes [29,30] as well as genes implicated in lysosomal function and actin cytoskeleton remodeling [31] when comparing expression levels in brain between schizophrenia patients and controls.

While statistical modeling at individual genes can likely better incorporate measurement variability and latent confounding [32,33], interpreting biological relevance of differentially expressed or methylated genes typically requires enrichment analyses within pre-defined and presumed functional gene sets, for example using the popular Gene Ontology tool [34]. Conversely, computational methods for inferring network structure can identify data-driven connections between genes, which was well-reviewed in Parikshak *et al.* [35•], but these approaches likely require large sample sizes and may be even more susceptible to latent confounding than single gene approaches [36].

These case–control studies can identify candidate molecular targets that may be differentially regulated in the brains of cases compared to controls, but, unlike genetic association studies, where risk genotypes are established at birth and unaffected by epiphenomena associated with illness, postmortem tissue gene expression levels represent cumulative effects of living with serious mental illness. For example, any differences in the expression of genes or their networks comparing patients to controls could represent effects of medication and drug abuse across decades of illness compounded with the potential effects of the agonal state and periods of time between death until tissue processing. Therefore disambiguating cause from consequence of illness in observed expression differences at the gene-level between patients and controls is difficult, and has been a point of debate in the field [2]. Incorporating genetic variation data can potentially better protect against confounding factors in postmortem brain studies as the genetic variation precedes illness [2]. Furthermore, while the vast majority of common risk variants for neuropsychiatric disorders are non-coding [1••], multiple reports have identified significant enrichment of these variants among enhancer and regulatory elements [3••,37] suggesting putative mechanisms of association.

Many of these postmortem human brain studies, particularly in normal subjects, therefore aim to associate genetic risk variants, for example identified in GWAS, with local expression (termed 'expression quantitative trait loci', or eQTLs [38•]), DNA methylation (termed 'methylation quantitative trait loci', or meQTLs [39]), and chromatin openness (termed 'DNase hypersensitivity quantitative trait loci' or dsQTLs [40]) levels in these samples to better understand the biological mechanisms underlying the genetic risk. While many of these QTLs may be specific to the most etiologically relevant brain region, a large fraction may be conserved across multiple brain regions [41] or even multiple tissue types [3••]. However, lack of QTL signals from risk variants — for example, only a fraction of the PGC2 loci for schizophrenia represented putative eQTL signals [1••], could suggest that large samples sizes from more diverse brain regions, cell types, or even developmental stages could further illuminate potential mechanistic insight into common variant risk.

Importance of considering cell type composition in postmortem brain data

The interpretation of results from postmortem human brain studies largely depends on the samples collected, processed, and assayed, as homogenate brain tissue samples vary in cellular composition, i.e. the relative proportion of each cell type, across samples due to individual variation, dissection issues, effects of disease, and/or subject age. The importance of considering cell type composition within heterogeneous tissue sources such as whole blood and homogenate brain tissue has been highlighted in epigenetics research over the past several years [42–44]. Failure to account for cellular composition in the analysis of heterogeneous tissue sources can result in widespread false positives and negatives [45]. Previous work has identified widespread epigenetic differences between neurons and glia using DNA methylation (DNAm) data [43,46], and false positives may arise when there are cellular composition differences associated with disease, with normal development or any other outcome of interest. For example, loss of neurons (or glia) resulting from disease may identify thousands of false positive loci associated with illness due solely to this changing composition. Therefore, studying tissue for brain disorders that cause, or result in, loss of biological variation in particular cell populations can be particularly susceptible to this confounding. Cell composition variance can confound studies of patients and control samples through non-uniformity of dissection, for example in disorders associated with cortical thinning (e.g. neurodegenerative disorders, schizophrenia), partial volume effects can confound the relative proportions of grey and white matter in the dissection. Conversely, if a disorder only affects one or several specific cell populations, then the presence of unassociated cell types may obscure the true biological signal, resulting in potential false negatives.

Cellular composition associates with both epigenetic and gene expression patterns in homogenate brain tissue and failure to account for this composition variability can result in widespread false positives and negatives. A seminal paper by Houseman *et al.* [42] first proposed a statistical method for computing the relative proportion of cell types from heterogeneous tissue source with DNAm data using blood as a primary example, and this framework has been utilized in post-mortem brain data [43,46] — these projects generated the required DNAm data on segregated cellular populations via NeuN staining followed by flow sorting [47] (NeuN+ cells are neuronal and NeuN− cells are non-neuronal). These composition estimation approaches will become especially important as large publically funded brain collections distribute tissue to a larger number of researchers, most of whom will likely not receive enough tissue for cell type-specific analyses [48]. Therefore, accurately estimating and controlling for cellular composition can lead to more direct comparability across multiple experiments using tissue from the same donors, for example in tissue quality control, matching in the design of studies, and subsequent adjustment in downstream analysis.

Moving towards interrogating individual cell populations

While measuring and adjusting for cellular composition within postmortem brain samples can likely reduce the likelihood of false positive discoveries resulting from composition changes associated with the disorder, it is difficult to statistically estimate cell type-specific effects in homogenate tissue samples [43]. While transgenic modeling in mice can permit the isolation of RNA from individual cell populations [49], isolating and interrogating cell types of interest from postmortem human samples has become a main challenge in the field. When the cell type of interest is known, there are (at least) three popular approaches for cell type-specific analyses in human samples — fluorescence-activated cell sorting (FACS) [47], laser capture microdissection (LCM) [50] and single cell sequencing [51]. While single cell sequencing is perhaps the most high-throughput of the three methods, it is still quite expensive to profile a large number of individual cells in a large number of samples, likely requires fresh tissue [51], and have numerous analytic challenges, as reviewed by Stegle *et al.* [52]. However, this approach does not require a priori knowledge of the cell type likely harboring the biologically meaningful molecular signal, which can be useful for hypothesis-generating experiments. Conversely, the other two approaches require defining the cell type of interest prior to data generation. FACS-based approaches are currently optimized to isolate neuronal and non-neuronal nuclei (not cells) using NeuN+ labeling [47] and this immunohistochemical approach is limited both in terms of cell selectivity (only neurons versus non-neurons) and also by the potentially compromised immunoreactivity of postmortem human brain. This FACS-based approach is being utilized by many projects in the psychENCODE consortium to characterize genomic signatures in both neuronal and non-neuronal cell populations [5]. Lastly, while LCM can perhaps have the best specificity, the approach is low throughput, can potentially induce degradation of biological material [53], and can only be used to identify easily distinguishable cell types. This approach has recently been used to characterize differences between schizophrenia and schizoaffective patients compared to unaffected controls in layers 3 and 5 pyramidal cells in the DLPFC, which identified downregulation of genes involved in the mitochondrial and ubiquitin-proteasome system [50]. While each cell isolation approach therefore has strengths and weaknesses, these three approaches can move from homogenate tissue to individual cells and populations which can increase specificity of genomic signals contributing neuropsychiatric disorders. Given the technological advances of these approaches in the past several years, I anticipate a larger number of datasets

will become more focused on cell type-specific data generation and analysis.

Conclusions

In this review, I have summarized utilizing publicly-available genomics datasets to better characterize underlying etiology of neuropsychiatric disorders such as schizophrenia. In particular, studying genetic risk variants in non-psychiatric controls can identify biological mechanisms of risk free from the confounding factors related to epiphenomena of illness. While the field has begun moving towards cell type-specific analyses, homogenate brain tissue with accompanying cellular profiles, can still identify useful hypotheses for more focused experiments, particularly when the dysregulated cell types are unknown. Overall, the future is bright, as technological advances, larger sample sizes, and focused research questions can continue to further leverage postmortem human brain research to better identify and understand the molecular etiology of neuropsychiatric disorders.

Conflict of interest

Nothing declared.

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