Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2016 January ; 171(1): 81–91. doi:10.1002/ajmg.b.32395.

RDoC and Translational Perspectives on the Genetics of Trauma-Related Psychiatric Disorders

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Abstract

Individuals with a history of child abuse are at high risk for depression, anxiety disorders, aggressive behavior, and substance use problems. The goal of this paper is to review studies of the genetics of these stress-related psychiatric disorders. An informative subset of studies that examined candidate gene by environment (GxE) predictors of these psychiatric problems in individuals maltreated as children is reviewed, together with extant genome wide association studies (GWAS). Emerging findings on epigenetic changes associated with adverse early experiences are also reviewed. Meta-analytic support and replicated findings are evident for several genetic risk factors; however, extant research suggests the effects are pleiotropic. Genetic factors are not associated with distinct psychiatric disorders, but rather diverse clinical phenotypes. Research also suggests adverse early life experiences are associated with changes in gene expression of multiple known candidate genes, genes involved in DNA transcription and translation, and genes necessary for brain circuitry development, with changes in gene expression reported in key brain structures implicated in the pathophysiology of psychiatric and substance use disorders. The finding of pleiotropy highlights the value of using the Research Domain Criteria (RDoC) framework in future studies of the genetics of stress-related psychiatric disorders, and not trying simply to link genes to multifaceted clinical syndromes, but to more limited phenotypes that map onto distinct neural circuits. Emerging work in the field of epigenetics also suggests that translational studies that integrate numerous unbiased genome-wide approaches will help to further unravel the genetics of stress-related psychiatric disorders.

Keywords

child abuse; psychiatric disorders; genetics; epigenetics; RDoC

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INTRODUCTION

Early life stress is associated with the development of a wide range of psychiatric problems. Maltreated children are at increased risk of experiencing major depression (MDD), Posttraumatic Stress Disorder (PTSD), other anxiety disorders, aggressive behavior, and substance use disorders [Grasso et al., 2009; Afifi et al., 2012]. In addition, child maltreatment can serve as a strong predictor of early onset of illness, increased comorbidity, and poor treatment response [Nanni et al., 2012; Teicher and Samson, 2013]. This paper reviews research on the genetics of stress-related psychiatric disorders, including a discussion of candidate gene and genome wide association studies (GWAS). In this manuscript, stress-related disorders include MDD, PTSD, other anxiety disorders, aggressive behavior, and substance abuse problems, as individuals with a history of child maltreatment are at elevated risk for these problems. The results of candidate gene by environment (GxE) and GWAS both provide evidence of pleiotropy; genetic risk factors for stress-related psychiatric diseases do not map on to distinct Diagnostic and Statistical Manual (DSM) disorders [APA, 2000, 2013]. The results of the GWAS also highlight the importance of epigenetic gene regulation mechanisms, so the last section provides a brief overview of emerging findings relevant in understanding the epigenetics of stress-related psychiatric disorders. Given the results of this review, the benefit of incorporating the Research Domain Criteria (RDoC) framework, and the value of translational studies examining the effects of early stress in model organisms is then highlighted in the closing section of this paper. The role of mitochondrial DNA in stress related psychiatric disorders [Flaquer et al., 2015], and the effects of early stress on telomere length [Ridout et al., 2015], are considered beyond the scope of this review and are not discussed in the current manuscript.

GxE Studies of Child Abuse and Psychopathology: Evidence for Pleiotropy

Following the seminal study of Caspi et al. [2002] showing that risk for antisocial behavior in individuals maltreated as children is moderated by *MAOA* genotype [Caspi et al., 2002], there have been over a hundred studies published which examined the moderating effects of various candidate gene variants on a range of mental health outcomes among individuals with a history of abuse. Consistent with other data in the field showing that SNPs do not map on to distinct DSM diagnoses, but rather individual SNPs are associated with a range of psychiatric disorders of childhood and adult onset [PGC, 2013], results of the GxE candidate gene studies demonstrate pleiotropy in the genetics of stress-related psychiatric disorders, with each candidate gene examined associated with a variety phenotypic traits. As depicted in Table I, the serotonin transporter gene (*5-HTTLPR*) and the *MAOA* gene have been found to moderate the risk for depression, PTSD or anxiety, aggression, and substance use problems in individuals with a history of abuse. These studies are briefly reviewed below. There is also evidence for pleiotropy in studies examining genetic variation at the catechol-O-methyltransferase (*COMT*) gene [Drury et al., 2010; Perroud et al., 2010; Humphreys et al., 2014], the brain derived neurotrophic factor (*BDNF*) gene [Wagner et al., 2010; Comasco et al., 2013; Min et al., 2013; Cicchetti and Rogosch, 2014; Nilsson et al., 2015], the corticotropin releasing hormone receptor (*CRHRI*) gene [Bradley et al., 2008; Heim et al., 2009; Polanczyk et al., 2009; Grabe et al., 2010; Nelson et al., 2010; Kranzler et al., 2011;

Laucht et al., 2013] and the FK506 binding protein 5 (*FKBP5*) gene [Binder et al., 2008; Xie et al., 2010; Appel et al., 2011; Bevilacqua et al., 2012; van Zuiden et al., 2012; Levran et al., 2014]. For illustrative purposes, however, the review below only focuses on studies examining the moderating effect of *5-HTTLPR* and *MAOA* genes, as these two candidate genes have been most extensively studied, with the broadest range of phenotypes examined.

The greatest number of GxE studies conducted to date has examined the moderating effect of the serotonin transporter gene (*5-HTTLPR*) on the development of depression following experiences of child maltreatment and other stressful life events [Caspi et al., 2003]. There have been several meta-analyses of these data [Munafo et al., 2008; Risch et al., 2009; Karg et al., 2011; Sharpley et al., 2014], and some controversy regarding the strength of this association [Munafo et al., 2009; Risch et al., 2009; Kaufman et al., 2010], but the largest of these meta-analyses which included 81 studies with 55,269 participants supports the conclusion that the s-allele of *5-HTTLPR* increases risk for depression following child maltreatment and other stressful life events [Sharpley et al., 2014].

This GxE interaction has been found to be enhanced in individuals possessing the met allele of the val66met polymorphism of the brain derived neurotrophic factor (*BDNF*) gene in five independent investigations [Kaufman et al., 2006; Grabe et al., 2012; Comasco et al., 2013; Cicchetti and Rogosch, 2014; Gutierrez et al., 2015], with only one study in the literature failing to replicate this three-way interaction [Aguilera et al., 2009]. In addition, the availability of a positive social support has been found to ameliorate risk for depression associated with a history of abuse and both *5-HTTLPR* and *BDNF* high-risk genotypes [Kaufman et al., 2004, 2006].

Far fewer studies have examined the moderating effect of *5-HTTLPR* on other outcomes, but research findings are generally consistent. The s-allele, which seems to be associated with reduced ability to buffer serotonin in the brain, has been associated with greater anxiety sensitivity [Stein et al., 2008] and higher risk of developing PTSD in individuals with a history of child maltreatment or other childhood adversities [Xie et al., 2009, 2012], with the findings less robust in African American cohorts [Xie et al., 2012; Walsh et al., 2014]. The s-allele has also been found to predict antisocial behavior in maltreated children and adolescents [Cicchetti et al., 2012; Nilsson et al., 2015], and aggressive behavior in adults who experienced childhood adversity or chronic stress [Reif et al., 2007; Conway et al., 2012], with the s-allele a less consistent predictor of the more complex multifaceted antisocial personality disorder phenotype in adult cohorts [Li and Lee, 2010; Douglas et al., 2011; Sadeh et al., 2013]. Among individuals with a history of maltreatment, the s-allele has also been associated with early initiation of alcohol use [Kaufman et al., 2007] and problematic cannabis use [Vaske et al., 2012].

In terms of GxE studies examining the *MAOA* gene, an early meta-analysis of five studies of male cohorts supported Caspi's original finding that the *MAOA* genotype conferring low versus high *MAOA* activity increases risk for the development of antisocial behavior in males maltreated as youth [Kim-Cohen et al., 2006]. This conclusion was recently again confirmed in a meta-analysis of 20 studies of male cohorts [Byrd and Manuck, 2014]. The

association, however, was not present in the meta-analyses of the 11 available studies conducted with female participants [Byrd and Manuck, 2014].

In terms of *MAOA* genotype and other psychiatric outcomes, three studies reported the *MAOA* genotype conferring low vs high *MAOA* activity also increases risk for depression in individuals with a history of child maltreatment [Cicchetti et al., 2007; Nikulina et al., 2012; Melas et al., 2013], although one study reported the opposite association with the high activity allele conferring risk for depression in individuals with a history of child maltreatment [Beach et al., 2010]. Findings predicting alcohol misuse are also somewhat contradictory, with one study finding the *MAOA* genotype conferring low versus high *MAOA* activity associated to increased risk for alcohol problems in the sexually abused women in the study [Ducci et al., 2008], another study reporting the finding limited to African American women [Nikulina et al., 2012], and a third study reported the association only among adolescent males, with the genotype conferring high *MAOA* activity increasing risk among the adolescent girls with a history of sexual abuse [Nilsson et al., 2011]. To the best of our knowledge, no studies examined the association between *MAOA* genotype and the development of PTSD in individuals with a history of child maltreatment. There is one preliminary report, however, that suggests the low-active *MAOA* allele shows a trend toward moderating risk for anxious traits in males with histories of aversive experiences in childhood [Baumann et al., 2013].

Pleiotropy may be due to overlapping symptoms across diagnoses [APA, 2000, 2013], or the high rates of comorbidity among disorders [Kessler et al., 1994; Kaufman and Charney, 2000; Kessler et al., 2012], which is true even among substance use disorders and other diagnoses that share no common symptoms [Kessler et al., 1997]. Alternatively, a central tenet of the RDoC initiative is that pleiotropy and comorbidity can occur because the various DSM diagnoses are associated with abnormalities in common inter-locking brain circuits [Etkin and Cuthbert, 2014]. Moving beyond DSM diagnoses has been recommended in the past [Gottesman and Gould, 2003], and using an RDoC framework that incorporates dimensional assessments of behaviors that map onto discrete brain circuits, research on the genetics of stress-related psychopathologies would likely be advanced [Cuthbert, 2014; Ford et al., 2014]. As noted by others [Kaffman and Krystal, 2012], clinicians can diagnose major depression by selecting five from a pool of nine possible criteria, many of which can deviate from baseline in either direction (e.g., decreased or increased sleep). This approach can result in two individuals diagnosed with depression with no common symptoms. This heterogeneity within a given diagnostic category is also believed to have contributed to inconsistency in research findings across investigations, and the slow progress in identifying robust genetic biomarkers associated with mental illnesses [Kaffman and Krystal, 2012].

As a methodological aside before moving on to discuss the GWAS data, in the large-scale meta-analysis of 5-*HTTLPR* GxE studies, the greatest effect size was observed in studies that utilized objective measures to assess child maltreatment experiences [Sharpley et al., 2014]. The importance of obtaining objective measures has been demonstrated in our research with maltreated cohorts as well; in one study when we compared rates of disclosure via direct parent and child interview to rates of abuse documented in child protective services records, parents and children failed to report over one-third of documented

incidents of physical and sexual abuse [Grasso et al., 2009]. Parents, however, were good informants about experiences of domestic violence, and at times reported incidents of past abuse not known to protective services. For our own research, we have developed reliable methods to integrate data from multiple informants and data sources to derive best-estimates of children's maltreatment and other adverse experiences [Kaufman et al., 1994; Holbrook et al., 2015].

GWAS: Additional Evidence for Pleiotropy and an Emerging Role for Epigenetic Mechanisms in the Etiology of Stress-Related Psychiatric Disorders

Genome-wide association studies (GWAS) move beyond *a priori* hypothesis of relevant risk factors, and offer the potential for the identification of novel genetic markers and molecular pathways relevant for understanding risk for the development of complex traits including psychiatric and substance use disorders. Despite moderate heritability estimates for depression, PTSD, aggressive behavior, and substance use disorders [Pickens et al., 1991; Coccaro et al., 1993; True et al., 1993; Bierut et al., 1999; Maes et al., 1999], the few GWAS published to date seeking to identify genetic markers of these psychiatric problems have identified only a few findings that have been genome-wide significant and replicated in independent samples [Maher, 2008; Lee et al., 2011; Thapar and Harold, 2014]. In addition, the amount of variance accounted for by significant SNPs is relatively small [Bierut, 2009; Rietschel and Treutlein, 2013]. It has been proposed that unexplained phenotypic variation is in part due to undetected gene by environment (GxE) interactions [Bookman et al., 2011; Mechanic et al., 2012; Hutter et al., 2013].

The most effective way to detect GxE interactions in GWAS data sets remains to be determined, but simulated and applied work has led to the development of novel analytic approaches [Murcray et al., 2009; Bookman et al., 2011; Mechanic et al., 2012; Hutter et al., 2013; Sohns et al., 2013; Almli et al., 2014a]. The most appropriate analytic methods appears to depend on the strength of G-E correlations [Sohns et al., 2013]. Characteristics of the sample, rates of environmental exposure, and measurement error in environmental and phenotypic assessments also impact power to detect GxE interactions [Bookman et al., 2011]. In addition, heteroscedasticity, defined as variability in outcomes that differs by the value of the environmental exposure, can inflate GxE estimates, leading to recommendations to utilize robust joint tests over traditional joint tests of gene and gene-environment interactions [Almli et al., 2014a].

PTSD, by definition, requires trauma exposure for the development of PTSD symptoms. There have been two published studies that examined over 3,000 candidate SNPs in association with the diagnosis of PTSD [Liberzon et al., 2014; Solovieff et al., 2014], and five published GWAS focused on PTSD [Guffanti et al., 2013; Logue et al., 2013; Xie et al., 2013; Liberzon et al., 2014; Solovieff et al., 2014; Almli et al., 2015; Nievergelt et al., 2015], with additional large-scale GWAS on the horizon with samples collected as part of the Psychiatric Genomics Consortium for PTSD [Logue et al., 2015].

In the one study that examined 3755 SNPs, the adrenergic beta2 receptor (*ADRB2*) gene was found in two independent samples to interact with childhood adversity (e.g., physical abuse, sexual abuse, emotional abuse, witnessing domestic violence between parents), to affect risk

for development of PTSD symptoms following adult trauma [Liberzon et al., 2014]. *ADRB2* is biologically plausible as a genetic risk factor as the β 2-adrenergic receptor is a major transducer of the sympathetic nervous system and the fight-or flight response. While *ADRB2* has to date not been linked with risk for any other psychiatric disorder, the β 2-adrenergic receptor is a member of the G protein–coupled receptor super-family and associates intracellularly with the calcium channel encoded by the *CACNA1C* gene [Bhat et al., 2012], and *CACNA1C* is one of the most replicated and strongest signals for psychiatric vulnerability identified to date, associated with risk for both bipolar disorder and schizophrenia in large GWAS [Alsabban et al., 2011; Ripke and Sanders, 2011; Liberzon et al., 2014].

In the second study that examined over 3,000 candidate SNPs [Solovieff et al., 2014], PTSD diagnosis was associated with a SNP (rs363276) in *SLC18A2*, a vesicular monoamine transporter. A haplotype analysis of nine SNPs in *SLC18A2* identified a risk haplotype, with the same risk haplotype associated with PTSD in an independent cohort. *SLC18A2* has been implicated in a number of neuropsychiatric disorders, including depression. Polygenic analyses were also conducted within this cohort. The findings in this cohort suggested that there are SNPs in common between PTSD and bipolar disorder [Solovieff et al., 2014], with associations between PTSD and the bipolar disorder polygenic risk score also reported in another independent GWAS sample [Nievergelt et al., 2015].

The first PTSD GWAS conducted reported an association between PTSD and the retinoid-related orphan receptor alpha (*RORA*) gene [Logue et al., 2013], a gene which has also been associated with multiple other psychiatric disorders including depression [Terracciano et al., 2010], bipolar disorder [Le-Niculescu et al., 2009], attention deficit hyperactivity disorder [Neale et al., 2008], and autism [Sarachana et al., 2011]. Variation in the *RORA* gene, in addition to predicting onset of PTSD symptoms [Logue et al., 2013], is associated with more persistent course of PTSD symptoms, with a significant GxE interaction also detected such that the impact of *RORA* genotype on course of illness was found to be most pronounced in individuals with a history of childhood physical abuse [Lowe et al., 2015].

The second PTSD GWAS reported genome wide significant associations at the *TLL1* Locus and a SNP (rs406001) in an intergenic region located approximately 630 kilobases from the gene Cordon-Bleu (*COBL*), with two other SNPs in close proximity in this intergenic region also strong predictors of the PTSD phenotype [Xie et al., 2013]. In follow-up analyses of an independent sample, these three intergenic SNPs were found to interact with a childhood history of abuse to predict PTSD symptoms [Almli et al., 2014b]. In addition, the SNP rs406001 in the intergenic region which met criteria for genome wide significance in the original study accounted for individual differences in brain imaging findings available on a subset of subjects, such that the that risk allele carriers evidenced alterations in white matter integrity in the uncinate fasciculus which serves as a primary connection between the amygdala and ventral aspects of the prefrontal cortex, with the uncinate fasciculus a region that is thought to play a role in extinction of learned fear [Almli et al., 2014b].

The third GWAS reported an association with PTSD at genome wide significance for SNP rs10170218, which is located within AC068718.1 on chromosome 2, a novel long intergenic

non-coding RNA (lincRNA) gene [Guffanti et al., 2013]. lincRNA is believed to exert a regulatory role on other genes, affecting protein expression, DNA binding, and transcriptional function [Guttman and Rinn, 2012].

The fourth GWAS reported a genome-wide significant association with PTSD and the phosphoribosyl transferase domain containing one gene (*PRTFDC1*), with a similar effect across ancestry groups, and association of *PRTFDC1* with PTSD showing some evidence for replication in an independent cohort [Nievergelt et al., 2015]. *PRTFDC1* has been reported as a possible tumor-suppressor gene, and to date has not been implicated in other investigations of the genomics of PTSD or other psychiatric disorders, so its potential role in the etiology of PTSD remains to be determined.

The fifth GWAS reported an association with PTSD at genome wide significance on chromosome four for SNP, rs717947, also in an intergenic region [Almli et al., 2015]. Genetic variation in this SNP predicted individual differences in methylation at nearby CpG sites, and the risk allele of rs717947 was associated with altered medial and dorsolateral prefrontal activation to fearful faces in the subset of subjects with available neuroimaging data [Almli et al., 2015]. Much is still to be learned of the role of intergenic genomic regions in gene regulation, and the role of epigenetic mechanisms in disease risk. The next section discusses the value of using translational research approaches to advance knowledge in this area.

Epigenetics of Stress-Related Psychiatric Disorders: Translational Studies

This section focuses on preclinical (e.g., animal) studies, as the role of epigenetic mechanisms was first elucidated in preclinical models, and a fewer number of postmortem epigenetic studies have been conducted in humans than in non-human animal studies examining the impact of early life stress.

Research by Meaney and colleagues provided the first evidence that maternal behavior could produce stable alterations of DNA methylation and chromatin structure, providing a mechanism for the long-term effects of early adversity on gene expression in the offspring [Weaver et al., 2004]. Utilizing a rat model of neglect, operationalized as decreased maternal pup licking and grooming and arched-back nursing (LG-ABN), reduced LG-ABN was associated with altered offspring epigenome at the glucocorticoid receptor (GR) gene promoter in the hippocampus. Offspring of mothers that showed low levels of LG-ABN were found to have increased DNA methylation of the GR gene when compared to offspring of “non-neglectful” mothers. These differences emerged early in life, were reversed with cross-fostering, persisted into adulthood, and were associated with altered histone acetylation and transcription factor (NGFI-A) binding to the GR promoter. Central infusion of a histone deacetylase inhibitor removed the group differences in histone acetylation, DNA methylation, NGFI-A binding, GR expression and hypothalamic-pituitary-adrenal (HPA) responses to stress, suggesting a causal relation among epigenomic state, GR expression and the maternal effect on stress responses in the offspring. This was the first study to establish that an epigenomic state of a gene could be altered through behavioral programming and early experience [Weaver et al., 2004].

Methylation in the promoter region of the gene is associated most usually with gene silencing. Over the past decade there have been at least 40 studies published examining the impact of early adversity on methylation in the promoter region of the GR gene [Turecki and Meaney, 2014] and numerous studies published examining the impact of early stress on promoter methylation of several other candidate genes [Klengel et al., 2014]. Given the cited recently published reviews that show early adversity leads to epigenetic changes in the promoter region of GR and other stress-related genes [Klengel et al., 2014; Turecki and Meaney, 2014], this research will not be reviewed in the current manuscript.

While most studies have examined the impact of early adversity on methylation changes in the promoter region of various candidate genes, an estimated 97% of methylation in the genome occurs in intergenic regions and across gene bodies [Maunakea et al., 2010]. The prevalence of methylation sites in intergenic regions is interesting given the growing number of significant GWAS associated variants identified in intergenic regions of the genome, and other reports that have found methylation in intergenic regions is implicated in multiple psychiatric diseases [Qureshi et al., 2010]. Many CpG islands in intergenic regions are enriched for factor binding sites and are involved in the three-dimensional organization of the genome and gene regulation [Hodges et al., 2011; Yang and Corces, 2011]. Transcription factor binding sites and chromatin insulators within intergenic regions are believed to mediate intra- and inter-chromosomal interactions, affecting gene expression at both proximal and distal locations [Yang and Corces, 2011]. As less than 2% of the over three billion DNA base pairs in human genome code for proteins, it is not surprising that a role in gene regulation and disease risk has emerged for intergenic regions of DNA. Novel methods have been developed to characterize the three-dimensional configuration of the genome, and a better understanding of the regulatory role of these three-dimensional changes will open up new frontiers in human brain research and psychiatric genetics [Mitchell et al., 2014].

Methylation is also only one mechanism by which lifetime experience such as trauma exposure can alter gene expression. Histone modifications and post-translational regulation of gene expression via non-coding RNA species are two additional epigenetic mechanisms [Turecki et al., 2014]. It appears that only methylation and histone modification epigenetic changes have been examined to date in preclinical studies examining the effects of early stress. A representative sample of preclinical studies are reviewed below with the data from these studies outlined in Table II which delineates the epigenetic mechanism, gene, direction of expression change, and brain region investigated in studies examining epigenetic changes in response to adverse experiences in early life.

As depicted in Table II, adverse early experiences are associated with both histone modification and DNA methylation changes in multiple brain regions implicated in depression, PTSD, aggressive behavior, and substance use problems. The greatest number of studies has examined gene expression changes in the hippocampus following various early adversity paradigms [Weaver et al., 2004; Tsankova et al., 2006; McGowan et al., 2011; Bagot et al., 2012]. These studies have found early adversity is associated with decreased glucocorticoid receptor (*Nr3c1*), brain derived neurotrophic factor (*Bdnf*), and glutamate decarboxylase 1 (*Gad1*) gene expression, and increased type one metabotropic glutamate

receptor (*Grm1*) gene expression. Early adverse experiences are also associated with decreased neurotensin receptor (*Nst*) expression in the amygdala [Toda et al., 2014], decreased *Bdnf* expression in the medial prefrontal cortex [Roth et al., 2011], and enhanced *Maoa* PFC gene expression [Marquez et al., 2013]. Gene expression changes have also been reported in the reward circuit following early stress, with decreases in tyrosine hydroxylase (*Th*) expression reported in the ventral tegmental area [Niwa et al., 2013] and decreased expression of dopamine receptor genes reported in the nucleus accumbens [Pena et al., 2014].

It has been argued that integration of numerous unbiased genome-wide and proteomic approaches will be necessary to fully understand the neuroepigenome and the extraordinarily complex nature of the human brain [Maze et al., 2014]. Simen and colleagues are among the only ones to have utilized this approach in their studies of the effects of early stress. They studied the effects of early stress using a model of maternal separation and early weaning (MSEW) in which mice were separated from the dams for 4 hr per day on postnatal days 2–5, and 8 hr per day on postnatal days 6–16, then weaned on postnatal day 17. After weaning, animals were group housed with same sex littermates [George et al., 2010; Bordner et al., 2011].

Genomic, epigenomic, and proteomic characterization of the PFC were completed on adult male MSEW and control mice on postnatal day 75 [Bordner et al., 2011]. Microarray analysis showed a total of 4,375 probes in 4,031 genes to be dysregulated as a consequence of early life stress. The significant genes were subjected to gene ontology analysis, and the most significant category affected by MSEW was genes involved in translation. These genes included many translation initiation factors (*Eif3i*, *Eif3k*, *Eif5a*, *Eif6*) and ribosomal components (*Rpl19*, *Rpl29*, *Rpl5*). RNA-sequencing pooled identified 1,239 significant genes with dysregulation of translation, with multiple initiation factors and ribosomal components. Many genes associated with myelin were also represented in the significant findings [Bordner et al., 2011]. In particular, genes characteristically expressed by mature, myelinating oligodendrocytes such as *Mag*, *Mbp*, *Mog*, *Omg*, and *Plp1* were found to be downregulated by RNA sequencing [Carlyle et al., 2012]. In addition, results of label free proteomics analyses identified numerous proteins showing differential expression, including the downregulation of myelin-related proteins including 2', 3'-cyclic nucleotide 3' phosphodiesterase, myelin basic protein, oligodendrocyte myelin glycoprotein, and reticulon 4, all of which were confirmed using targeted MRM proteomics. There was also evidence of a decrease of the GABAergic interneuron marker *Calb1*, concomitant with decreases in neuropeptide Y and somatostatin at the RNA level, suggesting effects of early life stress on inhibitory interneurons in the PFC of MSEW mice [George et al., 2010; Bordner et al., 2011; Carlyle et al., 2012].

These results—like all of the results discussed above—require replication and extension, with an added focus on circuit development as a fundamental unit for understanding behavior [Kaffman and Krystal, 2012]. The explosion of large-scale, high-throughput technologies has necessitated a shift away from reductionism and fueled the development of new computational tools [Chang et al., 2015; Schadt et al., 2005]. Methodological issues

relevant in executing these studies are reviewed elsewhere [Kaffman and Krystal, 2012; Maze et al., 2014; Klengel and Binder, 2015]. The complexity can be daunting.

Research Domain Criteria (RDoC) Perspectives

While the DSM has been an invaluable tool in establishing reliability of psychiatric diagnoses and creating a common language to facilitate communication about mental illnesses [Insel and Wang, 2010; Association, 2013], the validity of the DSM psychiatric nomenclature has come under considerable scrutiny [Regier et al., 2009; Keshavan and Ongur, 2014], and spurred initiation of the National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) project [Insel et al., 2010; Cuthbert and Insel, 2013]. As reviewed elsewhere [Kaufman et al., 2015], central tenets of the NIMH RDoC initiative include: (1) Mental illnesses are brain circuit disorders [Insel and Wang, 2010]; (2) Psychopathology is conceptualized in terms of component abnormalities in discrete, but frequently highly interconnected, brain circuits [Etkin and Cuthbert, 2014]; (3) Brain circuit abnormalities cut across traditional diagnostic boundaries [Etkin and Cuthbert, 2014]; (4) Behaviors linked to different brain circuits vary dimensionally from impairment to healthy functioning [Etkin and Cuthbert, 2014]; and (5) Brain circuit function varies across development and is significantly influenced by experience [Cuthbert, 2014]. The RDoC further assumes that diagnoses based solely on observable signs and symptoms are non-specific and inevitably reflect heterogeneity in terms of pathophysiology [Insel, 2014], and that in time, data from genetics and clinical neuroscience will yield meaningful biomarkers to augment clinical symptoms in guiding treatment [Insel et al., 2010].

The RDoC initiative derived a matrix to organize psychiatric symptoms that currently consists of five domains and a series of interrelated constructs. The domains and constructs were selected during a series of thoughtful workshops facilitated by NIMH over the past several years [NIMH, 2012]. In order for constructs to be included in the RDoC Matrix, evidence demonstrating that they are reliable and valid behavioral functions and are subserved by an identified neural circuit, was required [Cuthbert, 2014]. The five initial domains identified by the RDoC workshops include: negative valence (e.g., anxiety, loss), positive valence (e.g., reward), cognitive systems (e.g., attention, working memory), social processes (e.g., affiliation), and arousal/modulatory systems (e.g., sleep-wake). Over time it is likely that additional domains and constructs will be added to the matrix. It should be noted that the boundaries between the separate domains and constructs are not sharp, as research has demonstrated that the domains and constructs function interactively via highly integrated brain circuits [Ford et al., 2014].

The primary focus of RDoC is on neural circuitry, with levels of analysis progressing in one of two directions: upward from measures of circuitry to clinical symptomatology, and downward to the genetic and molecular factors that ultimately influence function [Insel et al., 2010]. The RDoC initiative promotes the examination of each construct across seven units of analyses: genes, molecules, cells, circuits, physiology, behavior, and self-reports. It also identifies paradigms that can be used to assess each construct.

There has been some concern expressed that the RDoC framework is reductionist, with an overemphasis on neural circuits and genetics, and minimal attention to contextual factors

[Frances, 2014; Parnas, 2014]. The incorporation of preclinical translational studies of fear extinction at both the behavioral and molecular level into treatments (e.g., exposure therapy and D-cycloserine), however, demonstrates the potential value of the integrated approach proposed by RDoC [Kaufman et al., 2015], and the burgeoning literature on neuroplasticity and epigenetics further highlights that this concern is unwarranted, as one cannot study neural circuits and genetics, without considering experience [Krystal et al., 2009; Turecki and Meaney, 2014].

In terms of future studies of the genetics of psychiatric disorder, RDoC suggests moving beyond DSM categorical diagnoses, incorporating dimensional approaches to the assessment of discrete domains, not highly heterogeneous clinical phenotypes, and including assessments from the other proposed units of analyses (e.g., physiology, circuits). As noted previously, the explosion of large-scale, high-throughput technologies has necessitated a shift away from reductionism and fueled the development of new computational tools [Schadt et al., 2005; Chang et al., 2015]. As Schadt et al. [2005] stated, "... future successes in biomedical research will likely demand a more comprehensive view of the complex array of interaction in biological systems and how such interactions are influenced by genetic background, infection, environmental states, life-style choices, and social structures more generally. This holistic view requires embracing complexity in its entirety, so that complex biological systems are beginning to be seen as dynamic, fluid systems able to reconfigure themselves as conditions demand." There is no area of medicine in which this holds more truth than in psychiatry.

Closing Remarks

A history of abuse need not lead to the development of psychiatric problems. Risk is altered by genetic factors, and can be ameliorated by positive factors (e.g., social supports) in the environment. There are meta-analytic support and replicated findings for several genetic risk factors identified which increase risk for psychiatric problems among individuals with histories of child abuse; however, this review highlighted that the genetic risk factors are pleiotropic: genetic factors are not usually associated with distinct psychiatric disorders, but rather diverse clinical phenotypes. In addition, emerging findings suggest an important role of epigenetic mechanisms, as adverse experiences early in life are associated with changes in gene expression of multiple known candidate genes, genes involved in DNA transcription and translation, and genes necessary for brain circuitry development, with changes in gene expression reported in key brain structures implicated in the pathophysiology of psychiatric and substance use disorders. There is also an emerging role for intergenic regions of the genome in conferring risk for stress-related psychiatric disorders

The finding of pleiotropy highlights the value of utilizing the Research Domain Criteria (RDoC) framework in future studies of the genetics of stress-related psychiatric disorders, and not trying to link genes to multifaceted clinical syndromes, but rather more limited behaviors or phenotypes that are thought to map onto distinct neural circuits. Emerging findings in the field of epigenetics also highlight the value of translational research studies that integrate numerous unbiased genome-wide approaches to further unravel the genetics of stress-related psychiatric disorders. Improved understanding of the genetic and

environmental factors that affect risk for psychiatric problems following early adversity, and the mechanisms by which these factors confer risk, will help to develop more effective, personalized approaches to prevent and treat disorders in vulnerable individuals.

ACKNOWLEDGMENTS

This work was supported by the NIH R01MH098073 (JK, JH); the National Center for Posttraumatic Stress Disorder–Veterans Affairs Connecticut (JG, JK); and the VA Cooperative Study #575B, Genomics of Posttraumatic Stress Disorder in Veterans (JG, JK).

Grant sponsor: NIH; Grant number: R01MH098073; Grant sponsor: National Center for Posttraumatic Stress Disorder–Veterans Affairs Connecticut; Grant sponsor: VA Cooperative Study #575B; Grant sponsor: Genomics of Posttraumatic Stress Disorder in Veterans.

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TABLE I**GxE Studies of Child Abuse and Psychopathology: Evidence for Pleiotropy**

Gene	Depression	PTSD/Anxiety	Aggression	Substance abuse
5-HTTLPR	↑↑↑	↑↑	↑↑	↑↑
MAOA	↑	↑	↑↑↑ *	↑

Codes: ↑↑↑, meta-analytic support for association; ↑↑, replicated finding; ↑, single study or greater number of supportive studies, some inconsistent findings.

* The meta-analyses only support the association in males.

TABLE II

Preclinical Studies: Brain Regions Showing Epigenetic Changes in Gene Expression in Response to Early Stress

Reference	Epigenetic mechanism	Site	Direction/Gene	Brain region
Weaver et al. [2004]	Histone acetylation	TF	↓ <i>Nr3c1</i>	Hippocampus
McGowan et al. [2011]	DNA methylation	Promoter	↓ <i>Nr3c1</i>	Hippocampus
Tsankova et al. [2006]	Histone methylation	Promoter	↓ <i>BDNF</i>	Hippocampus
Bagot et al. [2012]	DNA methylation	Promoter	↑ <i>GRM1</i>	Hippocampus
Zhang et al. [2010]	DNA methylation	Promoter	↓ <i>GAD1</i>	Hippocampus
Toda et al. [2014]	DNA methylation	Promoter	↓ <i>NTSR1</i>	Amygdala
Roth et al. [2009]	DNA methylation	Promoter	↓ <i>BDNF</i>	Prefrontal cortex
Marquez et al. [2013]	Histone acetylation	Promoter	↑ <i>MAOA</i>	Prefrontal cortex
Chen et al. [2012]	DNA methylation	Promoter	↑ <i>CRF</i>	PVN
Murgatroyd et al. [2009]	DNA methylation	Promoter	↑ <i>AVP</i>	PVN
Niwa et al. [2013]	DNA methylation	Promoter	↓ <i>TH</i>	VTA
Pena et al. [2014]	DNA methylation	Promoter	↓ <i>DAR</i>	NAc

Codes: BDNF, brain derived neurotrophic factor; TF, transcription factor; Nr3c1, glucocorticoid receptor; GRM1, type I metabotropic glutamate receptor; GAD1, glutamate decarboxylase 1; NTSR1, neurotensin receptor 1; CRF, corticotropin releasing hormone; MAOA, monoamine oxidase A; PVN, paraventricular nucleus of the hypothalamus; AVP, arginine vasopressin; TH, tyrosine hydroxylase; DAR, dopamine receptor.