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REVIEW

Epigenetic Mechanisms Underlying the Pathogenesis of Neurogenetic Diseases

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Abstract There have been considerable advances in uncovering the complex genetic mechanisms that underlie nervous system disease pathogenesis, particularly with the advent of exome and whole genome sequencing techniques. The emerging field of epigenetics is also providing further insights into these mechanisms. Here, we discuss our understanding of the interplay that exists between genetic and epigenetic mechanisms in these disorders, highlighting the nascent field of epigenetic epidemiology—which focuses on analyzing relationships between the epigenome and environmental exposures, development and aging, other health-related phenotypes, and disease states—and next-generation research tools (i.e., those leveraging synthetic and chemical biology and optogenetics) for examining precisely how epigenetic modifications at specific genomic sites affect disease processes.

Keywords Chromatin · DNA methylation · Epigenetic · Epigenome · Histone · Noncoding RNA

Introduction

For several decades, research in genetics and genomics has focused on mapping disease-associated genes and loci, through candidate gene and less biased genome-wide approaches, and on uncovering the effects of rare and more common allelic and structural variants on the risk of disease

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onset and progression and other phenotypic traits. More recently, with the expanding availability and decreasing costs of massive parallel sequencing and computational- and systems-level data analysis tools and techniques, exome (referring to the entire repertoire of protein-coding genes) and whole genome studies—often combined with transcriptomic data (i.e., expression quantitative trait loci analysis)—are increasingly being employed for these purposes [1]. It is widely believed that these powerful new methodologies will help to illuminate the genetic mechanisms underlying complex, heterogeneous, and often difficult to diagnose neurological and psychiatric diseases, which have largely remained elusive, and serve as the foundation for individualized precision medicine for patients with these disorders [2]. In fact, this era of molecular genetic diagnosis and targeted therapy has already arrived for other important classes of diseases, such as cancer [3,4], though many very important challenges remain.

This revolution has also arrived for nervous system disease applications, as evidenced by ongoing progress in the genetic classification and diagnosis of mitochondrial diseases [5], epilepsy syndromes [6], hereditary spastic paraplegias [7], amyotrophic lateral sclerosis [8], and other nervous system disorders [2]. A salient example of how these advances can be immediately clinically relevant and affect treatment is provided by the genetics of the childhood-onset motor neuron disease, Brown–Vialetto–Van Laere syndrome. It was recently discovered via exome sequencing that mutations in riboflavin transport pathways are responsible for causing this disorder in a subset of patients [9]; this finding led to the identification of a novel mechanistic therapy (i.e., high-dose riboflavin) that seems to be effective for treating this previously intractable neurodegenerative disorder [10]. Another example of how sequencing approaches can be pertinent for an acquired disease is the recently reported use of sequencing for pathogen detection in a 14-year-old boy with severe combined immunodeficiency, who presented with meningoencephalitis [11]. Cerebrospinal fluid analysis revealed evidence of *Leptospira*, a rare but treatable cause of infection. This finding was made within a clinically relevant timeframe, dramatically affecting his therapy and clinical outcome.

In addition to such advances, a critical insight provided by genetic and genomic approaches has been that, while some disease risk loci and causal variants are embedded in protein-coding genes and consequently disrupt the structure and function of corresponding proteins, a significant number (if not the majority) of disease-associated loci fall within noncoding genomic regions [12]. This finding suggests that crosstalk between epigenetic regulatory mechanisms (Table 1) and genetic variants present at these sites, which may represent noncoding RNA (ncRNA) genes, promoters, enhancers, and other functional genomic elements, underpins the onset, progression, and therapeutic responsiveness of these disorders. Thus, scientific and technological innovations are now focused on interrogating these additional layers of biological complexity [13].

In this review, we discuss our emerging understanding of the interplay that occurs between genetic and epigenetic mechanisms in the pathogenesis of nervous system disorders, highlighting the nascent field of epigenetic epidemiology and next-generation research tools for examining precisely how epigenetic modifications at specific genomic sites might impact disease processes.

Epigenetic Mechanisms

Epigenetic processes are control systems for modulating genomic structure and function in response to interoceptive and environmental stimuli [14–16]. The core mechanisms are DNA methylation and hydroxymethylation, histone post-translational modifications and chromatin remodeling, and ncRNA regulation (Table 1). These dynamic and highly interconnected processes are responsible for mediating the cell type-specific execution of genomic programs, such as long-term gene silencing, transcription, post-transcriptional RNA processing, translation, X chromosome inactivation, genomic imprinting, DNA replication and repair, and the maintenance of genomic integrity. Our appreciation for how and why these mechanisms are deployed in different contexts, both in health and disease, is still rudimentary but very rapidly evolving.

Nevertheless, it has become clear that epigenetic factors and mechanisms have key roles in promoting development, cellular diversity, plasticity, homeostasis, stress responses, aging, and transgenerational effects within the nervous system (and beyond) [14,17,18]. Therefore, it is not surprising that epigenetic processes are also implicated in the pathogenesis of a very broad array of nervous system diseases. Indeed, many studies are now focused on uncovering how exactly these processes influence disease pathophysiology. A major priority is to connect this emerging knowledge of epigenetics with our existing understanding of the, often complex, genetic mechanisms that underlie these disorders.

Gene Mutations and Other Genomic Features

The most direct link is provided by the increasing list of germline mutations in genes encoding epigenetic factors involved in each of the core epigenetic mechanisms that are responsible for causing a spectrum of nervous system diseases. One of the most prominent illustrations is provided by *MECP2*, which is mutated in Rett syndrome. Further, mutations in a number of genes encoding histone modification and chromatin remodeling proteins produce a significant proportion of recognized forms of syndromic and nonsyndromic intellectual and developmental disabilities (IDDs) [19]. For example, mutations in *CREBBP*, which has histone

Table 1 Core epigenetic mechanisms

Epigenetic mechanism	Description	Examples of associated epigenetic factors
DNA methylation and hydroxymethylation	Refers to the covalent modification of cytosine residues to form 5-methylcytosine and 5-hydroxymethylcytosine. These dynamic marks are present throughout the genome (i.e., at gene regulatory regions, gene bodies, and repetitive elements) and have context-specific roles (e.g., promoting transcriptional activation/repression)	DNA methyltransferase enzymes Methyl-CpG-binding domain proteins Ten–eleven translocation enzymes
Histone post-translational modifications and chromatin remodeling	Genomic DNA is wrapped around a histone protein (i.e., H2A, H2B, H3, H4) octamer forming a nucleosome. This basic unit of chromatin mediates the accessibility of DNA and its interactions with other nuclear factors (e.g., transcriptional regulators, RNA polymerases, other DNA sequences). Histone post-translational modifications alter the structure of the nucleosome and form combinatorial "codes" recognized by specific chromatin-binding proteins. The nucleosome and higher-order chromatin states are also subject to further repositioning and remodeling, respectively. Together, these evolving processes modulate genomic programs (e.g., transcriptional activation/repression, DNA replication/repair) in response to diverse stimuli	Histone modifying enzymes (e.g., histone deacetylases/acetyltransferases and histone demethylases/methyltransferases) SWI/SNF nucleosome remodeling complex Bromodomain proteins Chromodomain proteins Polycomb group proteins Trithorax group proteins
ncRNA regulation	These transcripts function as RNA molecules, not as translated proteins. Each class and subclass is associated with specific biogenesis pathways, mechanisms of action, and biological roles. miRNAs engage in post-transcriptional gene regulation. Piwi-interacting RNAs control the activity of transposable elements. Long ncRNAs are the most abundant and heterogeneous class, having the broadest range of regulatory functions	miRNAs Piwi-interacting RNAs Long ncRNAs Enhancer RNAs Retrotransposon-derived RNAs

ncRNA = noncoding RNA; miRNA = microRNA; SWI/SNF = Switch/Sucrose NonFermentable family

acetyltransferase (HAT) activity, result in Rubinstein–Taybi syndrome, an autosomal dominant IDD. Loss-of-function mutations in *RPS6KA3*, which has histone kinase activity, result in Coffin–Lowry syndrome, an X-linked IDD. Mutations in *ATRX*, a member of the SWI/SNF (Switch/Sucrose NonFermentable) family of chromatin remodeling proteins, result in alpha thalassemia X-linked intellectual disability syndrome. In addition, alterations in genes encoding ncRNA-related factors and ncRNAs, including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), are now being implicated in nervous system disease pathogenesis. Haploinsufficiency of the *DGCR8* gene, which encodes a miRNA-processing factor located within the 22q11.2 chromosomal region that is deleted in DiGeorge syndrome, likely contributes to the cognitive and behavioral phenotypes observed in this disease [20]. Deletion of *MIR17HG*, which encodes the *miR-17–92* cluster, causes Feingold syndrome 2, a disorder characterized by IDD, microcephaly, and other malformations [21]. Disruption of the *LINC00299* lncRNA gene also produces a form of IDD [22]. Similarly, a point mutation in the *SLC7A2-IT1* lncRNA gene is responsible for progressive encephalopathy with severe infantile anorexia (Ravine encephalopathy) [23].

Variations in genes encoding epigenetic factors, in genes targeted by epigenetic factors, and in associated regulatory regions (e.g., promoters, transcription factor binding sites, and miRNA response elements) can also modify the risk of nervous system disease onset and progression. For example, single nucleotide polymorphisms (SNPs) in *BRD2*, which encodes a chromatin-binding protein that recognizes acetylated histones, confer a significant degree of susceptibility to juvenile myoclonic epilepsy [24]. SNPs in the *ANRIL*/*CDKN2B-AS1* lncRNA gene on chromosome 9p21.3 are risk loci for a number of diseases, including ischemic and hemorrhagic stroke, intracranial aneurysms, plexiform neurofibromas, and Alzheimer disease (AD) [25,26]. By contrast, SNPs can influence miRNA-mediated gene regulation by creating, destroying, or otherwise modifying miRNA response elements in genes associated with nervous systems disease pathophysiology [e.g., AD, Parkinson disease (PD), multiple sclerosis (MS), schizophrenia, and depression] [27–29]. For example, a SNP in *FGF20*, which modulates PD risk, disrupts the *miR-433* response element [30]. Further, a variant in *SLITRK1* within the *miR-189* response element causes Tourette syndrome in a small percentage of patients [31].

Moreover, noncoding genomic sites can affect disease pathogenesis because these might represent additional

functional elements, such as enhancers and/or ncRNA genes [32–35]. In fact, enhancer activity (and associated long-range regulatory interactions) is likely to be an important cause of tissue- and cell type-specific vulnerability to genetic diseases [36]. For example, in facioscapulohumeral muscular dystrophy, two enhancers (i.e., *DUX4* myogenic enhancer 1/2) seem to account for muscle-specific pathology [37]. In myocytes, these enhancers are both physically associated with the *DUX4* promoter and have chromatin signatures that imply they are active and are thus responsible for promoting selective expression of the pathogenic form of *DUX4*. The recent characterization of large numbers of active enhancers across many human cell types and tissues [32], and the construction of cell type-specific chromatin connectivity maps revealing long-range genomic interactions [38], further suggest that genetic variation in enhancers and alterations in enhancer–promoter communications are extremely relevant to the risk of developing nervous system diseases including, for example, AD, PD, and MS [32].

In addition to the germline, the somatic genome also represents a burgeoning focal point for the convergence between genetic and epigenetic mechanisms in neurological and psychiatric disorders. Specifically, mobile genetic elements (i.e., transposable elements), which represent a significant proportion of the human genome, play an important role in the transcriptional landscape and promote neuronal genomic mosaicism [39–43]. In the brain, these transposable elements seem to mediate neural development, including neural cellular differentiation, homeostasis, and plasticity [43–45]. Importantly, the activity of these mobile genetic elements is modulated by epigenetic mechanisms and their deregulation is implicated in disease processes, including schizophrenia [46], Rett syndrome [47], ataxia telangiectasia [48], and others (Table 2).

Epigenetic Deregulation

In addition to the interplay that exists between genetic and epigenetic processes at the level of gene mutations and other genomic features, further crosstalk can occur with epigenetic mechanisms and factors being involved in disease-related cellular pathways and/or in modulating disease-associated genomic loci and gene products [14–16].

For example, one particularly interesting study uncovered an epigenetic mechanism interleaved within the cascades of oxidative stress, DNA damage, cell cycle reactivation, and apoptosis that are responsible for tau-mediated neurodegeneration [50]. The authors found that there is a general decrease in the levels of histone H3 lysine 9 dimethylation (H3K9me2), heterochromatin protein 1α (HP1α), and heterochromatin formation in brains from transgenic tau models of *Drosophila* and mice, as well as in hippocampal neurons from human AD specimens. Not only did the degree of chromatin relaxation correlate with the extent of tau-induced neurotoxicity, but modulation of heterochromatin formation through genetic manipulations also modified the neurodegenerative phenotype, both positively and negatively. They showed that this selective pathological process occurs downstream of oxidative stress and DNA damage and upstream of cell cycle reactivation. In fact, the consequence of chromatin relaxation seemed to be aberrant activation of subsets of developmental genes normally subject to heterochromatic silencing, including those with roles in cell cycle regulation, as well as others that hint at novel mechanisms, such as the piwi RNA-associated factors *Ago3* and its homolog, *PIWIL1*. Furthermore, this study demonstrated that a reduction in *Ago3* in transgenic *Drosophila* brain mitigates tau-mediated neurodegeneration. These intriguing observations suggest that transposable element deregulation might be involved in the pathogenesis of tauopathies. Furthermore, in ataxia telangiectasia, the nuclear accumulation of histone deacetylase 4 (HDAC4) and increased H3K27me3, mediated by polycomb repressive complex 2, contributes directly to neuronal cell death [51,52]. Many similar examples exist for linking the mechanisms underlying nervous system disorders with multiple layers of the epigenome [14–16,53–55].

Moreover, an increasing number of epigenetic epidemiology studies (see below) are now focused on cataloging the, often deregulated, epigenetic profiles present in neurological and psychiatric diseases (Table 3). Some of these approaches also aim to uncover relationships between underlying genetic sequence variants with epigenetic profiles at specific sites, referred to as epigenetic quantitative trait loci [71,72]. Connections between these two layers of information are evident in human brain and may be relevant for explaining the mechanisms by which risk alleles contribute to disease [73,74]. However, our understanding of these relationships is preliminary and largely centered on DNA methylation.

Epigenetic Epidemiology and Epigenome-wide Association Studies

Epigenetic epidemiology essentially refers to the integration of epigenetic and high-throughput epigenomic analyses into population-based epidemiological research, with the aim of understanding the causes of epigenetic variation and its effects on health and disease. This emerging field faces very significant challenges, including issues related to our rapidly evolving knowledge of biological mechanisms, cellular and tissue heterogeneity, complexities of study design and data interpretation, and selection of appropriate epigenomic techniques and technology platforms [75–78]. Nevertheless, examples of epigenetic epidemiology have started to emerge. In particular, epigenome-wide association studies focused on

Table 2 Examples of deregulation of mobile genetic elements in nervous system diseases

Disease	Link to mobile genetic element deregulation	Ref.
Ataxia telangiectasia	L1 retrotransposition efficiency and copy number are, respectively, increased in ATM-deficient cells and in patient-derived neuropathological specimens	[48]
Fukuyama congenital muscular dystrophy	Insertion of a SVA retrotransposon into the 3′-UTR of the fukutin gene causes disease by inducing aberrant splicing via SVA-mediated exon trapping	[49]
Progressive encephalopathy with infantile anorexia (Ravine encephalopathy)	Point mutation in a primate-specific repeat (L1PA8) within an *Alu* element, which is embedded in a brain expressed lncRNA, mediates disease	[23]
Rett syndrome	MeCP2 represses L1 retrotransposition in neuronal cells and, in reprogrammed cells from Rett syndrome patient tissues, L1 activity is increased	[47]
Schizophrenia	L1 copy number is increased in neurons from patient-derived prefrontal cortex specimens, and brain-specific L1 insertions seem to preferentially target genes involved in synaptic function or linked to schizophrenia	[46]

ATM = ataxia telangiectasia mutated; SVA = SINE-VNTR-Alu; 3′-UTR = 3′-untranslated region; lncRNA = long noncoding RNA; MeCP2 = methyl-CpG-binding protein 2; L1 = long interspersed element 1

identifying differential profiles of DNA methylation in tissues from relatively large cohorts of individuals are becoming increasingly common. These approaches have been used to analyze relationships between DNA methylation and environmental exposures, development and aging, other health-related phenotypes, and disease states. Yet, these represent only preliminary incursions into epigenetic epidemiology. More sophisticated and integrated paradigms for interrogating relationships between health and disease and additional layers of the epigenome that address the challenges raised above are still necessary and emerging [79]. Furthermore, planning, executing, and contextualizing these studies requires harmonization within frameworks for clinical and translational research and systems biology and network medicine, including those that are already in place—and those that are evolving—to examine other omics datasets (i.e., genome, transcriptome, microbiome, microvesicle/exosome, proteome, metabolome, lipidome, and exposome).

Environmental Exposures

Smoking is perhaps the best-studied environmental exposure that affects DNA methylation. There are robust correlations between maternal smoking during pregnancy and DNA methylation patterns found in placenta, umbilical cord, and offspring [80,81]. These include changes in overall levels of DNA methylation and those at neuroscience-relevant loci (i.e., exon 6 of *BDNF* [82]). These findings implicate epigenetic alterations in the mechanisms responsible for the adverse neuropsychiatric outcomes that are linked to maternal smoking. Similarly, there are associations between being a current or former smoker and specific DNA methylation signatures in blood and other tissues [83–86], particularly at genomic sites encoding factors involved in inflammation, immune function, and coagulation [87]. The underlying mechanisms for smoking-related DNA methylation alterations are likely to be complex and may include the effects of hypoxia, nicotine, DNA damage, and/or other processes.

Interestingly, one of the most significantly and reproducibly differentially methylated loci lies in *AHRR*, which is involved in aryl hydrocarbon receptor signaling. This critical pathway mediates xenobiotic and immune responses, and is implicated in nervous system autoimmunity and neuroinflammation [88]. This observation suggests a potential explanation for the links that exist between smoking and neurological and psychiatric disorders, including the increased risk of multiple sclerosis onset and progression, and of AD pathology and the inverse correlation found with PD pathology. These examples show how the impact of smoking and other exposures (e.g., diet, physical, chemical, psychosocial) on DNA methylation (and perhaps other epigenetic mechanisms) can be studied and used to better understand gene–environmental interactions important for fetal programming and evolution of brain diseases.

Development and Aging

An increasing number of studies have reported associations between development and aging and DNA methylation profiles in different brain regions, blood, muscle, saliva, and other tissues, including both cross-sectional and longitudinal analyses [89–94]. Importantly, these types of studies have not all accounted for the confounding effects of having samples with varying cellular compositions [95]. However, despite this limitation significant correlations between age and DNA methylation have been identified and independently validated, including tissue-specific and covariant patterns [89]. These profiles have even been used to construct quantitative models for accurately predicting the age of individuals and relative aging rates of tissues [96]. In brain, methylation marks seem to be differentially arrayed across genomic elements (e.g., CpG *vs* CpH sites) in regional-, cell type-, and sex-specific patterns

Table 3 Representative examples of genome-wide DNA methylation studies performed in nervous system disease patient-derived tissues

Disease	Tissues examined	Principal findings	Ref.
Alcohol dependence	Peripheral blood mononuclear cells from discordant sibling pairs	865 hypomethylated and 716 hypermethylated CpG sites, with *SSTR4* and *GABRP* exhibiting the most significant levels of hypo- and hypermethylation, respectively	[56]
AD	Postmortem frontal cortex specimens	Differential methylation at 948 CpG sites representing 918 genes, with a site in the *TMEM59* gene promoter showing the greatest discordance	[57]
ASD	Postmortem brain dorsolateral prefrontal cortex, temporal cortex, and cerebellum specimens	4 differentially methylated regions, including 3 in temporal cortex and 1 in cerebellum	[58]
ASD	Buccal epithelium	15 differentially methylated regions in 14 genes, which are all expressed in brain, encode proteins involved in synaptic function, and have previously been implicated in ASD pathogenesis	[59]
Depression	Buccal epithelium from monozygotic twin pairs discordant for adolescent depression and postmortem brain tissue from patients with MDD	2 DMPs identified, with the most significant level in *STK32C*	[60]
Down syndrome	Buccal epithelium	3300 differentially methylated CpG sites	[61]
Fragile X syndrome	Peripheral blood and induced pluripotent stem cells	Abnormal methylation present only at the *FMR1* gene locus	[62]
MS	Pathology-free brain regions derived from MS patients	Widespread differential methylation, with hypomethylation associated with genes involved in immune responses and hypermethylation with oligodendrocyte-specific genes (*MBP, SOX8*), including those mediating survival (*NDRG1, BCL2L2*)	[63]
MS	CD4+ lymphocytes from relapsing remitting MS patients	74 DMRs, with peak signal at *HLA-DRB1*, including 55 non-HLA CpG sites associated with genes previously implicated in MS pathogenesis	[64]
MS	CD4+ lymphocytes in disease discordant monozygotic twin pairs	No differentially methylated sites common between different twin pairs	[65]
PD	Postmortem brain and peripheral blood	2908 DMRs in brain and 3897 in blood, including loci previously implicated in PD pathogenesis, with significant covariance between profiles in brain and blood	[66]
PD	Postmortem cortex and putamen specimens	3 differentially methylated genes, including significant hypomethylation of *CYP2E1* in both brain regions	[67]
PSP	Peripheral blood	DMPs clustered within the 17q21.31 chromosomal region associated with the major genetic risk factor for PSP, the H1 haplotype	[68]
Schizophrenia	Peripheral blood from patients with first-episode schizophrenia	4641 DMPs corresponding to 2929 genes	[69]
Schizophrenia and bipolar disorder	Postmortem frontal cortex and anterior cingulate specimens	Widespread hypo- and hypermethylation, respectively, in frontal cortex and anterior cingulate compared with controls for both disorders, with a large proportion of DMRs distributed in intronic and intergenic regions	[70]

AD = Alzheimer disease; ASD = autism spectrum disorder; MS = multiple sclerosis; PD = Parkinson disease; PSP = progressive supranuclear palsy; MDD = major depressive disorder; DMPs = differentially methylated probes; DMRs = differential methylated regions; HLA = human leukocyte antigen

during development and aging [89–94]. Moreover, it is notable that methylation marks measured in easily accessible peripheral tissues, such as blood, can serve as reliable surrogates for those present in brain for certain subsets of genes [89].

While it has been suggested that age-related changes in DNA methylation arise because of epigenetic drift, referring to a stochastic process that reflects imperfect DNA methylation maintenance leading to epigenetic mosaicism and corresponding variegation in gene expression, these DNA methylation alterations are nonrandomly distributed [96–98]. The majority of the genome is hypomethylated with age, whereas promoters of developmental genes are preferentially hypermethylated. The biological consequences of these observations are not well characterized, but some evidence supports a compromise in stem cell functions and a loss of phenotypic plasticity [97]. One interesting systems biological approach focused on analyzing genes subject to age-associated epigenetic changes in the context of protein interaction networks and found that these factors have distinct network topological features, such as low centrality and connectivity, and they exhibit topological synergy with classes of genes known to be involved in longevity and disease-relevant gene networks [99]. These findings suggest novel mechanisms and molecular substrates of the aging process.

Overall, these examples illustrate how epigenetic profiles can evolve over the lifespan and may be exploited to provide insights into normative brain aging and vulnerability to neurodegenerative disorders that present later in life.

Other Health-related Phenotypes

These strategies have also been used to investigate connections between epigenetics and important health-related phenotypes, such as metabolic parameters. For example, a recent study performed using blood and adipose tissue from adults of European origin demonstrated that increased body mass index is associated with increased DNA methylation at the *HIF3A* gene locus [100]. The authors replicated this finding in 2 separate cohorts and correlated the body mass index-associated epigenetic information with genomic and transcriptomic data. Another analysis utilized leukocytes and identified differentially methylated sites in intron 1 of *CPT1A* that correlated strongly with levels of very low-density lipoprotein cholesterol and triglycerides [101]. The authors replicated this observation in a Framingham study cohort. One very interesting report employed epigenome-wide association studies coupled with metabolomic data to examine associations between DNA methylation and numerous metabolic traits in human blood [102]. This strategy uncovered two types of methylation-related metabolic phenotypes (metabotypes), one mediated by genetic factors and another independent of genetic effects and likely driven by environment and lifestyle. Together, these examples demonstrate how epigenetic information can complement and be integrated with the study of complex health-related traits.

Disease

An increasing number of studies have also focused on uncovering relationships between epigenetic variation and disease states, including cancer (for which such connections are best characterized), autoimmunity, and nervous system disorders [75–78]. In the latter case, these analyses have mostly been limited in scope, for example targeting relatively few genomic sites and small numbers of samples.

However, the next phase in conducting these types of studies is now arriving. For example, one particularly detailed analysis performed using blood focused on identifying genome-wide DNA methylation patterns associated with pain sensitivity in 100 individuals in various cohorts, including 25 pairs of pain sensitivity discordant monozygotic twins [103]. The authors found 9 differentially methylated regions highly correlated with pain sensitivity, which were located proximal to genes known to be involved in pain and nociception and also at other genomic loci (i.e., novel pain genes and intergenic regions). Differential methylation of the *TRPA1* gene promoter exhibited the most robust link with pain sensitivity. They also observed that these methylation profiles were stable over time, associated with genetic variants in *cis*, and correlated positively with methylation patterns in brain tissues and negatively with corresponding gene expression levels in skin. Additional analyses have similarly started linking genetic risk and epigenetic alterations in nervous system disorders including, for example, in tauopathies [68,104]. Further well-designed studies are also underway that focus on more sophisticated and integrated epigenomic examinations of nervous system disorders, such as AD [79].

Next-generation Epigenetic Research Tools and Therapeutic Approaches

Key challenges for the future lie in developing tools and techniques for functionally interrogating these epigenomic alterations at higher resolution (e.g., uncovering the biological effects mediated by noncoding genomic variants and by specific chromatin modifications at particular genomic sites) and, ultimately, for targeting the epigenome very selectively for therapeutic purposes. Several intriguing studies have demonstrated how powerful new approaches, such as synthetic and chemical biology and optogenetics, can be employed for these purposes.

Synthetic Biology

Synthetic biology-enabled technologies that have emerged over the last decade now permit high-precision and efficient genome editing (i.e., modification of the genetic code at target loci) and control of gene expression [105]. These include zinc finger (ZF), transcription activator-like effector (TALE), and RNA-guided [i.e., bacterial clustered regularly interspaced short palindromic repeat (CRISPR)-Cas (CRISPR-associated)] systems. These strategies utilize either customizable DNA binding proteins or RNA sequences that target user-defined sequences of interest within the genome and guide nucleases or other functional molecules to these sites. Coupling with nucleases leads to cleavage of genomic DNA at the target site, and endogenous DNA repair mechanisms can subsequently be exploited together with an exogenous template to permanently modify the sequence (i.e., introduce novel information into the genome). Alternatively, coupling with other functional molecules, such as transcriptional regulators, can be used for selective gene (and gene network) activation or repression.

Not only are these approaches valuable for studying disease-causing mutations in protein-coding genes (as was recently reported for PD [106,107]) and for gene therapy (as

reported for HIV [108]), but they are also being used to determine the impact of noncoding genomic sequence variants. One impressive study, in particular, utilized TALE nuclease-based gene editing to establish a causal link between a single nucleotide substitution in an intergenic region and mosaic variegated aneuploidy syndrome, which is characterized clinically by a high risk of childhood cancer, and pathologically by premature chromatid separation and constitutional aneuploidy [109]. This autosomal recessive disorder is caused by mutations in *BUB1B*; however, the authors identified only monoallelic mutations within this gene in several families with the syndrome, prompting further investigation. They subsequently identified a single nucleotide substitution in an intergenic region located upstream of the *BUB1B* transcription start site that cosegregated with the disorder. Utilizing a TALE nuclease-based methodology, they introduced this substitution into cultured human cells biallelically and consequently recapitulated the molecular pathology of the disease, confirming that the intergenic single nucleotide substitution is, in fact, a causal mutation.

Complementary strategies are being applied to control the expression of endogenous genes by targeting synthetic transcriptional modulators containing repressor or activator domains to specific genomic regulatory elements. One of the first of these methods, published in 2000, employed ZF proteins coupled with either a Krüppel-associated box repressor domain or a VP64 activation domain (derived from the herpes simplex virus protein, VP16) that were designed to bind to *erbB-2* and *erbB-3* in order to, respectively, down- and upregulate their expression in human cells [110]. Since that time, an increasing number of studies have focused on developing more advanced ZF, TALE, and CRISPR/Cas technologies for these purposes. Notable innovations are the use of several activators designed to bind to different sites within a single gene promoter region to synergistically induce high expression levels of the gene of interest [111–113] and the targeting of multiple genes simultaneously for multiplexed gene activation [113]. Interestingly, synthetic factors operate within the context of the chromatin landscape present at a specific genomic site, and their ability to influence locus-specific transcription can be potentiated by small molecules that inhibit epigenetic factors [e.g., valproic acid (VPA) or 5-aza-2′-deoxycytidine] [114].

Importantly, these programmable reagents are also being adapted to target epigenetic modifications in order to modulate chromatin environments and transcriptional activity at specific sites. For example, one group of investigators has reported the development of an interesting technology for promoting genomic locus-specific DNA methylation, using 2 different ZFs designed to bind to DNA flanking a target CpG site that are each fused with a component of a bifurcated DNA methyltransferase [115–117]. This approach aims to increase the local concentration of both methyltransferase fragments when the ZFs bind to the genome, leading to the assembly of an active methyltransferase enzyme only at the target site. In contrast, most other methods employing sequence-specific DNA binding proteins fused with DNA methyltransferase enzymes have led to significant off target methylation events [118,119]. These tools can potentially be used to repress a pathological gene either stably or reversibly, as these induced methylation marks are subject to removal by DNA methyltransferase inhibitors (i.e., 5-aza-2′-deoxycytidine). Other groups have focused on designing tools for locus-specific DNA demethylation. For example, one recent study reported successful genomic targeting of 5mC for hydroxymethylation/demethylation utilizing a TALE-based method [120]. The authors created fusion proteins containing TALE DNA binding domains linked with TET1 hydroxylase catalytic domains. These fusion proteins were engineered to induce locus-specific hydroxymethylation/demethylation at CpG sites associated with three genes (*KLF4*, *RHOXF2*, and *HBB*). This activity was validated in different human cell lines and occurred to the greatest extent within 30 base pairs of the TALE target-binding site in a fusion protein dose–responsive manner. When these epigenome editing events were targeted to promoter regions, they produced selective and significant increases in corresponding gene expression levels. The authors also demonstrated the feasibility of a complementary ZF–TET1 fusion protein-based approach for locus-specific hydroxymethylation/demethylation.

Additional strategies have focused on developing technologies for genomic locus-specific editing of activating and repressive histone modifications. For example, one study reported an approach for targeting of methylated histone proteins for demethylation [121]. The authors created fusion proteins containing TALE DNA binding domains linked with the LSD1 histone demethylase, which acts on mono- and dimethylated lysines at the H3K4 and H3K9 positions. The fusion proteins were designed to target a set of candidate enhancer regions that are characterized by the presence of H3K4 mono- and dimethylation (H3K4me1 and H3K4me2), as well as H3K27 acetylation (H3K27ac) marks. Introduction of the fusion proteins reduced H3K4me2 levels by 2-fold or more at the majority of enhancers that were targeted in a locus-specific manner, thereby inactivating these enhancers and causing downregulation of their proximally located target genes. Notably, these fusion proteins also decreased H3K27ac levels, reflecting either a direct effect of H3K4 demethylation or an indirect effect mediated by LSD1 interacting proteins and highlighting the complex crosstalk that exists between different epigenetic mechanisms.

Chemical Biology

Another approach for examining genomic locus-specific epigenetic modulatory events is to employ chemical biology. One interesting study showed, for example, how a bifunctional

small molecule might be used for precision targeting of chromatin regulators to a particular gene [122]. The authors employed a chemical-induced proximity system based on the ability of the small molecule, rapamycin, to interact physically with both FK506 binding protein (FKBP) and FKBP12-rapamycin binding domain of mammalian target of rapamycin. They designed 2 chimeric proteins, one with FKBP fused to ZF homeodomain 1 (ZFHD1) and the other with FKBP12-rapamycin binding domain fused to HP1α—a key player in the establishment of repressive higher-order chromatin that acts via recruitment of the H3K9 methyltransferases, SUV39h1/2 and histone-lysine *N*-methyltransferase (SETDB1). The authors also engineered the *Oct4* gene promoter to harbor a ZFHD1-binding site. Thus, in the presence of rapamycin, the two chimeric proteins form a complex, and the effector (i.e., HP1α) is thereby selectively tethered to the target site (i.e., ZFHD1 binding site in the *Oct4* promoter). The authors showed that this induces the H3K9me3 mark and DNA methylation and loss of H3K4me3 over a distance of 10 kb *in cis* forming a transcriptionally repressed heterochromatic domain.

Optogenetics

An alternative paradigm for examining the functional consequences of specific epigenetic modifications is the use of tools and techniques from the emerging field optogenetics, which enable precise temporal and spatial control of cellular processes by coupling light-sensitive proteins with various other molecules. A key study recently highlighted the feasibility of utilizing such optical methods to modulate transcriptional and epigenetic states in neuronal cells [123]. The authors engineered modular light-inducible transcriptional effectors (LITEs), comprised of customizable TALE DNA-binding domains fused with the light-sensitive cryptochrome 2 protein (CRY2) from *Arabidopsis thaliana* and a second module including the cryptochrome-interacting basic-helix-loop-helix (CIB1; the interacting partner of CRY2) fused with an epigenetic effector domain. Stimulation with light provokes a CRY2 conformational change leading to the recruitment of the CIB1 module and, in turn, to inducible genomic locus-specific epigenetic modifications. The authors developed LITEs targeted to the metabotropic glutamate receptor 2 and neurogenin 2 genes containing a spectrum of epigenetic effector domains that included those from HDACs, histone methyltransferases (HMTs), HAT inhibitors, and HDAC- and HMT-recruiting proteins, and demonstrated the ability to promote site-specific chromatin remodeling and also to modulate the expression of these genes. This LITE system allows optogenetic control of epigenetic regulatory events in various biological contexts.

Perspective

Studying the crosstalk that exists between genetic and epigenetic mechanisms is the next frontier for uncovering how and why nervous system diseases unfold, and for identifying novel diagnostic and therapeutic modalities targeting these disorders. We have called attention to this interplay, which can be mediated by disease-causing gene mutations and risk-modifying genomic variants, including those that are present in regulatory noncoding regions. Enhancers represent a prime example of such elements. Accordingly, the concept of enhancer malfunction or "enhanceropathy" offers an interesting paradigm for explaining the cell type- and tissue-specific manifestations found in certain disorders (i.e., selective cell death of different neuronal subtypes in AD, PD, and Huntington disease) [36]. Strategies for modulating enhancer function are already being explored and may provide the basis for innovative molecular treatments [124].

In addition, relationships between genetic and epigenetic mechanisms can also be mediated, broadly, at the level of epigenomic deregulation. Epigenetic epidemiology focuses on interrogating the causes and effects of these epigenetic alterations. However, the field is still nascent, encumbered by issues of methodology and data interpretation, and, as yet, centered primarily on DNA methylation. Nonetheless, epigenetic epidemiology—coupled with advanced tools and techniques for functionally manipulating genetic and epigenetic processes (i.e., those leveraging synthetic and chemical biology and optogenetics)—has tremendous potential for providing a more integrated view of the links between genetic factors, transgenerational effects of ancestral exposures, sex differences, developmental and age-related biological changes, environmental influences, and central–peripheral communications, and, thus, for illuminating the mechanisms that underpin the risk, onset, progression, and treatment responsiveness of neurological and psychiatric diseases [17,18].

We are at the vanguard of the era of epigenetic medicine, and these rapidly emerging insights will, no doubt, serve as the foundation for further development of advanced molecular diagnostics and individualized precision treatments that might even include site-specific epigenome editing (epigenome surgery).

Along with genetic and genomic testing, epigenetic profiling already provides clinically relevant information. For example, prevailing methods for diagnosing imprinting disorders, such as Prader–Willi syndrome and Angelman syndrome, employ a combination of genetic and epigenetic analyses. The DNA methylation status of the *MGMT* gene promoter in glioma, which mediates responsiveness to the alkylating agent temozolomide, is increasingly being utilized for making treatment decisions in selected populations (i.e., elderly patients with glioblastoma and those with anaplastic glioma lacking *IDH1/2* mutations) [125]. Also, the US Food

and Drug Administration recently approved a sensitive noninvasive screening assay for colorectal cancer that examines a panel of markers, including *KRAS* mutations and *NDRG4* and *BMP3* methylation status [126]. An array of additional epigenetic tests is either available or in development for a very broad range of diseases, including many nervous system disorders [127–134]. These assays are purported to have roles in risk stratification, screening, prognostication, customization of therapies, and monitoring treatment responses and disease recurrence; however, data from prospective trials is limited. Nonetheless, it is likely that such epigenetic diagnostic applications will continue to proliferate, be clinically validated, and become more technically sophisticated and integrated with other biomarkers because of the ongoing mechanistic and methodological innovations we have outlined here, as well as many others such as those enabling noninvasive tissue-specific *in vivo* epigenetic imaging and profiling [135].

Moreover, while a limited number of drugs targeting epigenetic factors are already commercially available, pharmaceutical pipelines are rich with additional epigenetic compounds in preclinical and clinical phases of development. The DNA methyltransferase inhibitors 5-azacytidine (Vidaza; Celgene Corporation, Summit, NJ, USA) and 5-aza-2′-deoxycytidine/decitabine (Dacogen; Otsuka Pharmaceuticals, Tokyo, Japan), and the HDAC inhibitors vorinostat (Zolinza; Merck & Co, White House Station, NJ, USA) and romidepsin (Istodax; Celgene Corporation) are approved by the Food and Drug Administration for treating myelodysplastic syndrome and cutaneous T-cell lymphoma, respectively. However, these agents are relatively nonspecific and associated with significant off-target effects and toxicity. It has also been demonstrated that some commonly used drugs (e.g., hydralazine, procainamide, and VPA) can affect epigenetic pathways and thereby influence disease processes (in preclinical studies) [136]. Thus, clinical trials have focused on repurposing these agents, either alone or in combination with other drugs, for various disorders. For example, preliminary studies have investigated whether VPA has disease-modifying activity in spinal muscular atrophy and amyotrophic lateral sclerosis [137–140]. The outcomes of such studies have been somewhat inconsistent, suggesting that more refined agents with better pharmacological profiles might offer superior results. Additional epigenetic compounds in development include novel and more isoform-selective inhibitors of HDACs, HMTs, HATs, histone demethylases, bromodomain proteins, and chromodomain proteins [141–144], as well as modulators of ncRNA pathways [133,134]. Several of these therapeutic efforts are pursuing neurological and psychiatric disease indications (i.e., highly selective HDAC1 and HDAC6 inhibitors) [145], though important challenges, such as those related to *in vivo* central nervous system exposure and long-term consequences (e.g., transgenerational effects), remain.

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