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Archival Report

A Glucocorticoid-Sensitive Hippocampal Gene Network Moderates the Impact of Early-Life Adversity on Mental Health Outcomes

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ABSTRACT

BACKGROUND: Early stress increases the risk for psychiatric disorders. Glucocorticoids are stress mediators that regulate transcriptional activity and morphology in the hippocampus, which is implicated in the pathophysiology of multiple psychiatric conditions. We aimed to establish the relevance of hippocampal glucocorticoid-induced transcriptional activity as a mediator of the effects of early life on later psychopathology in humans.

METHODS: RNA sequencing was performed with anterior and posterior hippocampal dentate gyrus from adult female macaques (n = 12/group) that were chronically treated with betamethasone (glucocorticoid receptor agonist) or vehicle. Coexpression network analysis identified a preserved gene network in the posterior hippocampal dentate gyrus that was strongly associated with glucocorticoid exposure. The single nucleotide polymorphisms in the genes in this network were used to create an expression-based polygenic score in humans.

RESULTS: The expression-based polygenic score significantly moderated the association between early adversity and psychotic disorders in adulthood (UK Biobank, women, n = 44,519) and on child peer relations (ALSPAC [Avon Longitudinal Study of Parents and Children], girls, n = 1666 for 9-year-olds and n = 1594 for 11-year-olds), an endophenotype for later psychosis. Analyses revealed that this network was enriched for glucocorticoid-induced epigenetic remodeling in human hippocampal cells. We also found a significant association between single nucleotide polymorphisms from the expression-based polygenic score and adult brain gray matter density.

CONCLUSIONS: We provide an approach for the use of transcriptomic data from animal models together with human data to study the impact of environmental influences on mental health. The results are consistent with the hypothesis that hippocampal glucocorticoid-related transcriptional activity mediates the effects of early adversity on neural mechanisms implicated in psychiatric disorders.

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Psychiatric disorders are multifactorial, involving interactions between genetic and environmental influences, especially during periods of neurodevelopment (1). There is compelling evidence for the importance of developmental processes. First, a genome-wide association study (GWAS) across multiple psychiatric disorders revealed enrichment for genes highly expressed during neurodevelopment (2). Second, childhood adversity is associated with alterations in the structure and connectivity of brain regions implicated in cognitive-emotional function (3–6) and significantly increases the risk for psychiatric disorders (7–13). Finally, all common neuropsychiatric disorders show an early age of onset that peaks between late childhood and postpubertal development (14,15). However, the molecular pathways that link childhood environmental experience to mental health outcomes remain unknown.

Studies in model systems and humans have identified the hippocampus as both highly sensitive to early adversity and associated with a range of psychiatric disorders (4,16,17). Glucocorticoids have been implicated as mediators of the effects of early adversity on the hippocampus and other corticolimbic structures that are associated with psychopathology (4,18,19). The hippocampus is enriched for glucocorticoid receptors and is a primary mediator of the cellular effects of chronic stress (18,20,21). In rodents, nonhuman primates, and humans, glucocorticoids affect structure and connectivity (4,22–26) as well as neurogenesis and synaptic remodeling

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(27–32). Glucocorticoids have been implicated in a range of mental disorders including affective disorders (27,33–37) and psychosis (38). Thus, increased exposure to glucocorticoids has been proposed as a mechanism for the effects of early-life stress on hippocampal development and risk for future psychopathology (4,18,25,39–41). The challenge is devising approaches to test this hypothesis in humans.

Psychiatric disorders are highly polygenic and thus are associated with changes in the expression of multiple genes that operate within networks to regulate biological pathways (42,43) underlying neural function and mental health (44). Therefore, a gene network approach is best positioned to inform us about relevant molecular mechanisms and processes (45) that underlie variations in brain health (46,47). Because transcriptomic data is tissue specific, gene network modeling also informs us about the regional differences in gene expression in relation to brain structure and function (48,49).

We reasoned that the identification of gene coexpression networks could be marshalled to test specific hypotheses in humans concerning the biological basis for psychiatric disorders. We focused on the proposed role of glucocorticoid-induced transcriptomic effects in the hippocampus as a mediator of the effects of childhood adversity on risk for mental disorders. We used RNA sequencing to identify hippocampal-specific, glucocorticoid-sensitive gene coexpression networks that were preserved across species (nonhuman primate and rodent models) (50,51). Then, we used the genes comprising a glucocorticoid-sensitive network to construct a polygenic score for analyses in humans. The polygenic score was comprised of single nucleotide polymorphisms (SNPs) in the network gene weighted by their association with gene expression. We hypothesized that a polygenic score generated from a glucocorticoid-sensitive network would moderate the association between early-life stress and later mental health outcomes. We also aimed to identify association patterns between variations in the polygenic score and brain structure in adults while accounting for the influence of early adversity using a multivariate analysis. We used available online datasets of postmortem human hippocampal samples to investigate whether our network from animal models has comparable gene expression in humans. Furthermore, we explored a potential epigenetic influence involving glucocorticoids using an available hippocampal DNA methylation microarray (52) reporting glucocorticoid effects on DNA methylation sites (see analysis overview in Figure S1).

METHODS AND MATERIALS

Animal Model

Adult (ages 7–20 years) female macaques (*Macaca fascicularis*) originating from Singapore or Vietnam were housed singly or in pairs and maintained with free access to water and a commercial diet enriched with fruits. Animals were divided into 2 experimental groups of similar age and received saline (n = 6/each cohort) or betamethasone (Diprosan, 0.3 mg/kg/day) injections (n = 6/each cohort) for 7 days. Only female macaques were used in this study due to the closure of the breeding program of a primate research center for which only females were available. On day 8, macaques were killed (~10 AM) using 7 to 10 mg/kg of intramuscular ketamine followed by 80 to 150 mg/kg of pentobarbital administration intravenously. The brain was immediately removed, frozen on dry ice, and sectioned for the anterior dentate gyrus (aDG) and posterior dentate gyrus (pDG). Circulating cortisol levels following betamethasone treatment were reduced by more than 90% of control values (25.9 $\pm$ 6.5 vs. 1.8 $\pm$ 1.7 μg/dL; $p < .0001$), reflecting the physiological efficacy of the treatment.

Adult female Long-Evans rats were housed (2/cage) under controlled conditions (12-hour light/dark cycle, 22 $\pm$ 2 °C, food ad libitum). Rats were divided into 2 groups with glucocorticoid exposure in drinking water for 6 weeks (postnatal day 75–110): 1) corticosterone group (corticosterone dissolved in 2.4% ethanol, 40 mg/L, n = 9) and 2) control group (2.4% of ethanol in the drinking water, n = 9). Rats were killed by rapid decapitation, and the brains were frozen for punch dissection of the dorsal and ventral DG (also see Supplemental Methods and Materials).

Weighted Gene Coexpression Network Analysis

The WGCNA R package (45,53) was used to identify gene networks showing similar glucocorticoid-induced expression patterns. We used the gene expression from Singapore macaques as a reference dataset to perform weighted gene coexpression network analysis (WGCNA) (pDG and aDG were analyzed separately). Module-trait associations were determined using the correlation between the module eigengene and glucocorticoid exposure (see Supplemental Methods and Materials for module preservation and module enrichment analysis).

Expression-Based Polygenic Score Calculation

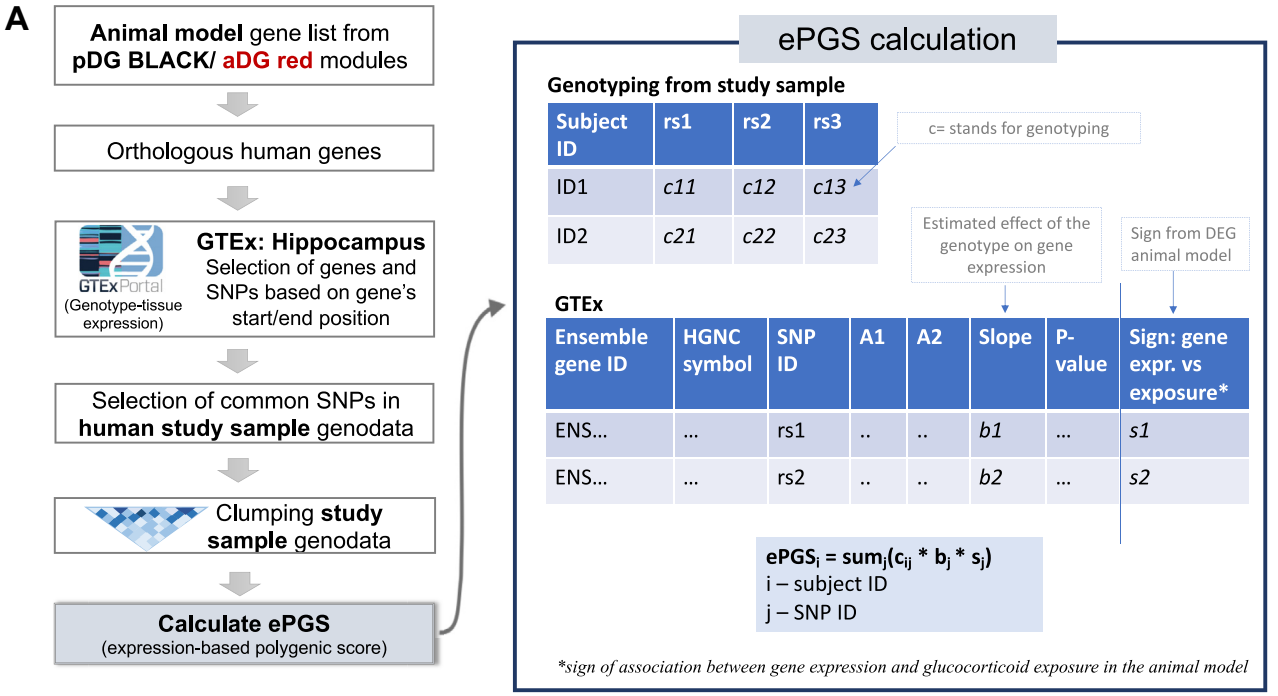
The expression-based polygenic score (ePGS) was created (also see Supplemental Methods and Materials) using the corresponding 475 ortholog human genes for the selected WGCNA module (black) from the pDG of macaques (Figure 1A) (48,54). We selected all SNPs within the black module and merged this list with SNPs from the Genotype-Tissue Expression (https://gtexportal.org/home/) (55,56) data in the human hippocampus. Alleles at a given *cis*-SNP were weighted by the estimated effect on gene expression also accounting for the sign of the correlation coefficient between gene expression and glucocorticoid exposure (log fold change from the RNA sequencing of Singaporean macaques).

Human Subjects

We studied 2 human cohorts of different ages to identify whether variation in the glucocorticoid-sensitive network from the animal model would predict psychopathology as a function of adversity exposure.

Adult Cohort. The UK Biobank is a large population-based cohort (57) with participants of ages 37 to 73 recruited through assessment centers between 2006 and 2010. The sample size in this study was 405,274 (218,539 females and 186,735 males) (Figure S2). Analyses were performed separately for women to analyze the specificity of the results according to sex and also to maintain consistency with the animal models that were used to generate the gene networks.

Child Cohort. Analyses were conducted separately in girls (Figure S2) from the ALSPAC (Avon Longitudinal Study of Parents and Children) (58–60), which included pregnant



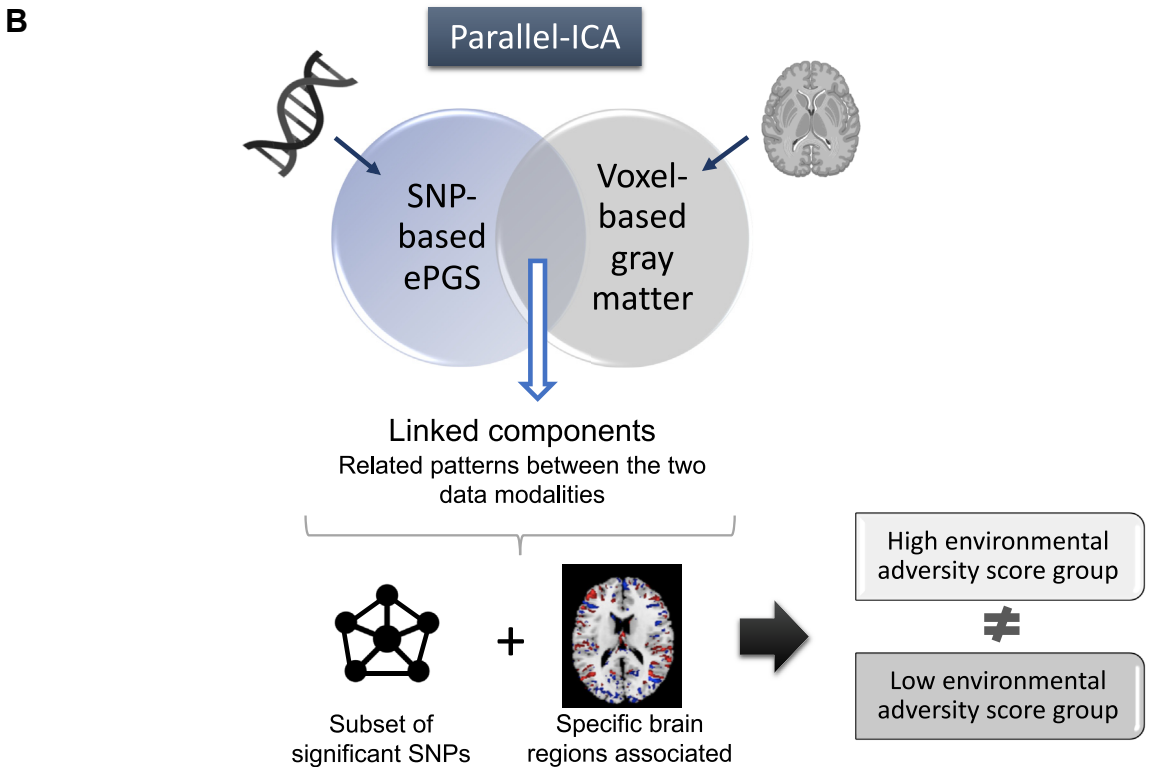


Figure 1. Schematic framework for the ePGS calculation and parallel ICA. **(A)** Flowchart showing the steps involved in the creation of the ePGS. First, the genes from weighted gene coexpression network analysis modules selected in the animal model (pDG black and aDG red modules) were converted to the orthologous human genes. We selected the genes and corresponding SNPs in GTEx and gathered all common SNPs between 1) SNPs from the human study samples (each one at a time) and 2) SNPs available in GTEx. Then, we kept these common SNPs in the study sample genodata and subjected them to linkage

women from the county of Avon in the United Kingdom (58–60). Analyses were conducted separately in girls (see Table 1 and Figure S2 for sample sizes). The full description of cohorts, genotyping, and phenotyping outcomes are described in Supplemental Methods and Materials and in Figure S2.

Early-Life Adversity Score

The early-life adversity scores for UK Biobank and ALSPAC study participants were created using a cumulative measure combining multiple forms of early-life adversity that are known to predict increased risk for psychopathology (61–65) calculated according to the methods previously described in developmental studies (64,66) (see Table S1 and Supplemental Methods and Materials for validation).

Enrichment Analysis for Glucocorticoid-Sensitive Sites

We compared genes from our network to a list of glucocorticoid-sensitive genes identified by Provençal *et al.* (52), who found glucocorticoid effects on DNA methylation. We selected the genes from our network containing CpG sites (434/475 human genes) annotated in the Illumina Human 450K methylation arrays (https://bioconductor.org/packages/release/data/annotation/html/IlluminaHumanMethylation450kanno.ilmn12.hg19.html). We selected 291 genes from this list that were also present in the hippocampal DNA methylation microarray used by Provençal *et al.* (52). We used Fisher's exact test at p value $< .05$ significance to investigate whether genes from the black module were enriched for glucocorticoid-induced DNA methylation genes (see Supplemental Methods and Materials). The prevalence of overlapped genes was compared against the null distribution and considered significant at a p value $< .05$.

Parallel Independent Component Analysis

Parallel independent component analysis (pICA) (67) was used to identify relations between clusters of interrelated data patterns from SNPs from the pDG ePGS-black and the whole-brain voxel-based gray matter density information from UK Biobank women (Figure 1B). We examined independent components from these 2 data modalities and their relations (68). pICA is a variant of ICA that separately extracts maximally independent components within each data modality while maximizing the association between them. It uses an entropy term based on information theory, thereby enhancing the interconnection by maximizing the linkage function in a joint estimation process (67,69). Because neuroimaging data available for women ages 40 to 70 years in the UK Biobank revealed a significant correlation between volume of gray matter and age ($r = -.062$, $p = <2.2 \times 10^{-16}$), pICA was performed by age blocks of 5 years to accommodate the age-related confound (see Figure 1B and Supplemental Methods and Materials).

Statistical Analysis

Logistic regression (for binary behavioral outcomes) and linear regression (for continuous outcomes) analyses were conducted to examine the main effects of ePGS (continuous variable) and adversity scores (continuous variable) as well as their interaction effects on several outcomes.

RESULTS

Gene Networks Associated With Glucocorticoid Exposure

While the risk for common neuropsychiatric disorders emerges from polymorphisms in multiples genes that confer transcriptional alterations (46,70), recent genomic approaches have emphasized the importance of gene networks for cellular functions (46). Gene expression from macaque hippocampus were analyzed using WGCNA to identify coexpression gene networks associated with glucocorticoid exposure and preserved between the independent Singaporean and Vietnamese macaque groups as well as in a rodent chronic glucocorticoid model. The preservation analysis using RNA sequencing from a rat model of chronic glucocorticoid exposure (6 weeks of corticosterone) aimed to examine the conservation of glucocorticoid-related modules between species and ensure that the selected modules were relevant for both short- and long-term exposure to elevated glucocorticoid levels.

WGCNA identified a total of 52 modules (coexpressed networks) in the pDG of Singaporean macaques. Using the module eigengene-trait analysis that measures the strength and direction of association between gene expression variation in the module and a condition, 6 of the 52 modules were significantly ($p < .05$) associated with betamethasone treatment (Figure 2A–C). Three modules (pale turquoise, orange, and black) showed significant positive correlations and 3 showed significant negative correlations (purple, cyan, and thistle2) between the module eigengene (first principal component of the module to assess variation in gene expression) and betamethasone exposure (Figure 2C).

We used the WGCNA module preservation function (71) to confirm the reproducibility of these modules for glucocorticoid sensitivity. We identified module preservation between the pDG Singaporean and the Vietnamese macaque cohort ($n = 6$/group) for 3 of the previously identified modules in pDG: black, purple, and cyan (Figure 2D). We performed a second preservation analysis using a dorsal DG of adult female rat model of chronic (6 weeks, $n = 9$/group) corticosterone exposure to

disequilibrium clumping. We used a count function of the number of alleles at a given SNP (rs1, rs2,…) weighted by the estimated effect of the genotype on gene expression, and the sum of these values from the total number of SNPs provided the ePGS score. **(B)** Multivariate analysis performed by parallel ICA. We analyzed the interrelated data patterns from 2 different data modalities, genetic and neuroimaging. For the genetic modality, the value given to each SNP derived from the ePGS (participant genotype × GTEx gene expression slope at each SNP comprised by the pDG ePGS-black) was selected. For the brain image modality, we used T1 images representing patterns of variation in gray matter density at the voxel level for each subject (UK Biobank). The influence of early-adversity exposure on significant linked components between the 2 modalities (genetic and brain imaging) was tested. aDG, anterior dentate gyrus; ePGS, expression-based polygenic score; GTEx, Genotype-Tissue Expression; ICA, independent component analysis; pDG, posterior dentate gyrus; SNP, single nucleotide polymorphism.

Table 1. Logistic and Linear Regressions and Simple Slope Analyses Using the ePGS Created From WGCNA Black Module in the pDG in Women (UK Biobank)/Girls (ALSPAC)

UK Biobank			
Outcome		Main Effect	
Main Effect of pDG ePGS-Black			
Diagnosis—psychotic disorders (n = 72,600; data-field = 20544)		β = 0.03, p = .58, pFDR = .90	
Diagnosis—depression (n = 72,660; data-field = 20544)		β = 0.004, p = .67, pFDR = .90	
ICD-10 F20–F29 schizophrenia, schizotypal and delusional disorders (n = 218,539; data-field = 41270)		β = 0.003, p = .94, pFDR = .94	
ICD-10 F30–F39 mood (affective) disorders (n = 218,539; data-field = 41270)		β = 0.01, p = .15, pFDR = .61	
Main Effect of Early Environmental Adversity Score			
Diagnosis—psychotic disorders (n = 44,519)		β = 0.39, $p < .001$, pFDR = 1.59×10^{-12}[c], f^2 = 0.02	
Diagnosis—depression (n = 44,548)		β = 0.31, $p < .001$, pFDR = 1.44×10^{-207}[c], f^2 = 0.03	
ICD-10 F20–F29 schizophrenia, schizotypal and delusional disorders (n = 44,640)		β = 0.56, $p < .001$, pFDR = 5.16×10^{-7}[c], f^2 = 0.04	
ICD-10 F30–F39 mood (affective) disorders (n = 44,640)		β = 0.37, $p < .001$, pFDR = 2.18×10^{-61}[c], f^2 = 0.02	
Outcome	Adversity × ePGS Interaction	Adversity Effect for Higher ePGS	Adversity Effect for Lower ePGS
Interactions Between pDG ePGS-Black × Early Environmental Score			
Diagnosis—psychotic disorders (n = 44,519)	β = 0.16, p = .002, pFDR = .01[a], f^2 = 0.004	β = 0.55, $p < .001$[b]	β = 0.22, p = .006[b]
Diagnosis—depression (n = 44,548)	β = 0.006, p = .53, pFDR = .71	–	–
ICD-10 F20–F29 schizophrenia, schizotypal and delusional disorders (n = 44,640)	β = 0.24, p = .02, pFDR = .048[a], f^2 = 0.008	β = 0.83, $p < .001$[b]	β = 0.21, p = .02[b]
ICD-10 F30–F39 mood (affective) disorders (n = 44,640)	β = −0.006, p = .80, pFDR = .80	–	–
ALSPAC			
Outcome		Main Effect	
Main Effect of pDG ePGS-Black			
SDQ peer problems—9 years 7 months (n = 2269)		β = 51.2, p = .44, pFDR = .47	
SDQ emotional symptoms—9 years 7 months (n = 2354)		β = −61.5, p = .47, pFDR = .47	
SDQ peer problems—11 years 8 months (n = 2153)		β = 137.5, p = .06, pFDR = .23	
SDQ emotional symptoms—11 years 8 months (n = 2209)		β = −77.5, p = .38, pFDR = .47	
Main Effect of Early Environmental Adversity Score			
SDQ peer problems—9 years 7 months (n = 1666)		β = 0.21, $p < .001$, pFDR = 3.67×10^{-15}[c], f^2 = 0.04	
SDQ emotional symptoms—9 years 7 months (n = 1722)		β = 0.17, $p < .001$, pFDR = 1.31×10^{-6}[c], f^2 = 0.02	
SDQ peer problems—11 years 8 months (n = 1594)		β = 0.16, $p < .001$, pFDR = 1.41×10^{-7}[c], f^2 = 0.02	
SDQ emotional symptoms—11 years 8 months (n = 1630)		β = 0.22, $p < .001$, pFDR = 2.10×10^{-9}[c], f^2 = 0.03	
Outcome	Adversity × ePGS Interaction	Adversity Effect for Higher ePGS	Adversity Effect for Lower ePGS
Interactions Between pDG ePGS-Black × Early Environmental Score			
SDQ peer problems—9 years 7 months (n = 1666)	β = 195.6, p = .003, pFDR = .01[a], f^2 = 0.005	β = 0.30, $p < .001$[b]	β = 0.14, $p < .001$[b]
SDQ emotional symptoms—9 years 7 months (n = 1722)	β = 114.9, p = .18, pFDR = .23	–	–
SDQ peer problems—11 years 8 months (n = 1594)	β = 190.4, p = .01, pFDR = .03[a], f^2 = 0.004	β = 0.25, $p < .001$[b]	β = 0.09, p = .05
SDQ emotional symptoms—11 years 8 months (n = 1630)	β = 108.5, p = .23, pFDR = .23	–	–

ALSPAC, Avon Longitudinal Study of Parents and Children; ePGS, expression-based polygenic score; FDR, false discovery rate; pDG, posterior dentate gyrus; SDQ, Strengths and Difficulties Questionnaire; WGCNA, weighted gene coexpression network analysis.

[a]Significant interaction between the pDG ePGS-black and early adversity score ($p < .05$, pFDR < .05).

[b]Significant simple slopes for higher- and lower-ePGS groups ($p < .05$).

[c]Significant main effect of pDG ePGS-black or adversity score ($p < .05$, pFDR < .05).

investigate the conservation of the glucocorticoid-related modules between species. Black and cyan modules were moderately preserved (Figure 2D) across species. We focused our analysis on the black module (563 genes, Table S2), which showed a stronger correlation and p value in the module-trait analysis (r = 0.71; p value = .01) (Figure 2C) than cyan (r = −0.61, p value = .05). The positive correlation with glucocorticoid exposure found in the black module revealed

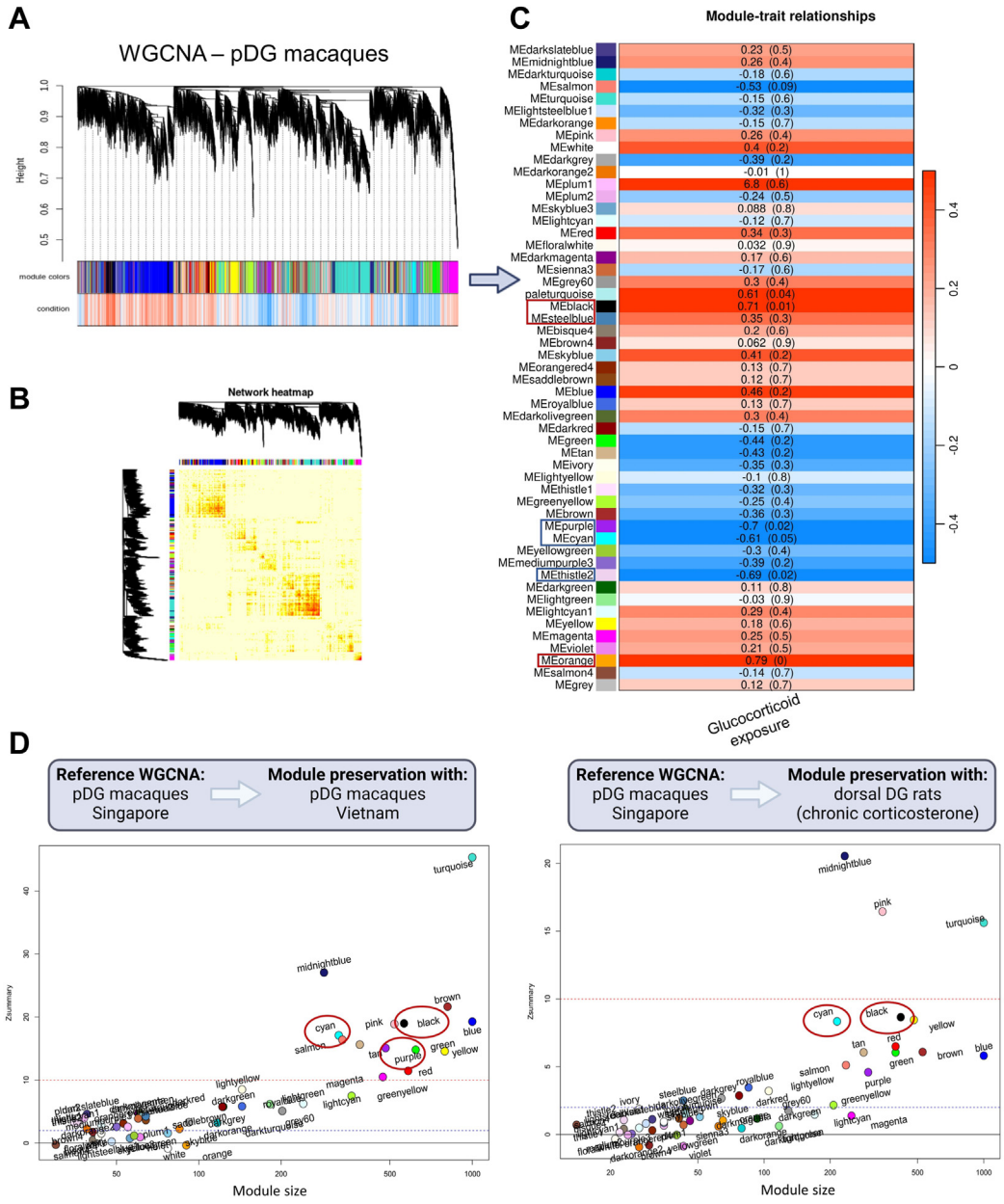


Figure 2. Identification of the coexpressed modules correlated with glucocorticoid exposure and preserved between different datasets in the pDG using WGCNA. **(A)** Hierarchical clustering dendrogram of WGCNA modules in the pDG of female macaques from Singapore. **(B)** Heatmap representing the topological overlap matrix for all genes in the WGCNA. Each row and column correspond to a gene. Darker colors represent higher coexpression interconnectedness (higher topological overlap). **(C)** The panel shows the correlation of WGCNA modules and betamethasone exposure by module-trait relationships analysis. Modules significantly associated with betamethasone (correlation < 0.5 and p value $< .05$) are indicated by rectangles. The red rectangles represent modules with significant positive correlations and blue rectangles represent modules with negative correlations with glucocorticoid exposure. **(D)** Zsummary statistics of module preservation analysis comparing the modules from pDG macaques from Singapore with pDG macaques from Vietnam, as well as with dorsal DG data from female rats after chronic exposure to glucocorticoids. Red circles represent the modules significantly correlated with glucocorticoid exposure and statistically preserved between the datasets. pDG, posterior dentate gyrus; ME, module eigengene; WGCNA, weighted gene coexpression network analysis.

that all genes in this network followed the same pattern of increasing gene expression with betamethasone exposure. Informatic analyses with the pDG black module showed significant enrichment for transcriptional activity, regulation of gene expression, and nervous system development (see Supplemental Methods and Materials for complete details), which is unsurprising because the glucocorticoid receptor acts as a ligand-gated transcription factor (72).

WGCNA for the aDG was similarly applied with the RNA sequencing data from the Singaporean macaques (Figure S3A–C). The WGCNA revealed 48 modules (Figure S3C), 7 of which were significantly associated with betamethasone. Three modules were positively correlated (darkolivegreen, skyblue3, and brown4) and 4 were negatively correlated (magenta, cyan, tan, and red) with glucocorticoid exposure (Figure S3C). Module preservation analysis using aDG RNA sequencing of Vietnamese macaques showed that only the red module was preserved between the 2 cohorts (Figure S3D). However, no preservation was found in the second preservation analysis using the RNA sequencing from the orthologous ventral DG (Figure S3D) of corticosterone-treated female rats. Because no modules were preserved across species, we focused subsequent analyses on the black pDG module. The aDG red module was used as a validation network for tissue specificity for the genetic score analysis in humans because it was a module that was positively associated with glucocorticoid exposure in a different brain region.

The Glucocorticoid-Related pDG Coexpression Network in Humans

We evaluated whether genes comprising our pDG black coexpressed network (475 orthologous humans' genes) showed comparable gene expression patterns in humans. To validate the applicability of this translational approach, we explored the correlation based on gene expression using data from human postmortem hippocampal samples from BrainSpan (http://www.brainspan.org) (56), which contains gene expression data from childhood (ages 1–11 years) and adulthood (21–40 years). In adult samples, 46% (of 473 human genes from our network) had high correlation patterns of gene expression (correlation > 0.5) in adult hippocampal samples from BrainSpan (Figure 3E). The high prevalence of genes with an expression correlation > 0.5 was significant at a p value $< .0001$ based on the null distribution of 10,000 permuted correlations of 473 genes randomly selected from BrainSpan-expressed transcripts. In the child samples, we observed 64% (p value $< .001$) of highly coexpressed genes (correlation > 0.5, considering 472 human genes from our network based on the null distribution of 10,000 permuted samples) (Figure 3D). These findings revealed that the macaque pDG black module showed gene expression profiles that significantly overlap with those in human hippocampal samples across the life span and especially during development. Indeed, the higher percentage of gene expression correlation patterns in childhood (64%) compared with adulthood (46%) revealed a significant difference as a function of age (Fisher's exact test, p value $= 2.2 \times 10^{-16}$, odds ratio = 0.71).

The Glucocorticoid-Related pDG Network and Human Mental Health

We explored the biological relevance of the glucocorticoid-sensitive pDG black network for various human mental disorders to provide the empirical basis for a focused analysis. We investigated whether the most central genes in the pDG network also contained SNP-associated genes from GWASs of psychiatric disorders or brain volume (Figure 3F). The pDG black network was significantly enriched for genes from the major depressive GWAS 2019 (73) (p false discovery rate–corrected [FDR] = .037, 131 overlapping genes corresponding to 27.6%), the suicide attempt in major depressive disorder GWAS 2019 (74) (pFDR = .037, 473 overlapping genes corresponding to 99.6%), the schizophrenia GWAS 2022 (75) (pFDR = .053, 474 overlapping genes corresponding to 99.8%), and the brain volume GWAS 2019 (76) (pFDR = .037, 473 overlapping genes corresponding to 99.6%). These associations are consistent with the known association between glucocorticoid exposure and both mood disorders and psychosis. Our gene network was also enriched for SNPs associated with brain volume, which is affected by glucocorticoid treatment in humans (22,24,77–80). These results guided the selection of outcomes in human cohorts to depressive and psychotic/schizophrenic conditions as well as brain volume.

A Polygenic Score Based on the pDG Glucocorticoid-Sensitive Network Moderates the Effect of Early Adversity on Mental Health

Our bioinformatic approach was intended to provide a platform by which to interrogate human data to examine specific hypotheses about the molecular mechanisms linking early adversity to mental health. Our focus was on the mediational role of hippocampal glucocorticoid signaling. Toward this aim, we used genes from pDG black network as the basis for the calculation of a polygenic score in humans (ePGS) (see Methods and Materials). The rationale for this approach assumes that a cumulative index of SNPs that are associated with expression in genes in the network should create a polygenic score that reflects the functional activity of the network and, according to our hypothesis, predict mental health outcomes as a function of early adversity. Thus, variations in our pDG ePGS-black network score reflect changes in gene expression associated with glucocorticoid exposure from the genes that comprise our network in the human hippocampus (higher scores represent greater expression in response to glucocorticoids).

Among UK Biobank adult women (n = 218,539), we observed no main effect of the pDG ePGS-black on the ICD-10 or self-reported diagnoses (Table 1). In contrast, and consistent with our hypothesis, we found a statistically significant interaction effect between the pDG ePGS-black and the early adversity score on the risk for "ICD-10 schizophrenia, schizotypal and delusional disorders" ($\beta = 0.24$, pFDR = .048, $f^2 = 0.008$) (Figure 4A) and "psychotic disorders" ($\beta = 0.16$, pFDR = .01, $f^2 = 0.004$) (Figure 4B). Adult women with a higher pDG ePGS-black score were at higher risk for each of these outcomes as a function of increased early adversity (Figure 4; Table 1). These findings indicate that the influence of early adversity on the risk for psychosis in adulthood was significantly moderated by a higher ePGS derived from the glucocorticoid-sensitive pDG black module (Figure 4A, B).

In ALSPAC, we found significant interaction effects in girls between the pDG ePGS-black and early adversity for the peer problems score from the Strengths and Difficulties Questionnaire at both 9 ($\beta = 195.6$, pFDR = .01, $f^2 = 0.005$) (Figure 4D) and 11 ($\beta = 190.4$, pFDR = .03, $f^2 = 0.004$) years of age (Figure 4E). Simple slope analysis showed that individuals with a higher ePGS score for glucocorticoid-responsive genes and who were exposed to greater early-life adversity showed

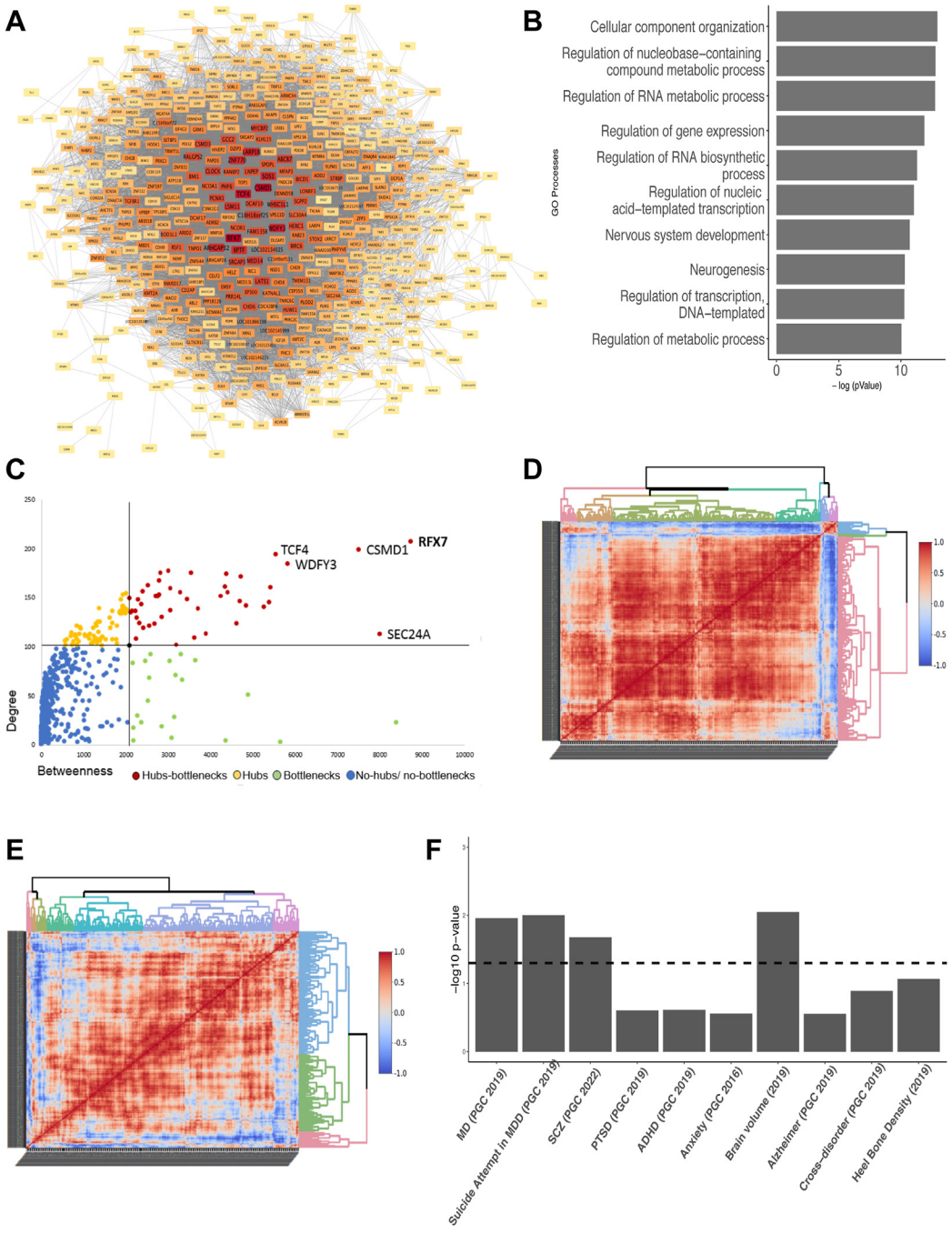


Figure 3. Functional characterization of the pDG black module. **(A)** Network structure of the pDG black module. Darker color represents the genes with the highest degree. **(B)** Enrichment analysis showing the significant biological processes involved in the pDG black gene network (Metacore, false discovery rate < .05) (see also Figure S5 for associated pathways). **(C)** Topological properties of the gene network showing hubs (degree higher than +1 SD above the mean), bottlenecks (betweenness higher than +1 SD above the mean), and hub-bottlenecks genes. Lines in black indicate mean +1 SD for degree and betweenness. **(D)** Heatmap for the human coexpression of the genes included in the pDG black module during infancy and **(E)** adulthood in postmortem human hippocampal samples. Genes from the same expression quantification tended to cluster together and are visualized in red (positive correlation) or blue (negative correlation) patterns. Clustering of genes was represented by branches in different colors, grouping genes with similar expression patterns. The heatmap of genes coexpressed showed 64% and 46% of highly coexpressed genes (considering correlation pattern higher than 0.5), respectively in both periods. The childhood period ranges from 1 to 11 years (n = 8) and the adulthood period ranges from 21 to 40 years of age (n = 6). Data for this analysis were extracted from BrainSpan. **(F)** Genome-wide association study enrichment. Spearman's correlation coefficients were applied between the $-\log_{10} p$ value for the single nucleotide polymorphism associated with genes in each genome-wide association study with the module membership of the genes in the black module. ADHD, attention-deficit/hyperactivity disorder; GO, gene ontology; MDD, major depressive disorder; pDG, posterior dentate gyrus; PGC, Psychiatric Genomics Consortium; PTSD, posttraumatic stress disorder; SCZ, schizophrenia.

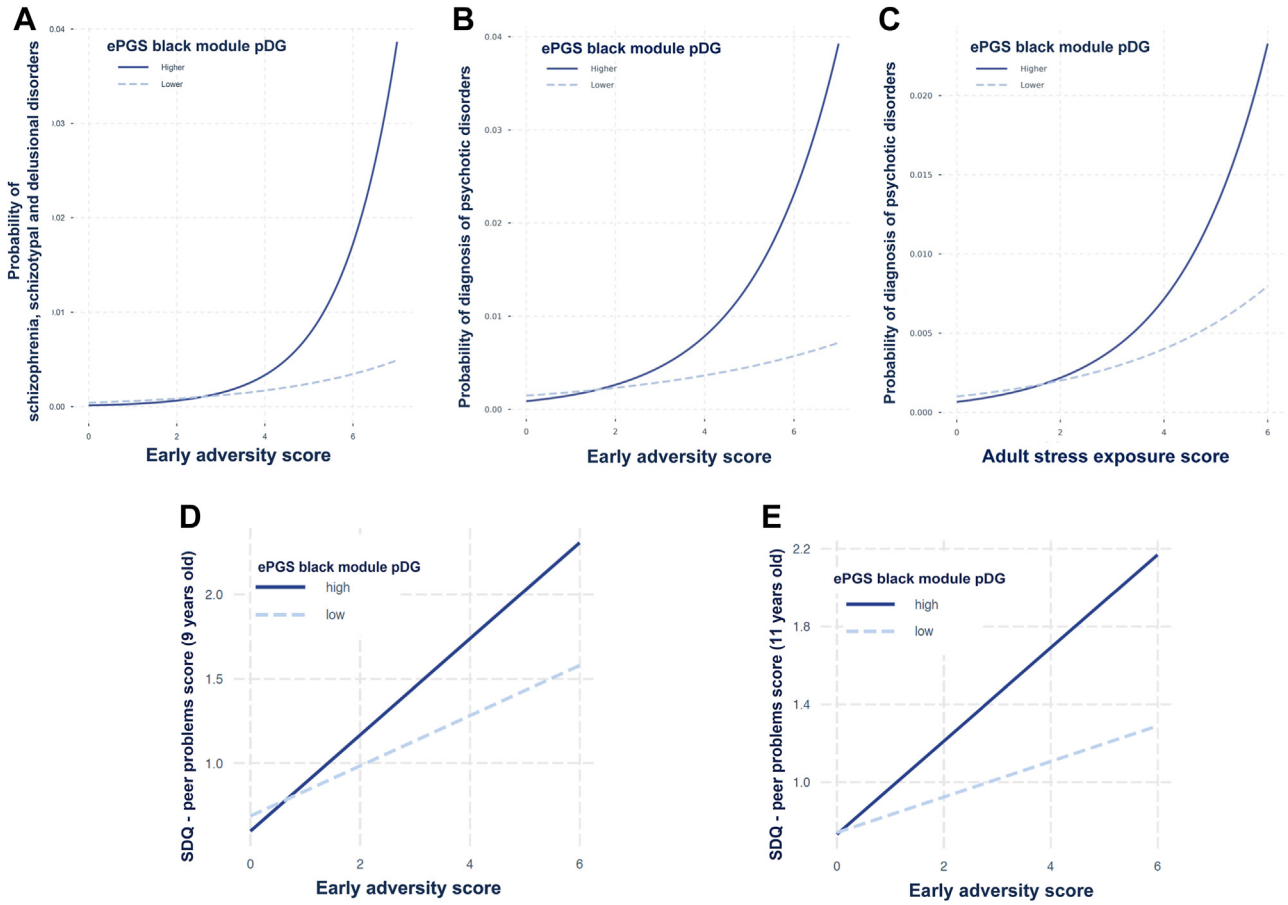


Figure 4. Phenotypic differences predicted by the pDG ePGS-black in the human cohorts. Significant interactions between early adversity exposure/adult stress exposure and the pDG ePGS-black were observed on **(A)** "ICD-10 schizophrenia, schizotypal and delusional disorder" ($\beta = 0.24$, $p = .02$, pFDR = .048, $n = 44{,}640$), **(B)** "psychotic disorders" diagnosis in the UK Biobank ($\beta = 0.16$, $p = .002$, pFDR = .01, $n = 44{,}519$), **(C)** "psychotic disorders" diagnosis in the UK Biobank ($\beta = 0.13$, $p = .0008$, pFDR = .03, $n = 36{,}245$), **(D)** SDQ for peer problems at 9 years of age in ALSPAC ($\beta = 195.6$, $p = .003$, pFDR = .01, $n = 1666$), and **(E)** 11 years in ALSPAC ($\beta = 190.4$, $p = .01$, pFDR = .03, $n = 1594$). High adversity exposure is associated with poorer outcomes as the ePGS increases for all analyses. Only female humans were selected in these analyses. ALSPAC, Avon Longitudinal Study of Parents and Children; ePGS, expression-based polygenic score; pDG, posterior dentate gyrus; SDQ, Strengths and Difficulties Questionnaire.

higher scores for peer problems at both ages (Table 1). Interestingly, peer problems in childhood strongly have been shown to predict later risk for schizophrenia (81–84), thus linking the findings with children to those from the UK Biobank.

Genetic-Neuroimaging Relations

We used multivariate analysis to combine genetic and neuroimaging data based on the established influence of glucocorticoids on brain morphology (18). pICA aimed to investigate patterns of associations between subsets of SNPs from pDG ePGS-black and specific features of whole-brain structure in UK Biobank participants at different ages while also accounting for the influence of early adversity exposure (Figure 1B). Because there is a significant correlation between age and the volume of gray matter in subjects from the UK Biobank ($r = -0.062$, $p < 2.2 \times 10^{-16}$), pICA was performed in bands of 5 years between 40 and 70 years.

Interestingly, a significant effect (Table 2) of the early-life adversity score was observed between genetic and imaging measures from all age bands except for 50 to 55 years. This finding reveals that the relationship between the SNP-based ePGS and gray matter volume was moderated by early-life adversity. Cerebellar regions, the parahippocampal gyrus, insula, medial frontal gyrus, and anterior and posterior cingulate cortex were the brain regions that were most strongly associated with the genetic components extracted from the whole ePGS-black network (Figure 5A; Table S3). We observed an overlap of 72 genes annotated from the significant SNPs across all age bands. Enrichment analysis (Metacore) for these genes showed a pronounced involvement with anatomical structure and system development processes, as well as nervous system development and morphogenesis (Figure 5C). These findings highlight a possible underlying process by which glucocorticoid may mediate the effects of early adversity on brain development and function.

Table 2. Significant Differences Between Loading Coefficients by Component Modality and Environmental Group Analyzed by Parallel ICA

Genetic and Neuroimaging Linked Components by Age Blocks	High Adversity Score, Mean (SD)	Low Adversity Score, Mean (SD)	*t* Value	*p* Value
Ages 40–45 Years				
MRI component 40	−0.00513 (0.01061)	0.00209 (0.01012)	−5.7085	2.6136×10^{-8}
Genetic component 11	−0.00030 (0.00419)	0.00246 (0.00380)	−5.5939	4.7915×10^{-8}
Ages 46–50 Years				
MRI component 14	−0.00232 (0.01163)	0.00173 (0.00971)	−3.8758	.00012
Genetic component 9	−0.00009 (0.00392)	0.00110 (0.00351)	−3.3227	.0010
Ages 51–55 Years				
MRI component 39	0.00245 (0.01022)	0.00080 (0.01124)	1.7132	.087
Genetic component 8	0.00039 (0.00333)	0.00039 (0.00375)	0.022	.982
Ages 56–60 Years				
MRI component 6	−0.00665 (0.01421)	−0.01023 (0.01352)	2.9695	.0031
Genetic component 7	0.00026 (0.00326)	0.00102 (0.00363)	−2.5717	.0104
Ages 61–65 Years				
MRI component 47	−0.00482 (0.00952)	−0.00269 (0.01053)	−2.3084	.021
Genetic component 21	0.00028 (0.00314)	−0.00088 (0.00323)	3.922	9.92×10^{-5}
Ages 66–70 Years				
MRI component 16	−0.00203 (0.01510)	−0.00878 (0.01453)	2.925	.004
Genetic component 21	0.00057 (0.00448)	−0.00264 (0.00381)	4.8586	2.44×10^{-6}

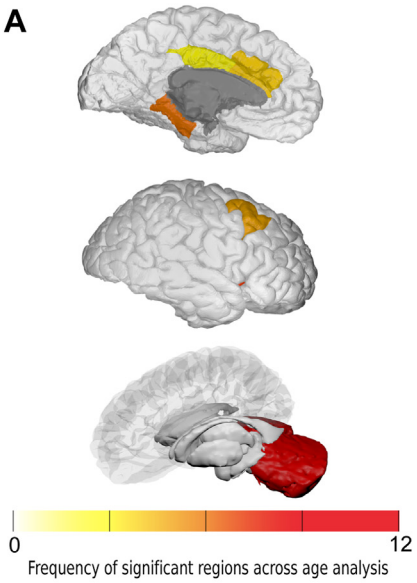
ICA, independent component analysis; MRI, magnetic resonance imaging.

Glucocorticoid-Sensitive pDG Network Is Enriched for DNA Methylation Sites

Our findings suggest that the glucocorticoid-sensitive gene network moderates the association between early adversity and brain structure and mental health outcomes. We explored the possibility that these effects might involve glucocorticoid-induced epigenetic remodeling. Glucocorticoids are known to alter DNA methylation in hippocampal neurons (52). We used an in vitro dataset from a human fetal hippocampal progenitor cell line repeatedly exposed to glucocorticoid during proliferation and neuronal differentiation, which altered DNA methylation profiles (52). We found that a strikingly high 61.3% (291/475 genes) of the human orthologous genes from the pDG black network overlapped with the genes from the study by Provençal *et al.* (52) that showed a significant glucocorticoid-induced change in DNA methylation in human hippocampal neurons (n = 27,812 probes mapped to n = 11,645 genes, Fisher's exact test, p value = 2.129×10^{-5}, odds ratio = 1.52). The distribution of the number of overlapping genes resulting from a permutation procedure (10,000×) provided the null distribution against which the observed pDG black network overlapped. The 61.3% prevalence of overlapped genes was significant at p value $< .02$, revealing the specificity of the findings from the pDG black network. This finding suggests that our gene network may mediate the effects of early-life adversity on mental health outcomes through epigenetic mechanisms.

DISCUSSION

The identity of the biological pathways that link environmental risk factors to psychopathology is a critical gap in our current understanding of the pathophysiology of mental disorders. We addressed this issue using an approach that integrates transcriptomic data from relevant model systems with human genomics to test specific hypotheses focused on biological mediators. In this study, we examined hippocampal glucocorticoid signaling as a mediator of the association between early adversity and psychopathology. We assumed that genes operate in networks and therefore used whole WGCNA to define a gene network in the pDG that was highly sensitive to chronic glucocorticoid exposure in nonhuman primates. We note that the pDG glucocorticoid-sensitive network was preserved across species despite different durations of glucocorticoid treatment (1 week for macaques and 6 weeks for rats). This finding suggests that this pDG network captures both long- and short-term glucocorticoid effects. Comparisons with relevant human databases (BrainSpan) verified that this gene network showed similar coexpression patterns in the human hippocampus throughout development, confirming the overlap with the human transcriptome. A computational approach was used to develop a polygenic score based on SNPs in the pDG glucocorticoid-sensitive network to create the ePGS. This polygenic score provided an informatic instrument to examine our hypothesis concerning the mediational role of hippocampal glucocorticoid-sensitive genes in the association between early adversity and mental health. The ePGS significantly moderated the association between early-life adversity and psychotic symptoms in adulthood or endophenotypes for psychosis in childhood. Likewise, we found evidence for an interaction between the glucocorticoid-sensitive ePGS and early-life adversity on brain volume. Our findings suggest that the association



B

Common genes across all ages				
AC097467.3	CORIN	LRP1B	PTPRK	TENM2
ACAN	CSMD1	MAGI2	RALGPS2	TENM3
ACOX3	DCAF10	MBD5	RBM20	THSD7B
ACTN2	DSCAM	MMP16	RUNX2	TMEM131
ADRA1A	EP300	MTMR3	SETBP1	TNRC6C
AFF3	EVI5	N4BP2	SGCG	TOP1
AKAP9	FAM120A	NAV3	SLCO2A1	TRPM3
ANO1	IGF1R	NCBP3	SLCO5A1	UNC13C
ARHGEF28	IQCB1	NCOA6	SMYD4	ZNF320
ATP10D	ITGAE	NFIB	SNX27	ZNF331
ATXN7L1	ITPRID1	NIN	SORL1	ZNF609
BICD1	KBTBD11-OT1	NUBPL	SOX5	ZNF618
BOD1L1	KDM1B	NXN	STRIP2	
CACNA1C	LARP1B	PIP5K1A	SUPT3H	
CNTN3	LARP4B	PRR16	SYT14	

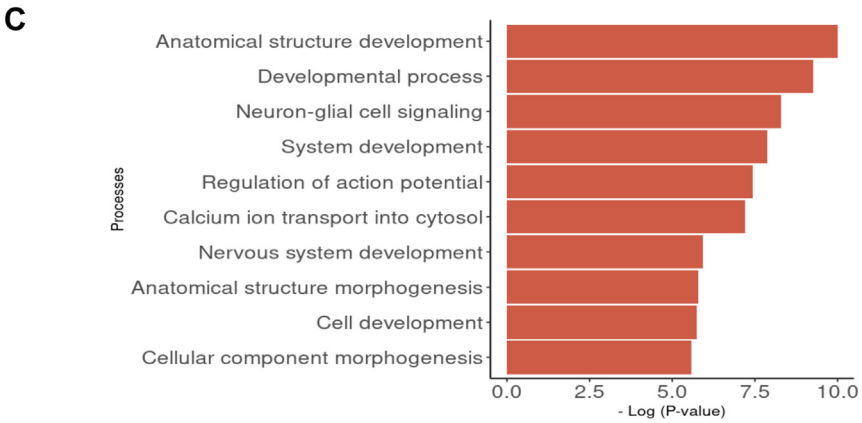


Figure 5. Summary representation of significant brain regions and single nucleotide polymorphisms for all age groups from significant linked components across genetic and magnetic resonance imaging data modality performed by parallel independent component analysis. **(A)** Brain regions of significant gray matter density variations associated with genetic information variation across all ages. Color scheme represents the frequency of regions across age analysis (including positive and negative associations), highlighting regions such as the cerebellum, parahippocampal gyrus, insula, posterior and anterior cingulate, and medial frontal gyrus for being the most associated with the genetic data. Brain images were generated using BrainPainter (https://brainpainter.csail.mit.edu/). **(B)** List of 72 genes derived from the significant subset of SNPs overlapped across all ages associated with gray matter density variation. **(C)** Enrichment analysis for the 72 overlapped genes associated with variations in gray matter density at all ages (MetaCore, false discovery rate $< .05$).

between glucocorticoids and both neurodevelopment and mental health (27,33–38) may be conditional to exposure to early-life stressors.

The clinical use of glucocorticoids has been associated with the development of psychiatric symptoms, including psychosis (4,85–90). Psychiatric disorders secondary to the use of corticosteroids have a specific code in the DSM-5 as substance/medication-induced mental disorders (91). There are indications of a female predominance in psychiatric complications with corticosteroid therapy (92). Glucocorticoid exposure during development is associated with an increased risk for psychotic symptoms (38). Childhood adversity increases the occurrence and severity of psychosis (93), thereby affecting hippocampal subfields in patients with first-episode schizophrenia (94,95). Interestingly, this effect is unique to females (94).

Glucocorticoids are known to alter DNA methylation in hippocampal neurons (52,96,97). A striking 61.3% of the pDG ePGS network overlapped with genes showing glucocorticoid-induced alterations in DNA methylation in human hippocampal cells (52). Such effects on DNA methylation may modify access of transcriptional regulators to DNA sites to change subsequent stress-induced transcriptional activity (52). These findings suggest that glucocorticoids may moderate the impact of early adversity through epigenetic modifications across the hippocampal pDG ePGS glucocorticoid-sensitive network.

Conclusions

In sum, we demonstrated a translational approach to exploring transcriptional changes within coexpressed genes significantly associated with glucocorticoid in replicate datasets and across species. Variations in the expression of this network, in association with exposure to early adversity, predicted stress-associated neuropsychiatric outcomes. While we believe that this translational approach has broad application, the specific findings have some limitations. First, our transcriptomic data included only female macaques due to limitations related to our colony. Although the human analysis included both sexes separately, the inclusion of glucocorticoid-associated transcriptomic analysis in males is warranted in future studies. Another potential limitation is that our study is based on bulk RNA sequencing, which merges contributions from composite cell types in the DG. Future studies using single-cell RNA sequencing in macaques will provide cell-type markers to extend these findings. The use of European cohorts in this study may be a limitation because the results may not necessarily generalize to other populations. These limitations notwithstanding, our study provides a novel approach for the use of transcriptomic data from relevant model systems for identifying gene-network signatures of individual differences in susceptibility to early environmental influences in humans.

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Data from the macaque gene expression RNA sequencing were deposited at the GEO repository under GEO: GSE15134. The codes generated during this study are available at https://github.com/MeaneyLab/WGCNA/blob/main/WGCNA%20script.R for weighted gene coexpression network analysis; https://github.com/MeaneyLab/PRSoS for expression-based polygenic score calculation; https://github.com/SilveiraLab/GWAS-enrichment-analysis for genome-wide association study enrichment analysis.

The data that support the findings of this study are available from the UK Biobank and ALSPAC management teams, but restrictions apply to the availability of these data, which were used under license for the current study and so are not publicly available. For the UK Biobank, data can be purchased; the study website contains details of all the data available at https://biobank.ndph.ox.ac.uk/showcase/. For ALSPAC, data can be purchased; the study website contains details of all the data available through a fully searchable data dictionary and variable search tool at http://www.bristol.ac.uk/alspac/researchers/our-data/.

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ARTICLE INFORMATION

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