Review

SEX DIFFERENCES IN DNA METHYLATION MAY CONTRIBUTE TO RISK OF PTSD AND DEPRESSION: A REVIEW OF EXISTING EVIDENCE

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There are well-established sex differences in the prevalence of certain mental disorders. Work in animal models has provided us with an emerging understanding of the role that epigenetic factors play in establishing sex differences in the brain during development. Similarly, work in animal models, and a more limited but growing literature based on human studies, has demonstrated that DNA methylation (DNAm) changes occur in response to environmental stress, with some of these occurring in a sex-specific manner. In this review, we explore whether DNAm plays a role in contributing to the observed sex differences in prevalence of mental disorders in which stress contributes significantly to their etiologies, specifically posttraumatic stress disorder (PTSD) and depression. We propose that investigating sex differences in DNAm among genes known to influence brain development may help to shed light on the sexually dimorphic risk for, or resilience to, developing PTSD and depression. Depression and Anxiety 30:1151–1160, 2013. *© 2013 Wiley Periodicals, Inc.*

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INTRODUCTION

There are well-established sex differences in the prevalence of certain mental disorders. For example, the burden of mood and anxiety disorders is far greater among women than men[1] and the male:female ratio of autism spectrum disorders is on the order of 4:1.[2] What accounts for sex disparities in the prevalence of these and other mental disorders is currently unknown. Work in animal models has provided us with an emerging understanding of the role that epigenetic factors play in establishing sex differences in the brain during development.[3–5] Similarly, work in animal models, and a more limited but growing literature based on human studies, has demonstrated that DNA methylation (DNAm) changes occur in response to environmental stress, with some of these changes occurring in a sex-specific manner (e.g.,[6,7]). In light of these established and emerging themes in the literature, we explore here whether epigenetic factors, in particular DNAm, play a role in contributing to the observed sex differences in prevalence of mental disorders in which stress contributes significantly to their etiologies, specifically depression and posttraumatic stress disorder (PTSD).

One of the strongest predictors of risk for onset of depression and PTSD is female sex. Data from the nationally representative National Comorbidity Survey indicate that lifetime prevalence of depression in the United States is twice as high in females versus males,[8] women have a higher hazard rate for depression compared to males from age 10 to 50,[8] and the lifetime prevalence

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of PTSD is twice as high in females versus males,[9] a finding supported by the identification of female sex as a risk factor for PTSD by two meta-analyses.[10,11] Although these sex differences in risk of PTSD and depression are well established, their etiology is not well understood. Three competing, but not mutually exclusive, hypotheses have been advanced for understanding the causal mechanisms that mediate sex differences in psychopathology including (i) different types or patterns of risk factors between males and females that are the consequence of being either male or female; for example, differences in the ways that brains become organized during development in males versus females; (ii) differential exposure to the number or severity of risk factors; for example, the greater exposure among males to head injury;[12,13] and (iii) differential susceptibility or sensitivity to risk;[14] for example, the vulnerability to dysthymia among adult women, but not men, who carry the high-activity *MAOA* allele and were exposed to childhood physical abuse.[15] In the case of PTSD and depression, existing evidence supports more than one of these three hypotheses.

For example, established risk factors for depression include exposure to childhood sexual abuse.[16,17] Childhood sexual abuse is more common in girls than boys;[18] thus, the increased exposure to this potent stressor could plausibly account for the higher rates of depression among women during adulthood (hypothesis (ii)). On the other hand, sex-specific sensitivities to other established risk factors for depression, including divorce or separation, work-related problems, and difficulties with interpersonal relationships, suggest the plausibility of hypothesis (iii), as women are more sensitive to difficulties associated with personal relationships and men more sensitive to divorce/separation and/or work-related problems.[19] Yet, even after accounting for differences in sensitivity to these stressful life events, as well as differences in exposure rates to these events (hypothesis (ii)), the sex difference in the prevalence of depression is not fully accounted for.[19]

A similar picture emerges in analyses of PTSD. Exposure to interpersonal violence, such as adult sexual assault, is a strong predictor of PTSD risk.[9] Meta-analysis indicates that the risk of PTSD is equal in men and women following experiences of adult sexual assault;[11] yet since women in general are exposed to greater rates of sexual assault,[9,20] it is plausible that this greater exposure rate accounts for at least some of the heightened risk of PTSD onset in women (hypothesis (ii)). Nevertheless, in general, women in the U.S. show a more than twofold increase in risk for PTSD following trauma,[21] and this twofold increased risk of PTSD persists across a wide range of trauma exposures, including accidents, nonsexual assault, combat or war, disaster or fire, witnessing death or injury, and serious illness or unspecified injury.[11] Together, these results suggest that women and men show differential susceptibility or sensitivity to risk (hypothesis (iii)). However, the mechanisms underlying this differential susceptibility of PTSD risk by sex, and the etiology of these differences, remain unknown.

In the following review, we propose that investigation of hypothesis (i) above—namely, that observed differences are due to differences in the pattern of risk factors that exist in males and females—may help to shed light on the etiology of sex differences in PTSD and depression. Specifically, we suggest that sex differences in DNAm, particularly those established during brain development, provide a plausible biologic mechanism to explain the heightened risk of PTSD and depression onset in females. This working hypothesis proposes that sex-specific differences in DNAm that arise during neurodevelopment lay the groundwork for subsequent sex differences in reactions to stress. Under this model, we envision these sex-specific methylation patterns are actual risk factors for mental disorders,[22,23] rather than markers of disease presence, although we recognize that some of these patterns are also modified by social contexts (e.g., single sex vs. mixed litters) that are known to affect anxiety-like behaviors.[23] Below, we synthesize existing literature on sex differences in DNAm levels during brain development based on work conducted in animal models. Given the emerging evidence for DNAm effects in association with stress, and the major influence of stress on both PTSD and depression, we also consider the evidence for stress-induced DNAm dysregulation in human and animal studies, provide an overview of known DNAm differences associated with PTSD and depression, and conduct exploratory analyses of sex differences in DNAm associated with these two mental disorders. Published studies demonstrating sex-specific epigenetic regulation associated with brain development or mental disorders cited throughout this chapter are summarized in Table 1.

Although we highlight primarily studies that specifically examine epigenetic sex differences in the brain, important work has also been done using peripheral blood from living human subjects. DNAm can be tissue-specific,[24] suggesting that epigenetic signatures in the blood may not reflect those within the brain, nor be associated with behavioral or psychological changes. Previous work, however, has demonstrated associations between peripheral DNAm and neural imaging,[25,26] transcriptomics,[27] and behavioral and psychiatric states.[28–30] Additionally, Klengel and colleagues have recently demonstrated a putative mechanism by which epigenetic responses to an environmental stimulus could be integrated across physiological systems: glucocorticoids induce similar DNAm changes at a particular genetic locus in peripheral blood cells and in neuronal progenitor cells.[25] Likewise, the authors reported an association between neuroanatomy of the hippocampus and a specific peripheral blood DNAm state,[25] suggesting that in some instances peripheral measures of DNAm have strong relevance to brain-related traits.

TABLE 1. Summary of sex-specific epigenetic regulation associated with brain development or mental disorders

Rodents					
			Major finding		
Gene name	Author	Year	Early postnatal period	Juvenile period	Adulthood
Esr1	Schwarz	2010	POA: Higher DNAm at two promoter CpG sites in females versus males (PN1).	POA: No sex-specific DNAm differences (PN20).	POA: Higher DNAm in females versus males at an alternate promoter CpG site (PN60).
			MBH: Higher DNAm at two promoter CpG sites in females versus males (PN1).	MBH: No sex-specific DNAm differences (PN20).	MBH: No sex-specific DNAm differences (PN60).
	Kurian	2010	POA: NA	POA: Higher DNAm at two promoter CpG sites in males versus females (PN8).	POA: NA
Esr2	Schwarz	2010	POA: No sex-specific DNAm differences (PN1).	POA: No sex-specific DNAm differences (PN20).	POA: Higher DNAm of one promoter CpG site in males versus females (PN60).
			MBH: No sex-specific DNAm differences (PN1).	MBH: Higher DNAm at one promoter CpG site in males versus females (PN20).	MBH: Higher DNAm at two alternate promoter CpG sites in females versus males (PN60).
Dnmt3a	Kolodkin	2011	A: Higher gene and protein expression in females versus males (PN1).	A: No sex-specific expression differences (PN10).	A: NA
			POA and MBH: No sex-specific expression differences (PN1).	POA and MBH: No sex-specific expression differences (PN10).	POA and MBH: NA
Mecp2	Kurian	2007	A and VMH: Higher gene and protein expression in females versus males (PN1).	A and VMH: No sex-specific expression differences (PN10).	A and VMH: NA
			POA: No sex-specific expression differences (PN1).	POA: Lower gene expression in females vs. males (PN10).	POA: NA
Crh	Sterrenburg	2011	PVN: NA	PVN: NA	PVN: Higher total DNAm in stressed adult females versus female controls; no corresponding effect in males.
			BST: NA	BST: NA	BST: Lower total DNAm in stressed versus nonstressed males; no corresponding effect in females. Sex-specific DNAm changes in response to stress at four CpG sites.
			CeA: NA	CeA: NA	CeA: Lower total DNAm in stressed females versus stressed males. Sex-specific DNAm changes in response to stress at five CpG sites.
Humans					
WNT1 *NR4A2* *LMX1B* *LHX5* *FOSB*	Mill	2008	FC: Higher DNAm of *WNT1*, *NR4A2*, and *LHX5* and lower DNAm of *FOSB* and *LMX1B* in females affected by major psychosis versus female controls. Similar patterns were not seen in males affected by major psychosis versus male controls. See Table S3 of original manuscript for full list of genes.		
SLC6A4	Beach	2010	LB: Elevated DNAm across promoter region in abused males. Elevated DNAm at specific CpG sites in abused females, but not males.		
	Philibert	2008	LB: Higher DNAm and lower mRNA expression in females versus males.		
Global methylation	Essex	2011	BC: Altered DNAm in adolescence associated with paternal stress during infancy and preschool years in girls, but not boys.		

A, amygdala; BC, buccal cell; CeA, central amygdala; FC, frontal cortex; LC, lymphoblast cell lines; MBH, medial basal hypothalamus; PN, postnatal day; POA, pre-optic area of the hypothalamus; PVN, paraventricular nucleus of the hypothalamus; BST, bed nucleus of the stria terminalis; VNMH, ventromedial hypothalamus.

DNA METHYLATION VARIATION MAY CONTRIBUTE TO SEX DIFFERENCES IN MENTAL DISORDERS

Epigenetic factors are plausible biologic candidates for mediating sex differences in risk of PTSD and depression. Broadly speaking, epigenetic factors refer to those mechanisms that regulate gene function without the alteration of underlying DNA sequence.[31] In contrast to DNA sequences, which are largely fixed, epigenetic factors are known to change in response to individuals' physical, biological, and social exposures in a manner that influences the long-term regulation of gene expression.[32–35] DNAm in particular is one of the major and best-studied epigenetic mechanisms to date. DNAm occurs in vertebrates through covalent modification of DNA, whereby methyl groups are typically coupled to cytosine residues when cytosine and guanine are separated by a phosphate (i.e., at a CpG site).[36] This chemical modification at specific DNA sequences regulates DNA accessibility, which in turn alters the transcriptional activity of the surrounding loci. In many cases, increased methylation in specific gene regions (e.g., promoters) is associated with reduced transcriptional activity and, therefore, gene expression.[37] Evidence that DNAm is involved in the molecular pathology of mental health disorders, including in the developing brain, has been well documented (reviewed in[38]).

Emerging work in animal models has begun to document the role that DNAm plays in establishing sex differences in the brain during development. This growing literature provides evidence supporting hypothesis (i) discussed above, namely that different patterns/risk factors exist between the sexes and, moreover, that these factors emerge across development. For example, sexual differentiation in the rodent brain is enacted by a process of masculinization in males during a sensitive period between embryonic day 18 and postnatal day 10 (PN10) via testosterone produced by the testes.[4] This hormonally mediated sexual differentiation occurs when testosterone reaches the brain and is converted in part to estradiol, which then acts through the nuclear receptors estrogen receptor (ER) alpha (encoded by *ESR1*) and ER beta (encoded by *ERS2)* to initiate male brain organization. Importantly, CpG sites in *ESR1* and *ESR2* show sex-specific DNAm levels that interact with hormones at critical periods of development to effect downstream trajectories of brain development, which differ in males and females.

The preoptic area (POA), for example, is a brain area known to be sexually dimorphic in rodents and important to male sexual behavior. Male rats have higher levels of methylation at two CpG sites within the *ESR1* promoter in the POA compared to females toward the end of the sensitive period (PN8);[39] in contrast, newborn females have significantly higher methylation levels compared to males at two alternate CpG sites in the *ESR1* promoter region.[5] Experimental treatment of female pups with estradiol confirms that the hormonal milieu of male pups induces increased methylation at one of the two male-associated CpG sites,[39] and reduces methylation at both of the female-associated CpG sites.[5] By 3 weeks of age, these *ESR1*-related sex differences are no longer apparent; yet the activating effect of hormones that circulate during adulthood induces an additional set of ER-related sex differences in promoter methylation not only in the POA (*ESR1*)[5] but also the mediobasal hypothalamus (MBH, *ESR2*).[5] Importantly, DNAm differences in these brain regions are also associated with gene expression differences that vary by sex, suggesting that sex-specific epigenetic variation is associated with downstream functional effects.[39] Together, these results suggest that a brief and temporary exposure to neonatal hormones may enact sex-specific effects on brain development through DNAm in ER regulatory regions, with differences that, in some instances, only become apparent in adulthood.[4,5,40,41]

Brain region-specific, developmentally transient sex differences in gene expression and regulation have been observed for additional hormone-responsive molecular processes with relevance to epigenetic mechanisms. DNA methyltransferase 3A (*DNMT3A*) and DNA methyltransferase 1 (*DNMT1*) are genes that encode two of the major enzymes responsible for, respectively, de novo methylation of CpG sites early in development and maintenance of CpG methylation during cell division. As such, sex-specific differential expression of DNMTs has important implications for understanding a variety of diseases. In female neonatal rats (PN1), *DNMT3A* gene and protein expression levels are higher in the amygdala—but not in the POA or MBH—compared to male neonatal rats;[42] by PN10, this sex difference in *DNMT3A* expression is no longer apparent. In contrast, no sex differences are observed in any brain region for *DNMT1* gene or protein expression at either time point.[42] Importantly, females treated with either estradiol benzoate or dihydrotestosterone at PN0 and PN1 show a decrease in *DNMT3A*—but not *DNMT1*—gene expression levels in the amygdala, confirming that the temporal and regional sex differences in *DNMT3A* are hormonally regulated.[42]

Similarly, transient sex differences in gene and protein expression levels of methyl CpG binding protein 2 (*MeCP2*) have been detected in neonatal rat brains. *MeCP2* is a member of the methyl CpG binding domain (MBD) family of proteins and is capable of binding specifically to methylated DNA, thereby recruiting histone deacytelases (HDACs) to induce chromatin condensation and/or block transcription factor binding,[43] both of which reduce gene expression. Gene and protein expression levels of *MeCP2* are lower in males versus females in both the amygdala and ventromedial hypothalamus (VMH), but not the POA, at PN1[44] and as with the *DNMT3A* example described above, these differences are no longer apparent at PN10.[44] Although,

to our knowledge, hormonal influences on *MeCP2* have not been directly tested and/or reported (but see, however, recent work implicating stress hormones in the regulation of Rett syndrome, an *MeCP2*-linked phenotype[45]), the detection of an expression difference in the immediate postnatal period is consistent with the period in which male rats have a surge in testicular testosterone, which may regulate the differential expression of *MeCP2*. Importantly, another methyl CpG binding protein—*mbd2*—is expressed at equal levels between the sexes through PN10,[44] indicating a specific role for *MeCP2* in effecting sexual differentiation in the rat brain.

In humans, profiles of epigenetic changes by sex during brain development, akin to those described above for rodents, are not yet readily available. Nevertheless, some inferences can be drawn from existing literature based on clinical studies. The X-linked *MeCP2* gene has been implicated in brain-related disorders, in particular Rett syndrome, a neurodevelopmental disorder diagnosed predominantly in females as mutations in this locus are generally thought to be lethal in males. More recently, aberrant *MeCP2* DNAm patterns have been linked to autism[46] with male autism patients showing a slight increase in DNAm in an upstream regulatory region of *MeCP2* that was not observed in females with or without autism.[46] In addition, despite the current dearth of direct evidence for sex-specific epigenetic profiles of brain development in humans, indirect evidence does exist for the influence of sex-specific patterns in frontal cortex DNAm levels on risk for mental disorders. For example, sex-specific methylation patterns have been reported in genes involved in neuronal development in postmortem studies of brains obtained from individuals with major psychosis (MP, schizophrenia and bipolar disorder). Genes implicated in this sex-specific epigenetic dysregulation include *WNT1*, a locus involved in the Wnt signaling pathway known to be important in neurodevelopment,[47] which shows significantly higher methylation in female (but not male) MP patients versus controls. The same study also identified increased DNAm levels in *NR4A2*, whose expression controls differentiation of dopaminergic neurons,[48] in female MP patients, as well as additional neurodevelopmental genes (i.e., *LMX1B*, *LHX5*, *FOSB*) that showed contrasting patterns of DNAm in female—but not male—MP patients versus controls. More generally, sex differences in epigenetic factors influencing brain development have recently been identified as a potential source of the sexually dimorphic risk for, or resilience to, developing neurological and mental health disorders later in life, including attention deficit hyperactivity disorder, autism spectrum disorder, and Rett syndrome.[3] Although PTSD and depression have yet, to our knowledge, to be specifically included in such inferences of risk, the stark divergence between the sexes in prevalence of these disorders, discussed above, suggests that they are prime candidates to be considered in such etiologic models.

STRESS-INDUCED EPIGENETIC DYSREGULATION

In addition to providing evidence for epigenetic factors that influence brain development in a sex-specific manner, work in animal models has provided ample evidence of the potential for epigenetic changes to occur in response to external stressors, particularly those that occur early in life. The earliest work in this area did not specifically examine the sex specificity of these epigenetic changes; however, results from these studies convincingly established that epigenetic changes occur in response to external stressors with relevance to mood anxiety disorders such as PTSD and depression. For example, foundational work by Meaney and colleagues[34] exploited the naturally occurring variations in maternal care among rats to investigate the emergence of DNAm differences during the early postnatal period: rat pups born to mothers who provide a high degree of licking and grooming, as well as arched back nursing, show reduced levels of hippocampal methylation, and increased gene expression at the *NR3C1* glucocorticoid receptor locus—an important modulator of the stress response and integral component of the hypothalamic–pituitary–adrenal (HPA) axis—compared to pups born to mothers who provide less licking and grooming and nonarched back nursing.[34] These differences emerge during the first week of life and persist until adulthood, and are concordant with differences in HPA axis responses to stress during adulthood, indicating a developmentally sensitive period during which behavioral responses later in life may be epigenetically programmed.[34] Similar findings regarding the effects of early life stress (ELS) on DNAm in rodents have been reported for additional stress-relevant loci in other brain areas. In some cases (e.g., *ERS1* in the medial POA, brain-derived neurotrophic factor (*BDNF*) in the prefrontal cortex [PFC]), the direction of effect mirrors that observed in *NR3C1*, that is, exposure to ELS is associated with increased DNAm[35,49] whereas in others, the effect is in the opposite direction (e.g., *Avp* in the PVN).[50] Taken together, these results confirm that stress-induced changes in DNAm occur across multiple loci and in multiple brain regions, producing phenotypes associated with increased stress reactivity that can persist into adulthood.

More recently, investigators have begun to examine epigenetic differences associated with mood anxiety disorders in which stress is known to play a role in their etiology, such as depression and anxiety disorders. Importantly, the etiology of these epigenetic differences is not limited to ELS exposures. For example, in an animal model of PTSD, adult male rats have been used to investigate the effects of chronic psychosocial stress on brain DNAm in the *BDNF* gene. Among rats subjected to the stress paradigm, significant hypermethylation in the *BDNF* promoter region was identified compared to nonstressed controls in the dorsal CA1 region of the hippocampus,[51] a brain region important to learning and memory and implicated in PTSD etiology in

human studies.[52] Similarly, investigation of an additional HPA axis relevant gene, corticotropin-releasing hormone (*Crh*, also known as *CRF)*, revealed that, in male mice, chronic social defeat experienced during adulthood induces *Crh* promoter region demethylation in hippocampal DNA.[53] Importantly, this reduced *Crh* DNAm is only observed among adult mice susceptible to the chronic stressor, that is, those mice that avoided interacting with an unfamiliar mouse following the chronic stressor; notably, these effects were observed 2 weeks after the chronic stress exposure, indicating relatively long-term effects of social defeat on *Crh* DNAm levels. That these results were obtained from adult rats indicates that DNAm is an active process throughout the life course, modifiable by environmental exposures even in the mature central nervous system.

More recent work including both males and females has established that chronic variable mild stress produces sex-specific effects in *Crh* methylation assessed in DNA from the PVN in 2-week-old rats. Total DNAm across the *Crh* region showed that methylation was consistently higher in stressed females but not stressed males,[7] and analysis of individual CpG sites showed that these effects were specific to females at two particular CpG sites within the promoter region. Sex-specific *Crh* DNAm differences were also observed in the bed nucleus of the stria terminalis and the central amygdala, with each region showing differential methylation in unique CpG sites.[7] These differences may thus reflect epigenetic mechanisms that mediate adaptation to stress in a sex-specific manner.

In humans, as in animal models, few studies have examined the sex specificity of DNAm differences in response to stress. Nevertheless, recent findings from the human-based literature echo those from animal models in demonstrating that epigenetic changes occur in association with external stressors that have relevance to mood anxiety disorders. For example, among adult male suicide victims with a history of childhood abuse, increased DNAm has been observed at the *NR3C1* locus (analagous to the same locus in rats), when compared to suicide victims without such a history and other postmortem controls.[54] This pattern of relative *NR3C1* hypermethylation was echoed by two additional studies in humans: one in which increased maternal depressed/anxious mood in the third trimester showed an association with increased DNAm at a predicted nerve growth factor-inducible protein A (NGFI-A) binding site in cord blood-derived DNA, and with increased infant salivary cortisol stress responses at 3 months,[55] and another, more recent study, which identified increased whole blood-derived DNAm levels among healthy adults who reported exposure to childhood adversity, including childhood maltreatment, parental loss, and poor parental care.[56] ELS has also been linked to DNAm changes at the serotonin transporter (*SLC6A4*) locus in DNA derived from epstein barr virus (EBV)-transformed lymphoblastoid cell line samples drawn from the Iowa Adoption Study; specifically, child abuse was associated with significantly elevated methylation levels across the entire promoter region in abused versus nonabused males and, among abused versus nonabused females, at specific CpG sites.[57,58] Genome-scale investigations have also detected epigenetic differences associated with ELS exposure: compared to adolescents whose mothers were unexposed to high stress levels during their first year of life, adolescents whose mothers reported high stress levels during their infancy showed higher DNAm levels at 139 CpG sites in buccal cell-derived DNA.[6] Results from this study identified important developmental and sex-specific effects of adversity, with increased maternal stress associated with differential DNAm during adolescence in both sexes whereas increased paternal stress during the preschool years was significantly associated with altered DNAm during adolescence in girls only.[6]

Together, these data from animal and human studies suggest that early life experiences, and especially adverse early life experiences, have the potential to alter epigenetic gene regulation and downstream gene function with lasting physiologic, behavioral, and psychological implications. Emerging work further suggests that at least some of these stress-associated DNAm changes show distinct patterns between the sexes.

DNA METHYLATION DIFFERENCES ASSOCIATED WITH PTSD AND DEPRESSION

As with work in animal models, recent human-based studies have moved beyond investigations of ELS to examine epigenetic differences associated with commonly occurring mood anxiety disorders such as PTSD and depression. Here, population-based and/or cohort studies have provided important initial evidence of DNAm differences associated with these disorders. Early work based on Iowa Adoption Study derived samples showed that *SLC6A4* mRNA expression was significantly associated with both DNAm in the CpG island promoter region and *5-HTTLPR* genotype, with a trend suggesting the *l* allele was associated with decreased methylation,[59] and that *SLC6A4* promoter region DNAm was significantly higher, and mRNA expression levels significantly lower, in females compared to males.[60] Although the later work did detect the suggestion of a significant difference between those with versus without a history of major depression, the finding was only marginally ($P < .07$) significant.[60] Subsequent *in vivo* work provided stronger evidence of an *SLC6A4* methylation-depression association. Examination of DNAm levels from buccal cells in a prospective cohort of Australian adolescents found a joint effect of methylation and *s* allele carriage on risk for depression, such that adolescents with the highest tertile of methylation in a subregion of the promoter-associated CpG island in the *SLC6A4* locus and who also carried one or more *5-HTTLPR s* alleles were at approximately fivefold higher risk for persistent depressive symptoms. In contrast, work investigating the

role of epigenetic factors in vulnerability/resilience to trauma found that the long, *l* allele, in combination with high methylation levels in the *SLC6A4* CpG island promoter region, predicted more unresolved loss or trauma among adults.[61] The opposing direction of these findings compared to those reported for depression suggests the possibility that different combinations of DNAm and DNA sequence at the *SLC6A4* locus may show divergent patterns depending on the outcome of interest (e.g., depression versus trauma); however, additional study variables, such as age and DNA source, cannot be ruled out as contributors to these opposing patterns based on the few currently available studies.

In addition to these candidate gene examples, recent genome-scale work has demonstrated that DNAm profiles across multiple genes distinguish between those with versus without depression and PTSD in samples drawn primarily from urban, predominantly African-American population-based studies. Using whole blood-derived DNA samples from a longitudinal study of adult Detroit residents, investigators found that presence/absence of lifetime depression status was associated with DNAm differences in genes implicated in brain development/neurogenesis, tryptophan metabolism, and lipoprotein-related functions—that is, pathways or processes previously implicated in the etiology of this disorder.[62] Similarly, recent genome-scale work performed on studies conducted in Detroit and Atlanta has provided evidence that DNAm profiles differ between those with versus without PTSD, with differences found predominantly in immune system related genes[28,29] and in genes relating to developmental processes in general, and neurogenesis in particular.[28] The Atlanta-based study has also shown that PTS symptoms are positively correlated with blood DNAm levels at the adenylate cyclase activating polypeptide 1 (pituitary) receptor type I (*ADCYAP1R1*) locus,[30] a gene involved in moderating the stress response in both the central and peripheral nervous systems.[63] Finally, recent work on longitudinal samples drawn from U.S. military service members has identified increased DNAm in LINE-1 repetitive sequences among service members without PTSD post- versus predeployment, a finding that was more pronounced when the sample was restricted to males[64], and increased DNAm in Alu repetitive sequences in pretrauma PTSD cases versus controls predeployment, a finding that was more pronounced when the sample was restricted to females.[64] These repetitive sequences comprise a large fraction of the human genome and are indicators of global DNAm patterns.

Though these data provide some evidence of a biologic embedding of posttraumatic stress, it is likely that there are also both molecular and environmental features that render some individuals more susceptible to PTSD. Potential epigenetic examples of this come from both the Detroit- and Atlanta-based studies. In the former, *SLC6A4* methylation status was tested for interaction with number of potentially traumatic events (PTEs) to predict PTSD.[65] Results showed a significant interaction whereby *SLC6A4* methylation modified the effect of cumulative trauma on risk for PTSD, such that individuals with increased numbers of PTEs were at increased risk for PTSD at lower methylation levels; however, individuals with more PTEs were protected from this disorder at higher methylation levels, and among individuals with fewer PTEs, this predicted pattern was reversed.[65] In the Atlanta-based study, genotype and DNAm of *COMT* was assessed for their relation to fear inhibition in PTSD.[66] Results showed that the Met/Met genotype—associated with less efficient COMT function in the PFC—was associated with DNAm at multiple CpG sites, and that higher methylation at two of these sites was associated with impaired fear conditioning in a fear-potentiated startle paradigm.[66] These examples suggest that individuals' preexisting methylation levels at certain loci may moderate their experience of stress and trauma in a manner salient to PTSD etiology, suggesting potential molecular signatures of increased risk for—or resilience to—this disorder.

EXPLORATORY ANALYSIS

In addition to the above-described published studies, we have undertaken an exploratory analysis of the nature and extent of differential methylation between males and females in our subsample of 100 participants in the Detroit study with available methylation data. Following removal of ~1,500 CpG sites annotated to the X or Y chromosome on Illumina's HM27 Beadchip microarray, we detected significant (uncorrected $P < .05$) sex differences in the trauma-exposed/PTSD-free subsample in 625 unique genes. Functional annotation clustering analyses[67] indicated that the three most overrepresented biologic functions/pathways among these genes included membrane, lipoprotein, and regulation of hormone secretion (Table 2). In contrast, among the trauma-exposed/PTSD subsample, we detected significant differences in methylation level by sex in 496 genes, with the Wnt signaling pathway, membrane, and neurological system process ranking as the top three most overrepresented functions associated with these genes; notably, the Wnt signaling pathway has been implicated in the etiology of mood disorders.[68,69] In addition, many of the genes known to contribute to sex differences in brain development,[70] or that are believed to serve as coactivators of sex steroids in the brain,[71,72] also show sex-specific patterns relating to PTSD, for example *HDAC1* and *DNMT3B*, which show significant DNAm differences between males with versus without PTSD, and *DNMT3L* and *UBE3A*, which show significant DNAm differences between females with versus without PTSD. Moreover, preliminary multiple linear regression analyses of the *SLC6A4* locus (assessed at CpG site cg2258413 on the HM27 BeadChip) show that PTS severity is significantly associated with both DNAm ($P = .035$) and childhood abuse ($P = .022$) in females, but not males ($P = .65$ and $P = .12$, respectively). Although

TABLE 2. Functional annotation cluster analyses of genes[a] showing significant[b] differences in DNAm by sex

Comparison	Cluster	Number of genes	Enrichment score[c]
Males versus females without PTSD	Membrane	247	2.27
	Lipoprotein	43	2.21
	Regulation of hormone secretion	27	2.1
Males versus females with PTSD	Wnt signaling pathway	13	1.18
	Membrane	191	1.16
	Neurological system process	46	0.88

[a]All genes are identified in terms of DAVID IDs;[67] genes can appear in more than one cluster.
[b]Defined as $P < .05$.
[c]Defined as the geometric mean of the annotation cluster P values, in negative log scale.

the extent of DNAm concordance between blood and brain in these specific examples is unknown, recent work suggests that there is considerable within-individual correlation between DNAm levels in these two tissues.[73] Taken together with the above-described animal and human studies, these exploratory results suggest that sex differences in DNAm profiles across multiple genes and at specific candidate loci may plausibly contribute to sex differences in the prevalence of PTSD and depression.

OUTLOOK

Although a majority of American men and women are exposed to trauma in their lifetime, only a minority go on to develop PTSD.[74] Depression is the most commonly occurring mood disorder in the United States and is predicted to be the leading cause of disease burden worldwide by the year 2030.[75] Collectively, mental disorders such as PTSD and depression result in impairment of proper role functioning that is significantly worse than in a number of commonly occurring chronic medical disorders, such as diabetes and heart disease.[76] The high burden of disease associated with PTSD and depression, and the stark sexual dimorphism in the prevalence of these disorders between men and women, highlights the need to gain an improved understanding of their etiology to improve chances for prevention and treatment. In the context of earlier work outlining strategies to understand causal mechanisms contributing to sex differences in psychopathology,[14] here, we have briefly discussed the following three competing hypotheses that have been advanced to explain the mechanisms mediating the observed sex differences in PTSD and depression onset: that the differences may be due to different patterns of risk factors between the sexes, that they may be due to differential exposure to risk factors, or that they may be due to differential susceptibility to risk factors. We have proposed in particular that a focus on the first of these hypotheses—namely investigating sex differences in DNAm in genes influencing brain development—may help to shed light on the sexually dimorphic risk for, or resilience to, developing PTSD and depression later in life. Though we have limited our discussion in this review to the role of DNAm, we recognize the potential importance of other mechanisms of epigenetic regulation that are marked by sex differences important to brain development—namely, histone modifications[77] and miRNA expression.[78] Although this area of research is still in its infancy, emerging work across multiple disciplines, as synthesized in this review, suggests that the time is ripe for pursuing this avenue of inquiry.

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