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Epigenetic Research in Neuropsychiatric Disorders: the "Tissue Issue"

Kelly M. Bakulski[1], **Alycia Halladay**[2,3], **Valerie W. Hu**[4], **Jonathan Mill**[5,6], and **M. Daniele Fallin**[7,8]

[1]Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan, USA

[2]Autism Science Foundation, New York City, New York, USA

[3]Department of Pharmacology and Toxicology, Rutgers University, New Brunswick, New Jersey, USA

[4]Department of Biochemistry and Molecular Medicine, School of Medicine and Health Sciences, George Washington University, Washington, DC, USA

[5]University of Exeter Medical School, University of Exeter, Exeter, UK

[6]Institute for Psychiatry, Psychology and Neuroscience, King's College London, London, UK

[7]Department of Mental Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA

[8]Wendy Klag Center for Autism and Developmental Disabilities, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Abstract

Purpose of Review—Evidence has linked neuropsychiatric disorders with epigenetic marks as either a biomarker of disease, biomarker of exposure, or mechanism of disease processes. Neuropsychiatric epidemiologic studies using either target brain tissue or surrogate blood tissue each have methodological challenges and distinct advantages.

Recent findings—Brain tissue studies are challenged by small sample sizes of cases and controls, incomplete phenotyping, post-mortem timing, and cellular heterogeneity, but the use of a primary disease relevant tissue is critical. Blood-based studies have access to much larger sample sizes and more replication opportunities, as well as the potential for longitudinal measurements, both prior to onset and during the course of treatments. Yet, blood studies also are challenged by cell-type heterogeneity, and many question the validity of using peripheral tissues as a brain

Correspondence to: M. Daniele Fallin.

Compliance with Ethical Standards

Conflict of Interest

Dr. Kelly M. Bakulski, Dr. Alycia Halladay, Dr. Valerie W. Hu, Dr. Jonathan Mill, and Dr. M. Daniele Fallin declare that they have no conflict of interest.

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biomarker. Emerging evidence suggests that these limitations to blood-based epigenetic studies are surmountable, but confirmation in target tissue remains important.

Summary—Epigenetic mechanisms have the potential to help elucidate biology connecting experiential risk factors with neuropsychiatric disease manifestation. Cross-tissue studies as well as advanced epidemiologic methods should be employed to more effectively conduct neuropsychiatric epigenetic research.

Keywords

Neuropsychiatric disorders; DNA methylation; Epigenetics; Tissue; Blood

Introduction

The study of epigenetic variation, which is well established in cancer research and plant biology, has become increasingly integrated into the epidemiology and potential etiology of other common human diseases. Epigenetics, which refers to regulatory information not contained in the DNA sequence itself, includes DNA methylation and hydroxymethylation, histone modifications, chromatin structure, and some forms of RNA [1]. These marks are part of a gene expression regulation system that developmentally controls the spatial and temporal regulation of gene expression [2] and provides complementary information to the DNA sequence. Importantly, epigenetic marks can be dynamic, reversible, and susceptible to environmental insult or nutritional supply [3, 4]. This area of research has stimulated the fields of epidemiology and medicine as potential mechanisms mediating gene-environment interaction and for phenotype heterogeneity among genetic disorders [5]. Even in cases where epigenetics is not mechanistically related to disease, epigenetic marks may be an important biomarker of historic exposure [6], as has been shown with famine exposure during the Dutch Hunger Winter [7]. Alternatively, epigenetic marks may represent an early biomarker of pathogenesis that can be used to target early interventions with clinical applications [8]. In particular, neuropsychiatric disease research has been stimulated by the potential for epigenetic mechanisms to help elucidate the biology connecting experiential risk factors with psychiatric disease manifestation [9], in addition to the potential for understanding phenotype heterogeneity for a given genetic risk. The emerging field of neuroepigenetics has identified epigenetic underpinnings of learned behavior and central nervous system development [10]. Further, many psychiatric disorders are thought to be neurodevelopmental in origin, and given the role of epigenetic processes in cellular differentiation and development, the study of epigenetic variation may inform understanding of disease mechanisms and risk.

Given this potential, studies have pursued epigenetic measurement in epidemiologic and clinical studies, as summarized in recent reviews specific to autism spectrum disorder [11], bipolar disorder [12], schizophrenia [13, 14], post-traumatic stress disorder [15], substance abuse disorder [16], and other neuropsychiatric disorders. Epigenetic marks are being investigated for their potential mechanistic disease role as either a mediator or a modifier of environmental or genetic risk, or as a biomarker of exposure or disease (Fig. 1). These cutting-edge projects are also forging the path for what is and what is not feasible and fruitful in the epidemiology of neuropsychiatric disease. A major debate in this regard has

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been the utility of peripheral tissue samples, such as blood, for the study of disorders that primarily manifest in the brain, a challenge we refer to as the “tissue issue.” Peripheral tissues provide many opportunities for large sample sizes and multiple replication opportunities, while target-tissue brain studies are limited to post-mortem sampling and relatively small sample sizes. While epigenetics involves many potential mechanisms of gene regulation, including histone modification, miRNA expression, and chromatin remodeling, this article focuses on DNA methylation. DNA methylation is most frequently investigated in epidemiology because DNA is easy to collect and is already archived in large numbers, allowing researchers to leverage existing genetic epidemiology samples. In this article, we address the potential benefits of epigenetics research in neuropsychiatric disorders, the pragmatics as well as advantages and limitations of using brain vs peripheral tissues, the importance of sample timing, which is feasible in human studies, and suggest practical approaches moving forward.

Utility of Brain Tissue

Epigenetic marks are critical features of cellular differentiation and cellular phenotype; therefore, epigenomic signatures are specific to types of tissues and cells. Neuropsychiatric disorders are primarily diseases of the brain; thus, brain samples are logically the most appropriate tissue source for epigenetic study. For this reason, many studies examining epigenetic marks for neuropsychiatric disease have focused on brain material (see Table 1 for examples). In fact, neuropsychiatric diseases may affect a specific subset of cells in a very specific region of the brain. It is not yet possible to perform in vivo epigenetic studies of the brain to capture information during the critical time points of development, especially in humans. Only post-mortem brain samples are available for epigenetic measurement, and even post-mortem brain samples for research are scarce for most developmental psychiatric disorders including autism, schizophrenia, and bipolar disorder and do not even exist for more common conditions like anxiety. Perhaps the biggest challenge in using brain tissues is in obtaining control tissue samples from an adequate number of unaffected individuals matched for factors such as age, sex, and exposures. The recent PsychENCODE project recognizes these issues and aims to compile a public resource of multi-layered “omic” and regulatory data on healthy and diseased human brains throughout different stages of development. Despite these efforts, the current availability and applicability of primary tissue for epigenetic analysis is limited for most neuropsychiatric disorders.

Challenges of Brain-Based Research

While brain tissue may at first glance appear to be the “go ld standard” for epigenetic analysis in neuropsychiatric disorders, the timing of acquisition, cell type, and sample sizes actually available present many limitations. First, there is limited information about the specific brain regions (and cell types) across developmental time points that are likely to be of primary importance in the pathogenesis of particular disorders. There are marked functional differences across brain regions, and these are reflected in epigenetic differences that impact gene function [17•]. Also, for some disorders such as autism and schizophrenia, connectivity between brain regions may be of primary importance, rather than specific regional states [18]. Therefore, an argument for tissue specificity should really be cast as an argument for brain region—and cell type—specificity, and current approaches to brain

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epigenetic measurement are only beginning to reach this granularity [19, 20]. With respect to cell type, a portion of epigenomic marks are known to be cell-type specific, and the brain is a heterogeneous mix of neurons and glia, including astrocytes, oligodendrocytes, and microglia. Thus, if individuals vary in the proportion of various cell types, this will result in DNA methylation differences when bulk sections of tissues are homogenized. Methods have been developed to deconvolute brain tissue DNA methylation data into coarse cell-type proportions (neuron vs glia) [21, 22], helping in part to address this source of confounding. Further, emerging research shows that single neurons or subtypes may have highly specific transcriptional profiles [23, 24] as well as individual DNA sequences from accumulated damage during active transcription [25], potentially reflecting developmental lineage and function [26] or memory [27, 28]. This implies that epigenetic measurement from aggregated cells even of the same type may not be fully informative. Currently, microdissection or sorting of brain tissue into pure cell populations, or further into single cells, is challenging and not practical for large-scale studies, though new technical developments may increase the feasibility across populations. In addition, epigenetic marks are analyzed in one brain region at a time (see Table 1), and efforts to map as well as match epigenetic patterns across the brain with particular behaviors have not been possible.

Second, the nature of post-mortem sampling has implications for interpretations of epigenetic measurement, similar to those encountered in the gene expression literature [29]. Fundamentally, these brains are sampled after the disease has occurred, which distorts the prospective timing clarity needed for etiological studies and raises concerns that the epigenetic patterns detected are a consequence of disease rather than part of the cause. Further, there may be bias in biological signals due to cause of death [30], tissue pH [31], or pre-mortem agonal state [32]. The effect of dying itself may have unknown effects on the gene regulation process. The limitations imposed by post-mortem sampling can be somewhat mitigated by comparison with non-human models where pre-symptomatic or even embryonic brain samples can be obtained, but causal connections may simply not be possible from current human brain tissue designs. Careful sampling and storage and integration of measures of pre- and postmortem factors in epigenetic analyses [33, 34] may also help distinguish cause of death from disease-related patterns.

Third, even if the limitations above can be overcome, a major problem is the limited availability of suitable material and a lack of replication potential. Most studies therefore have low power to detect or replicate effects, especially if disease-associated epigenetic changes are subtle. A related methodological issue is the suitability of case and control brains included in epigenetic studies. Often in brain biorepositories, case definitions are loose and deeper diagnostic phenotype data are not available. Case classification may often be based on symptoms obtained from family members post-mortem, rather than direct clinician confirmation as is available in studies with living participants. In addition, the absence of disease symptoms or related co-morbidities is not always confirmed in controls. Parsing neuropsychiatric disorders into subphenotypes with potentially shared features and etiology is an emerging area of research that is often not a possibility for post-mortem annotations when the sample sizes are often low. Further, important potential confounder variables or effect modifiers are typically limited. Controls are often skewed to older individuals, a potentially important problem for epigenetics where age appears to be highly

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correlated with at least DNA methylation [35]. Environmental exposure data are typically not collected in brain bank archives, and our tools to estimate retrospective exposures from archived samples, pinpointing a pre-disease state, are limited. To be viable moving forward, brain-based epigenetic work in neuropsychiatric disorders will need large time-consuming efforts to collect and compile large numbers of appropriate brain samples from affected individuals and controls with available clinical, demographic, and risk factor data, which require coordination across multiple groups and brain repositories for different neuropsychiatric disorders.

Many creative alternatives to brain-based models of epigenetics have been explored. Transformed lymphoblastoid cell lines that have been archived for genetic studies are an important existing source of genetic material linked with phenotype. The transformation process of B cells with Epstein-Barr virus (EBV) involves epigenetic reprogramming; however, so, the results of these studies must be interpreted cautiously [36]. Nonetheless, many neuropsychiatric disease DNA methylation associations, including autism, have been identified in transformed lymphoblastoid cell lines [37, 38], and these observations warrant further inquiry. Another convenient source for epigenetic discovery in neuropsychiatric disease research are buccal or saliva samples. Buccal cells are derived from the ectoderm during development, similar to the central nervous system, are non-invasive to collect, and a recent study suggests that buccal cells are more informative of tissue differential methylation signatures than blood [39]. Saliva samples are mixtures of white blood cells and buccal epithelial cells and may be arduous to collect from children with neurodevelopmental disorders, as several milliliters are required. The most mechanistically promising option is the use of induced Pluripotent Stem Cells (iPSCs) derived from cases and sex- and age-matched controls which can be differentiated into neuronal progenitor cells (NPCs) and neuroblasts. This model allows for direct experimentation of pharmaceutical agents, environmental exposures, and nutrients on human neural cells and model brain organoids, customized to the genetic backbone relevant for a disorder [40]. The generation of neuroblasts and other target tissue models from iPSCs, however, is mediated by epigenetic reprogramming, which is often incomplete, meaning the cells retain epigenetic information from their cell type of origin [41, 42]. The iPSC reprogramming process would also erase exposure or disease epigenetic marks from the original patient, which would restrict neuroblast studies of early exposure or disease. Extensive heterogeneity has been observed between iPSC lines and even within clones of the same line [43]; thus, replication across studies may be challenging. Currently, the production of iPSCs is very labor intensive, although cell culture models could potentially be developed for investigation across an epidemiologic sample. In terms of epidemiologic scale, whole blood remains the most obvious surrogate tissue for epigenetics studies.

Practicality of Leveraging Blood in Epidemiology

Peripheral blood is currently the most abundant type of sample used for epidemiologic and clinical research. A clear advantage of blood samples is the relatively non-invasive collection that is easy to obtain, often at the time patients are undergoing blood draws for clinical evaluations. The amount of DNA extracted from standard pediatric peripheral blood samples (3 mL) is generally sufficient for multiple genetic and DNA methylation experiments. One

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advantage of blood samples is that many neuropsychiatric disorder research laboratories and large specimen biorepositories, such as the Psychiatric Genomics Consortia, several NIH repositories, the Autism Genetic Resource Exchange (AGRE), and the Simons Simplex Collection for example, already have DNA samples isolated from blood with paired detailed phenotyping data, often on multiple family members. The convenience and availability of blood as a tissue for epigenetic inquiry in existing epidemiologic studies with strong neuropsychiatric phenotyping and existing environmental exposure data are an important practical research consideration.

The use of peripheral blood in epigenetic epidemiologic studies affords several methodological advantages. First, blood can be obtained prior to disease onset to establish appropriate timing for testing epigenetic marks as an etiologic factor in disease. Observation of early marks is potentially actionable through future intervention studies. Second, blood can be sampled serially prior to disease and during the disease process. The use of longitudinal samples allows for an estimation of change in epigenetic marks over time, potentially related to disease risk. Third, causal modeling options are available for studies with appropriate temporal sequence. Fourth, large sample sizes are often achievable, yielding the statistical power to observe modest effect sizes. Fifth, as the most common type of tissue measured in epidemiologic settings, results can be compared or even combined across studies. Sixth, family-based design options are possible, which is particularly useful for relatively rare psychiatric disorders that are clustered in families [44]. Similarly, blood samples can be used in studies targeting and following individuals at high or low risk of disease. Finally, the identification of blood-based biomarkers of disease may have translational utility in clinical settings.

Challenges of Blood-Based Neurospychiatric Epigenetic Research

Epigenetic marks are critical features of cellular phenotype such that epigenetic differences across tissue types are expected [45, 46]. Thus, the relevance of blood epigenetic findings must be carefully considered when trying to make inferences about specific mechanistic pathways for brain-related disorders. Levels of DNA methylation at specific CpG sites are often not comparable across tissues, and inter-individual variation in DNA methylation at the majority of CpG sites on the Illumina 450 K array, the most widely used tool in epigenetic epidemiology, is only weakly correlated across blood and brain tissues (cortex and cerebellum) [47••]. Further, principal component analysis of paired brain and blood DNA methylation data from unaffected individuals separates tissue type in the first component, explaining more of the variance than between-person variability, although both intra- and inter-individual variability are detectible via such an analysis [48]. However, epidemiologic data are typically looking to identify associations between epigenetic marks and disease (or environmental exposures), such that the *actual* level of methylation is less relevant than the conservation of inter-individual variation across tissues [47••]. Another concern is that hydroxymethylcytosine, another DNA modification that is of increasing interest for neuropsychiatric disease because it is particularly enriched in the brain, occurs at extremely low levels in the blood [49]. Because common DNA methylation quantitation methods do not distinguish between DNA methylation and DNA hydroxymethylation, observed methylation levels in blood may not reflect the methylation state relevant in the brain.

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As in studies of post-mortem brain tissue, cellular heterogeneity is an important potential confounder when quantifying DNA methylation in blood samples. A whole blood sample is composed of a heterogeneous population of neutrophils, lymphocytes, monocytes, and other cell types. The proportion of white cells in an individual at any time can be influenced by factors including bacterial or viral infection, inflammation, dietary intake, stress, medication, or other environmental exposures. Ideally, epigenetic epidemiology analyses would be performed on sorted cell populations, but this is not possible from frozen archived blood samples or previously isolated DNA from blood derivatives. Cell-type estimation algorithms have been developed for adult and cord blood [50•, 51, 52] and can be used to partially control for such heterogeneity in association analyses [53]. Expanding blood cell reference panels to populations with variable age, sex, and race would further improve estimation strategies.

Argument for Blood-Based Neuropsychiatric Epigenetic Research

Epigenetic studies are performed to investigate both disease mechanisms as well as to identify associated biomarkers of either exposure or disease (Fig. 1). Blood-based epigenetics studies in neuropsychiatric disorders have shown promising associations with respect to both mechanisms and identification of disorder biomarkers (Table 1) and continue to be useful in each regard for multiple reasons.

First, neuropsychiatric disorders may have mechanistic underpinnings in tissues beyond the brain, including blood. Many neuropsychiatric disorders, such as autism spectrum disorder [54], schizophrenia [55], and bipolar disorder [56], have been associated with inflammation and altered immune response. Furthermore, if one looks at a neuropsychiatric disorder as a "systems" disorder (especially as individuals with autism spectrum disorder often present with gastrointestinal and immune system problems), peripheral tissues may be used as a window to detect disrupted pathways (metabolic or signaling) that we may carry to the brain. Altered immune processes and similar phenotypes that are associated with neuropsychiatric diseases may be well measured in blood samples.

With additional respect to mechanistic insights, blood-based epigenetics may be a proxy for brain epigenetics in certain circumstances. There are now examples where inter-individual variation in DNA methylation is correlated between blood and regions of the brain, although this varies across the genome and may be driven in part by underlying DNA sequence variation [17•, 47••]. Genetic influences on DNA methylation—e.g., via methylation quantitative trait loci (mQTLs) [47••, 57, 58] and genotype-driven allele-specific DNA methylation [59]—are often consistent across tissues [60], although even these effects can be tissue-specific [61]. Disease-specific studies have probed target-surrogate tissue correlations at specific loci. For example, frontal cortex DNA methylation differences in Parkinson's disease are associated with those found in blood [62]. There are also useful cross-tissue proxy examples from the non-neurological literature, such as cancer [63] and loss of imprinting [64]. Future studies will continue to refine our understanding of cross-tissue epigenetic similarities over developmental time and in specific cell types.

Information on correlation between target and surrogate tissue DNA methylation can be exploited for epidemiologic study design and analyses. For example, among variably

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methylated CpG sites in the brain, a subset (7.9 %) of sites was correlated (rho > 0.59) between temporal lobe and blood [65]. These blood-brain correlated sites were used to a priori filter blood CpG discovery analysis in a schizophrenia case-control study. Although these specific CpG sites may be markers of DNA sequence variation, future studies may use similar methods to reduce the number of attempted comparisons and focus analyses on potentially brain informative regions. A module of CpG sites is commonly susceptible to aging in both brain and blood [66], which in addition to indicating that aging processes are consistent across tissues, may represent another subset of informative surrogate tissue markers. Many DNA methylation patterns are highly conserved across tissues [67], and recent studies propose statistical methods to predict methylation of target tissue using methylation profiles measured in surrogate tissues [68], particularly among CpG sites with substantial variation. With further understanding of locations of non-methyl modifications at CpG sites (e.g., hydroxymethyl), we may better understand and predict cross-tissue differences and similarities and leverage that knowledge in our epidemiologic design and analyses.

Surrogate tissue epigenetics research has important applications for disease biomarkers. Early biomarkers of neuropsychiatric disease may be particularly useful where early interventions are possible [69]. Cord blood or placenta DNA methylation may predict later neurodevelopmental disorders in childhood, and monitoring these early biomarkers of disease could help target at risk children for treatment. Blood-based markers may also be informative for monitoring disease progression and treatment effectiveness.

Epigenetic marks as biomarkers of exposure are relevant for determining exposure-disease associations in situations where direct chemical exposures have already been flushed from the body [6]. Many environmental toxicants have been associated with global or gene-specific epigenetic change [70]. Work is underway to identify unique epigenetic signatures of chemical exposure, including exposures to air pollution particulate matter [71] and mercury [72], or nutrient intake, such as folate supplements [73]. A recent meta-analysis across 13 cohorts demonstrated that the in utero exposure to smoking is associated with a consistent DNA methylation signature at infancy and in childhood [74]. Future studies could measure DNA methylation in children and estimate smoking exposure during gestation. In mice, glucocorticoid exposure was associated with corresponding DNA methylation differences in blood and hippocampus at the *Fkbp5* candidate gene [75], while bisphenol A exposure is associated with parallel hippocampus and blood changes in *Bdnf* [76]. Stress-responsive methylation at the *COMT* gene in rat pre-frontal cortex has been further correlated with methylation in lymphocytes [77]. Chemical and CpG site specificity of environmental epigenetic biomarkers will continue to be investigated and refined.

Conclusions

Going forward, it is important that epigenetic epidemiology studies recognize the pragmatic reality of their tissue sample and be straightforward about the intention, utility, and scope of possible inferences. Blood and brain-based epidemiologic studies can provide potential insights to neuropsychiatric disease research, though each has limitations (Table 2). Where possible, we advocate for a combined approach of paired blood and brain research. This can

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be effectively demonstrated in relatively few examples to date [65, 78, 79]. Cross-tissue analyses should be encouraged in human and animal models. More tissue banks may consider archiving blood alongside brain tissues to increase these opportunities. Indeed the recent TARGET initiative from the National Institute for Environmental Health Sciences has been developed to better understand surrogate versus target tissue epigenetic measures. We further advocate for researchers to exploit epidemiologic design options available for blood-based research settings. These include taking advantage of longitudinal samples, adequate phenotype information, environmental exposure and covariate data, large sample sizes, and replication opportunities. In addition, methodological approaches to enhance causal inference can be used [80], such as mediation and moderation analyses that examine specific roles of epigenetic marks in exposure-based or genetic causes of disease [81, 82]. Ideally, epidemiologic studies would obtain either directly measured cell-type counts from whole blood, which can be done using routine hospital assays, or prioritize sorting blood samples into specific cell types at time of collection, which may require specialized training and equipment. In cases where neither is possible, we recommend cell-type estimation and adjustment using cell-type epigenetic reference panels. Future studies may also consider the impact of aggregate (global) epigenetic differences versus genome-wide site-specific features that have rarely been estimated in the same samples.

Multiple lines of evidence across neuropsychiatric disorders point to a role for epigenetic alterations in these diseases. There are pragmatic reasons to examine blood epigenomes and evidence that this approach can be informative for multiple purposes. Future studies in blood and brain will hopefully replicate and expand upon the current literature, taking advantage of emerging methodological developments, though these studies will likely maintain some of the current limitations. In our first “at bat” in conducting neuropsychiatric epigenetic epidemiology research, we may have only reached first base, but the potential and opportunities are such that we should keep improving and swinging for a home run.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Utility of epigenetic marks for neuropsychiatric disease research. Figure adapted from [83–85]

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Table 1

Examples of human genome-wide neuropsychiatric epigenetics publications using target (brain) or surrogate (blood) tissues with evidence for association.

Approach	Source	Tissue	Neuropsychiatric disorder	Sample	Methylation assay
Target	(Nagarajan et al. 2006) [86]	Prefrontal cortex	Autism spectrum disorders	19 cases, 14 controls (males)	Candidate gene
Target	(Mill et al. 2008) [87]	Frontal cortex	Schizophrenia	35 cases, 35 controls	Genome-wide (12,000 CpG islands)
Target	(Abdolmaleky et al. 2011) [88]	Frontal lobe	Bipolar and schizophrenia	35 BPD, 35 SCZ, 35 control	Candidate gene (Bisulfite sequencing *HTR2A*)
Target	(James et al. 2013) [89]	Cerebellar cortex	Autism spectrum disorders	13 cases, 13 controls	Candidate gene
Target	(Ladd-Acosta et al. 2014) [90]	Temporal cortex, prefrontal cortex, cerebellum	Autism spectrum disorders	7 cases, 10 controls	Genome-wide (Illumina 450 k)
Target	(Zhu et al. 2014) [91]	Cerebellum, cerebral cortex	Autism spectrum disorders	54 cases, 43 controls	Candidate gene
Target	(Nardone et al. 2014) [92]	Prefrontal cortex, cerebral cortex	Autism spectrum disorders	12 cases, 12 controls	Genome-wide (Illumina 450 k)
Target	(Wockner et al. 2014) [93]	Frontal cortex	Schizophrenia	24 cases, 24 controls	Genome-wide (Illumina 450 k)
Target	(Pidsley et al. 2014) [94]	Prefrontal cortex, matched cerebellum	Schizophrenia	21 cases, 23 controls, 179 fetal sanokes	Genome-wide (Illumina 450 k)
Target	(Jaffe et al. 2016) [95•]	Dorsolateral prefrontal cortex	Schizophrenia	191 cases, 35 fetal, 300 controls	Genome-wide (Illumina 450 k)
Target	(Hannon et al. 2016) [61]	Fetal whole brain	Schizophrenia	173 fetal brain samples (testing for enrichment of meQTLs at SCZ genetic loci)	Genome-wide (Illumina 450 k)
Target	(Pandey et al. 2016) [96]	Prefontal cortex	Suicide	52 suicide victims, 27 non-suicidal psychiatric, 24 normal controls	Gene and protein expression of *SKA2*
Surrogate	(Nguyen et al. 2010) [38]	Lymphoblastoid cell line	Autism spectrum disorders	3 discordant MZ twin pairs, 2 discordant sib pairs	Genome-wide (CpG island array 8.1 K)
Surrogate	(Carrard et al. 2011) [97]	Peripheral blood	Bipolar and schizophrenia	58 BPD, 40 SCZ, 67 controls	Candidate gene (High resolution melt of *5HTR1A*)
Surrogate	(Dempster et al. 2011) [98]	Peripheral blood	Schizophrenia	22 discordant twin pairs	Genome-wide (Illumina 27 K)
Surrogate	(Rusiecki et al. 2013) [99]	Peripheral blood	PTSD	75 cases, 75 controls	Candidate gene (Pyrosequencing n = 5)
Surrogate	(Nishioka et al. 2013) [100]	Peripheral blood	Schizophrenia	18 first-episode cases, 15 controls	Genome-wide (Illumina 27 K array)
Surrogate	(Zhang et al. 2013) [101]	Peripheral blood	Alcohol dependence	518 cases, 369 controls	Genome-wide (Illumina Golden Gate CpG island)
Surrogate	(Aldinger et al. 2013) [102]	Lymphoblastoid cell line	Autism spectrum disorders	Women, 4 cases, 6 controls (discovery), 13 cases, 13 controls (replication)	Genome-wide (Illumina 27 K)
Surrogate	(Wong et al. 2014) [103]	Peripheral blood	Autism spectrum disorders	25 discordant MZ twin pairs	Genome-wide (Illumina 27 K)

Approach	Source	Tissue	Neuropsychiatric disorder	Sample	Methylation assay
Surrogate	(Dempster et al. 2014) [104]	Buccal cell	Adolescent depression	18 Discordant MZ twin pairs	Genome-wide (Illumina 450 K)
Surrogate	(Fisher et al. 2015) [105]	Buccal cell	Childhood psychotic symptoms	24 Discordant MZ twin pairs	Genome-wide (Illumina 450 K)
Surrogate	(Kang et al. 2015) [106]	Peripheral blood	Major or minor depressive disorder	309 breast cancer patients	Candidate gene (Pyrosequencing of *BDNF*)
Surrogate	(Kim et al. 2016) [107]	Peripheral blood	Anxiety, depression, hostility	538 community dwelling older men	Candidate gene (Pyrosequencing n = 7 immune genes)
Surrogate	(Kahl et al. 2016) [108]	Peripheral blood	Major depressive disorder	52 cases, 18 controls, longitudinal 6 weeks	Candidate gene (Bisulfite sequencing n = 2)
Surrogate	(Montano et al. 2016) [109••]	Peripheral blood	Schizophrenia	Discovery: 689 cases, 645 controls; Replication: 247 cases, 250 controls	Genome-wide (Illumina 450 k)
Combined	(Gregory et al. 2009) [79]	Superior temporal gyrus, Peripheral blood	Autism spectrum disorders	8 cases, 8 controls brain, 20 cases, 20 controls blood	Candidate gene
Combined	(Sabunciyan et al. 2012) [78]	Prefrontal cortex discovery, lymphoblastoid cell line follow-up	Major depressive disorder	39 cases, 27 controls	Genome-wide (CHARM); Candidate gene (Pyrosequencing validation n = 17 DMRs, cell line n = 1 DMR)
Combined	(Walton et al. 2016) [65]	Filter by correlated sites in paired temporal lobe and blood, discovery in blood	Schizophrenia	12 brain and blood, 111 cases, 122 controls	Genome-wide (Illumina 27 K)

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Table 2

Rationale/challenges for tissue type

Tissue type	Disease relevance	Timing/inherent design bias	Availability/sample size	Technical challenges
Blood	• Surrogate tissue • May be best source for environmental epigenetic biomarker • Evidence for correlation of inter- individual variation patterns for blood and brain • Evidence for expression and methylation discrimination of disease phenotype	• Potential for samples prior to onset • Ability to monitor longitudinal changes in epigenetic marks • Potential to assess effectiveness of interventions targeted towards epigenetic alterations	• Convenient, highly available • Large sample sizes possible • Many replication opportunities	• Cell-type heterogeneity
Brain	• Primary tissue • May be best source for disease biomarker • Specific region, cell-type target unclear for most disorders	• Only post-mortem (post-onset) samples available. • Bias due to cause of death and pre-mortem agonal state • Appropriate controls and confounder information rare	• Rare resource, limited sample sizes possible • Limited or no replication possible	• Cell-type heterogeneity • Region heterogeneity