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Molecular Windows into the Human Brain for Psychiatric Disorders

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Abstract

Delineating the pathophysiology of psychiatric disorders has been extremely challenging but technological advances in recent decades have facilitated a deeper interrogation of molecular processes in the human brain. Initial candidate gene expression studies of the postmortem brain have evolved into genome wide profiling of the transcriptome and the epigenome, a critical regulator of gene expression. Here, we review the potential and challenges of direct molecular characterization of the post-mortem human brain, and provide a brief overview of recent transcriptional and epigenetic studies with respect to neuropsychiatric disorders. Such information can now be leveraged and integrated with the growing number of genome-wide association databases to provide a functional context of trait-associated genetic variants linked to psychiatric illnesses and related phenotypes. While it is clear that the field is still developing and challenges remain to be surmounted, these recent advances nevertheless hold tremendous promise for delineating the neurobiological underpinnings of mental diseases and accelerating the development of novel medication strategies.

Introduction

Every day, nearly half a billion people worldwide struggle to manage their psychiatric disorders that cloud cognition, dampen or sensitize their emotions, alter perception, erase their memories, induce delusions and compromise their communication skills. The estimated cost of the global disease burden of mental illnesses tops that of other medical diseases in

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Conflict of Interests

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western societies[1, 2] with an enormous toll on the individual, their family and communities that has had crippling economic, medical and social consequences. Depression, anxiety and substance use disorders constitute the largest group of mental disorders in most western societies with an opioid epidemic currently gripping the USA killing approximately 100 people daily and suicide being one of the leading causes of death worldwide. Psychiatric illnesses span all ages from childhood disorders such as autism or schizophrenia emerging in young adulthood and Alzheimer's disease expressing in later stages of life. Each has unique features but there are overlapping struggles with societal stigmas, misconceptions about the disorders and the loss of quality of life. Perhaps the greatest challenge is that despite the devastating impact of these disorders, effective treatments are still lacking. Thus afflicted persons have limited options to regain control of their minds and lives.

Decoding the mystery of psychiatric illnesses has been the Holy Grail for scientists and clinicians hoping to uncover their biological underpinnings and to develop medications and eventual cures. Such goals are hampered by the complexity of the brain and the multidimensional nature of psychiatric disorders that are highly heterogeneous even within one diagnosis and with overlapping symptomatologies among disorders. Additionally, diagnoses still rely predominantly on clinical interviews with no biological markers, which altogether makes it challenging to expand neurobiological knowledge about these disorders. Animal models are extremely important for delineating causal relationships with behavior but have inherent limitations, being developed based on hypothesis of a particular underlying pathology though the pathophysiology of these human diseases is still unknown. Such challenges and the advancement of molecular techniques have shifted more research attention in recent years on human studies and translational strategies.

Indeed, as human disorders, psychiatric illnesses necessitate concerted efforts for direct investigation of the human brain. Here, we review the potential and challenges of human postmortem molecular strategies to expand knowledge about the underlying neuropathology of psychiatric disorders and provide a narrow overview of some of the published neurobiological findings that might offer clues regarding disease neuropathology and for targeting future medication strategies. We focus on gene expression and epigenetic strategies that provide windows into alterations of transcription and its regulation (Figure 1), given the importance of gene disturbances and the environment contributions to psychiatric vulnerability and disease course.

Transcriptional studies of the post-mortem human brain

The case for post-mortem human transcriptional profiling

Transcriptional studies have been at the forefront of the molecular exploration of the post-mortem human brain. However, initial attempts to dissect genes and related protein networks that underlie disease in the human postmortem brain were met with significant criticism and skepticism. Critics argued that the rapid degradation of RNA, combined with relatively long (hours or even days) post-mortem intervals (PMIs), would be prohibitive for extracting high quality RNA and for conducting quantitative transcriptional experiments. However, by minimizing PMI (<24 hours), optimizing transport and storage conditions, and by careful sterile and RNase-free practices during brain dissection and RNA extraction, the field has

been able to demonstrate consistent success in obtaining high quality RNA from post-mortem human brains. Consequently, transcriptional profiling, both in an unbiased and a hypothesis-driven manner, continues to play a central role in our improved understanding of the molecular underpinnings of neuropsychiatric conditions.

In vitro cell culture and *in vivo* animal models are invaluable for mechanistic studies, but the direct assessment of the post-mortem human brain remains critical to understand underlying neurobiology and to also drive the development and optimization of various disease models. This has been strongly emphasized by a number of studies pointing to human-specific transcriptional profiles particularly evident in certain brain regions[3–8]. For example, whereas gene expression in some brain areas like the caudate nucleus appears to maintain relatively strong conservation, the frontal cortex displays a large number of differentially expressed genes when compared even to non-human primates[7, 9]. Intriguingly, human-specific co-expression networks in the frontal cortex are enriched for genes involved in neuronal differentiation and process formation, which underlie neuronal functional activity and brain plasticity[7]. That the prefrontal cortex shows great species differences is not surprising considering the marked relative expansion of this brain region during human evolution and its role in higher cognitive function that distinguishes humans from other species. Even in brain areas with greater transcriptional conservation among species there are still noted differences in gene expression patterns that can have important implications. For example, in the caudate nucleus the *PDYN gene*, which encodes the opioid neuropeptide prodynorphin that regulates emotion, memory and motor function, and in which the regulation of its transcription has been favored in human evolution[10] has far higher expression in humans particularly within the patch (striosome) organization of the striatum that is predominantly aligned to limbic/emotion neuronal networks.[11, 12] Moreover, not only is the expression of the kappa opioid receptor, which binds dynorphin-like agents, up to 100-fold higher in the human compared to the rodent brain, it is also expressed in different neuronal populations between the species [13, 14]. The discrete anatomical expression and human-specific regulatory changes of numerous genes emphasize the importance of obtaining greater insights regarding the molecular anatomical organization of the human brain.

The increased focus to expand the molecular understanding of the human brain in regard to psychiatric disorders has led to close to a hundred postmortem human datasets deposited currently in GEO (Supplementary Table 1), several brain banks and publicly available resources (Table 1) plus hundreds of publications. This review cannot provide an in-depth evaluation of all the molecular data that exist for each psychiatric disorder, but we provide below a glance to what potentially has been gleaned to guide future focused investigations.

Hypothesis-driven and unbiased transcriptional studies of substance use disorders

Molecular studies relevant to psychiatric disorders have paid significant attention to substance use since animal models that dominate the literature have better predictive validity for this pathology compared to other psychiatric illnesses. Decades of preclinical and *in vivo* neuroimaging research have documented that drugs of abuse increase dopaminergic signaling from the midbrain to, for example, the ventral striatum (nucleus accumbens)[15–17], and alter glutamate release in corticostriatal projections[18–26]. Persistent synaptic alterations

in the mesocorticolimbic circuitry play a central role in neuronal maladaptations that underlie reward, craving, drug-seeking and relapse even after protracted periods of abstinence. As such, a large body of literature has focused on delineating molecular alterations that drive synaptic plasticity, such as membrane trafficking and alterations of the surface expression of neurotransmitter receptors[27–30], and changes in intracellular signaling pathways both pre- and post-synaptically[31–34]. Since gene expression changes are at the core of drug-induced molecular adaptations[19, 35, 36], they have comprised an important area of investigation in the post-mortem human brain of addicted individuals.

Most of the literature relates to hypothesis-driven molecular approaches in interrogating specific genes using real time polymerase chain reaction or in situ hybridization histochemistry. Such studies have proven fruitful in substantiating dysregulation of known neurobiological systems associated with substances of use.[37–59] A more unbiased exploration of the whole transcriptome has increasingly become more feasible and widely utilized in recent years. Through these efforts, several genome-wide human datasets have been generated and made available to the public (Supplementary Table 1). In our assessment of human heroin users using microarray to profile gene expression in the nucleus accumbens, a brain region central to reward and emotion, we revealed marked abnormalities related to glutamatergic neurotransmission[19]. Intriguingly, these glutamatergic impairments strongly related to epigenetic dysregulation in long-term heroin users[19]. Transcriptome analyses of corticolimbic brain areas of cocaine users or other substances of abuse also emphasized significant alterations of genes associated with synaptic plasticity[60, 61] and epigenetic processes[62].

Though genome-wide interrogations of human substance abusers have validated disturbances of neurobiological systems previously documented in candidate gene strategies, other genes and gene networks have also been identified using the discovery approaches. For example, we determined that a large portion (approximately 20%) of dysregulated genes in the striatum of heroin users were targets of the transcription factor ELK1[63]. We confirmed ELK1 impairment on the protein level and showed that striatal ELK1 in human heroin abusers is associated with genetic variants of *ORPM1*, the gene encoding for the mu opioid receptor, in a genotype dose-dependent manner. Moreover, ELK1 expression correlated with the documented history of heroin use in the human subjects, an effect reproduced in the animal model[63]. ELK1 had not been of any focus in the field driven mainly by animal studies in which the transcription factor CREB has been predominantly highlighted. However, the strong relationship to *OPRM1* suggests that ELK1 as a master transcriptional regulator might have significant relevance for opioid abuse vulnerability.

The observation that ELK1 levels in heroin abusers correlated with the history of drug use highlights another important challenge of human postmortem studies. Namely dissociating the acute and long-term drug effects. A critical aspect of human studies that impacts interpretation of data is that some of the observed molecular changes may be due to acute pharmacological effects of the drugs and therefore not necessarily reflective of the chronic, pathological maladaptations that underlie the repeated use of drugs in individuals with substance use disorders. The combination of toxicological analysis and information regarding the history of drug use can help to address this limitation. Indeed, differential

effects of acute and long-term drug exposure have been observed both on transcriptional and epigenetic levels[19, 63]. As such, it was possible to distinguish an opposite relationship between ELK1 levels in the striatum of heroin users based on acute opioid toxicology versus their years of heroin use.

To date, the majority of transcriptome studies related to illicit drugs have focused on cocaine and heroin use. Most investigations are, however, confounded by a high degree of polysubstance use among these populations, which makes it inherently challenging to assess the individual contribution of different substances. Clearly, studying individuals who had predominantly used one drug type is ideal. Nevertheless, studies thus far have revealed a large number of differentially expressed genes in multiple brain regions using high-throughput microarrays[61, 64–66] or RNA-seq[62, 67] approaches. Interestingly, the affected neural gene networks appear largely unique with limited overlap among different abused substances. For instance, a recent study identified significant reduction in the expression of numerous genes encoding proteins involved in presynaptic neurotransmitter release in heroin abusers, a finding not observed in a cocaine-abusing cohort[68]. Conversely, a striking decrease in myelin-related genes observed in cocaine abusers was not evident in their cohort of heroin subjects. Overall, little overlap in gene expression profiles was seen by Albertson and colleagues between the two drug-abusing cohorts: out of the approximately 39,000 detected transcripts, only 25 were significantly changed in both cocaine and heroin abusers, with nearly a half of these being altered in opposite directions[68]. Despite the small sample size of that study (n=7–10/group), the alterations of genes expressed in different directions are consistent with prior findings. Specifically, *ACTN2* (Actinin Alpha 2), which codes for actin-binding cytoskeletal proteins, was increased in cocaine users and decreased in heroin subjects in line with increased and reduced spine density characteristic of cocaine and heroin exposure, respectively. Similarly, cocaine users had increased *PDYN* (prodynorphin) expression that was decreased in heroin users, a direction of change documented in the previous candidate-gene-approach human studies of these stimulant[69] and opioid[70] drugs. Similarly, transcriptional impairments were also largely non-overlapping in the hippocampus between cocaine and alcohol users[62]. The differences in transcriptional alterations induced by different classes of drugs may be due to the distinct pharmacological actions and pharmacokinetics of each drug, the time period after drug use at which the subjects are studied, differences in the causes of death and small sample sizes for many of the existing studies. Decisive conclusions will require a larger number of transcriptome investigations of subjects with substance use disorders for each drug before common and unique features of the different drug classes can be fully uncovered.

Among all substances of abuse, the largest number of transcriptome studies relate to alcohol use disorder. Several genome-wide microarray and RNA-seq datasets spanning multiple brain regions are publically available. They comprise investigation of the hippocampus, where McClintock and colleagues identified alterations of stress-response pathways in chronic alcohol users[71]. In the nucleus accumbens, Mamdani et al. described mRNA and miRNA co-expression networks enriched for genome-wide association studies (GWAS) signals from the COGA (Collaborative Studies on Genetics of Alcoholism) database[72]. In the basolateral amygdala, alcohol use was shown to affect a broad range of genes with many systems involved in synaptic transmission, neurotransmitter transport, structural plasticity,

metabolism, energy production, transcription and RNA processing and the circadian cycle. A number of the impairments such as down-regulation of excitatory amino acid transporters GLAST, GLT-1 and AMPA glutamate receptor 2 genes revealed by this microarray were confirmed at the protein level[73]. In the PFC, Farris and colleagues identified gene co-expression networks associated with alcohol dependence and lifetime alcohol consumption. These networks were enriched for GWAS signals of alcohol dependence and included many genes related to neurophysiological targets and signaling mechanisms affected by ethanol. The dataset also provided intriguing new insights into alcohol biology by highlighting a potential role of alternative splicing - expression of a human-specific isoform of the voltage-gated sodium channel subunit *SCN4B* was significantly correlated to lifetime alcohol consumption[74]. Thus, there appears to be significant transcriptional disturbances throughout the brain associated with the history of alcohol use and to the genetic risk of alcohol. Such insights could be leveraged to determine mechanistic causality and potential new treatment targets.

Beyond substance use disorders – transcriptional window of psychiatric disease

A common neurobiological feature of mental illnesses is pathology of synaptic plasticity that is not only evident in relation to drug addiction but in disorders that span the entire development and aging of the brain that is perhaps reflective of the sensitivity of these dynamic and adaptive processes. Transcriptome analyses have validated such plasticity disturbances and offer insights about other biological processes that may have important implications for disease etiology and treatments. A recent publication from Daniel Geschwind and colleagues, for example, identified shared and disease-specific transcriptomic perturbations across autism, schizophrenia, bipolar disorder, depression and alcoholism[75]. Intriguingly, dysregulation of synaptic plasticity was common for many of these disorders. Most studies to date have, however, focused on specific patient populations and are reviewed below.

Autism spectrum disorder (ASD)—This spectrum of developmental disorders that occurs early in life is not fatal but afflicted individuals do incur a high incidence of injuries leading to death at relatively younger age compared to the general population[76]. Of the few existing post-mortem brain collections, microarray strategies have thus far been the discovery approach conducted most often to profile gene expression patterns in ASD. For example, Liu and colleagues examined developmental gene expression trajectories in the cerebral cortex of individuals with ASD as well as of controls and of non-human primates[77]. Among a large number of developmentally disrupted networks that were detected in autism, genes of only one, which mainly included synaptic genes, were enriched in autism-linked mutations. Intriguingly, the same gene set exhibited more developmental expression changes unique to the human brain than any other developmental pattern disrupted in autism[77]. Since the clinical and biological complexity of neuropsychiatric disorders often results in a lack of consensus between findings of individual studies, meta-analyses of published transcriptomic datasets can be invaluable to identify pathways that are consistent across populations and studies. Emphasizing this potential, a recent meta-analysis of over 1000 microarray profiles from several independent studies determined that many of the genes that were most robustly affected across studies were not previously underscored in ASD literature[78]. In addition to

highlighting genes such as *PDYN* that have been shown to be relevant to other neuropsychiatric vulnerability,[43, 79] but never really emphasized previously in ASD despite validation in replication cohorts[80], the meta-analysis revealed several novel genes suggesting a strong contribution of mitochondrial dysfunction to ASD[78]. These previously unreported genes are of significant interest since they emphasize a common transcriptome ASD signature that can be further explored mechanistically and that could offer potential future therapy development.

Affective disorders—Synaptic plasticity in brain regions that regulate reward, emotional expression and executive control strongly influence mood. Genome-wide transcriptional studies have begun to shed light on gene expression profiles associated with mood disorders, and a recent study examining gene expression and exon usage within the dorsolateral prefrontal cortex (dlPFC) of suicide victims with major depressive disorder (MDD) revealed low expression of genes involved in the regulation of glutamatergic neurotransmission.[81] Dysregulation of neuroplasticity in the PFC of depressed patients was also reported by other RNA-seq and microarray studies[82]. Psychiatric disorders are often characterized by sex differences in their prevalence, symptomatology and treatment response such as in MDD that occurs twice as much in females than in men. In a fascinating new study, Labonte and colleagues reported that depression and stress susceptibility in males and females is associated with different genes and neural pathways, potentially relevant to the well-known sexual dimorphism in MDD prevalence[83]. The authors performed a comprehensive characterization of transcriptional profiles across six brain regions and discovered major rearrangement of gene expression networks with limited overlap between genders. Key regulators identified in both sexes (*DUSP6* in females and *EMX1* in males) were manipulated in the mouse PFC and, in addition to recapitulating transcriptional remodeling, resulted in increased stress susceptibility[83]. Such insights could be useful in helping to develop treatment strategies for MDD selectively tailored to either sex, since a ‘one size fits all’ strategy has not proven effective for meeting the therapeutic needs of most patients with the disorder in which sex is such an important factor in the expression of the disorder and associated suicide attempts. It is important to emphasize that the spectrum of mood disorders is not as yet captured in regard to brain transcriptional signatures since limited post-mortem populations exist for anxiety disorders though molecular data are actively being obtained for MDD and suicide. However, recent attention has been focused on developing PTSD brain collections.

Schizophrenia (SCZ)—Studies of subjects diagnosed with schizophrenia constitute the largest numbers of postmortem brain studies to date (Supplementary Table 1) and not surprisingly, the vast majority focused on the prefrontal cortex given the cognitive impairments that are characteristic of this disorder. A recent meta-analysis of six independent transcriptional studies of SCZ patients identified marked sex differences with many more differentially expressed genes altered in the PFC of male patients compared to females[84]. Such findings might point to distinct underlying molecular pathology in male and female SCZ subjects that could have important implications for the well-know sex differences in SCZ patients including the age of onset as well as clinical symptom profiles. Another meta-analysis of gene co-expression networks in the post-mortem PFC of SCZ

patients and controls identified altered expression of gene networks related to biological processes such as synaptic transmission, oxidative phosphorylation, myelination, and immune function[85].These findings are consistent with previous candidate gene studies, thus verifying the prior focus on those biological functions for the disease. The large number of schizophrenia brain collections has helped to develop large consortiums needed to drive discoveries. One such Public-Private partnership, the CommonMind Consortium has sequenced RNA from the dlPFC of hundreds of schizophrenic and control subjects. This dataset was recently used to elucidate the functional impact of polygenic risk in SCZ, in which Fromer and colleagues identified ~700 differentially expressed genes and observed that 20% of SCZ risk loci could affect gene expression[86]. Five of the SCZ risk loci overlapped individual genes, which thus represent ideal candidates for targeted interventions. The value of this unbiased transcriptional profiling was strongly emphasized by follow-up mechanistic experiments, in which altering the expression of some of these candidate genes (*FURIN, TSNARE1, CNTN4*) in a zebrafish model affected neurodevelopment, and a knockdown of *FURIN* in human neural progenitor cells yielded abnormal neuronal migration[86]. Impairment of neuronal migration has been highly implicated in schizophrenia, a disorder that is hypothesized to incur its initial neurobiological insult during early fetal development as part of its etiology.

Combining genome-wide gene expression data with genome-wide association studies (GWAS) is a particularly powerful tool of human molecular studies to identify the most functionally relevant and potentially ‘druggable’ risk variants. The availability of GWAS datasets for schizophrenia can thereby be leveraged in combination with RNA-seq data. Using this approach, researchers have revealed the involvement of ion channels and calcium-related processes in SCZ risk[87]. In addition, large-scale transcriptional datasets are particularly amenable for network analyses that have the potential to uncover new aspects of underlying disease biology. For example, a weighted gene co-expression network analysis (WGCNA) of transcriptomic profiles (microarray) of cerebrocortical regions identified oligodendrocyte, microglial, mitochondrial, as well as neuronal modules associated with SCZ[88]. The involvement of oligodendrocyte-related gene expression changes was in line with earlier microarray findings from 15 different brain regions in SCZ and control subjects, indicating that gene classes associated with oligodendrocytes and myelin function were among the most profoundly affected differentially expressed genes[89]. Overall, the availability of these large collections of postmortem brain specimens from patients with schizophrenia are poised to provide the most rigorous data regarding the neurobiological underpinnings of schizophrenia not to mention valuable information regarding the molecular organization of the normal (healthy control) brain.

Alzheimer’s disease (AD)—AD is a devastating disorder at the end of the psychiatric neurodevelopmental spectrum. In contrast to other mental disorders where there are minimal neuropathological biological markers to guide diagnoses, this neuropsychiatric disorder is characterized by hyperphosphorylated tau, neurofibrillary tangles and amyloid plaques consisting of beta amyloid aggregates. Nevertheless, etiology and effective treatment options remain elusive, necessitating novel insights through leveraging genome-wide transcriptional profiling of the post-mortem human brain. Some novel findings have been reported by a

recent transcriptome meta-analysis that revealed a central role for sex steroids in the degeneration of hippocampal neurons in AD[90]. Furthermore, RNA-seq identified significant transcriptional alterations associated with late-onset AD related to myelination and innate immune response[91, 92]. A recent integrative network analysis of 19 brain regions identified further molecular signatures of AD. Wang and colleagues analyzed a large-scale single-cell gene expression dataset from 1053 postmortem brain samples across 125 individuals with dementia and AD[93]. The neurobiological pathways detected by these analyses included actin cytoskeleton, axon guidance, and nervous system development. Analysis based on disease severity suggested that many of the gene expression changes occurred early in the progression of disease, making them potential treatment development targets and unlikely to be mere bystanders of neurodegeneration[93]. Another investigation measured expression levels of ~25,000 transcripts in hundreds of brain samples from the cerebellum and temporal cortex of autopsied subjects with AD and other brain pathologies and then conducted an expression genome-wide association study (eGWAS) using ~200,000 SNPs located near the tested transcripts[94]. This study detected SNP-transcript associations for disease-related variants, demonstrating significant contributions of genetic factors to human brain gene expression and thus assigning functional relevance to these variants[94]. Large transcriptional databases and large populations coupled with GWAS data will be of significant value to expanding knowledge regarding disease risk and potentially personalized precision treatment strategies.

Epigenetic strategies to unmask the psychiatric genome

Epigenetics encompasses the regulation of gene expression that occurs during development or in response to environmental influences that do not involve alterations in DNA sequence. Epigenetic mechanisms include DNA methylation (DNAm) or hydroxymethylation (DNAhm) at cytosine residues (mC or hmC), histone post-translational modifications, histone variants, changes in nucleosome positioning, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) [95–97]. It is recognized that epigenetic remodeling is essential for developmental processes, including tissue and cell specification [98]. In addition, epigenetic mechanisms mediate the effects of environmental influences on gene expression during the development and throughout adult life, influencing such processes as synaptic plasticity or acquisition and consolidation of memory [99]. Epigenetic dysregulation has also been implicated in impaired cognition and neuronal death [100, 101]. Therefore, during the last decade numerous studies have explored the potential role of epigenetic modifications in psychiatric and neurological diseases. Several recent reviews provide a comprehensive coverage of epigenetic alterations detected in postmortem human brain of patients with various brain disorders [101–107]. Here we focus on studies, which have employed genome-wide profiling of epigenetic modifications that mark major classes of non-coding genomic regions and are important in the emergence of psychiatric and neurologic diseases.

Epigenetic regulation of gene expression via non-coding genomic regulatory elements

Over the last few years, GWASs have made significant progress in identifying genetic risk factors for many psychiatric disorders, including SCZ, bipolar disorder (BD) and ASD, providing evidence that these disorders involve both common and rare risk variants [108]. The

common theme that has emerged from these studies has been that of polygenicity of SCZ, BD, and ASD, as variants in many genes influence risk in the population. Most of the risk variants identified so far fall into non-coding regions of DNA (introns and intergenic regions). Unlike genetic variations in coding sequences where the functional implications are often apparent, the effects of non-coding variants are difficult to interpret. Recent advances in genome-wide sequencing and large-scale studies (e.g., ENCODE and RoadMap projects) has enabled the scientific community to recognize that non-coding DNA comprises numerous genomic regulatory elements (GREs) that are involved in the regulation of transcription through interactions with regulatory proteins (e.g., transcription factors and transcription co-regulators), chromatin architecture, and non-coding RNAs [109–114]. Depending on their function, GREs are classified into different groups, including promoters, enhancers, and insulators, with enhancers being the most numerous GREs (the human genome harbors ~400,000 putative enhancers vs. ~70,000 promoters [115]). GREs can function across considerable genomic distances, making it difficult to annotate them on the genome [116, 117]. The collective effort by many investigators worldwide has established that different classes of GREs are distinguished by specific epigenetic marks. Although other epigenetic mechanisms are also important for the regulation of gene expression, two key processes that influence the activity of GREs are DNAm and post-translational modifications of histone tails.

Various epigenomic studies have illustrated that different classes of GREs are characterized by unique chromatin states formed by a combination of multiple post-translational histone modifications within the nucleosomes (the basic units of DNA packaging in eukaryotes) [112, 118]. Such histone marks combined with other modifications (e.g., DNAm/hm levels) [119–121] have proven a useful measure for active GREs. In most cases, enhancers of active genes display a high level of mono- or di-methylation on histone H3 lysine 4 (H3K4me1/2) but are devoid of H3K4me3, whereas promoters show the opposite pattern. In addition to H3K4me1/2, two mutually exclusive modifications on H3K27 residues co-segregate with active or inactive/poised enhancers [122, 123]. Active enhancers are enriched with the H3K27ac mark deposited by histone acetyltransferases [124], while poised enhancers are enriched with the H3K27me3 mark deposited by Polycomb repressive complex 2 and associated with transcriptionally repressed regions [125]. DNAm is frequently associated with transcriptional repression in such cellular processes as X chromosome inactivation, genomic imprinting and silencing of repetitive DNA elements [126, 127]. However, a number of recent studies suggest that the function of mC (in both CG and non-CG contexts) as well as its oxidized derivatives such as hmC could be far more complex than previously thought, and that depending on the cell type and genomic location, (h)mC could recruit a wide variety of transcriptional modulators and even be compatible with transcriptional activation in certain biological contexts [120, 128–132]. Notably, studies in neurons have revealed large-scale changes in DNAm during development and in response to neuronal activity [130, 133], suggesting the contribution of dynamic DNAm to these processes [100, 134, 135].

GREs can also be annotated by chromatin accessibility. This approach maps genomic regions of open chromatin, as these sites are depleted of nucleosomes and are more readily accessible to transcription factors and co-regulators. Open chromatic regions have been detected through sequencing of the DNase I hypersensitive sites (DNase-seq) [136, 137] and

more recently by Assay for Transposase-Accessible Chromatin (ATAC) using sequencing (ATAC-seq) [138, 139]. The latter method probes DNA accessibility using hyperactive Tn5 transposase that inserts sequencing adapters into accessible regions of chromatin. Sequencing reads are then used to identify regions of increased accessibility in order to map transcription-factor binding sites and nucleosome position. The ATAC-seq method proved to be a fast low-input alternative to DNase-seq for assaying chromatin accessibility genome-wide[140, 141].

Whereas the primary sequence of the human genome is largely preserved in all human cell types, the epigenomic landscape of each tissue and cell type can vary considerably, contributing to distinct gene expression programs and biological functions [142]. To better understand how the epigenome contributes to cellular function, lineage specification, and the onset and progression of disease, recent studies of the NIH-supported Roadmap Epigenomics Mapping Consortium (REMC) created >100 reference epigenomes (including histone marks, DNA methylation, DNA accessibility, and RNA expression) spanning diverse cell and tissue types from clinically unremarkable donors [114]. In addition to other tissues, eight different regions of the adult brain, fetal brain as well as neuronal progenitors and brain-derived primary cultures were examined by REMC. REMC data are publicly available (Table 1).

REMC studies resulted in high-resolution maps of GREs that were annotated across all tissues. The analyses demonstrated the usefulness of the regulatory annotations for interpreting human genetic variation and disease. In an unbiased sampling across many GWASs, the authors found that genetic variants associated with complex traits (including brain disorders) are highly enriched in epigenomic annotations of trait-relevant tissues. The GWAS enrichments were strongest for enhancer-associated marks, which is consistent with their highly tissue-specific nature. Tissue and cell-type specificity of enhancers allows adaptations to occur within particular tissues and cells without invoking pleiotropic effects that are associated with changes to genes. The enrichment of disease-associated variants within GREs and the fact that this enrichment is stronger in the tissue most relevant to a particular disorder have been confirmed by several recent investigations [137, 143–145]. These findings emphasize that epigenetic annotations of GREs in relevant tissues/cells are extremely valuable in the study of human disease. Notably, among other findings, REMC studies showed that epigenetic landscapes of many embryonic stem cells (ES)-derived cell lines are closer to pluripotent states than corresponding somatic states[114]. Therefore, although ES-derived neural lines are important for testing mechanistic hypothesis, the REMC results clearly demonstrate that epigenetic regulation of gene expression in healthy and diseased brain should be investigated using human brain specimens, thus highlighting the paramount importance of postmortem brain research.

Covalent modifications of RNA

Similarly to the epigenetic modifications of DNA, over 100 known covalent base modifications are found on almost all types of RNA, including mRNA, tRNA, rRNA and snRNA [146]. This exciting discovery provides yet another dynamic and reversible biological mechanism for regulating transcription. Among the RNA modifications, the most abundant

and the most extensively characterized is N6-methyladenosine (m^6A) in mammalian mRNA [147, 148]. Using next-generation sequencing, ~120,000 m^6A peaks in over 12,000 genes have been identified in the human transcriptome [149]. It was also demonstrated that m^6A tags are enriched near stop codons and in 5′ untranslated regions (UTRs), and to a lesser extent in introns and long internal exonal regions [147, 148, 150]. m^6A is enzymatically added to mRNA molecules by heterodimer of METTL3–METTL14 in complex with several other proteins, and enzymatically removed from mRNA molecules by FTO and ALKBH5 [151–153]. In addition, numerous m^6A-reader proteins have been identified, including fragile X mental retardation protein (FMRP) [154]. As such, m6A might play a significant role in psychiatric disorders.

It has been shown in mouse brain that, compared to other tissues, m^6A mRNA methylation is high and increases during development [148]. In addition, m^6A levels and patterns are highly diverse in different regions of the adult brain [155], and based on single-cell RNA-seq data, all known m^6A-modifying enzymes and readers are expressed in the major brain cell types including neurons and glia as well as their subtypes [156]. Recent work from the Hongjun Song lab found that m^6A is critical for perinatal and early postnatal cortical neurogenesis in the mouse brain and in human induced pluripotent stem cell-derived organoids [157]. This study also showed enrichment of human-specific m6A tagging of transcripts related to brain-disorder risk genes. However, to the best of our knowledge, studies of m^6A RNA methylation in human postmortem brain have not been published to-date, probably because the existing assays require large amounts of input material. Precise quantification of the m^6A modification dynamics in the human brain will be crucial to elucidate the importance of this mechanism for brain function and disease.

Hierarchical organization of chromatin structure

Recent advances in genomic science have shown that chromatin is organized into hierarchical 3D structures that play an important role in gene regulation [158–160]. In particular, to direct gene activity, specific interactions are formed between gene promoters and distal enhancers. The interacting elements can be situated at a very large distance from each other, yet they communicate by looping out the intervening sequences and engage in direct contacts that are facilitated by the recruited transcription factors [161]. Also, chromosomes are structurally demarcated into large topologically associated domains (TADs) that encompass ~ 1 megabase of genome and act to reduce the contact between the GREs to within each of these domains [162, 163]. To-date, only few studies have examined 3D genome annotations in the human brain. High-resolution 3D maps of chromatin contacts during human corticogenesis have been recently obtained, identifying hundreds of novel enhancer–promoter interactions [164]. In this study, brain tissue-relevant chromatin contacts were used to inform the biological interpretation of SCZ risk variants. A recent exciting paper from the Schahram Akbarian lab examined the role of a H3K9 methyltransferase SETB1 in FACS-separated neuronal nuclei [165]. The study showed that SETB1 regulates epigenetic landscape and gene expression of a large neuron-specific TAD; this domain encompasses >70 genes at the clustered protocadherin (cPcdh) locus and is conserved in humans and mice. This work demonstrates the importance of cell-type specific approaches

in obtaining mechanistic understanding of locus-specific 3D chromatin-determined gene expression regulation in differentiated brain cells.

Epigenetic studies of schizophrenia (SCZ)

As of today, SCZ has received the most attention in genome-wide epigenetic studies of the post-mortem human brain. There is strong evidence that the etiology of SCZ is determined by both genetic background and environmental influences [166–169], the latter being the domain of epigenetic studies. Notably, a large scale GWAS analysis by the Psychiatric Genomics Consortium has identified 145 SCZ-associated loci, the vast majority of which were located outside of gene coding sequences and were significantly enriched in enhancers active in the brain [170, 171].

Initially, a large number of human postmortem epigenetic studies in SCZ focused on DNAm analysis of promoters of individual genes, suggesting evidence for DNAm alterations in *RELN, GAD1, BDNF, COMT*, and other genes (see [172, 173], as well as [105–107] and references therein). The progress in experimental approaches allowed researchers to shift the focus to genome-wide profiling of DNAm in human brain[174–180]. Wockner et al [176] profiled DNAm in 24 SCZ and 24 control subjects and detected changes at 4641 CpG sites at 2929 unique genes, confirming many previously found associations (*NOS1, AKT1, DTNBP1, DNMT1, PPP3CC*, *SOX10)*. A recent study from the Jonathan Mill lab [179] explored DNAm differences in PFC and cerebellum between SCZ and control postmortem brain samples. The authors detected multiple differentially methylated positions (DMPs) and regions (DMRs), with the most significant alterations uncovered in the PFC, which were successfully validated in a replication cohort. Notably, the genes associated with the detected DMPs and DMRs were found to be enriched for neurodevelopmental pathways. Furthermore, the SCZ-associated DMPs showed a significant overlap with CpG sites that undergo highly dynamic methylation changes during human fetal brain development [181]. Subsequently, this group [180] studied DNAm in 4 brain regions (PFC, striatum, hippocampus and cerebellum) from 41 SCZ patients and 47 controls, detecting multiple disease-associated alterations that were consistent across three of the four studied brain regions (not including cerebellum), as well as multiple sites of DNAm variation associated with SCZ polygenic risk score. These studies suggest significant DNAm alterations in the brains of SCZ patients that have just started to be uncovered.

In contrast to DNAm studies, the profiling of histone modifications in human postmortem SCZ brain has received significantly less attention. One recent study [182] focused on the role of *GAD1* gene expression regulation in SCZ, and uncovered concurrent changes in gene expression, the activity of a distal enhancer that was measured by the H3K27ac marks, as well as altered strength of the looping interaction between this enhancer and the promoter. Notably, using the REMC's annotations of enhancers that employed the measurements of multiple histone modifications in the brain, it was also shown that a SCZ GWAS SNP near the *CACNA1C* gene is situated within a distal regulatory element that directly interacts with the gene promoter [183]. Moreover, the risk allele was found to affect the promoter activity in a luciferase assay.

In addition, the above-mentioned approach of open chromatin profiling using ATAC-seq has emerged as a powerful low-input method of detecting active promoters and enhancers. A recent study by Fullard and colleagues [141] described its application to flow cytometry-sorted neuronal and non-neuronal nuclei. Whereas only samples from clinically insignificant subjects were used, the detected open chromatin regions helped assign functional roles to many non-coding SCZ risk variants, suggesting that the ATAC-seq approach may prove to be of great value in future studies of epigenetic alterations in brain disorders.

Epigenetic studies of autism spectrum disorder (ASD)

In contrast to SCZ, a smaller number of publications describing human genome-wide epigenetic profiling on ASD is available (reviewed in [184, 185]). Among them are DNAm studies employing the Illumina 450K arrays [186, 187], which uncovered significant autism-associated alterations at multiple genomic regions, some of which overlapped between the two reports. A recent DNAm profiling [188] employing Reduced Representation Bisulfite Sequencing provided evidence for autism-associated increase in the levels of the methyl-CpH modification, a subtype of DNAm that is specifically enriched in neurons [130, 189] and is established in early postnatal stage of brain development [130, 190]. Also, Shulha and colleagues profiled the H3K4me3 modification, indicative of active promoters, using chromatin isolated from FACS-separated neuronal nuclei (16 ASD, 16 controls) [191]. This work found recurring alterations in variable subsets of cases, located at genes with important roles in neurodevelopment and cognition. A recent seminal study from the Shyam Prabhakar and Daniel Geschwind labs [192] generated genome-wide H3K27ac profiles in a cohort of ASD and matched control individuals (45 ASD, 49 controls) using postmortem tissue from 3 regions: PFC, temporal cortex and cerebellum. They observed that the activity of over 5,000 enhancer or promoter loci was altered in autism and the nearby genes were enriched for GO categories of synaptic transmission, epilepsy, behavioral abnormality, histone acetylation, and immunity. Notably, the H3K27ac enrichment in ASD subjects was detected not only near genes expressed in adult brain, but also near genes upregulated at 1 year after birth, a developmental stage associated with the processes of neuronal maturation and synapse formation.

Epigenetic studies of substance use disorders

As of today, few studies have assessed epigenetic impairments directly in the addicted post-mortem human brain. One of the first investigations was carried out by the teams of Deborah Mash and David Goldman[62] and showed genome-wide enrichment of H3K4me3 in the promoter regions of protein coding genes of the hippocampus of cocaine abusers and individuals with alcoholism. There was a significant overlap of the H3K4me3 disturbances between cocaine and alcohol users indicating common epigenetic perturbations associated with the use of these addictive substances. We also recently observed increased enrichment of H3K27ac in the dorsal striatum of human heroin users compared to matched controls[19]. Intriguingly, acute morphine toxicology and heroin use history showed negative and positive correlations with H3K27ac, respectively, suggesting potentially different or even opposing epigenetic states during acute versus long-term heroin use. Strikingly, H3K27ac was strongly enriched at genes related to glutamatergic neurotransmission and is associated with a more open state of chromatin (identified by ATAC-seq) that drives the expression of these

genes, well-known to underlie drug-induced synaptic plasticity. Of note, these findings were closely translatable to heroin self-administering rats, where the administration of JQ1 (a small molecule blocking members of the BET family of histone acetylation readers) led to decreased self-administration and drug-seeking behaviors[19]. These findings highlight specific epigenetic dysregulation in heroin abusers associated with synaptic plasticity and identify JQ1-related compounds as promising candidates for targeted clinical interventions in opioid use disorder.

Epigenetic studies of affective disorders

A number of postmortem studies have explored epigenetic alterations in the brains of patients with MDD and of suicide victims with MDD. The majority of these studies explored DNAm in the promoters or gene bodies of individual candidate genes (reviewed in [103, 193, 194]). In particular, DNAm changes, that often involved hypermethylation of promoter regions accompanied by a decrease in gene expression, were detected in genes associated with stress response (e.g. *NR3C1* that encodes glucocorticoid receptor GR), neurotrophic signaling (e.g., *BDNF* and its receptor TrkB/*NTRK2*), and the polyamine system (e.g., *SAT1*). Genome-wide profiling of MDD patients with and without suicide also identified numerous promoter DNAm differences that were often inversely correlated with gene expression [195–198] as well as coordinated changes in DNAm of multiple genes [199]. Importantly, a seminal paper by McGowan et al., revealed that hyperactivity of the hypothalamic pituitary adrenal (HPA) axis observed in depressed suicide victims with childhood abuse as compared to those without childhood abuse might be partly explained by decreased hippocampal expression of *NR3C1* resulting from an increase in DNAm of its promoter [200]. This suggests that early-life adversity increases suicide risk through long-term epigenetic regulation of specific genes. This hypothesis was supported by a recent postmortem study of depressed suicides in which a history of childhood abuse was associated with cell-type-specific changes in DNAm of oligodendrocyte-specific genes as well as with global impairment of the myelin-related transcriptional program [201]. These effects were absent in the depressed suicide decedents with no history of childhood abuse. Another study suggested that DNAm status of *SKA2* mediates vulnerability to suicidal behavior and PTSD through dysregulation of the HPA axis in response to stress [202].

In addition to DNAm, histone modifications have also been implicated in the etiology of MDD (reviewed in [203]). For example, the reduction of histone acetylation in the brain of MDD patients was observed in regulatory regions of *CAMK2A* and *RAC1,* genes that are involved in synaptic function and plasticity [204, 205]. Histone methylation changes were also demonstrated at promoters of several genes including *BDNF* and genes that encode synapsin proteins [206, 207].

Postmortem studies also suggested that epigenetic mechanisms could also play an important role in bipolar disorder as well as in anxiety disorders [208–210] (also see [101, 211] for review). For example, the Infinium HumanMethylation450 array DNAm profiling of human hippocampus in schizophrenic, bipolar and control subjects (N=8 per group) revealed epigenetic and transcriptional alterations in the GAD1 regulatory network, associated with the function of GABAergic neurotransmission[209]. Future work in larger cohorts of subjects

probing different epigenetic modifications will be needed to understand how this misregulation contributes to disease.

Epigenetic studies of Alzheimer's disease (AD)

In the field of the epigenetics of neurodegenerative disorders, the largest progress has been made in epigenome-wide studies of AD (for a recent review, see [212]). However, the studies are mostly limited to exploring DNAm. An important example is an Illumina 450K DNAm profiling study (N = 122) by Lunnon and colleagues [213], which detected significant hypermethylation of the *ANK1* gene in entorhinal cortex (the brain region displaying earliest pathological signs in AD), PFC, and superior temporal gyrus, but not in cerebellum or blood. The findings were replicated in three independent cohorts. Similarly, De Jager and colleagues [214] assessed DNAm in a large cohort of AD and control individuals (N = 708), detecting alterations at many CpG sites, including those within the *ANK1* gene, which were mirrored by changes in gene expression of *ANK1*.

In a very recent postmortem study of the lateral temporal lobe of AD patients [215], the Shelley Berger lab examined the genome-wide enrichment of the histone acetylation mark H4K16ac which has been implicated in preclinical models of aging and cellular senescence [216, 217]. The authors discovered that while normal aging leads to H4K16ac enrichment, AD leads to significant losses of H4K16ac in the proximity of genes linked to aging and AD. In addition, the study discovered an association between the genomic locations of significant H4K16ac changes with genetic variants identified in prior AD GWAS and eQTLs. The relevance of discrete epigenetic disturbances associated with certain disorders remains to be explored in regard to their potential to inform the development of novel treatments in the future.

Challenges and Future Directions

As with most scientific strategies, challenges and limitations still exist with molecular studies of the postmortem human brain. A number of these have been mentioned throughout the review and in Table 2. Nevertheless, the experiences gained over the past few decades have clearly helped to address potential confounds through optimization of tissue processing, advancement of techniques, consideration of rigor and reproducibility (e.g., adding replication cohorts). One limitation that is still is not well integrated into postmortem studies is the clinical phenotyping of subjects which depending on the psychiatric illness can be extremely challenging to obtain from clinical records. Improved availability of complete patient records, pre-mortem and/or next of kin interviews could significantly aid the establishment of definite clinical diagnoses.

The large size of the brain and the still enormous expense of sequencing modalities continue to be a limitation for expanding molecular insights across the entire human brain outside the normal list of usual suspects of neuroanatomical regions studied (e.g., PFC and striatum). Moreover, even within brain regions frequently examined, the targets are often based on hypotheses driven from animal research, so discrete anatomical subregions derived from human pathological insights are still not used to guide translational investigations. For instance, most animal studies of the amygdala relevant to psychiatric disorders focus on the

basal and central nuclei with limited molecular knowledge about the multiple other subnuclei. However, molecular characterization of the amygdala in individuals diagnosed with psychiatric diseases revealed disturbances of *PDYN* gene expression in cortical subnuclei of MDD and bipolar disorder subjects (but not schizophrenia subjects),[218] an impairment also evident in heroin abusers particularly in the periamgdala cortical subregion. [50] Using an biased biobehavioral imaging strategy,[219, 220] we determined the important relevance of the periamgdala cortical *Pdyn* neurons to specifically regulate the extended amygdala circuit, regulate peripheral stress corticosterone levels and induce anhedonia phenotype that altogether emphasize a role in mood and anxiety.[50] Post-mortem molecular neuropathological interrogation of the human brain is therefore an important first step to guide translational approaches to subsequently provide functional insights. The challenge is to have the financial resources to profile the molecular signatures of multiple complex subregions throughout the human brain that could have functional relevance for behaviors linked to psychiatric disorders. Some information, however, will be obtained from public resources such as the Allen Brain Atlas (Table 1) as they continue to enhance the resolution of gene expression data available about the normal human brain.

Due to the extreme cellular heterogeneity of the brain, an important future direction will also be to expand the use of FACS (fluorescence assisted cell sorting)/FANS (fluorescence assisted nuclear sorting), laser-capture microdissection (LCM) or other new techniques for transcriptional profiling of single cell populations which will help to discern the contribution of specific cell types and uncover subtle molecular impairments masked by unaffected cell populations. LCM-dissected pyramidal neurons were, for example, used to study glutamate receptor splice variants in SCZ[221], while FACS-sorted neurons from the medial PFC revealed previously uncataloged transposable elements in the DNA, long interspersed nuclear elements-1 (LINE1) insertions, some of which were validated by PCR[222]. Strikingly, LINE1 insertions in cocaine samples were enriched in gene ontologies and pathways previously associated with cocaine addiction[222], pointing to an interesting genetic mechanism that could drive transcriptional alterations underlying substance use. In a more recent study, Ribeiro and colleagues performed RNA sequencing on neuronal nuclei isolated from post-mortem dlPFC of cocaine abusers and healthy controls[223]. The authors identified an AP-1 transcription factor regulated gene expression network in dlPFC neurons which was associated with cocaine use disorder and contained several differentially expressed hub genes, many of which were GWAS hits for traits that might involve dysfunction of brain reward circuitry (obesity) or dlPFC (bipolar disorder, schizophrenia)[223].

Indeed, regulatory changes affecting particular cell types cannot be reliably inferred from data obtained from bulk (cellular heterogeneous) brain specimens that combine signals from all cell types. Such masking of cell-type-specific signals is particularly relevant for low abundance cell types. Although the majority of transcriptional and epigenetic work has been performed in bulk tissue specimens, many investigators have begun to shift efforts toward studies in individual cellular populations. This direction initiated by Akbarian and colleagues have demonstrated the successful separation of neuronal and non-neuronal nuclei in frozen postmortem human brain specimens using FANS and antibodies against pan-neuronal marker RNA-Binding Protein RBFOX3 (also known as NeuN) [224]. Those and subsequent studies allowed detailed investigations demonstrating significant differences in

epigenetic landscapes not only between neuronal and non-neuronal cells [130, 189, 225, 226] but also between major neuronal subtypes [227]. Moreover, specific markers amenable for FANS-sorting of different populations of glial nuclei (e.g., SOX10 oligodendrocytes) have been also identified [228]. Notably, regions marked by brain cell-type-specific epigenetic modifications were found to be enriched for common risk variants identified in GWASs of psychiatric diseases [189, 227]. It is expected that data obtained from cell-type-specific epigenetic studies will help to further refine risk variant-GRE interactions towards relevant cell types in which the pathological effects of the variants can be further tested in future work. For example, as documented in Figure 2, our recent studies (Kozlenkov, Dracheva, Hurd unpublished) show that genes encoding two major opioid receptors, *OPRM1* and *OPRD1,* differ in their expression in glutamate and GABA neurons. Whereas *OPRM1* is expressed in both neuronal subtypes, *OPRD1* has significantly higher expression in GABA versus glutamate cells (Figure 2, left panel). Importantly, the intronic *OPRD1* SNP that predicts treatment outcome for opioid dependence in African-Americans (*rs678849*)[229], is localized within a putative enhancer, which is predicted (based on enrichment of H3K27ac, an active enhancer mark) to be more active in GABA than in glutamate neurons (Figure 2, right panel). Additionally, among four polymorphisms that alter *OPRM1* expression in normal human brain tissue (eQTLs) and that were found to be associated with heroin addiction in European American and African American cases from the Urban Health Study (meta-analysis $p<0.01$)[230], one SNP (*rs3778150*) is localized within a putative enhancer region that is active in glutamate but not in GABA neurons (Figure 2, right panel). The ability to characterize regulatory regions across the genome in a cell-type-specific manner in the human brain provides unparalleled possibilities of elucidating regulatory mechanisms that orchestrate the regulation of gene expression associated with genetics and neuropsychiatric traits. An atlas of chromatin accessibility based on ATAC-seq of neurons from multiple regions throughout the human brain is currently being made available as a resource for the field (**B**rain **O**pen **C**hromatin **A**tlas; BOCA).

The recent advances of techniques to allow single cell resolution of the molecular repertoire within multiple cell types of the human brain will no doubt continue to grow exponentially. In a very recent study, Xiaogun Wang and collaborators analyzed approximately 2,300 single cells in the developing human PFC from early (week 8) to mid-gestation (week 26) using RNA-seq to characterize the complex molecular diversity of the cortical landscape[231]. They identified 35 subtypes of cells within 6 main classes and were able to track the developmental trajectory of these cells emphasizing the power of this approach. Extending this strategy to brain specimens from individuals with psychiatric disorders is clearly of key interest in the near future. Additional strategies in the pipeline such as high dimensional multiomic analyses in the same specimens will allow for the integration across multiple levels of biological systems to better inform the relationship between the epigenome, transcriptome and proteome that will expand our understanding of the relevance of discrete molecular perturbations to cell function and disease. In addition to single cell RNA-seq, a variety of single-cell epigenomic assays have recently been developed (reviewed in [232, 233]). These methods allow the assessment of open chromatin, chromosome conformation, and DNA methylation, among others, providing complementary approaches to transcriptome profiling for classifying cell types based on differences in their epigenomic landscapes.

Thus, future single cell profiling of healthy and diseased brain might employ multi-omic techniques, combining transcriptomics, epigenomics, and proteomics in single cells to further increase the power of molecular approaches to help discriminate cell subtypes and understand differences that contribute to disease [234].

Several other issues should be considered in future epigenetic investigations of postmortem brain tissue. First, although the recent whole-genome (or genome-wide) studies in the human brain have been mostly concentrated on DNAm and H3K4me3 (indicative of active promoters) or H3K27ac (indicative of active enhancers) histone modifications, other epigenetic marks (e.g., H3K27me3 or H3K9me2/3) are important in uncovering the regulatory potential of genomic DNA [235, 236]. Second, because the field of epigenetic regulation of gene expression is still evolving, several different epigenetic markers should be simultaneously assessed in order to obtain more reliable annotations of regulatory elements and chromatin state in each particular tissue/cell type. These are clearly challenging strategies for even studies of animal models but still important to also conduct in the human post-mortem brain.

Gaining insight into how GREs influence disease risk requires production of comprehensive maps of gene expression and regulatory regions (e.g., enhancers and promoters) in both healthy (i.e., clinically unremarkable) and diseased human brains. As emphasized above, such studies should be preferably performed in cell-type- and discrete brain-region-specific manner. Also, given that mental illnesses have a neurodevelopmental origin, the developmental trajectories of GRE activity need to be explored. The recently established PsychENCODE Consortium (https://www.synapse.org/#!Synapse:syn4921369/wiki) aims to accelerate discovery of the GREs in human brain, and to elucidate their role in the molecular pathophysiology of psychiatric disorders. The Consortium is currently producing a public resource of genome-wide RNA-seq, ChIP-seq and ATAC-seq data on tissue and cell-type specific samples from approximately 1000 phenotypically well-characterized healthy and diseased human post-mortem brains with antemortem diagnoses of SCZ, bipolar disorder, and ASD [237]. Undoubtedly the PsychENCODE will be a valuable resource for driving future targeted research efforts.

Ultimately, the establishment of multi-site consortia is critical in order to achieve large sample sizes needed to enhance statistical power. However, many factors need to be considered in establishing these cohorts in order to obtain reliable and reproducible data. Detailed clinical phenotyping along with assessment of comorbid disorders (depression, post-traumatic stress disorder, anxiety, mood disorders, etc.) and agonal factors (cause and manner of death) are important. Moreover, studies will need to include systematic reporting of many variables, such as those highlighted in Table 2, in order to support future meta-analysis of molecular studies. Such cohorts will also be ideal to elucidate the molecular signatures within multiple brain regions of the same individual, which as of today has only been performed in a limited number of studies[113, 238, 239]; as most investigations have focused on one or two brain areas. Being able to identify the transcriptomic and epigenomic networks within and between discrete neural circuits (as opposed to individual brain regions) will provide a profound shift in understanding the molecular underpinnings of psychiatric disorders that can inform the development of improved therapeutic strategies.

Conclusions

Overall, the field has come far despite the initial skepticism that it would not be possible to study molecular mechanisms in the postmortem human brain. A large and growing body of evidence has emphasized not only that it is indeed feasible, but that the pursuit of neurobiological knowledge through direct studies of the human brain has even been an important drive for molecular technological advances. However, it is clear that we are still in the infancy of discovery science, and conclusive findings that can completely move the field forward regarding disease etiology, biological diagnosis and new treatment strategies are yet to be actualized. We are nevertheless moving in the right direction as state-of-the-art techniques continue to be developed and large consortia of human brain collections are being established.

While it has proven challenging until recently (and still does for some psychiatric disorders) to gather large sample sizes to identify strong candidates that meet the criteria for genome-wide significance, the large consortia and multi-site collaborations, such as the genotype-tissue expression (GTEx) project, CommonMind Consortium and PsychENCODE, will help to achieve this goal [113, 238, 240, 241]. Large datasets nevertheless have not negated the importance of small studies in which replication is conducted or coupled with translational animal models to improve interpretation of the findings. Indeed, various small population studies conducted over the years indicate that molecular alterations detected in discrete brain areas are reproduced. Moreover, while there appears to be largely distinct patterns of the molecular signatures associated with specific psychiatric disorders, some common disturbances involve biological processes linked to synaptic transmission and myelin and epigenetic mechanisms. While we await the future generation of multi-omic data based on different cell types in multiple regions of the postmortem human brain, it is important for the field to interrogate genes that have already been identified from current validated strategies to begin to garner more in-depth knowledge about their potential functional relevance to disease and behavior and for which druggable targets of these genes/gene networks and related proteins can be explored. Elucidating molecular signatures of the human brain for mental illnesses is already feasible to begin to guide the development of potential novel medication strategies critical to meet the need of the millions of individuals who suffer from psychiatric disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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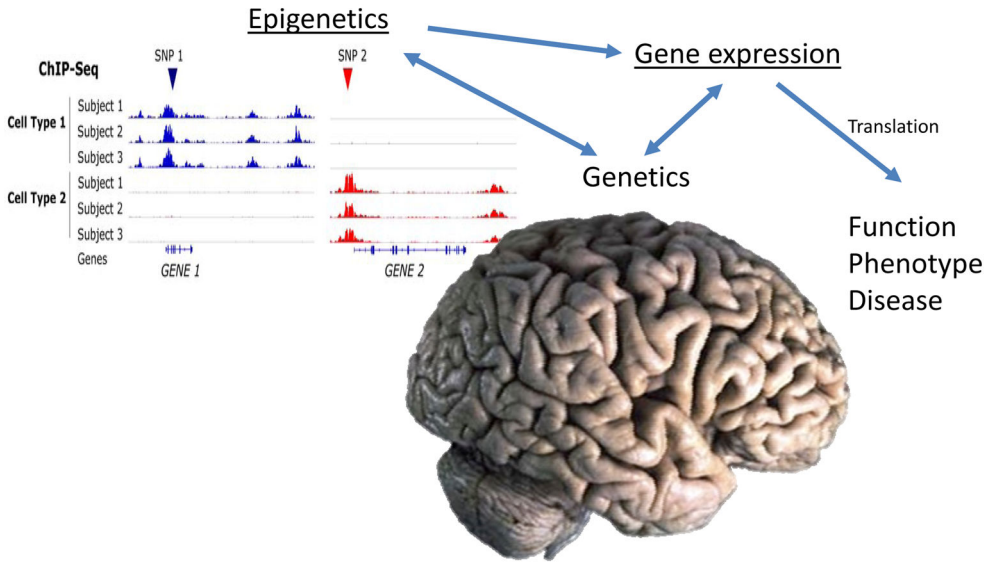

Figure 1. Molecular phenotyping of the post-mortem human brain has progressed along with technological advancements

Gene expression that initially was assessed in a low-throughput and hypothesis-driven manner using qPCR or *in situ* hybridization histochemistry for individual genes, can now be profiled genome-wide employing microarray or RNA-sequencing technologies. The epigenetic landscape (comprised of DNA methylation and hydroxymethylation, histone post-translational modifications, nucleosome positioning, microRNAs, and long non-coding RNAs as well as hierarchical 3D structures of the chromatin) mediates the effects of environmental influences on gene expression during development and throughout adult life. Epigenetic modifications mark non-coding regulatory elements (such as promoters and enhancers) and can now be assessed using multiple whole-genome strategies, including DNA bisulfite sequencing, ChIP-seq and ATAC-seq. These datasets can then be integrated with GWAS findings to infer the functional significance of risk variants. Lastly, due to extreme cellular heterogeneity of the brain and because many epigenetic marks differ between the cell types, an important future direction is to obtain transcriptional and epigenetic profiling of different cell populations and single cells, which are now feasible to carry out with postmortem human brain specimens.

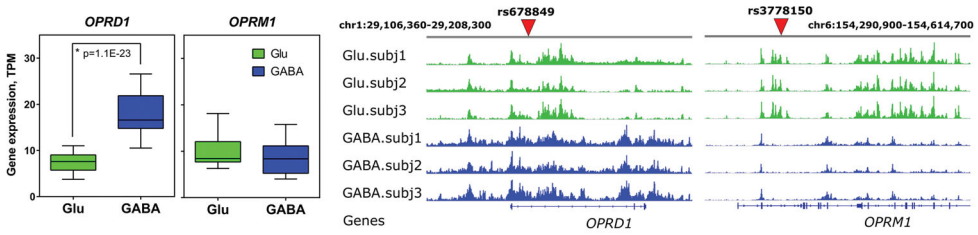

Figure 2. *OPRM1* and *OPRD1* expression (RNA-seq, *Left*) and H3K27ac enrichment profiles (ChIP-seq, *Right*) in GABA and Glu nuclei from human PFC

Left, RNA-seq **data** from 10 individuals are analyzed. ***Right***, H3K27ac data are shown for 3 individuals. Two SNPs (red triangles) implicated in opioid addiction are situated within cell-type specific H3K27ac peaks. For each of the two genes, the signals are presented in the same scale across cell types and biological replicates.

Table 1

Human brain gene expression datasets and brain banks

Name	URL	Description
Gene Expression Omnibus	https://www.ncbi.nlm.nih.gov/geo/	Repository of high-throughput gene expression data
NIH Roadmap Epigenomics Project	http://www.roadmapepigenomics.org/	Public resource of human epigenomic data
Psychiatric Genomics Consortium	http://www.med.unc.edu/pgc	Genome-wide genomic data for psychiatric disorders
Allen Human Brain Atlas	http://human.brain-map.org/	Multi-modal atlas mapping gene expression
NIH Neurobiobank	https://neurobiobank.nih.gov/	Six U.S. repositories
BrainNet Europe	http://www.brainnet-europe.org/	19 European brain banks
UK Brain Banks Network	https://www.mrc.ac.uk/research/facilities-and-resources-for-researchers/brain-banks/	Searchable directory for UK cohorts
Australian Brain Bank Network	http://www.austbrainbank.org.au/	4 Australian brain banks
Brain Bank for Aging Research	http://www.mci.gr.jp/BrainBank/index.cgi	Japan

Table 2

Standardized reporting of factors important for postmortem human studies.

Variable
Age
Sex
Inclusion/exclusion criteria
Drug use history (substances, years of use, former overdoses)
Psychiatric history
Cause of death
Manner of death
Toxicology (urine, blood; illicit and prescription drugs)
Comorbidities (psychiatric and general, head trauma)
Post-mortem interval (PMI)
Brain storage time and condition
Dissection method
Brain pH
RNA integrity number