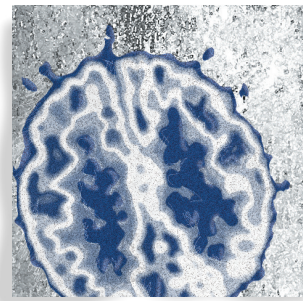# Basic research

Epigenetic approaches to psychiatric disorders

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Psychiatric diseases place a tremendous burden on affected individuals, their caregivers, and the health care system. Although evidence exists for a strong inherited component to many of these conditions, dedicated efforts to identify DNA sequence-based causes have not been exceptionally productive, and very few pharmacologic treatment options are clinically available. Many features of psychiatric diseases are consistent with an epigenetic dysregulation, such as discordance of monozygotic twins, late age of onset, parent-of-origin and sex effects, and fluctuating disease course. In recent years, experimental technologies have significantly advanced, permitting in-depth studies of the epigenome and its role in maintenance of normal genomic functions, as well as disease etiopathogenesis. Here, we present an epigenetic explanation for many characteristics of psychiatric disease, review the current literature on the epigenetic mechanisms involved in major psychosis, Alzheimer's disease, and autism spectrum disorders, and describe some future directions in the field of psychiatric epigenomics.

Epigenetics, complex disease, and the brain

In general, epigenetics refers to the regulation of DNA sequences that does not involve alteration of their actual base composition. Transcription and numerous other genomic functions are epigenetically controlled via heritable, but potentially reversible, changes in modification of DNA and histones (acetylation, methylation, phosphorylation, etc),[1] and epigenomics is the application of these processes across the genome. The normal functioning of genomes is tightly connected to their epigenetic regulation, and epimutations can be harmful in the presence of impeccable DNA sequences. The epigenetic theory of complex non-Mendelian disease is based on three key postulates. Firstly, an organism's epigenetic status is far more dynamic than its DNA sequence, and may be altered by a number of factors, such as environment, developmental programs,[2] or even as a result of stochasticity.[3] Secondly, certain epigenetic signals may be inherited transgenerationally with DNA sequence[4] and may account for heritability of some traits

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and diseases.[5] Thirdly, epigenetic regulation is required in the maintenance of proper genomic function, for example, regulation of gene activity, inactivation of parasitic DNA elements, and chromosomal segregation.[6] Epigenetic factors greatly affect phenotype—even genes that are free of mutations may become harmful if they are not expressed at the appropriate time and at the required level. Combined, these points provide a solid, mechanistic basis for a cohesive interpretation of various epidemiological, clinical, and molecular features of complex diseases.

The molecular epigenetic mechanisms are complex and highly intertwined. At the most basic level, methyl groups may be bound to cytosines at the C_5 carbon, usually within cytosine/guanine dinucleotides (CpG), which are established and maintained by the DNA methyltransferase (DNMT) family of enzymes. This is believed to be the most stable epigenetic mark, due to the covalent nature of the modification.[7] Additionally, another DNA modification, hydroxymethylcytosine, has very recently been discovered in Purkinje neurons and other cells of the brain, and it may also play a role in epigenetic regulation of neural function.[8]

DNA is wrapped around octamers of basic histone proteins, each consisting of a core and N-terminus, to form nucleosomes. Numerous modifications of these proteins influence the condensation of chromatin, which can be open (transcriptionally active) or closed (inactive). Histone acetyltransferases (HATs) acetylate lysine residues on the N-terminal tail of histone proteins, neutralizing the positive charge of the protein and decreasing its affinity for DNA. As a result, the chromatin relaxes and the transcription machinery gains access to previously restricted sites.[9] Acetyl groups can be removed by histone deacetylases (HDACs), resulting in chromatin condensation and transcriptional inactiva-

tion.[10] The presence of an N-terminal methyl-CpG-binding domain (MBD) allows proteins, such as methyl-CpG-binding protein 2 (MeCP2), to bind methylated sites on DNA and complex with HDACs and the corepressor SIN3A. The complex facilitates histone deacetylation and downstream gene silencing from the methylated CpG site. Histone methylation can result in either gene activation or repression, depending on the specific lysine or arginine that is modified.[11] Another family of enzymes, the histone demethylases, such as lysine-specific demethylase 1 (LSD1), are capable of removing this methyl group from the lysine residues of histone and nonhistone proteins.[12]

A hallmark of non-Mendelian disease, discordance of monozygotic (MZ) twins, has traditionally been attributed to differential environmental factors activating a disease state in one of the genetically predisposed cotwins[13]; however, very few of these factors have been identified. Alternately, MZ twin discordance may be due to the partial stability of epigenetic factors, as disease-relevant epigenetic dissimilarity can accumulate quite readily between cotwins.[5,14,15] Another non-Mendelian peculiarity, sexual dimorphism, is the differential susceptibility to a disease between males and females. It is observed in many psychiatric conditions, such as Alzheimer's disease, schizophrenia, alcoholism, and mood and anxiety disorders.[16] Although the exact mechanism by which they predispose or protect from a disease is currently unknown, there is a great deal of evidence that sex hormones exert control of gene expression via epigenetic modifications; thus it is hypothesized that sexual dimorphism in many disease states may be the result of sex hormone-induced differences in the epigenetic status of key genes.[17,18] Furthermore, the degree of risk for acquiring certain complex diseases may depend on the sex of the affected parent, as in schizophrenia,[19] Alzheimer's disease (AD),[20] autism,[21] and bipolar disorder (BD).[22] Genomic imprinting, an epigenetic mechanism in which differential epigenetic modification of genes occurs depending on their parental origin,[23] is thought to be the source of such parent-of-origin effects. Diseases affecting cell growth, development, and behavior may result from disruption of the normal imprinting pattern.[24]

In the epigenetic model of complex disease, it is assumed that a primary epigenetic disruption takes place during the maturation of the germline, and this pre-epimutation increases an organism's risk of acquiring a disease. The

pre-epimutation may be tolerated and it may not be sufficient to cause the disease itself, but with time, perhaps even decades, small misregulations add up until a threshold is crossed and the individual experiences phenotypic changes that meet diagnostic criteria for a clinical disorder. The age of disease onset may depend on the effects of tissue differentiation, stochastic factors, hormones, and likely some external environmental factors (nutrition, infections, medications, addictions, etc).[6,25,26] Severity of epimutations may fluctuate over time, due to their reversible nature, known to clinicians as "remission" and "relapse." It is also possible that epimutations may regress back to the norm with aging, which presents partial recovery, eg, reduction of psychopathology in elderly psychiatric patients.

Although there are very few studies investigating the role of epigenetic factors in psychiatric diseases, there is an increasing body of experimental evidence that epigenetic signals play a critical role in neuronal development, differentiation, and communication, as well as synaptic plasticity in general[27]; these processes are fundamental for normal brain activity, such as learning and memory.[28,29] The known epigenetic modifiers, Polycomb (PcG), and Trithorax (TrxG) proteins, have been shown to influence synaptic plasticity,[30,31] and cascade activation during memory formation in the mitogen activated protein kinase (MAPK) pathway appears to trigger H3K14 acetylation.[32] Additionally, pharmacologic inhibitors of epigenetic processes have had documented effects on long-term potentiation (LTP), an increase in efficiency of synaptic transmission, in the mammalian brain. DNMT inhibitors, such as zebularine, impair induction of LTP in mouse hippocampus,[33] while HDAC inhibitors (HDACi), such as sodium butyrate and trichostatin A (TSA), have been shown to enhance LTP in rat hippocampus[32] and amygdala.[34] Taken together, this theoretical and experimental evidence suggest that epigenetic regulation is essential for neural and brain functioning, and putative epimutations may play a role in etiopathogenesis of complex psychiatric disease.

Psychiatric epigenetics and epigenomics

Major psychosis

Major psychosis is a classification that encompasses both schizophrenia (SZ) and BD—two conditions that seem to be related etiologically.[35] SZ is a multifactorial disease characterized by disordered thinking and concentration that results in psychotic thoughts (delusions and hallucinations), inappropriate emotional responses, erratic behavior, as well as social and occupational deterioration,[36] while BD represents a category of mood disorders, in which affected individuals experience episodes of mania or hypomania interspersed with periods of depression, and may also suffer from delusions and hallucinations. Thus far, traditional gene- and environment-based approaches have not been very successful in deciphering the clinical, molecular, and epidemiological aspects of psychosis, such as MZ discordance (41% to 65% for SZ,[37] ~60% BD[38]), sexual dimorphism, parent-of-origin effects, fluctuating disease course with periods of remission and relapse, and peaks of susceptibility to the disease that correspond to periods of major hormonal changes in the organism.[25] Classically, psychosis research was aimed at defining genetic and environmental risk factors, but despite significant evidence of a heritable component derived from twin and adoption studies,[39,40] many molecular genetic findings have not been replicated, and significant heterogeneity and small effect sizes are thought to plague genetic association studies.[41]

Recently, the first epigenomic study of major psychosis utilizing CpG-island microarrays was released by Mill et al,[42] providing a large-scale overview of DNA methylation differences in the brain associated with SZ and BD. DNA extracted from the frontal cortex was subjected to enrichment of the unmethylated fraction using the methylation-sensitive restriction enzymes, and adaptor ligation coupled with PCR amplification. The amplicons (multiple copies of the unmethylated genomic DNA) were interrogated on 12 192 feature CpG-island microarrays. The data was normalized, assigned raw *P* values based on a *t* statistic, and then converted to false discovery rates (FDR). Indeed, in cortex they discovered differences at loci involved in glutamatergic and γ-aminobutyric acid (GABA)-ergic neurotransmission, brain development, mitochondrial function, stress response, and other disease-related functions, many of which correspond to psychosis-related changes in steady-state mRNA. In relation to the glutamatergic hypothesis, a lower degree of DNA methylation was observed in SZ and combined male psychosis (SZ and BD) samples at two glutamate receptor genes, *NR3B* and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor-subunit gene *GRIA2*; the dysregulation of AMPA and N-

methyl-D-aspartic acid (NMDA) receptors is an etiological component of major psychosis, and it has been shown that *GRIA2* expression is altered in the prefrontal cortex and striatum of SZ patients.[43] Hypomethylation was also detected at the vesicular glutamate transporter (*VGLUT2*) in SZ females, and at secretogranin II (*SCG2*), which encodes a neuronal vesicle protein that stimulates glutamate release. A higher degree of methylation was observed in SZ females at *VGLUT1*, a transporter protein that is downregulated in SZ brains,[44] and the glutaminase enzyme, *GLS2*, in SZ males, which has previously been shown to exhibit altered expression in cases of SZ.[45] In synergy with glutamatergic pathways, GABAergic pathways also show dysregulation in cases of major psychosis. Detected disruptions in such pathways included hypermethylation at the RNA-binding regulator of GABA(B) receptors, *MARLIN-1*, in SZ, BD, and psychosis females, the G protein-coupled inwardly rectifying potassium channel linked to GABA neurotransmission, *KCNJ6*, in SZ and psychosis males, as well as the *HELT* locus in SZ and BD females, which is known to determine GABAergic over glutamatergic neuronal fate in the mesencephalon. Several other intriguing loci were highlighted, such as the hypermethylation at *WNT1*, a gene critical for neurodevelopment that is differentially expressed in SZ brains,[46] in females affected with major psychosis, and at *AUTS2* in SZ males, which spans a translocation breakpoint associated with autism and mental retardation. A highly significant hypermethylation was detected in both male and female samples at two loci: *RPP21*, which encodes a component of ribonuclease P, a complex that forms t-RNA molecules via 5'-end cleavage, and *KEL*, which encodes the Kell blood-group glycoprotein and causes McLeod Syndrome when incorrectly expressed; SZ symptoms are manifested as part of McLeod Syndrome. Network and gene ontology (GO) analyses were performed in order to determine relationships between the functionally linked pathways from the microarray dataset. The network analysis revealed a lower degree of modularity of DNA methylation "nodes" in the major psychosis samples, indicating that there is some degree of systemic epigenetic dysregulation involved in the disorder. From the GO analysis, several categories were highlighted, including those involved in epigenetic processes, transcription, and development, as well as brain development in female BD and SZ samples, and in those related to stress response in male BD samples.[46] To date, this is the largest and most comprehensive epigenomic study of major psychosis—the data presented supports epigenetic mechanisms underlying broader hypotheses of major psychosis and uncovers some new avenues for future exploration.

Both SZ and BD have also been examined using the candidate gene approach, as epigenetic downregulation of genes is emerging as a possible underlying mechanism of the GABAergic neuronal dysfunction in SZ. One of the more intensively investigated SZ-related genes is *RELN*, which is involved in neuronal development and cell signaling, and has been found to be hypermethylated in cases of SZ.[47] However, no differences were observed at this locus in a replication attempt,[46,48] and the focus seems to be shifting to other candidate genes, namely the 67 kDa glutamate decarboxylase (*GAD67*, aka *GAD1*) and *DNMT1*. GAD67 catalyzes the conversion of glutamic acid to GABA. In cases of SZ, the levels of this enzyme and several others involved in GABAergic neurotransmission, such as *GAD65* and GABA plasma membrane transporter-1 (*GAT-1*), display decreased mRNA levels, as determined by real-time quantitative polymerase chain reaction (qPCR) and in situ hybridization.[49-52] In addition to aberrant methylation at this locus, an analysis of the microarray collection of the National Brain Databank (USA) has shown that decreased GAD67 mRNA levels strongly correlated with upregulated *HDAC1* in the prefrontal cortices of SZ subjects.[53] Oddly enough, at the *GAD67* promoter, SZ patients have been shown to display an ~8-fold deficit in repressive chromatin-associated DNA methylation.[54] In the prefrontal cortex of 41 SZ patients, another histone modification, H3-(methyl)arginine 17 (H3meR17) was found to exceed control levels by 30%, and this was associated with downregulated metabolic gene expression.[55] So, while it is apparent that histone modifications are involved in the development of SZ, their exact mechanism is not entirely clear. Hypermethylation of *GAD67* is believed to occur via DNMT1,[56,57] a maintenance methyltransferase enzyme that is upregulated in the GABAergic neurons and peripheral blood lymphocytes of SZ patients, along with the *de novo* methyltransferase, DNMT3a.[56,58] Interestingly, nicotine has been shown to decrease DNMT1 mRNA expression in cortical and hippocampal GABAergic neurons in mice—this decrease results in *GAD67* promoter demethylation, and is inversely related to an upregulation of cortical GAD67 protein.[59] This information is highly relevant, as SZ patients tend to smoke tobacco at a rate that is 2- to 4-

fold higher than in the general population,[60] and are possibly drawn to the nicotine content for its effects on the aforementioned pathway.

Less information is available on BD; genomic imprinting has been suggested by statistical genetics, but molecular approaches have not yielded the imprinted disease genes.[61] A recent study applied methylation-sensitive representational difference analysis (MS-RDA) to lymphoblastoid cells derived from twins discordant for BD.[62] One detected gene, named peptidylprolyl isomerase E-like (*PPIEL*), was hypomethylated in BD-affected twins, while a region of the spermine synthase (*SMS*) gene was hypermethylated versus unaffected twins; it has yet to be determined if either of these regions are biologically and functionally significant. In combined studies of epigenetics and DNA sequence, some interesting developments have been observed. It has recently been shown that rare G variants of a G/A polymorphism in the potassium chloride co-transporter 3 gene (*SLC12A6*) may represent risk factors for BD.[63] Eventually, it was discovered that variants containing the G allele were methylated at the adjacent cytosine, and this accompanied a decrease in gene expression in human lymphocytes.[64] This hints at a functional link between epigenetics and genetic variation, and the association with BD is believable, as *SLC12A6* mutations underlie another psychiatric disorder, Andermann syndrome, which is an autosomal recessive motor-sensory neuropathy associated with developmental and neurodegenerative defects.[65] It is interesting to note that BD provides a unique opportunity to investigate epigenetic variation between two extreme forms of the same disease—depression and mania. A study design of this variety would unfortunately be limited to the use of peripheral blood, buccal epithelial cells, and fibroblasts as experimental tissues, but nonetheless, it would be incredibly interesting to determine the state of the epigenome during manic and depressive states, in the same individual when the same genetic and environmental impacts are present.

Alzheimer's disease

AD is a neurodegenerative disorder and the most common form of dementia in the elderly; it is characterized by the accumulation of intracellular neurofibrillary tangles (NFT) and extracellular amyloid plaques in the brain.[66] AD often presents with psychiatric symptoms such as memory loss, mood swings, and irritability that increase in severity as the disease progresses. While the phenotype of this condition is well documented, the molecular mechanisms largely remain unknown. The majority of AD research focuses on dysregulation of fibers and proteins, such as epsilon4 allele of apolipoprotein E (APOE), but little ground has been gained in regards to determining the actual origins of their dysfunction.[67] In the rare early-onset form of AD (EOAD), genetic factors play a more defined role, with mutations in amyloid-beta precursor protein (*APP*) and the presenilin genes (*PSEN1*, *PSEN2*) showing a clear connection to the disease.[68] However, since EOAD does not represent the majority of all cases, accounting for only ~5% of the total,[69] this genetic model is not normally applicable.

Similar to other complex diseases, late-onset AD (LOAD), the more common form of the illness that affects individuals over 65 years of age, demonstrates a considerable number of non-Mendelian features. Some of these anomalies include dominance of sporadic over familial cases,[70] discordance of MZ twins,[71] differential susceptibility and course of illness in males and females,[16,18] parent-of-origin effects[72] and, clearly, the late age of onset that is not easily explained by genetic causes alone. Consistent with the epigenetic hypothesis, abnormal levels of folate and homocysteine, signs of dysregulated methylation maintenance, have been detected in the brain of AD subjects. LOAD is a particularly interesting target from the epigenetics of aging perspective, as the epigenome may become deregulated in old age.[73] Using a MethyLight approach, it was shown that a large number of genes increase in methylation with age in control subjects, including several implicated in AD and SZ (*GAD1*, *PSEN1*, *BDNF*, *DRD2*, *GABRA2*, *HOXA1*, *NTF3*, *LDLR*, and *S100A2*), whereas *Alu* and other repetitive elements showed a significant decrease in DNA methylation that was limited to the first decade of life.[74] Of the fifty loci investigated, two displayed significant changes in methylation status with age in AD subjects: *SORBS3* gained methylation over time and is more likely to be methylated in AD patients, while *S100A2* displays a complex chronology, but results in a slow, stochastic methylation decrease later in life (*ibid*). *SORBS3* encodes a neuronal/glial cell adhesion molecule and *S100A2* encodes a calcium binding protein from the S100 family. As part of normal brain aging, S100A2 protein accumulates in corpora amylacea, or polyglucosan bodies; subjects with neurodegenerative disorders experience a much greater accumulation of corpora

amylacea,[75] and this is consistent with the eventual decrease in *S100A2* methylation.[74]

In a study dedicated to DNA methylation analysis of AD candidate genes, it was found that the twelve analyzed loci were epigenetically different in the brains of LOAD cases versus controls, particularly at the locus for transcription factor A (*TFAM*), a key activator of mitochondrial transcription in mammals. Other candidates, such as *PSEN1*, *APOE*, *DNMT1*, and *MTHFR*, displayed an enhanced "distance" in LOAD subjects.[76] This concept of distance is part of the theory of "epigenetic drift," in which an affected individual has an epigenetic status at some gene(s) that is distanced from the norm, and this distance increases with age.[76] Of the CpG-rich regions analyzed, the majority were unmethylated, and it appears possible that very small alterations in methylation level could accumulate over time, ultimately affecting gene regulatory functions and causing disease. Age-related alteration of methylation status is a global phenomenon, not necessarily limited to particular disease susceptibility genes. Another study examined the methylation changes in 807 arbitrarily selected genes from two cohorts from Utah and Iceland, taking DNA samples at two timepoints from each subject, spaced either 11 or 16 years apart. In these two populations, they observed time-dependent changes in global DNA methylation within the same individual, with 8% to 10% of individuals in showing changes that were greater than 20 percent; both gains and losses of methylation were detected.[77] Similarly, the Boston Normative Aging Study measured DNA methylation in the blood of 718 elderly subjects (55 to 92 years of age) over a span of 8 years. A progressive loss of DNA methylation in repetitive elements was found, particularly in Alu repeats, and this linear decline highly correlated with time since the first measurement.[78] A seemingly innocuous early-life epigenetic change in some critical gene involved in AD etiology, for example, the amyloid precursor protein (APP) locus, could potentially become pathologic when subjected to epigenetic drift as the subject ages. Although the molecular mechanisms leading to early-life methylation disturbances have not yet been identified, the possibility of early epimutation and epigenetic drift should not be ignored as an etiological candidate for LOAD.

Autism spectrum disorders

Autism and related developmental disorders, such as Asperger's and Rett syndromes, fall under the broader class of autism spectrum disorders (ASD), where "spectrum" reflects the observed continuum of severity or impairment experienced. These disorders become apparent in young children and persist into adulthood, with deficits in social cognition regarded as the most characteristic feature of ASD, leading to restrictions in social communication.[79] While autism itself is believed to have a particularly strong inherited basis relative to other developmental psychiatric syndromes,[80] DNA sequence factors in the etiology of ASD are still largely unknown.[81] Evidence supports a contribution of imprinted genes in ASD, as well as paternal transmission (reviewed in ref 82), and perhaps the combination of this information and the lack of identified genetic markers will stimulate future epigenetic and epigenomic studies of ASD.

Rett syndrome (RTT), a division of ASD, has been extensively studied and arises from loss of function mutations at the locus for methyl-CpG-binding protein-2 (*MeCP2*), a transcriptional repressor that silences methylated genes[83] and may participate in RNA splicing.[84] Mouse models have been very useful in delineating the relationship between disturbances to *MeCP2* and the disease.[85] In mice, deletion of MeCP2 mimics RTT syndrome, leading to locomotor impairments and reductions in brain size.[86,87] Mice with a truncated MeCP2 protein, similar to that of RTT patients, developed many features of RTT, such as tremors, motor impairments, hypoactivity, increased anxiety-related behavior, seizures, kyphosis, and stereotypic forelimb motions; these mice also presented hyperacetylation on histone H3,[88] illustrating that chromatin abnormalities exist in this disorder. In astrocytes cultured from a mouse model of RTT, MeCP2 deficiency causes significant abnormalities in *BDNF* regulation, cytokine production, and neuronal dendritic induction. Whereas previous experiments have only focused on neurons, this evidence suggests that astrocytes may also represent therapeutic targets for RTT.[89]

The classic form of autism also appears to be connected to *MeCP2* expression. Coding mutations affecting the protein are rarely detected in autism, but significantly increased *MeCP2* promoter methylation has been found in autistic male frontal cortex compared with controls, and this inversely correlated with protein expression[90]; aberrant promoter methylation at *MeCP2* has also been detected in female brain DNA.[91] Similarly, loss of methyl-CpG binding protein 1 (MBD1), leads to autism-like behavioral deficits in mice, namely reduced social

interaction, learning deficits, anxiety, defective sensory motor gating, depression, and abnormal brain serotonin activity.[92] Also, a novel mutation has been discovered in the Jumonji AT-rich interactive domain 1C (JARID1C) gene of a child with autism. While very preliminary, this discovery is interesting, as *JARID1C* is believed to be a histone demethylase specific for di- and trimethylated histone 3 lysine 4 (H3K4), as well as a transcriptional repressor for the ASD-associated genes *SCN2A*, *CACNA1H*, *BDNF*, and *SLC18A1*.[93] Finally, another interesting hypothesis relating epigenetics to ASD concerns the observation that autistic children exhibit improved behavior communication during febrile episodes.[94] It may be the case that fever restores the modulatory functions of the intact, but dysregulated locus coeruleus-noradrenergic (LC-NA) system that is present in ASD. The fact that the state of the LC-NA system can be switched back and forth, combined with evidence that imprinted genes within the LC-NA are tightly epigenetically regulated and susceptible to environmental interference,[95] suggests that dynamic epigenetic remodeling processes may regulate the malfunctioning pathways in ASD.[96]

Epigenetic treatment opportunities

Epigenetic drug strategies are currently employed to treat a collection of cancer subtypes, and these medications are now being considered in the treatment of psychiatric disease, as well. The DNMT inhibitor, doxorubicin, has been used to increase reelin and GAD67 expression in neuronal precursor cells, and it was shown that reelin gene expression correlated with the dissociation of DNMT1 and MeCP2 from its promoter, as well as an increased level of histone H3 acetylation.[97] Other studies have shown that HDAC inhibition enhances learning and memory following neurodegeneration induced by traumatic brain injury,[98] and also shows some therapeutic efficacy in rodent models of neurodegenerative conditions, such as Huntington's disease,[99] multiple sclerosis,[100] and Parkinson's disease.[101] One of the downstream effects of HDAC inhibition is upregulation of p21,[102] a cyclin-dependent kinase inhibitor that appears to play an important protective role against oxidative stress and DNA damage.[103] Valproate, a compound utilized for its anticonvulsant and mood-stabilizing properties, also exhibits HDAC activity and has been successfully implemented as a treatment for epilepsy,[104] BD,[105] and, less

commonly, SZ.[106] Like valproate, it has been discovered that several drugs have previously unknown epigenetic modifying properties, and the list continues to grow. While such medications are promising, their pleiotropy, transient effects, and nonspecific alterations to the entire epigenome limit them for the time being.

A substantial challenge to the field of epigenomics of psychiatric and other diseases involves the identification and verification of inhibitors for specific histone-modifying enzymes. Once developed, these compounds should provide higher therapeutic efficiency versus the nonspecific therapeutics that are currently in use, such as suberoylanilide hydroxamic acid (SAHA). The development of small, targeted molecules to specific disease-causing epimutations may resolve some of these issues but, of course, the molecules themselves must first be identified. Alternately, discovery of the downstream effects of epimutations in vivo may nominate particular proteins, to which drug interventions can be applied in a more traditional style, using molecules to exert agonistic and antagonistic effects on the protein products of epigenetically misregulated genes. Knowledge of the three dimensional structures of DNA- and histone-modifying enzymes is mounting and, through the use of fragment-based drug design and ligand motif-based libraries,[107] virtual screening technologies may soon become a feasible option. In the search for target-specific ligands, high-throughput screening of small organic molecule libraries is a useful tool.[108] A recent study utilized a 125 000 small molecule library to screen for specific inhibitors against histone lysine methyltransferases (HMTases). The compound discovered was BIX-01294 (diazepinquinazolinamine derivative), an incredibly specific inhibitor of the target enzyme, euchromatic G9a HMTase, that was able to significantly lower promoter-proximal H3K9me2 marks in mouse embryonic stem cells.[109]

In addition to small molecules, RNA and proteins may also be utilized in the design of effective epigenetic drugs. One strategy focuses on RNA interference (RNAi), in which endogenously produced small interfering RNAs (siRNAs) are incorporated into an RNA-induced silencing complex (RISC) that targets and destroys homologous mRNA, thus preventing protein production.[110] A siRNA with the ability to knock down beta-secretase (*BACE1*) in Huntington's and AD has been developed, as has one against the *SCA1* gene in spinocerebellar ataxia.[111] However, before these RNAs can become effective treatment options, the issues of

nonspecific silencing of partially homologous genes, safe delivery, and inhibition of microRNA (miRNA) must first be resolved. Although the exact mechanisms by which RNAi affects local chromatin structure, gene silencing, and heterochromatin assembly is unknown,[112] it still holds much promise as a therapeutic technique. Another promising technology utilizes zinc-finger proteins (ZFPs), which can recognize specific DNA sequences and bind to short stretches of DNA (~9–18 basepairs), depending on their particular domains.[113] This feature could theoretically allow targeted ZFPs, attached to a DNA- or histone-modifying enzyme,[114] to bind an epimutated site and permit the enzyme to correct the misregulation at that location alone. The damaging global epigenetic effects observed with current drugs would not occur, in this case.

The ability to target etiological disease epimutations and identify epigenetic biomarkers for psychiatric diseases would be another incredibly beneficial development. Biotechnologies are advancing at an amazing rate, and already allow for genome-wide detection of the patterns of DNA methylation and histone modifications. Fully mapped epigenomes in different tissues and cells will facilitate the discovery of disease epimutations and the mechanisms of their pathological action, thus providing the basis for etiological treatment.

Concluding remarks

The role of epigenetic mechanisms in psychiatric diseases is only beginning to solidify, but it is already evident in major psychosis, AD, ASD, and several other conditions not described in this review, such as Rubinstein-Taybi syndrome,[115] addiction,[116,117] Huntington's disease,[118] and Fragile X syndrome.[119] Maintenance of DNA methylation and histone modifications is crucial for normal neurodevelopment and functioning of the brain—dysregulation of these components is highly deleterious to the subject and can predispose to any of the aforementioned disease phenotypes. Previous studies of psychiatric conditions have concentrated on the contributions of genetic and environmental factors but, while DNA sequence and external influences may play an important role in disease etiology, the impact of gene regulation via epigenetic mechanisms on neural function also cannot be ignored. Rather, the interplay between epigenetics, DNA sequences, and environment should become the focus of future work, adopting such concepts as differentially methylated "epi-alleles"[120] and environmental effects on DNA methylation and chromatin modifications.[2] As technologies advance, next-generation sequencing and comprehensive microarrays will become much more affordable, allowing researchers to perform larger, more in-depth epigenomic studies. Perhaps, in the near future, identification of epigenetic biomarkers and operationalization of new, effective diagnostics and treatments will become feasible for psychiatric and various other complex diseases. ❏

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Aproximaciones epigenéticas a los trastornos psiquiátricos

Las enfermedades psiquiátricas determinan un enorme costo para los individuos afectados, sus cuidadores y el sistema de atención de salud. Aunque existe evidencia de un fuerte componente hereditario para muchas de estas condiciones, los esfuerzos dedicados a identificar las causas en base a las secuencias de ADN no han resultado especialmente productivos y son muy pocas las opciones de tratamientos farmacológicos que están clínicamente disponibles. Muchas características de las enfermedades psiquiátricas son concordantes con una falta de regulación epigenética, como la discordancia de los gemelos monocigóticos, la edad tardía de aparición, los efectos del sexo y de los padres biológicos y el curso fluctuante de la enfermedad. Recientemente las tecnologías experimentales han avanzado de manera significativa, permitiendo estudios a fondo del epigenoma y de su papel en el mantenimiento de las funciones genómicas normales, como también en la etiopatogenia de las enfermedades. En este artículo se presenta una explicación epigenética para muchas características de la enfermedad psiquiátrica, se revisa la literatura actual acerca de los mecanismos epigenéticos involucrados en las principales psicosis, la Enfermedad de Alzheimer, y los trastornos del espectro autístico, y se describen algunas líneas a futuro en el campo de la epigenómica psiquiátrica.

Approches épigénétiques des troubles psychiatriques

Les maladies psychiatriques pèsent considérablement sur les individus atteints, leurs soignants et sur le système de santé. Même s'il existe des arguments pour une forte héritabilité de beaucoup de ces troubles, les efforts portés sur l'identification des causes liées à une séquence ADN n'ont pas été très productifs et très peu de traitements pharmacologiques sont disponibles. De nombreuses caractéristiques des maladies psychiatriques concordent avec une dysrégulation épigénétique, comme une discordance entre jumeaux monozygotes, un début tardif, des effets liés au sexe et aux origines parentales et une évolution fluctuante de la maladie. Ces dernières années, des avancées significatives des technologies expérimentales ont permis d'étudier en profondeur l'épigénome et son rôle dans le maintien des fonctions génomiques normales comme dans l'étiopathogenèse de la maladie. Nous présentons dans cet article une explication épigénétique pour de nombreuses caractéristiques des maladies psychiatriques, nous analysons la littérature actuelle sur les mécanismes épigénétiques impliqués dans les psychoses majeures, la maladie d'Alzheimer et les troubles autistiques et nous donnons quelques perspectives dans le domaine de l'épigénomique psychiatrique.

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