**Comparing Associations between Psychopathology-Related Outcomes and Stress-Sensitivity and Psychiatric Polygenic Risk Scores in the Adolescent Brain Cognitive Development (ABCD) Study**

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**Introduction**

Transient changes in cortisol levels following an acute stressor can be adaptive. Typically, a stressful event activates the hypothalamic-pituitary-adrenal (HPA) axis and causes the hypothalamus to release corticotropin-releasing hormone which stimulates release of adrenocorticotropic hormone and the subsequent release of cortisol from adrenal cortex (Palamarchuk et al., 2023). Cortisol binds to glucocorticoid receptors (GRs), creating negative feedback and decreasing HPA axis activity to return to homeostasis (Jimeno & Rubalcaba, 2023). However, chronic or sustained stress can provoke longer-term, maladaptive changes in cortisol responses with negative physical and psychological consequences (Hoffman et al., 2024; Palamarchuk et al., 2023; Zorn et al., 2017). Repetitive stress exposure is a risk factor for psychological disorders such as anxiety, major depressive disorder (MDD), and schizophrenia (Zorn et al., 2017), and HPA axis hyperactivity has been linked to anxiety and depression (Palamarchuk et al., 2023). Abnormal HPA axis responsiveness was also associated with post-traumatic stress disorder (PTSD)-like behavior, including changes in fear extinction and relapse, hippocampal volume, and rapid eye movement sleep in offspring of rats with unusually exaggerated or blunted responses to cortisol (Monari et al., 2024). Specific changes in HPA axis activity can differ based on sex, psychiatric disorder, and whether the disorder is active or in remission (Zorn et al., 2017). For example, women with a current MDD diagnosis had lower baseline cortisol and decreased cortisol stress responses to stress compared to controls, while men with a current MDD diagnosis displayed increased baseline cortisol but no difference in cortisol responses to stress. For subjects in remission, however, baseline cortisol levels for lower in women but not men, and neither men nor women had significant alterations in cortisol responses to stress. Also, women, but not men, with anxiety had decreased cortisol responses to stress, and conversely, men, but not women, with social anxiety disorder had increased cortisol responses to stress compared to controls. Both men and women with schizophrenia had decreased baseline cortisol levels (Zorn et al., 2017).

Disrupted HPA axis function in individuals with psychiatric disorders has also been linked to changes in gene expression. Although there were no differences in baseline gene expression in human induced pluripotent stem cell-derived glutamatergic neurons from combat veterans with PTSD and without PTSD, exposure to the GR agonist hydrocortisone provoked differential expression of 402 genes between cells derived from patients with and without PTSD, and these genes were enriched in postmortem brain tissue from individuals with PTSD (Seah et al., 2022). Additionally, a polygenic risk score (PRS) created based on hippocampal gene changes in female macaques following chronic administration of the glucocorticoid betamethasone moderated the relationship between exposure to early life adversity and diagnoses of adult psychotic disorders (Arcego et al., 2024).

Penner-Goeke et al. (2022) leveraged HPA axis responses to the GR agonist dexamethasone to create an experimentally-derived stress-sensitivity genetic score associated with physiological stress responses and differentially expressed genes in postmortem brain tissue of individuals with psychiatric disorders. Beginning with a set of 3,662 SNPs linked to dexamethasone responsiveness in a prior genome-wide expression quantitative trait locus (eQTL) analysis (Arloth et al., 2015), they identified 547 of these dexamethasone-responsive SNPs which were in regulatory elements (SNP-DREs). Transcripts associated with these SNP-DREs were enriched in postmortem cortical brain tissue from individuals with schizophrenia, autism spectrum disorder, MDD, bipolar disorder, and alcohol and substance use disorders. Penner-Goeke et al. (2022) then used Mendelian randomization based on a meta-GWAS of psychiatric disorders to select 79 of these SNP-DREs causally linked to psychiatric illness and created a weighted functional gene score (FGS) based on the SNP-DREs. Baseline cortisol levels did not differ based on FGS, but subjects with higher FGS had higher cortisol levels 30 minutes after completing a social stress task compared to subjects with lower FGS. Higher FGS were also associated with increased eyeblink startle response magnitude and decreased startle habituation in a fear conditioning task. SNP-DREs have therefore been linked to psychiatric disorders via differential gene expression in postmortem tissue and physiological stress-sensitivity markers. A logical extension of this work is to examine whether PRS derived from the SNP-DREs used to create the FGS can be linked directly to psychiatric diagnoses and psychopathology. *RQ1:*  What relationship, if any, is there between an experimentally-derived stress-sensitivity PRS and psychiatric diagnoses and symptoms of psychopathology?

To do so, the present investigation will use data from the Adolescent Brain Cognitive Development (ABCD) Study, an attractive data source due to its large sample size, demographically diverse subject pool, and extensive data collection.

The proposed stress-sensitivity PRS is unique in that it captures experimentally derived genetic-based sensitivity to cortisol, specifically (?), whereas PRS are typically created based on summary statistics from large GWAS, agnostic to mediating biological pathways This standard approach is well-established, and prior work using data from the ABCD Study has identified significant relationships between psychopathology and PRS for a wide variety of disorders including panic disorder, schizophrenia, MDD, ADHD, and externalizing disorders (Lahey et al., 2024; Qiu & Liu, 2023; Teeuw et al., 2023; Wainberg et al., 2022). However, PRS created based on GWAS summary statistics rely on associations between genetic variants and the target trait which can make it difficult to identify causal variants and their associated underlying mechanisms. The stress-sensitivity PRS capitalizes on results of an experimental manipulation, thus illuminating a mechanistic pathway between the identified variants (SNP-DREs) and outcomes significantly associated with the PRS. The second research question in this study compares the experimentally-derived stress-sensitivity PRS and standard, GWAS-derived PRS. *RQ2:* How does the variance in psychiatric diagnoses or symptoms of psychopathology explained by an experimentally-derived stress-sensitivity PRS compare to that explained by PRS for psychiatric disorders calculated based on GWAS summary statistics?

In addition to genetic influences on stress-related psychiatric disorders, there is strong evidence for the role of social and environmental stress exposure in the development of psychopathology. Likelihood of developing a psychiatric disorder increases as exposure to adversity increases, and children exposed to adverse experiences are approximately twice as likely to develop a mental disorder compared to unexposed peers (McLaughlin et al., 2019). Stress exposure is often assessed using self-report or interview based measures such as the Adverse Childhood Experiences Questionnaire (ACE-Q), the Life Events Scale (LES), or the PTSD module of the Kiddie Schedule for Affective Disorders and Schizophrenia (Felitti et al., 1998; Tiet et al., 1998; Townsend et al., 2020). Frequently, the number of stressful experiences to which an individual has been exposed is summed to generate a measure of cumulative stress exposure. While the specific types of adverse events identified in each measure vary, cumulative adverse event scores are generally associated with increased psychopathology. Higher scores on the ACE-Q were linked to increased levels of depression, drug abuse and alcoholism, and suicide attempts (Felitti et al., 1998). Increased negative lifetime event exposure quantified in the ABCD Study by the LES was associated with increased emotion-driven impulsivity, psychotic-like experiences, and externalizing and internalizing symptoms (Barnhart et al., 2022; Karcher et al., 2022; Weiss et al., 2023). Finally, exposure to larger numbers of potentially traumatic events as measured with the KSADS PTSD module tended to increase risk for depression, anxiety, PTSD, oppositional defiant disorder, conduct disorder, attention-deficit hyperactivity disorder (ADHD), and suicidality (Thompson et al., 2022).

Broader family- and community-level factors can add to individual stress exposure and further increase risk for psychopathology. School environmental factors such as engagement were significantly related to symptoms of internalizing, externalizing, anxiety, and depression in the ABCD Study (Qiu & Liu, 2023; Thapaliya et al., 2021). Lower levels of household income and parental education and higher neighborhood poverty as measured with area deprivation index were also associated with increased externalizing (Maxwell et al., 2021; Teeuw et al., 2023). The exposome is designed to capture individual-level stressors such as cumulative adverse events as well as family- and community-level stressors, and higher exposome scores have been associated with increased psychopathology. For example, individuals with higher exposome scores based on 348 environmental variables from the ABCD Study tended to have higher levels of self- and parent-reported psychopathology. When combined with basic demographic information, a model including these exposome scores was able to capture 38.2% of the variance in the psychopathology p-factor (Hoffman et al., 2024; Moore et al., 2022).

In addition to additive genetic and environmental effects on psychopathology, numerous studies have demonstrated a role of gene by environment interactions in psychiatric symptoms and diagnoses. For example, internalizing and externalizing scores for youth in the ABCD Study were best explained by models including genome-exposome interactions where the exposome measured cumulative negative life events and proximal contextual factors such as school risk and parental monitoring (Choi et al., 2022). MDD PRS and anxiety PRS also interacted with measures of stress such that there was a stronger relationship between PRS and depression or anxiety symptoms, respectively, for subjects with increased loneliness, long-term difficulties, greater numbers of stressful life events, and decreased social support (Wang et al., 2023). Interactions between a multilocus genetic profile score (MGPS) based on four key HPA axis genes (FKBP5, NR3C1, NR3C2, and GRHR1) and environmental factors were also associated with symptoms of depression and anxiety in a sample of Han Chinese adolescents. Higher levels of childhood maltreatment were linked with increased comorbid depression and anxiety symptoms in individuals with high levels, but not those with low levels, of this HPA axis-related MGPS (Cao et al., 2024). Similarly, only subjects with high HPA axis-related MGPS displayed stronger interactions between childhood maltreatment, exposure to recent interpersonal stress, and symptoms of depression (Sun & Cao, 2024). Given the impact of gene by environment interactions on psychopathology, the final research question of this study explores potential interactions between the stress-sensitivity PRS and environmental factors. *RQ3:* How do environmental factors as defined by the exposome or by a cumulative adverse event score affect the relationships between stress-sensitivity or psychiatric disorder PRS and emotional and behavioral problems?

Overall, the proposed study uniquely approaches the relationship between genetics and psychopathology by using an experimentally-derived stress-sensitivity PRS. By directly investigating the relationship between the stress-sensitivity PRS and psychopathology (RQ1), it extends prior work which indirectly linked these variables based on changes in postmortem brain tissue of individuals with psychiatric disorders and physiological markers of stress reactivity which differed based on subject PRS. This study also takes a novel approach to examining links between genetics and psychiatric outcomes by comparing the experimentally-derived stress-sensitivity PRS with more traditional PRS created based on GWAS summary statistics (RQ2). Finally, the present study furthers our understanding of the role of gene by environment interactions on psychopathology by analyzing the combined effects of the stress-sensitivity PRS and two different types of environmental measures (RQ3).

**Methods**

**Sample description**

The ABCD Study is an ongoing, longitudinal study which sampled adolescents from across the United States. Subject composition is similar to national demographic composition in terms of race, ethnicity, urbanicity, and sex (Compton et al., 2019). Data are available for 11868 adolescents at baseline when participants were 9-10 years old, and sample attrition has been relatively low (participants at year one: 11220, year two: 10973, year three: 10336). While the full data set for the year four follow-up visit has not been released (data currently available for 3718 participants), based on prior attrition, information should be available for about 9826 adolescents . The proposed study will use outcome measures from year four follow-up visits, as preliminary data suggest that prevalence for psychopathology is much greater in year four compared to earlier timepoints. For example, current diagnoses of GAD in year four were 2.8 times larger than those in year two, and current diagnoses of MDD in year four were about four times larger than those in year two. In year four, youth participants were 52.82% male, 47.15% female, and 0.02% intersex. Youth parent-reported race was: 79.44% White, 16.10% Black/African American, 0.04% Alaska Native, 0.15% Native Hawaiian, 0.02% Guamanian, 0.09% Samoan, 0.26% other Pacific Islander, 0.92% Asian/Indian, 1.96% Chinese, 1.45% Filipino, 0.75% Japanese, 0.94% Korean, and 0.38% Vietnamese. Combined family household incomes pre-tax were as follows: 1.86% less than $5,000; 1.69% $5,000 through $11,999; 1.43% $12.000 through $15,999; 3.25% $16,000 through $24.999; 4.14% $25,000 through $34,999; 6.56% $35,000 through $49,999; 10.84% $50,000 through $74,999; 12.64% $75,000 through $99,999; 32.92% $100,000 through $199,999; and 16.03% $200,000 and greater.

**Genetics**

Genetic material was collected primarily through saliva, though some participants provided blood samples. Genotyping was performed with Affymetrix Axiom Smokescreen Arrays.. The ABCD Data Analysis, Informatics, and Resource Center performed quality control which included removal of variants with more than 10% missingness and removal of subjects with more than 20% missing calls or excessive relatedness (Fan et al., 2023).

**Measures**

***Child Behavior Checklist (CBCL)***

The CBCL is part of the Achenbach System of Empirically Based Assessment and measures emotional and behavioral problems in youth (T. M. Achenbach, 2009). Responses to 113 items are grouped into eight subscales (rule-breaking, aggression, withdrawn/depressed, anxious/depressed, somatic, attention problems, thought problems, and social problems) as well as composite scores for internalizing (withdrawn/depressed, anxious/depressed, and somatic subscales), externalizing (rule-breaking and aggression subscales), and total problems (all subscales). Scores are t-scored based on a mean of 50 points and a standard deviation of 10 points. Values between 65 and 69 are considered subclinical, while scores of 70 or more suggest clinically significant problems. Externalizing, internalizing, and total scores have high internal consistency (Cronbach’s α: .94) and high test-retest reliability (r = .92) (T. Achenbach, 2011). CBCL scores in the ABCD Study reflect caregiver assessments as it was not administered to youth.

***Kiddie Schedule for Affective Disorders and Schizophrenia Computerized Version for DSM-5 (KSADS-COMP)***

The KSADS-COMP is a standardized interview with items based on DSM-5 criteria for psychiatric disorders including MDD, ADHD, PTSD, and a variety of anxiety disorders (Kobak et al., 2013). It has good internal reliability (Cronbach’s α = .91) and convergent validity with clinician-administered scales such as the CBCL (Townsend et al., 2020). It also has adequate test-retest reliability (κ = .63 to 1.00) (Kaufman et al., 1997). The present study considers both past and present diagnoses and includes information from both youth and caregiver reports when available. ADHD and PTSD items were not administered to youth.

**Ancestry**

Principal component analysis was performed on unpruned ABCD data with the conservative Hardy-Weinberg flag using PLINK (Purcell et al., 2007). Prior work identified the first eight principal components (PCs) as the optimal number to account for ancestry in this sample (J. Zhu and M. Hyat, personal communication, February 2024). Samples were separated into three ancestry groups (African, American admixed, and European) using a random forest model derived from combining the ABCD dataset with the HapMap3 reference dataset, and using population labels from the HapMap3 to assign continental super-population level ancestry labels with a probability threshold of 0.7 (J. Zhu and M. Hyat, personal communication, February 2024). After applying this threshold, genetic data was available for 3307 participants (2299 European ancestry, 517 African ancestry, 491 American admixed ancestry) in the currently released version of year four data.

**Genetic Relatedness**

To account for genetic relatedness between participants, genetic relatedness matrices (GRMs) were calculated for each ancestry using PLINK (Purcell et al., 2007) based on unpruned ABCD data (J. Zhu and M. Hyat, personal communication, February 2024).

**Polygenic Risk Scores (PRS)**

Stress-sensitivity PRS used for the preliminary results included here were generated with PLINK (Purcell et al., 2007) based on summary statistics from Penner-Goeke et al. (2023). Briefly, for each PRS, a data frame containing risk alleles and their associated effect sizes for each SNP was provided to PLINK, and PLINK then calculated the sum of the risk alleles for each participant weighted by effect size.

Final results will compare stress-sensitivity PRS to PRS for MDD, ADHD, PTSD, and anxiety disorders calculated based on summary statistics from Howard et al. (2019), ADHD Working Group of the Psychiatric Genomics Consortium (PGC) et al. (2019), Nievergelt et al. (2024), and Otowa et al. (2016) respectively. To improve PRS accuracy across ancestry groups, final results will use a more sophisticated method to generate PRS for anxiety, MDD, PTSD, and ADHD (i.e., PRS-Csx (Ruan et al., 2022), BridgePRS (Hoggart et al., 2024), or SBayesRC (Zheng et al., 2024), which incorporate summary statistics across GWAS from multiple ancestry populations and/or biological annotations of SNPs to refine effect size estimates per SNP). These techniques are not applicable to the stress-sensitivity PRS because it was generated experimentally based on results from individuals of unknown ancestry.

**Analysis**

Using the *R* package GENESIS (Gogarten et al., 2019), linear regression will be performed with scores from each of the eight subscales, externalizing, internalizing, and total problems on the CBCL as outcomes. Logistic regression was also be performed with lifetime diagnosis of MDD, ADHD, PTSD, and any anxiety disorder other than specific phobia as outcomes. Study site and genetic relatedness (quantified with GRMs) will be modeled as random effects. Subject sex, age, and the first eight ancestry PCs will be fixed effect covariates. Analysis code will be available in a Github repository.

**Preliminary Results: RQ1**

***Psychopathology-related Symptoms and Behavior***

For individuals of European ancestry, stress-sensitivity PRS was nominally significantly associated with somatic-related symptoms on the CBCL somatic subscale (uncorrected p-value = 0.022, FDR corrected p-value = 0.92), with an increase of 0.281 points for each standard deviation increase in stress-sensitivity PRS. Neither any other CBCL subscale nor total problems were significantly or nominally associated with stress-sensitivity PRS. Compared to average values, girls’ scores were 1.00 point higher on the internalizing (FDR corrected p-value = 0.000045), 0.49 points higher on the anxious-depressed (FDR corrected p-value = 0.00055), and 0.40 points higher on the somatic (FDR corrected p-value = 0.0037) subscales and 0.26 points lower on the aggression subscale (FDR corrected p-value = 0.010). Age did not significantly impact any CBCL scores.

For subjects of African ancestry, CBCL scores did not significantly differ based on stress-sensitivity PRS, sex, or age.

Finally, for individuals of American admixed ancestry, stress-sensitivity PRS was nominally associated with changes in withdrawn-depressed and total problem CBCL scores. A one standard deviation increase in stress-sensitivity PRS was linked to a decrease of 0.67 points on the withdrawn-depressed subscale (uncorrected p-value = 0.026, FDR corrected p-value = 0.20) and a decrease of 1.31 points on total problems (uncorrected p-value = 0.023, FDR corrected p-value = 0.20). Stress-sensitivity PRS was not significantly associated with any other CBCL scores. Sex was nominally significantly associated with some CBCL scores. Compared to average values, girls’ internalizing, anxious-depressed, and somatic scores were 1.12 points (uncorrected p-value = 0.024, FDR corrected p-value = 0.091), 0.54 points (uncorrected p-value = 0.032, FDR corrected p-value = 0.095), and 0.63 points (uncorrected p-value = 0.018, FDR corrected p-value = 0.091) higher, respectively. Age was not significantly related to any CBCL scores.

***Lifetime Psychiatric Diagnoses***

For individuals of European ancestry, there were no significant relationships between stress-sensitivity PRS or age and any diagnosis. Compared to average values, girls had an increase of 1.48 in odds of receiving a lifetime anxiety diagnosis (FDR corrected p-value = 0.00000000016), an increase of 1.63 in odds of receiving a lifetime MDD diagnosis (FDR corrected p-value = 0.00000000016, and a decrease of 0.72 in odds of receiving a lifetime ADHD diagnosis (FDR corrected p-value = 0.0089). Sex was not significantly associated with lifetime PTSD diagnosis.

For subjects of African ancestry, stress-sensitivity PRS, sex, and age were not significantly associated with likelihood of receiving a lifetime anxiety, ADHD, MDD, or PTSD diagnosis, with the exception that girls had a nominally significant (uncorrected p-value = 0.025, FDR corrected p-value = 0.38) increase of 1.44 in odds of receiving a lifetime MDD diagnosis compared to average values.

Finally, for subjects of American admixed ancestry, stress-sensitivity PRS was not significantly associated with the likelihood of receiving any lifetime diagnosis. For girls, odds of receiving a diagnosis of anxiety or MDD were 1.66 points (FDR corrected p-value = 0.011) or 1.75 points (FDR corrected p-value = 0.011) higher on average, respectively. Odds of receiving a lifetime diagnosis of ADHD were also 0.44 points lower on average for girls, but this difference did not survive FDR correction (uncorrected p-value = 0.046). Sex did not significantly affect likelihood of receiving a lifetime PTSD diagnosis. A one standard deviation increase in age was nominally associated (uncorrected p-value = 0.0063, FDR corrected p-value = 0.094) with an increase of 2.69 points in odds of receiving a lifetime MDD diagnosis. Age did not affect likelihood of receiving a lifetime diagnosis of anxiety, MDD, or PTSD.

**Discussion**

**Anticipated findings**

***RQ2***

For individuals of European ancestry, based on prior work in the ABCD Study, MDD PRS is expected to be significantly associated with anxious/depressed, somatic, social, thought, attention, and rule-breaking, but not aggressive or withdrawn/depressed, CBCL subscale scores (Wainberg et al., 2022) as well as total problems scores (Hoffman et al., 2024). Because MDD PRS is expected to be significantly associated with its composite subscale scores, we also expect MDD PRS to be significantly related to internalizing scores as well as MDD, PTSD, and anxiety, which are all internalizing disorders. Given that the externalizing subscale is composed of the rule-breaking and aggressive subscales, MDD PRS is not expected not be significantly related to externalizing scores or to diagnoses of ADHD, an externalizing disorder. The nominal significance between the stress-sensitivity PRS and the CBCL somatic subscale in individuals from European ancestry reported as preliminary results may reach significance following FDR correction in the full data set, which would be consistent with the predicted significant relationship between MDD PRS and somatic subscale scores. However, unlike MDD PRS, stress sensitivity PRS is not expected to be significantly related to any other CBCL subscale scores based on our preliminary findings.

In European ancestry subjects, Wainberg et al. (2022) found ADHD PRS to be significantly associated with only the attention subscale in the CBCL, and we therefore expect ADHD PRS in this study to be linked to the attention subscale and ADHD diagnoses. This differs from the lack of significant relationship between the stress sensitivity PRS and ADHD diagnoses or CBCL attention subscale scores based on our preliminary findings. We expect neither ADHD PRS not stress-sensitivity PRS to be associated with any other CBCL scores or psychiatric diagnoses based on our preliminary data and findings from Wainberg et al. (2022).

We expect to replicate results from Hoffman et al. (2024) who identified a significant relationship between PTSD PRS and CBCL total problems in individuals of European ancestry (but did not report results on CBCL subscale scores or diagnostic rates). This would contrast with our preliminary results which failed to identify a significant association between stress-sensitivity PRS and PTSD diagnoses or CBCL total problems.

Across individuals of all ancestries, Qiu and Liu (2023) did not find significant relationships between PRS for overall anxiety, social anxiety, panic, or phobia and externalizing or internalizing CBCL scores, and we expect to replicate these results. This would be consistent with our preliminary results indicating a lack of relationship between stress-sensitivity PRS and any CBCL score.

Published work on the relationship between PRS for psychiatric disorders and psychopathology is much more sparse for individuals of non-European compared to European ancestries. For example, the expected results for MDD and ADHD PRS described above are based on work from Wainberg et al. (2022), who restricted their analysis to individuals of European ancestry only due to “regrettably poor performance of polygenic risk scores calculated from European GWAS to predict psychopathology in non-white individuals.” While a recently-published report on PRS and psychopathology in the ABCD Study included participants of non-European ancestry, the method used to calculate PRS did not specifically account for differences in linkage disequilibrium based on ancestry, and results were not reported separately based on ancestry. This could potentially explain the lack of significant relationship between MDD PRS and CBCL internalizing score or between ADHD PRS and CBCL externalizing score reported in this study, which contrasts with the findings from Wainberg et al. (2022). Noteably, Hoffman et al. (2024) examined the link between MDD PRS and CBCL total problems scores in individuals of African ancestry and failed to find a significant relationship. We expect to replicate this result. However, they did not examine CBCL subscale scores, psychiatric diagnoses, or results for individuals of admixed American ancestry.

The generally worse performance of PRS for individuals of non-European compared to European ancestry is largely believed to reflect methodological limitations, largely due to lower representation of diverse ancestry groups in existing GWAS (Hoggart et al., 2024; Ruan et al., 2022; Zheng et al., 2024), rather than genuine genetic differences in causal variants (Hu et al., 2023). Because the stress-sensitivity PRS is experimentally determined rather than reliant on GWAS in which European ancestry individuals are typically over-represented, we expect the stress-sensitivity PRS to explain more of the variation in HPA axis-related psychopathology compared to PRS derived from GWAS summary statistics for individuals of non-European ancestry. We note, however, that the previously described discrepancies between the expected results for MDD, PTSD, and ADHD PRS compared to stress-sensitivity PRS point to the role of genetic variants unrelated to HPA axis activity in psychopathology.

***RQ3***

Previous work suggests that gene by environment interactions affect psychopathology measures from participants in the ABCD Study. Choi et al. (2022) found that best-fitting models of CBCL externalizing scores included genetic, environment, and gene by environmental effects for individuals of European, African, or American Admixed ancestry. Best-fitting models of CBCL internalizing also included main effects of genes and environment and a gene by environment interaction for subjects of European and African ancestry but not those of American Admixed ancestry. Environmental effects included cumulative exposure to negative life events and proximal contextual factors such as school environment. A recent study from Rea-Sandin et al. (2024) also identified significant effects of gene by environment interactions on psychopathology of youths in the ABCD Study, with family cultural values significantly interacting with additive genetic effects to influence parent-reported youth externalizing, but not internalizing, symptoms. We therefore expect to find significant interactions between stress-sensitivity PRS and environmental measures, though these results may vary based on subject ancestry as in Choi et al. (2022).

**Limitations**

The sample used to perform the eQTL analysis which formed the basis of the stress-sensitivity PRS was small and homogenous (164 Caucasian men from Munich, Germany), which potentially limits its external validity (Arloth et al., 2015). Additionally, ancestry was not reported for the subjects in which the stress-sensitivity PRS was significantly linked to physiological stress responses (Penner-Goeke et al., 2023). However, unlike most PRS which are created based on GWAS summary statistics and more susceptible to inaccuracies due to linkage disequilibrium differences based on ancestry, the stress-sensitivity PRS was experimentally developed and may therefore be more likely to accurately capture causal variants. As discussed above, causal variants are often consistent across different ancestry groups, though this is not always the case (Hu et al., 2023).

Another potential limitation of the study relies on the release schedule of ABCD Study data. PRS effect sizes tend to be small, necessitating large samples to detect effects. At this time, approximately half of the data from ABCD Study year 4 follow-up visits has been released, and usable data is currently available for 3718 participants. Acquiring data for the remainder of ABCD Study participants will be an important step in increasing detection power in this study.

**Potential implications**

The proposed study approaches the relationship between genetic variants and psychopathology from the unique angle of an experimentally-derived PRS. It aims to Illuminate the potential utility of this experimentally-derived PRS to identify HPA axis-related genetic variants linked with psychiatric disorders. More broadly, it may demonstrate the utility of developing PRS experimentally to suggest more direct, mechanistic pathways between genes and psychiatric disorders.

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