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Genome-wide alteration of histone H3K4 methylation underlies genetic vulnerability to psychopathology

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Abstract

Dysregulated histone methylation has emerged as a recurring theme in multiple neuropsychiatric disorders. However, it is yet unclear whether altered histone methylation is associated with etiologic mechanisms or are an outcome of disease manifestation. In this study, we examined the GWAS data sets of three major psychiatric disorders, schizophrenia (SCZ), bipolar disorder (BIP), and major depression disorder (MDD), which represents a total of 231,783 cases and 425,444 controls, to clarify the relationship. Our gene-set enrichment analysis results identified statistically significant association of genes involved in three histone methylation biological processes with the three adult-onset psychiatric disorders, which is mainly driven by the Histone H3K4 methylation pathway (GO:0051568). Further analysis of Histone H3K4 methylation pathway genes revealed a widespread role of the genes in brain function and disease; 29 (52%) and 41 genes (73.2%) were associated with at least one brain-related trait or brain disorder, respectively. Spatiotemporal gene expression analysis suggests these pathway genes play a critical role during the prenatal period and are consistent regulators in the cerebral cortex throughout the lifespan. *AUTS2*, *DNMT1*, and *TET2* are genes of particular interest due to their pervasive role in various aspects of brain function. Our findings support a critical etiologic role of H3K4 methylation genes shared across SCZ, BIP, and MDD, providing new direction for the development of epigenetically-focused drugs targeting common causal factors of these devastating disorders.

Keywords

H3K4 methylation; epigenetics; psychopathology; genome-wide association studies; brain-disorder etiology; cross-disorder analysis

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Compliance with Ethical Standards

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INTRODUCTION

Psychiatric disorders are a leading cause of the major worldwide health burden. In the US alone, more than 47.6 million adults, corresponding to nearly 20% of the adult population, live with mental illness (Substance Abuse and Mental Health Services Administration 2019). No cure exists, and thus many of the patients suffer from lifelong disability, impaired quality of life, and higher rates of medical comorbidity and mortality (Smeland et al. 2019). Yet, the quest to improve psychiatric care has been hindered mainly by our limited understanding of the etiologic mechanisms and difficulties in disease classification (Network and Pathway Analysis Subgroup of Psychiatric Genomics Consortium 2015). Indeed, psychiatry is the only medical field in which no biologic or pathologic link is used in nosology, diagnosis, and treatments. The evolving nature of disease classification, extensive polygenicity, and pervasive pleiotropy have created major challenges in understanding the disease etiology and developing new treatments.

In this study, we aim to clarify the etiologic role of histone methylation pathway genes in major psychiatric disorders. Histone methylation acts as a central feature of dynamic gene regulation by enabling rapid and precise adaptation of responses to abruptly changing environments (Cross-Disorder Group of the Psychiatric Genomics Consortium 2019). DNA forms a chromatin structure by wrapping around a histone octamer that contains two copies of the histone variants H2A, H2B, H3, and H4. Here, histones undergo different forms of modifications at various N-terminus sites of histone tails, specifically lysine and arginine, resulting in a covalent modification that can either increase or decrease gene expression (Figure 1) (Cedar and Bergman 2009; Jambhekar *et al.* 2019). In recent years, various forms of dysregulated histone methylation have emerged as a recurring theme in multiple psychiatric disorders (Abrahams *et al.* 2013). Changes in histone 3 lysine 4 (H3K4) methylation have been reported at multiple neuronal signaling genes for schizophrenia (SCZ) and major depressive disorder (MDD) (Collins *et al.* 2019; Shen *et al.* 2014) and for synapsin genes in bipolar disorder (BIP) and MDD (Cruceanu *et al.* 2013). O'Dushlaine and colleagues (Network and Pathway Analysis Subgroup of Psychiatric Genomics Consortium 2015) reported the association of the H3K4 methylation pathway genes with major psychiatric disorders, along with neuronal and immune pathways, but the finding has not been replicated. Post-mortem schizophrenic brain studies have reported increased levels of histone 3 lysine 27 trimethylation (H3K27me3) (Akbarian 2010; Akbarian and Huang 2009; Peter and Akbarian 2011). H3K27me3, specifically the region of a truncated form of tropomyosin-related receptor kinase type B called *Trk B-T1*, has also been observed in patients with depression and suicide victims (Dalton *et al.* 2014). Despite these intriguing findings, it is unclear whether altered histone methylation is associated with disease onset or is an outcome of the disease manifestation itself.

Here we performed GWAS-based gene-set enrichment analysis to examine association of histone methylation genes in psychiatric disorders. We focus on three adult-onset psychiatric disorders, SCZ, BIP, and MDD: all of which share a group of symptoms, such as mood instability, emotional withdrawal, and cognitive impairment (e.g., sustained attention deficits) (American Psychological Association 2013; Zhu *et al.* 2019), some known biological processes such as calcium channel activity (American Psychological Association

2013; Heyes *et al.* 2015; Zhu *et al.* 2019), and a significant level of genome-wide genetic risk (Cross-Disorder Group of the Psychiatric Genomics Consortium 2019). We specifically examined: (1) whether risk genes associated with psychiatric disorders are enriched among genes involved in specific histone methylation pathways; (2) whether this association is specific to psychiatric disorders, or other neuropsychiatric, cognitive, and behavioral traits; and (3) whether the associated histone methylation genes show heightened gene expressions specific to certain brain regions, cell-types, or during specific developmental stages.

MATERIALS AND METHODS

Genome-wide association study (GWAS) datasets

GWAS summary association statistics of three adult-onset psychiatric disorders were downloaded from the Psychiatric Genomics Consortium: schizophrenia (SCZ; N=40,675 cases and 64,643 controls), bipolar disorder (BIP; N=20,352 cases and 31,358 controls), and major depressive disorder (MDD; N=170,756 cases and 329,443 controls). The compiled studies represented a total of 425,444 healthy volunteers and 231,783 patients (Supplementary Table 1). Chromosome coordinates of all association summary statistics were standardized to the reference genome GRCh37/hg19. We removed rare SNPs (minor allele frequency (MAF) < 1%), non-biallelic SNPs, the ones without rs IDs, or not in 1000 Genomes Project Phase 3. SNPs of which the alleles do not match the 1000 Genomes data on both strands were also excluded. Palindromic SNPs were removed if no strand information is available and the allele frequency difference between the two alleles were less than 15%.

Gene-set enrichment analysis of Histone Methylation pathway genes

We downloaded Gene Ontology (GO) gene sets from the MSigDB database (v.7.1). We found 47 GO gene sets related to histone methylation (Supplementary Table 2). Gene information including the genomic locus, strand, gene type, description, and biological function was obtained from the NCBI Entrez (Supplementary Table 3). We limited genes to chromosome 1–22, X, and Y, with Entrez IDs. Next, we estimated the number of causal risk variants underlying each psychiatric disorder using MiXeR (Frei *et al.* 2019). While incorporating the effects of linkage disequilibrium (LD) structure, MAFs, sample sizes, and cryptic relationships, the method estimated the number of disease-risk variants as 8.3k, 6.4k, and 14.9k for SCZ, BIP, and MD, respectively. Disease-associated risk loci for each disorder were then generated using PLINK (Chang *et al.* 2015) LD clumping ($r^2=0.4$, kb=500). We conducted gene set enrichment analyses of disease-associated risk loci for each psychiatric disorder using INRICH (Lee *et al.* 2012), GWAS-specific analysis method that adjusts for various genomic confounding factors including SNP LD, gene density, and sizes. For specificity, we restricted gene-set enrichment analysis to 61 gene sets of which the number of genes ranges from 5 and 1000. Meta-analysis of gene set enrichment analysis results across disorders was conducted using the Fisher's method implemented in the R *metap* library.

Investigation of cognitive, behavioral, and brain disorders

For methylation gene sets with significant association with three psychiatric disorders, we examined whether any brain-related traits have been associated with the annotated genes using the GWAS catalog data. First, we downloaded the full GWAS catalog data (v1.0.2; downloaded on Jan/2020). We searched “mapped genes” or “reported genes” columns, which list gene symbols that are mapped to or reported for the strongest SNP of associated risk loci. The GWAS catalog data included genotype-phenotype association data for 3,643 traits. Following the GWAS catalog, we defined the seven domains of brain-related traits using the European Bioinformatics Institute Experimental Factor Ontology (EFO) data. Domains of brain-related traits were defined as: (1) mental process (e.g., intelligence); (2) emotion (e.g., irritability); (3) behavior (e.g., risk-taking behavior); (4) psychiatric disorder (e.g., anxiety disorder); (5) brain diseases (e.g., Alzheimer’s disease); (6) brain-related measurement (e.g., brain volume measurement); and (7) multi-domain studies that analyze traits in different domains together (e.g., schizophrenia and intelligence). There were 94 brain-related traits in the seven domains. For each of the trait-associated genes, we examined how many of the genes overlap with our methylation gene sets. We calculated the statistical significance of the overlap by randomly selecting the same number of genes 10,000 times and by counting how many of the random overlap is at least the same as the original overlap observed for the methylation gene set. False discovery rates of 10% was used to identify statistically significant overlaps.

We also investigated various bioinformatics databases that provide rare-variant-based evidence for gene-phenotype associations. The resources we used include: OMIM (Online Mendelian Inheritance in Man) (Amberger *et al.* 2019), ClinVar (Landrum *et al.* 2018), GeneCards (Stelzer *et al.* 2016), and the Genetic Testing Registry (GTR) (Rubinstein *et al.* 2013). We also examined disease-specific databases, SFARI (Abrahams *et al.* 2013), VariCarta (Belmadani *et al.* 2019), and SZBD (Wu *et al.* 2017), and literatures (Rhoades *et al.* 2019) that report sequencing-based evidence for Histone H3K4 methylation pathway-associated genes. SFARI and VariCarta gene databases are dedicated to integrating information about autism spectrum disorder (ASD), which showed significant genome-wide genetic correlations with SCZ, BIP, and MD (Cross-Disorder Group of the Psychiatric Genomics Consortium 2019). Both databases utilize peer-reviewed publications to build a library of in-depth knowledge about genes associated with ASD. SZBD provides a collection of 7,377 schizophrenia-associated genes identified through integration of various sources, including CNVs, exons, GWAS, and literature.

Spatiotemporal brain gene expression analysis

We also performed temporal analysis of brain-expressed genes using the Human Brain Transcriptome (<https://hbatlas.org/pages/hbtd>) (Kang *et al.* 2011). This database provides spatial and temporal gene expression analysis of over 1,340 specimens gathered from the 16 areas of the post-mortem human brain: cerebellar cortex, mediodorsal nucleus of the thalamus, striatum, amygdala, hippocampus, and 11 areas of the neocortex. Study samples span individuals of both genders and all ethnicities from 4 post-conceptual weeks of age through adulthood and are divided into 15 period groups. Each of our genes of interest were entered into the database, and spatiotemporal output was downloaded and analyzed

for expression patterns. For gene expression analysis, we observed the trends of the six different represented brain areas and defined High Prenatal as a decrease of 1 in signal intensity in four or more brain areas after birth, while High Postnatal was defined as an increase of 1 in signal intensity in four or more brain areas after birth. Expression patterns without changes significant enough to meet high or low expression thresholds were defined as Uniform Throughout. We further analyzed spatiotemporal data using the Brain Expression Spatiotemporal pattern (BEST) (<http://best.psych.ac.cn/>) (Guo *et al.* 2019). This database provides spatial and temporal co-expression data for brain-related genes based on eight post-mortem brain datasets, including the Allen Brain Atlas (Hawrylycz *et al.* 2012) and BrainSpan (Miller *et al.* 2014). The average expression level of each input gene is calculated with either a 16×10 or 25×9 spatiotemporal matrix, with the first number representing brain regions and the second number representing time periods (Johnson *et al.* 2016). Co-expression networks and heatmaps are based upon 525 RNA-seq data samples from BrainSpan and illustrate the top 20 input genes by network connectivity in the top 5 enriched clusters by adjusted p-value (Langfelder and Horvath 2008).

Brain cell-type-specific data analysis

We examined whether methylation-related genes are expressed in the brain using the GeneCards database. For brain-expressed genes, we investigated whether they show cell-type specific expression activities in the brain using the web-based data resource (<http://celltypes.org/brain/>). This database provides RNA expression data (MacArthur *et al.* 2017) for six brain cell-types: astrocyte, endothelial, microglia, neuron, oligodendrocyte, and OPC. We used human cortical sample data generated by Darmanis *et al.* (2015). Exon-level analysis was conducted by entering the number of exons for each gene, which was obtained from the Ensembl database (Hunt *et al.* 2018). To measure the statistical significance of cell-type-specific gene expression, we have run a permutation test as follows. In each simulation run, gene expression data were randomly permuted across cell-types for each gene. This reserves the number of single cell-type gene expression for each gene, while randomizing the cell-type membership. Then for each cell-type, we counted the number of genes that were expressed in the cell-type. This was repeated 1000 times. For each of the six cell-types, the statistical significance of enrichment was measured by comparing the number of originally observed gene expression to those counted in permuted datasets.

RESULTS

Enrichment of H3K4 methylation pathway genes in three-adult-onset psychiatric disorders

We assembled genome-wide association study (GWAS) datasets for three adult-onset psychiatric disorders, SCZ, BIP, and MDD, which represent a total of 231,783 cases and 425,444 controls (Supplementary Table 1). Among 47 Gene Ontology (GO) terms related to histone methylation, we found statistically significant association of three GO biological process (BP) and one cellular component (CC) gene sets across the three psychiatric disorders (FDR<1%). The top fifteen findings are summarized in Table 1 (full results in Supplementary Table 2). Three significant BP gene sets included: (1) “*Histone H3K4 methylation*” (GO:0051568, gene-set association meta-analysis p -value = 2.51×10^{-5} , FDR q = 0.0012); (2) “*Histone Methylation*” (GO:0016571, p = 2.82×10^{-5} , q = 0.0013); and (3)

“*Regulation of Histone Methylation*” (GO:0031060, $p = 8.77 \times 10^{-5}$, $q = 0.0041$) (Figure 2a). We found no significant enrichment for gene sets under GO Molecular Functions (Figure 2b). Association of the top three histone methylation pathway gene sets was more notable for two mood disorders, BIP and MDD, compared to SCZ (Supplementary Table 3). Comparison of the top three pathway genes indicated that association of the latter two gene sets, “*Histone Methylation*” and “*Regulation of Histone Methylation*” was largely driven by genes involved in “*Histone H3K4 methylation*” (supplementary Figure 1). Cross-disorder comparison showed that 50 out of 56 genes shared across SCZ, BIP, and MDD showed significant levels of enrichment. 43 of those 50 genes were implicated in at least two disorders, and 29 out of 50 genes were implicated in all three disorders. 18 of these 29 overlapping genes play a role in the epigenetic processes of chromatin compaction ($N = 3$) or the methylation of DNA ($N = 3$), histone ($N = 5$), or histone lysine ($N = 7$) (Figure 3). We thus focus further analyses on “*Histone H3K4 methylation*”. Excluding the MHC regions (chr6:25–35Mb) resulted in consistent findings. (Supplementary Table 4).

Implication of H3K4 methylation genes in brain-related traits

We hypothesized that pleiotropic association of Histone H3K4 methylation pathway (GO:0051568) genes across three adult-onset psychiatric disorders may indicate their broad impact on brain function and structure. To test the hypothesis, we examined whether common single nucleotide polymorphisms (SNPs) in Histone H3K4 methylation pathway genes had shown association with brain-related traits using the GWAS catalog data (MacArthur *et al.* 2017). We used European Bioinformatics Institute Experimental Factor Ontology (EFO) to define seven domains of brain-related traits: (1) mental processes (e.g., intelligence); (2) emotion-related phenotypes (e.g., irritability); (3) behaviors (e.g., risk-taking behavior); (4) psychiatric disorders (e.g., anxiety disorder); (5) brain diseases not including psychiatric disorders (e.g., Alzheimer’s disease); (6) brain-related measurement (e.g., brain volume measurement); and (7) multi-domain phenotypes that combine traits in distinct domains together (e.g., schizophrenia and intelligence).

We found implications of Histone H3K4 methylation genes in all of the seven domains, most notably, cognition, behavior, emotion, brain morphology, and mental health (Supplementary Table 5). We summarize brain-related traits associated with more than one H3K4 methylation gene in Table 2. Notable association was found for *intelligence* with which 10 out of 56 Histone H3K4 methylation pathway genes had been implicated (17.6%). Simulation studies indicated this overlap is highly unlikely to occur by a random chance ($p = 1.19 \times 10^{-3}$). Significant associations were also found for risk-taking behaviors (overlapping gene $N = 6$, $p = 3.99 \times 10^{-4}$), neuroticism ($N = 4$, $p = 2.99 \times 10^{-4}$), and reaction time measurement ($N = 3$, $p = 2.99 \times 10^{-4}$). As expected, multiple Histone H3K4 methylation pathway genes had shown genome-wide significant association in the GWAS catalog with adult-onset psychiatric disorders, including BIP ($N = 4$, $p = 1.99 \times 10^{-4}$), SCZ ($N = 3$, $p = 9.99 \times 10^{-5}$), and insomnia ($N = 3$, $p = 1.99 \times 10^{-4}$).

We also examined various bioinformatics resources and published literature to check whether rare inherited or *de-novo* exonic mutations in Histone H3K4 methylation genes had contributed to the risk of mental health conditions. The results are summarized in

Supplementary Table 6. We found 41 out of 56 Histone H3K4 methylation pathway genes have been implicated in at least one brain disorder (73.2%), with *DNMT1* (DNA methyltransferase 1) having the highest number of associated disorders (N=17). *DNMT1* showed association with narcolepsy, hereditary sensory neuropathy, neuropathy, dementia, bipolar disorder, autosomal dominant cerebellar ataxia, hereditary sensory and autonomic neuropathy type 1, mood disorder, epilepsy, fetal alcohol spectrum disorder, Rett syndrome, autism, pervasive developmental disorder, Charcot-Marie-tooth disease/*DNMT1*-related disorder1, and SCZ (Rubinstein *et al.* 2013; Stelzer *et al.* 2016; Wu *et al.* 2017). A cross comparison of the disorders associated with the 56 Histone H3K4 pathway genes showed some striking pathological patterns. Out of 56 genes, 9 were associated with a form of mental retardation (16.1%), 10 were associated with Kleeftstra syndrome (17.6%), 11 were associated with microcephaly (19.6%), 14 were associated with schizophrenia (25%), and 16 were associated with Kabuki syndrome (28.6%).

Spatiotemporal and cell-type-specific brain expression activity of histone H3K4 methylation genes

We examined how expression of 56 H3K4 methylation genes are regulated in different brain areas throughout the lifespan using the BrainSpan data (Kang *et al.* 2011). The postmortem brain data were gathered from the cerebellar cortex, mediodorsal nucleus of the thalamus, striatum, amygdala, hippocampus, and 11 areas of the neocortex. Overall, among 52 H3K4 methylation genes for which gene expression data was available, 35 genes showed higher expression during the prenatal period compared to postnatal (67.3%), while 1 genes showed an opposite trend (1.9%). The remaining 16 genes showed uniform expression throughout the lifespan (30.8%) (Supplementary Table 7). The cerebral cortex exhibited higher general uniformity throughout the lifespan across all examined genes when compared to other brain areas. *AUTS2* (activator of transcription and developmental regulator *AUTS2*), *TET2* (tet methylcytosine dioxygenase 2), and *DNMT1*, which were associated with the highest levels of brain-related traits and psychiatric disorders, all exhibited higher prenatal expression compared to postnatal. Co-expression analysis derived from BEST correlated to these spatial and temporal findings for all genes for which data was available (Supplementary Figure 2, Supplementary Figure 3) (Guo *et al.* 2019).

We examined the levels of expression for each of the 56 H3K4 methylation genes in six specific brain cell-types: astrocytes, endothelial cells, microglia, neurons, oligodendrocytes, and oligodendrocyte progenitor cells. We found no enrichment of single cell-type-specific expression for H3K4 methylation genes ($p > 0.05$). Out of the 47 genes for which data was available, close to half of the genes were expressed in at least five cell-types, including *AUTS2* and *TET2* (47.8%) (Supplementary Table 8).

Discussion

Genetic and epigenetic regulatory mechanisms, particularly histone methylation, play a critical role for brain development and function. Previous studies have shown abnormal H3K4 methylation disrupts normal skeleton and neurological development, which can lead to conditions with cognitive defects such as Kabuki syndrome and microcephaly

(Jambhekar *et al.* 2019). Using GWAS data representing more than 657,000 individuals, we showed that genes involved in Histone H3K4 methylation (GO:0051568) are shared between three major adult-onset psychiatric disorders: MDD, BIP, and SCZ. Brain gene expression data analysis indicated that these genes are specifically highly expressed during prenatal encephalic development, suggesting genetically-driven variations in Histone H3K4 methylation may increase MDD, BIP, and SCZ susceptibility through disruption of important neurodevelopmental mechanisms. We did not find statistically significant association of other types of histone methylation mechanisms, including H3K27, H3K9, and H3K36, despite prior studies suggesting their role in psychiatric disorders (Akbarian 2010; Akbarian and Huang 2009; Peter and Akbarian 2011; Hyun *et al.* 2017; Jambhekar *et al.* 2019).

We found robust evidence supporting the implication of Histone H3K4 methylation pathway genes in various brain disorders and traits. Overall, 29 out of 56 Histone H3K4 methylation pathway genes had at least one publication reporting genotype-phenotype association with brain-related traits (52%). Two genes, *AUTS2* and *TET2*, were found to be of particular interest. *AUTS2* was associated with 18 brain-traits, which encompass all seven domains we studied, while *TET2* was associated with 11 brain-traits in six domains, including behavior, brain-related measurement, emotion, mental process, multi-domain, and psychiatric disorder. Both genes were disproportionately associated with behavioral traits (7 of 18 for *AUTS2* and 5 of 11 for *TET2*) related to sleep, smoking, coffee consumption, and risk-taking behaviors. Other traits with which both *AUTS2* and *TET2* showed association are related to mental processes, including intelligence, reaction time, and self-reported educational attainment. Of the 41 genes implicated in at least one brain disorder, *DNMT1* ranked highest with association to 17 individual disorders. *AUTS2* was associated with 14 disorders (Supplementary Table 6, Supplementary Table 9).

Three H3K4 methylation genes, *AUTS2*, *DNMT1*, and *TET2*, which are highlighted based on their association with broad brain-related traits, play vital functional roles in early brain development. *AUTS2* consists of the polycomb group (PcG) multiprotein PRC1-like complex which modifies histones and chromatin structures during prenatal encephalic development. It shows significant expression in the frontal, parietal, and temporal regions of the fetal brain and several neuronal subtypes to include glutamatergic, GABAergic, and tyrosine hydroxylase (TH)-positive dopaminergic neurons (Oksenberg and Ahituv 2013; Stelzer *et al.* 2016). Spatiotemporal data and association of *AUTS2* copy-number variation with multiple neurodevelopmental disorders suggest a role in neuronal maturation or neocortical differentiation (Oksenberg and Ahituv 2013), which is consistent with single cell data analyses. In our brain single cell-data analysis, expression of *AUTS2* was evident in various brain cell types including endothelial cells, neurons, oligodendrocytes, oligodendrocyte progenitor cells, although markedly high expression was most notable in astrocytes (Darmanis *et al.* 2015) (Supplementary Table 8). There is strong evidence for links between abnormalities in astrocyte function and SCZ, with cell-specific hyperresponsiveness and morphological abnormality, brain-area specific changes in astrocyte density, and reduction in glutamate transporters in astroglia as just some aspects associated with the disorder (McCullumsmith *et al.* 2016; Tarasov *et al.* 2019). A strong body of research has emerged over the last 15 years showing that astrocytes are capable of

producing and reacting to neuroactive signaling with neurons and that they heavily influence critical formation and function of synapses and neural circuitry in early life. In correlation to our spatiotemporal data on *AUTS2*, astrocyte activity peaks during prenatal periods and continues to be high during post-natal synaptogenesis and synaptic pruning (Petrelli *et al.* 2016). Studies have shown that forebrain regulation of protein-protein interaction (PPI) and sensorimotor gating is dependent on functional neural circuitry, and disruption of such circuitry is a feature of some SCZ phenotypes (Swerdlow and Light 2018). Disruption in neurocircuitry has also been evidenced by fMRI in MDD, BIP, and other mood disorders (Horowitz-Kraus *et al.* 2018; Wessa and Lois 2015), suggesting *AUTS2* disruption in astrocytes could contribute to brain-disorder etiology through dysregulation of developmental synapse formation.

The protein encoded by *TET2* is involved in histone H2B GlcNAcylation through recruitment of OGT to transcription start sites of active genes containing CpG sites. *TET2* also plays an active role in DNA cytosine demethylation through the multi-step conversion of methyl-cytosine to 5-hydroxymethylcytosine (5hmC), 5hmC to 5-formylcytosine (5fC), and 5fC to 5-carboxylcytosine (5caC). *TET2* contributions to the demethylation of cytosine has broad developmental implications, as methylation of the C5 position of cytosine bases are heavily involved in transcriptional regulation (Stelzer *et al.* 2016). Unlike *AUTS2*, we found specifically higher expression of *TET2* in microglia compared to other cell-types. Microglia primarily serve to counteract brain-tissue damage and pathogens through macrophage, and microglial phagocytic activity is highly involved in the postnatal synaptic pruning of presynaptic and postsynaptic elements (Petrelli *et al.* 2016). Thus, the same SCZ, BIP, and MDD disruption of neural circuitry associated with dysregulated astrocyte function could be impacted by changes in microglia function. This correlates to our data showing consistently high *TET2* expression in both the prenatal period and the microglial cell-type, evidencing a link between gene disruption and brain-disease etiology.

DNMT1 produces an enzyme that transfers methyl groups to genomic DNA cytosine and is highly involved in maintaining methylation patterns following DNA replication. *DNMT1* genetic mutation leads to protein variation, resulting in dysregulated methylation of other genes and subsequent development of *DNMT1*-associated neurological disorders, and recent research suggests such dysregulation can be corrected by a *TET2-DNMT1* complex which downgrades abnormally elevated levels of CpG site methylation during DNA repair following oxidative stress (Baets *et al.* 2015; Stelzer *et al.* 2016; Zhang *et al.* 2017). Prior research has identified altered methylation patterns of specific genes in cases of SCZ, MDD, and BIP (Chen *et al.* 2017; Hannon *et al.* 2016; Ruzicka *et al.* 2015), evidencing the shared etiological role of prenatal *DNMT1* expression in these disorders.

Our study highlights the shared etiology of H3K4 methylation during early brain development across SCZ, MDD, and BIP. Yet much progress remains to be made, as genetic contributors to dysregulation of histone methylation are known to be compounded by environmental perturbations including maternal immune activation and exposure to neuroactive drugs (Dong *et al.* 2018; Nestler *et al.* 2016). At this time, treatment of SCZ, BIP, and MDD is largely limited to behavioral interventions and symptom management (Collins *et al.* 2019, Miklowitz *et al.* 2020, Annette *et al.* 2020). However, as contemporary

advances are made into highly-selective pharmacological interventions that can cross the blood-brain-barrier, direct enzymatic manipulation becomes an increasingly possible treatment option for dysregulated H3K4 methylation in psychiatric disorders (Ricq *et al.* 2016).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representation of locations and effects of known H3 N-Terminal tail methylation sites. Methylation of n-terminal tails can take place at various lysine sites and occur as either mono-, di-, or trimethylation. Whether histone lysine methylation increases or decreases gene expression varies by gene, lysine, and methylation type, as in the case of gene-specific instances of histone 3 lysine 9 (H3K9) where mono-methylation results in activation but di- or trimethylation result in repression

Figure 2.

Analysis of GO Biological Processes related to histone methylation. Threshold for significance is indicated with a dashed red line at $-\log(0.0007)$. **a** Top 10 BF gene sets ordered by extent of association based on $-\log(p)$. The most significant gene-set is *Histone H3K4 methylation*, followed by *Histone Methylation*, and *Regulation of Histone Methylation*. **b** Top 10 MF gene sets ordered by extent of association based on $-\log(p)$. No MF gene sets meet the criteria for significance.

Figure 3.

Cross-disease comparison of genes associated with three significant GO Biological Processes. Shows a strong correlation between SCZ, BIP, and MDD. Each circle in the diagram represents gene sets for one of the three diseases. N-values represent a number of genes, with n-values in areas where circles overlap representing genes common to two or all three diseases. 29 genes are associated with all three, and these genes are categorized by functional mechanism in the text to the right of the diagram

Table 1.

Pathway analysis results of histone methylation genes across SCZ, BIP, and MDD

Category	Gene set	metaP	FDR
bp	HISTONE H3 K4 METHYLATION	2.51E-05	1.18E-03
	HISTONE METHYLATION	2.82E-05	1.33E-03
	REGULATION OF HISTONE METHYLATION	8.77E-05	4.12E-03
	REGULATION OF HISTONE H3 K4 METHYLATION	2.80E-03	1.32E-01
	NEGATIVE REGULATION OF HISTONE H3 K9 METHYLATION	3.41E-03	1.60E-01
	NEGATIVE REGULATION OF HISTONE METHYLATION	3.50E-03	1.65E-01
	HISTONE H3 K9 METHYLATION	9.07E-03	4.26E-01
	POSITIVE REGULATION OF HISTONE METHYLATION	9.66E-03	4.54E-01
	REGULATION OF HISTONE H3 K9 METHYLATION	3.48E-02	1.00E+00
	HISTONE H3 K9 DEMETHYLATION	4.03E-02	1.00E+00
cc	HISTONE METHYLTRANSFERASE COMPLEX	4.68E-04	2.20E-02
mf	HISTONE METHYLTRANSFERASE ACTIVITY	1.42E-03	6.68E-02
	METHYLATED HISTONE BINDING	2.35E-03	1.10E-01
	HISTONE LYSINE N METHYLTRANSFERASE ACTIVITY	6.98E-03	3.28E-01
	HISTONE DEMETHYLASE ACTIVITY	3.74E-02	1.00E+00

Table 2.**Brain-related Traits Related to Histone H3–K4 Methylation**

Domain	Brain-related Traits	Number of Genes	Genes
behavior	alcohol consumption measurement	3	ARID4A, AUTS2, TET3
	coffee consumption measurement	2	AUTS2, TET2
	neuroticism measurement	4	HIST1H1C, HIST1H1E, KMT2A, SETD1A
	risk-taking behavior	6	AUTS2, GATA3, HISTH1E, KMT2E, TET2, TET3
	smoking initiation	2	AUTS2, DNMT3B
	smoking status measurement	5	AUTS2, DNMT3B, HISTH1E, SETD3, TET3
	social interaction measurement	2	GCG, TET2
brain disease	Parkinson's disease	2	SETD1A, SMAD4
brain-related measurement	brain volume measurement	2	AUTS2, HIST1H1E
	chronotype measurement	2	AUTS2, KMT2D
mental process	intelligence	10	AUTS2, HIST1H1C, HIST1H1E, KMT2D, KMT2E, PRMT6, RTF1, TET2, WDR82, ZNF335
	reaction time measurement	3	AUTS2, KMT2D, TET2
	self-reported educational attainment	2	AUTS2, TET2
multi-domain	well-being measurement	2	AUTS2, KMT2A
psychiatric disorder	bipolar disorder	4	AUTS2, KMT2D, PYGO2, WDR82
	schizophrenia	3	ASH2L, KMT2E, WDR82
	schizophrenia/autism spectrum disorder	6	HIST1H1C, HIST1H1D, HIST1H1E, KMT2E, NELFE, WDR82