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Archival Report

Multiomics Analysis of the Molecular Response to Glucocorticoids: Insights Into Shared Genetic Risk From Psychiatric to Medical Disorders

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ABSTRACT

BACKGROUND: Alterations in the effects of glucocorticoids have been implicated in mediating some of the negative health effects associated with chronic stress, including increased risk for psychiatric disorders and cardiovascular and metabolic diseases. In this study, we investigated how genetic variants influence gene expression and DNA methylation in response to glucocorticoid receptor (GR) activation and their association with disease risk.

METHODS: We measured DNA methylation (n = 199) and gene expression (n = 297) in peripheral blood before and after GR activation with dexamethasone, with matched genotype data available for all samples. A comprehensive molecular quantitative trait locus (QTL) analysis was conducted, mapping GR-response methylation (me)QTLs, GR-response expression (e)QTLs, and GR-response expression quantitative trait methylation (eQTMs). A multilevel network analysis was employed to map the complex relationships between the transcriptome, epigenome, and genetic variation.

RESULTS: We identified 3772 GR-response meCpGs corresponding to 104,828 local GR-response meQTLs that did not strongly overlap with baseline meQTLs. eQTM and eQTL analyses revealed distinct genetic influences on gene expression and DNA methylation. Multilevel network analysis uncovered GR-response network trio QTLs, characterized by SNP–CpG-transcript combinations where meQTLs act as both eQTLs and eQTMs. GR-response trio variants were enriched in a genome-wide association study for psychiatric, respiratory, autoimmune, and cardiovascular diseases and conferred a higher relative heritability per SNP than GR-response meQTL and baseline QTL SNPs.

CONCLUSIONS: Genetic variants modulating the molecular effects of glucocorticoids are associated with psychiatric as well as medical diseases and not uncovered in baseline QTL analyses.

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Glucocorticoids (GCs) are the main effectors of the hypothalamus-pituitary-adrenal axis and play a crucial role in mediating the acute stress response. They are also implicated in the negative health effects of chronic stress, which include increased risk for a variety of psychiatric disorders, cardiovascular diseases, diabetes, and cancer (1). Studies have shown that chronic exposure to GCs can increase morbidity and even reduce life span (2,3). In addition, synthetic GCs are effective in the treatment of different medical disorders but are also accompanied by side effects, both in short-term and chronic administration (4). GCs exert their influence on all tissues, including the immune system, where they acutely suppress the immune response (5). This immunosuppressive effect is leveraged in the treatment of autoimmune disorders. On the other hand, chronic stress has been associated with a decrease in the function of this inhibition and is often associated with a proinflammatory profile, which is a risk factor for a number of medical and psychiatric disorders (6). However, large interindividual differences are reported in the consequences of acute or chronic stress exposure as well as side effects experienced from GCs.

GCs exert their effects via mineralo- and glucocorticoid receptors (GRs), which are types of nuclear steroid hormone receptors. These receptors directly bind to gene regulatory elements at the DNA level and can either stimulate or repress gene transcription, leading to local epigenetic alterations (7). Individual variations in systemic response to GC exposure can be mediated in part by altered effects at the level of gene regulation. Using massively parallel reporter assays in cell lines and stimulated expression quantitative trait locus (eQTL) analyses in peripheral blood, we have previously reported that genetic variants can alter the effects of GCs at gene regulatory elements (8). These variants have been linked to a spectrum of outcomes, including altered risk for psychiatric disorders, variation in amygdala reactivity to threat, startle response, and cortisol response to a psychological stressor (8–10) as well as stress-related changes in brain physiology, such as the peak latency of the hemodynamic response function in limbic brain

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regions (11). In addition to genetic variation, the activity of GC response elements is also influenced by the local epigenetic landscape, including at the level of DNA methylation (DNAm) (7,12). Furthermore, it has been shown that GR activation can lead to changes in DNAm at its direct binding sites, mainly through DNA demethylation (13–15). Beyond the effects of transcription factors on chromatin accessibility and DNAm at gene regulatory elements (16), which are likely main mediators of the extensively explored environmental influences on DNAm (17), DNAm patterns can also be influenced by genetic variants known as methylation QTLs (meQTLs). A recent study highlighted the association between DNAm and single nucleotide polymorphisms (SNPs) in a large meta-analysis, mapping the role of meQTLs in shaping the epigenome (18). Furthermore, tissue-specific meQTLs have been identified (19), and studies that have focused on cell type–specific meQTLs have provided insights into the cell-specific effects of genetic variants on DNAm patterns (20,21). In addition to influencing baseline DNAm levels, genetic variation may alter the above-described impact of GR activation on DNAm in various tissues (13,14). However, to date, no study has systematically explored how common genetic variation can moderate this impact, which is the primary focus of this article.

As indicated above, the regulation of genes by GCs, and consequently its impact on disease risk or treatment outcomes, is multifactorial, involving genetic, epigenetic, and transcriptional factors. Multiomics approaches have emerged as powerful tools to disentangle such complex regulation (22), allowing for a comprehensive investigation of the molecular landscape that contributes to disease risk because these different levels are interrelated (23,24). For example, we have shown that lasting GR activation–induced alterations in DNAm within gene regulatory regions can influence the subsequent effects of GR activation on gene expression levels (18). This supports that changes in DNAm in response to stress-related stimuli can impact cellular responses and potentially contribute to the pathogenesis of various diseases.

In this study, we conducted the first multiomics analysis investigating the genetic moderation of the effects of GR activation via the agonist dexamethasone on changes in DNAm and gene expression. By integrating stimulus-dependent molecular QTLs into a comprehensive network analysis, we jointly analyzed DNAm and gene expression in response to GR activation in the context of common genetic variations. This analysis revealed a multiomics signature associated with GC-induced responses in peripheral blood as well as novel connections between functional, molecular GC effect–modulating variants, and disease risk variants.

METHODS AND MATERIALS

For detailed methods, see the Supplement.

Study Samples

The study involved 202 Caucasian participants from the Max Planck Institute of Psychiatry. Of these, 88 individuals were diagnosed with major depressive disorder (dataset GSE64930). Blood samples were collected at baseline and 3 hours after a single oral dose of 1.5 mg dexamethasone. The study was approved by the ethics board of the Ludwig Maximilians University (approval No. 244/01) and conducted in accordance with the Declaration of Helsinki.

DNAm Profiling

In our study, DNAm was evaluated using Illumina EPIC v1 Methylation arrays, with a total of 404 blood samples analyzed, comprising 202 samples for each of the 2 time points (baseline and GR response). Data processing involved several steps performed on both time points together, which led to a final dataset comprising 740,357 CpGs across 398 samples and 199 individuals.

Differential DNAm Analysis

A linear mixed effects model identified differentially methylated positions (DMPs) at a false discovery rate (FDR) of $<.05$, accounting for sex, age, body mass index, depression status, estimated white blood cell count, and genotype principal components. Differential methylation regions were identified using the ENmix R Bioconductor package and the comb-p function with a distance of 1000 bp and a seeded p value of .05.

Genotype Data and Imputation

Human DNA from EDTA blood samples was genotyped using Illumina Human610-Quad (n = 79) and OmniExpress (n = 120) BeadChips followed by quality control and imputation as previously described (9). The final dataset included 5,617,712 SNPs across 199 samples.

meQTL Analysis

The *cis*-meQTL analysis focused on evaluating the association of SNP-CpG pairs within a $\pm$1-Mb region at baseline utilizing standardized methylation changes (differences in DNAm postdexamethasone treatment relative to baseline standardized against the baseline). The latter are termed GR-response meQTLs. Significant meQTLs were identified at FDR $<.05$ using MatrixEQTL (25).

Gene Expression Data

Baseline and GC-induced gene expression in blood from 199 individuals were assessed using Illumina HumanHT-12 BeadChips (GSE64930) (9). This data underwent quality control processing for both time points together, resulting in a dataset encompassing 11,944 transcripts from 398 samples.

Expression Quantitative Trait Methylation Analysis

Expression quantitative trait methylation (eQTM) analysis was performed to assess the relationship between DNAm and gene expression. We examined 11,944 transcripts and 740,357 CpG sites within a $\pm$1-Mb window. For GR-response eQTMs, standardized methylation and expression values were correlated using MatrixEQTL, adjusting for covariates including those used in meQTL analysis and surrogate variables derived from gene expression data. Significant *cis*-eQTMs were identified at an FDR threshold of $<.05$.

Expression Quantitative Trait Analysis

We leveraged previously identified *cis*-GR-response eQTL results reported in (9) from the same cohort, which were based

on data from 297 individuals. Only a subset of 202 individuals (199 included in the meQTL analysis) had matched methylation data available. In the current analysis, we focused on identifying overlapping variants by comparing expression SNPs (eSNPs) from the eQTL analysis with meQTL methylation SNPs (meSNPs).

Functional Genomic Annotation and Characterization

Differentially methylated CpGs and GR-response meQTLs were annotated for genomic features using the minfi R package and the University of California Santa Cruz (UCSC) Genome Browser data from the R package ChIPseeker (26). meQTLs were also annotated for GR binding sites, and chromatin state enrichment was assessed using ChromHMM annotation (27,28).

Multiomics Network Inference and Analysis

Multiomics network inference and analysis were performed using KiMONo (29), which incorporated standardized changes in expression and methylation together with genetic variants and established relationships.

Gene Ontology Enrichment Analysis

For the Gene Ontology enrichment analysis, we used FUMA GENE2FUNC (30) with default parameters. The background list consisted of the 11,994 transcripts from our dataset.

Gene Overlap With Genes Induced by GR Activation in the Mouse Brain

To compare GR-response network trio QTL genes, specifically SNP-CpG-transcript combinations where meQTLs function as both eQTLs or eQTMs, we utilized GR-response genes in mouse brain tissue data from DiffBrainNet (31). Orthologs were mapped by using the R package orthologsBioMART. The significance of overlap was determined using Fisher's exact test in the R package GeneOverlap.

Genome-Wide Association Study Enrichment Analysis

We performed genome-wide association study (GWAS) enrichment analysis for GR-response trio SNPs. Linkage disequilibrium (LD)–independent SNPs were identified using PLINK (version 1.90b5.3) and matched to GWAS variants across psychiatric, metabolic, immune, and other phenotypes. GR-response meSNPs served as background. Enrichment was assessed with 1000 permutations, calculating empirical p values and odds ratios (ORs) with FDR correction (10%).

Mediation Analysis

Mediation analysis was conducted using the R package mediation to assess the indirect effect of SNPs on gene expression via DNAm. The model controlled for potential confounders.

Heritability Estimation

SNP-based heritability for 6 diseases (asthma, bipolar disorder, inflammatory bowel disease, multiple sclerosis, schizophrenia, stroke) was estimated using LD score regression version 1.0.1 and GWAS summary statistics. GR-response trio variants (n = 172) and GR-response meSNPs were assessed for their contribution to disease heritability using partitioned heritability.

RESULTS

We conducted a multiomics analysis assessing gene expression in 297 individuals and DNAm in 199 individuals, with genome-wide genotypes available for all participants. Blood samples were collected before and after dexamethasone administration.

Uncovering In Vivo GC-Induced Differential DNAm

We identified 3280 GR-response DMPs (Figure 1C and Table S1), with 76.4% hypomethylated and 23.6% hypermethylated. Most GR-response DMPs were located outside of CpG islands (72.3%), differing from the EPIC array's distribution (55.5%, Wilcoxon p value = .03). GR-response DMPs were not significantly different from the EPIC array's distribution in terms of genomic location (Figure 1D, E; Supplement). Differential methylation region analysis identified 4230 significant regions (Table S2), with 84.8% overlapping with DMPs.

Overlapping and Distinct Mechanisms of Genetic Control: Contrasting GR-Response meQTLs With Baseline meQTLs

We identified 104,828 GR-response meQTLs involving 3772 CpGs and 88,585 SNPs. Only 12 meCpGs were shared with DMPs (Table S3 and Figure 2A). The average distance between GR-response meSNPs and meCpGs was 105 kbp. Among the GR-response meCpGs, 69% overlapped with baseline meCpGs (n = 2618 CpGs) (Table S4). Examples of GR-response meQTLs with and without a baseline meQTL are illustrated in Figure 2B and C. GR-response meCpGs were primarily located in open sea regions (60.1%). GR-response meSNPs were located in introns (54.6%) and distal intergenic regions (32.7%), differing from baseline meSNPs (Wilcoxon p value = .05) (Figure 2D–F; Supplement). Furthermore, GR-response meCpGs were more frequently located within GC response elements (25.1%) than baseline meCpGs (20.3%) (Figure 2G, H).

Validation of baseline meQTLs using Genetics of DNAm Consortium (GoDMC) data showed a 77.6% replication rate.

Consistent Regulatory Patterns of GR-Response meQTLs Across Blood Cell Types

To understand the functional implications of GR-response meQTLs, we examined their distribution across functional genomic regions using ChromHMM epigenetic states in blood and T and B cell lines. Our analysis indicated that GR-response meCpGs were more frequently found in promoters and enhancers than GR-response meSNPs (Figure 3A, B). Additionally, we found that 44.6% of GR-response meCpG sites were associated with SNPs localized to the same chromatin state. Interestingly, neither GR-response meCpGs nor meSNPs showed significant differences in the distribution of epigenetic states across single blood cell types (pairwise Wilcoxon p value $>$ .05) (Figure 3A, B).

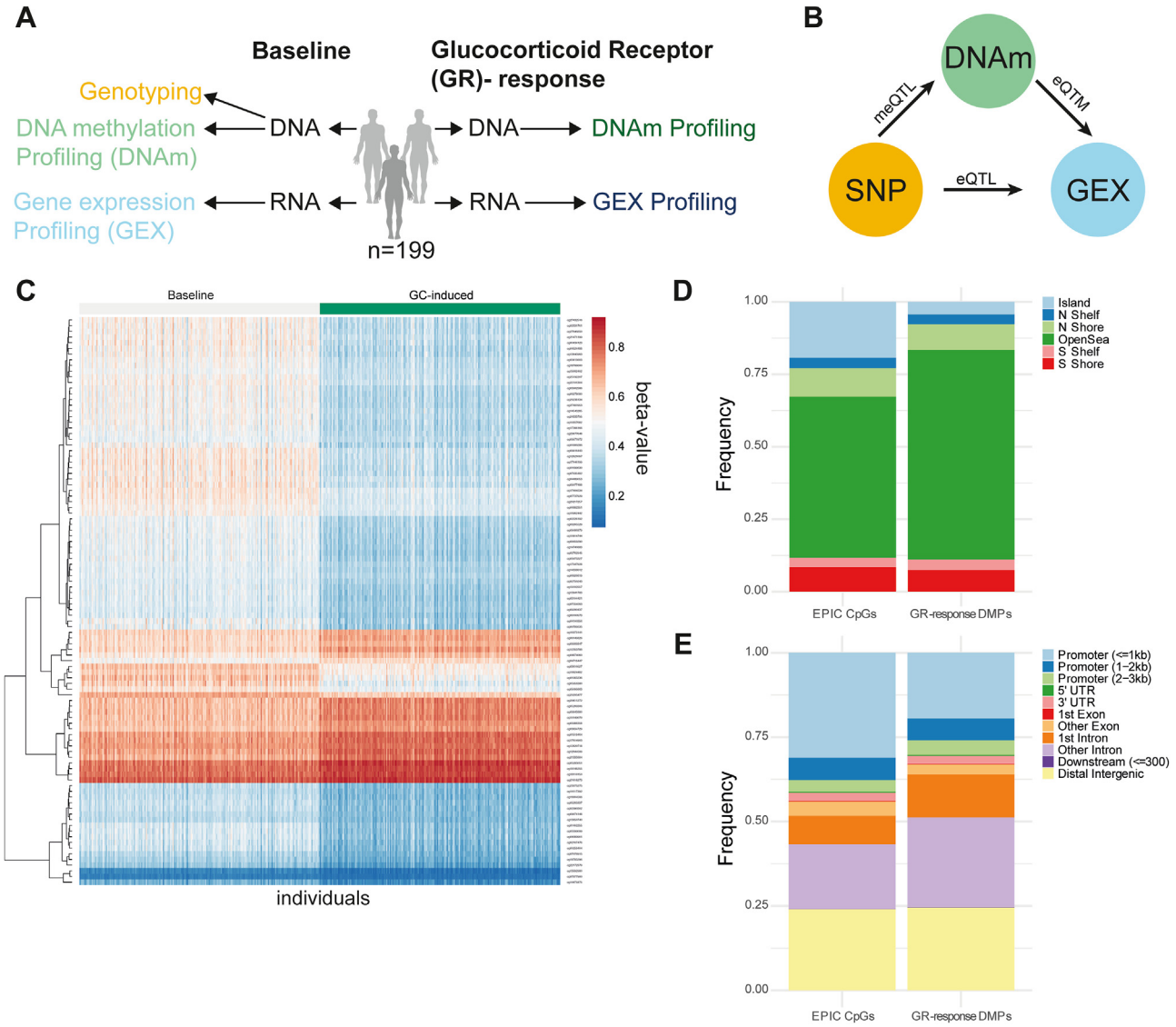
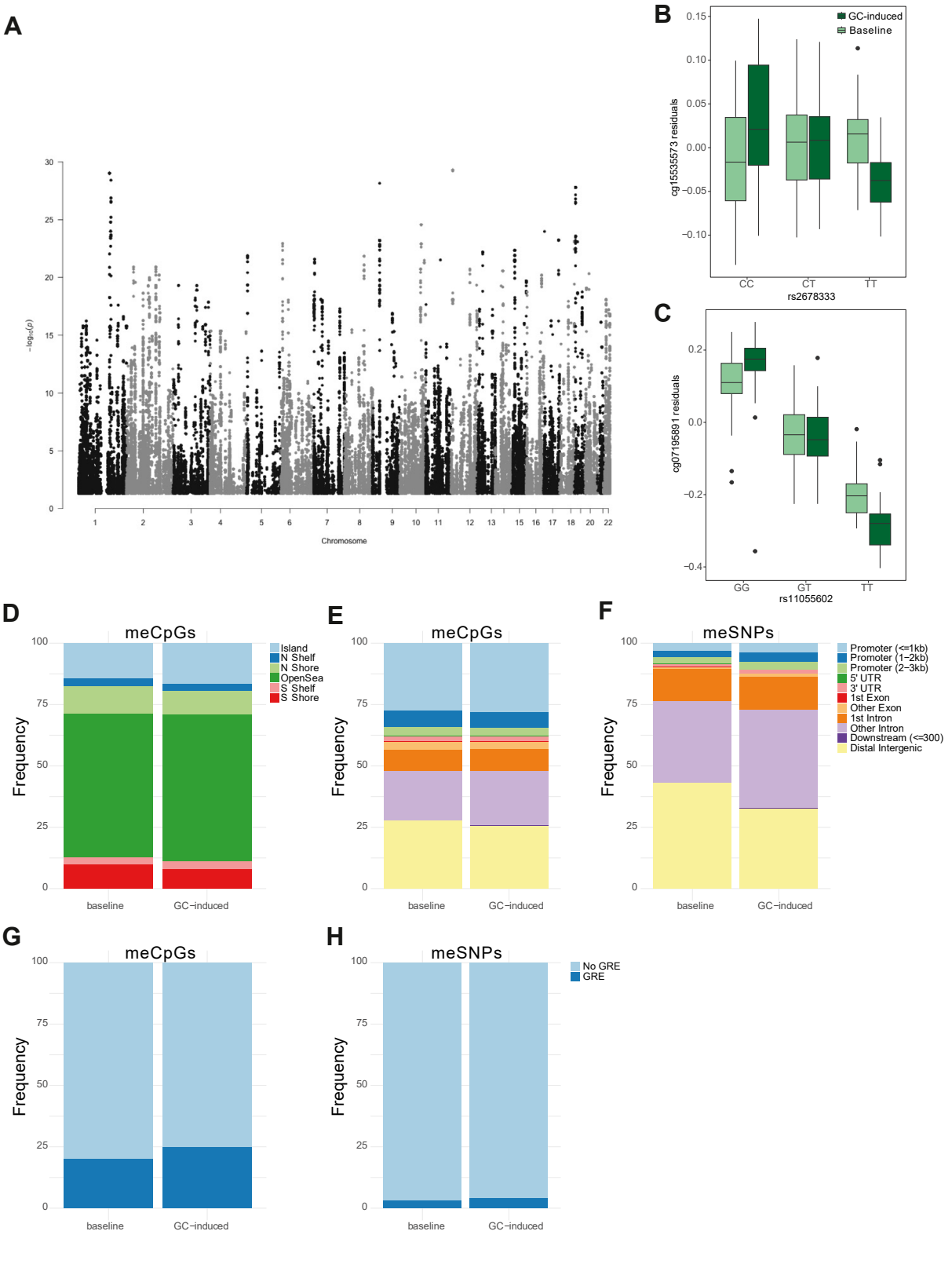


Figure 1. Overview of the GR-response meQTL study. **(A, B)** The stepwise experimental design used to investigate the genetic effects on the GR response in human whole blood involves several key steps: 1) treatment of 199 individuals with 1.5 mg dexamethasone per os; 2) transcriptome and methylome measurements were obtained from the entire cohort at 2 time points, baseline and 3 hours posttreatment; this included DNAm patterns assessed using the Illumina MethylationEPIC BeadChip, aligning with the time point of messenger RNA expression measures (same blood draw with Paxgene RNA and EDTA tubes); 3) genotype profiling was performed, utilizing a subset of our previously published GR-response eQTL analysis, to map GR-response meQTLs; 4) GR-response eQTM analysis was conducted to explore the relationships between gene expression and methylation; and 5) multiomics network inference and analysis were carried out, integrating the data to understand the genetic impact on GR responses. **(C)** Mean methylation profiles of DMPs (n = 3280, false discovery rate 5%) in 199 individuals, illustrating changes between baseline and GC-induced conditions. The top 100 DMPs are depicted. **(D, E)** Annotation of DMPs: **(D)** CpG islands; N(S) shore, 2-kb-long regions flanking both sides of a CpG island; N(S) shelf, 2 kb upstream/downstream of the farthest limits of the CpG shores, excluding CpG islands and CpG shores; OpenSea, encompassing the remaining genomic regions, with a significant difference in distribution of GR-response DMPs compared with the EPIC array following GC stimulation (Wilcoxon p value = .03). **(E)** Gene locations are indicated for a comprehensive understanding of the genomic context of the identified GR-response DMPs, with no significant difference in distribution compared with the EPIC array (Wilcoxon p value = .76). DMP, differentially methylated position; eQTL, expression quantitative trait locus; eQTM, expression quantitative trait methylation; GC, glucocorticoid; GR, glucocorticoid receptor; meQTL, methylation QTL; SNP, single nucleotide polymorphism; UTR, untranslated region.

Because cell type–specific meQTLs have been reported, we further explored the cell-type specificity of GR-response meCpGs by reanalyzing GR-response meQTLs without adjusting for blood cell compositions. This revealed 102,051 meQTLs, of which 90.6% overlapped with those identified in the original analysis that included white blood cell compositions (Figure 3C). These findings suggest that GR-response meQTLs do not show strong cell type–specific effects in peripheral blood.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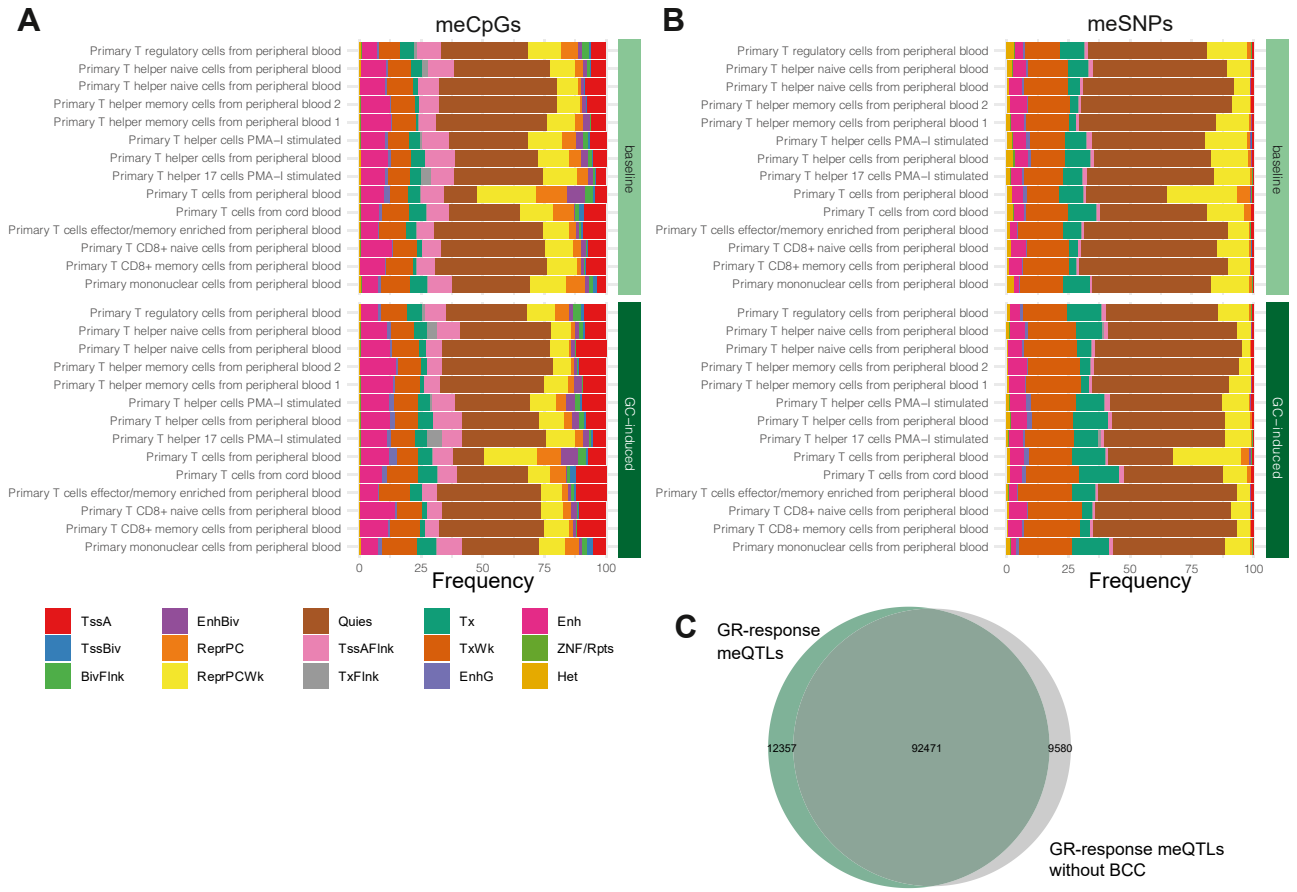


Figure 3. GR-response meQTLs and blood cell types. **(A, B)** This shows histone mark enrichment for GR-response meCpGs **(A)** and GR-response-meSNPs **(B)** across 15 chromatin states in 18 primary blood cell types. The top panel illustrates the distribution in baseline meQTLs, with GR-response meCpGs being more frequent in promoters (17.6% vs. 2.3% in GR-response meSNPs) and enhancers (14.8% vs. 6.9% in GR-response meSNPs). The bottom panel focuses on GR-response meQTLs, noting that 44.6% of meCpG sites are associated with SNPs in the same chromatin state but different positions. Analyzed chromatin states include TssA (active TSS), TssAFlnk (flanking active TSS), TxFlnk (transcr. at gene 5′ and 3′), Tx (strong transcription), TxWk (weak transcription), EnhG (genic enhancers), Enh (enhancers), ZNF/Rpts (ZNF genes and repeats), Het (heterochromatin), TssBiv (bivalent/poised TSS), BivFlnk (flanking bivalent TSS/Enh), EnhBiv (bivalent enhancer), ReprPC (repressed PolyComb), ReprPCWk (weak repressed PolyComb), and Quies (quiescent/low). **(C)** A Venn diagram highlights the overlap between GR-response meQTLs adjusted for white blood cell counts and those without adjustment. 102,051 meQTLs (86,834 meSNPs and 3839 meCpGs) were identified without BCC adjustment, of which 90.6% (n = 92,471 meQTLs) were found to be common with the original GR-response meQTL analysis that accounted for white BCCs. BCC, blood cell count; GC, glucocorticoid; GR, glucocorticoid receptor; meCpG, methylation CpG site; meQTL, methylation quantitative trait locus; meSNP, methylation single nucleotide polymorphism.

Figure 2. GR-response meQTL analysis. **(A)** Manhattan plot displaying the results of the GR-response meQTL analysis, illustrating the distribution of 104,828 local GR-response meQTLs (88,585 unique GR-response meSNPs) across the genome. **(B, C)** Boxplots showing the residualized beta methylation values of significant GR-response meQTLs as examples. Methylation levels are stratified based on the genotypes of the meSNPs. **(B)** A GR-response meQTL (rs2678333-cg15535573) located on chromosome 12 with a postdexamethasone-specific effect (false discovery rate = 8.24×10^{-15}). **(C)** A GR-response meQTL (rs11055602-cg07195891) located on chromosome 12, exhibiting effects both before and after dexamethasone treatment, with a notable decrease in individuals with major genotype and an increase in individuals with the minor allele genotype in postdexamethasone in comparison to baseline (false discovery rate = 5.1×10^{-30}). **(D, E)** Genomic characteristics of GR-response meCpGs and baseline meCpGs in relation to **(D)** CpG islands and **(E)** nearby genes. Various genomic regions were analyzed: CpG islands; N(S) shore, 2-kb-long regions flanking both sides of a CpG island; N(S) shelf, 2 kb upstream/downstream of the farthest limits of the CpG shores, excluding CpG islands and CpG shores; OpenSea, encompassing the remaining genomic regions. The distribution of GR-response meCpGs across the features in **(D)** and **(E)** did not show statistically significant differences between baseline and GR stimulation (Wilcoxon p value $>$ .05). **(F)** Characteristics of GR-response meSNPs are detailed in relation to the genomic location of nearby genes, with a significant difference in distribution between baseline and GR stimulation with Wilcoxon p value = .05. **(G, H)** GR-response and baseline meCpGs and meSNPs annotated for GRE proximity. The distribution of meCpGs and meSNPs did not show statistically significant differences between baseline and GR stimulation (Wilcoxon p value $>$ .05). GC, glucocorticoid; GR, glucocorticoid receptor; GRE, glucocorticoid response element; meCpG, methylation CpG site; meQTL, methylation quantitative trait locus; meSNP, methylation single nucleotide polymorphism; UTR, untranslated region.

Absence of Shared Genetic Control: No Overlap Between Genetic Variants Moderating GC-Induced DNAm and GC-Induced Gene Expression Changes

To investigate the genetic control of gene expression and DNAm, we compared the patterns of GR-response meQTLs and GR-response eQTLs (Table S5). By leveraging our previously identified *cis*-GR-response eQTL results (9), with 717 GR-response eQTL transcripts and 10,078 GR-response eQTL(e)SNPs across 297 individuals, we discovered a total of 9688 non-overlapping eSNPs. Strikingly, a significant proportion (96%) of these eSNPs were exclusively detected by GR-response eQTL analysis and not in the GR-response meQTL analysis (Figure 4A). Among the GR-response meSNPs, only 0.44% (n = 390) were also identified as GR-response eSNPs impacting 591 meQTLs. In contrast, our baseline analysis revealed a substantial overlap of 89% (n = 150,057) between our previously reported baseline eSNPs (n = 167,885) and meSNPs (n = 2,274,829).

Large Number of Associations Between GC-Induced DNAm and GC-Induced Gene Expression

To explore the relationship between GC-induced changes in DNAm and changes in gene expression, we conducted an eQTM analysis using the gene expression data from our sample. Employing the same analytic approach as for GR-response meQTLs, we identified a substantial number of 28,688 *cis*-GR-response eQTM associations (consisting of 14,364 GR-response eQTM CpGs and 4039 GR-response eQTM transcripts) in our dataset at an FDR of 5% (Table S6). However, only a small proportion of 162 GR-response eQTM

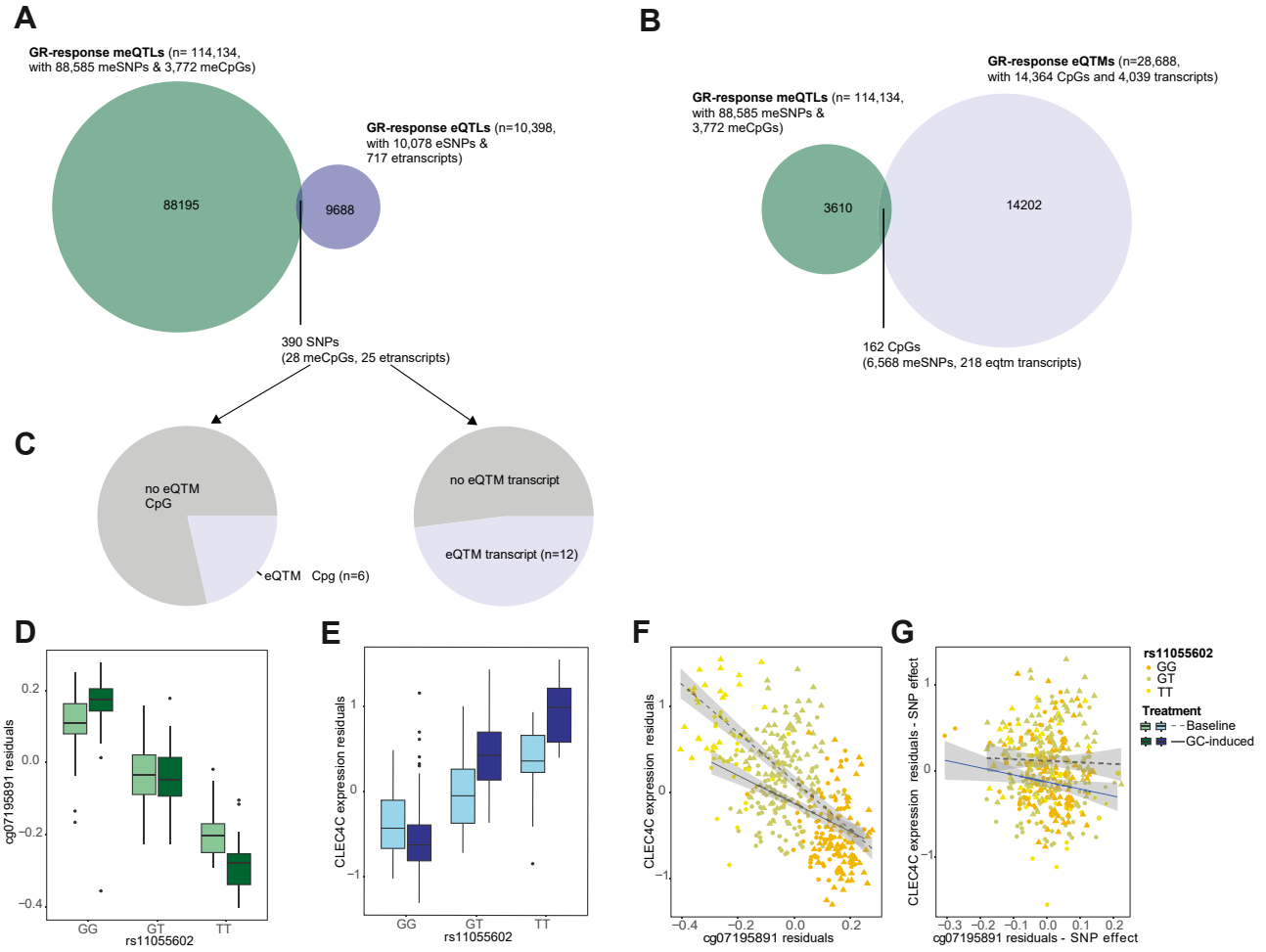


Figure 4. Integrative multiomics analysis. **(A)** Venn diagram representing the intersection of GR-response meSNPs and GR-response eSNPs. Our analysis uncovered 591 GR-meQTLs, including 390 SNPs and 28 CpGs, that share SNPs with GR-response eQTLs. **(B)** Venn diagram representing the intersection of GR-response meCpG and GR-response eQTM CpG sites. **(C)** Pie chart showing the distribution of eQTM transcripts and eQTM CpG sites that overlap with the CpGs and transcripts from the shared GR-response meSNPs and GR-response eSNPs overlap. **(D, E)** Boxplots displaying the residualized beta methylation values and expression levels for the GR-response trio hit *CLEC4C*, stratified by SNP rs11055602, with this locus acting as an eQTL, eQTM, and meQTL. **(F)** Scatterplot illustrating the relationship between residualized methylation and expression, highlighting the SNP effect. **(G)** Scatterplot showing the relationship after adjusting for the SNP effect, indicating that the correlation at baseline disappeared postadjustment. eQTL, expression quantitative trait locus; eQTM, expression quantitative trait methylation; eSNP, expression SNP; GC, glucocorticoid; GR, glucocorticoid receptor; meCpG, methylation CpG site; meQTL, methylation QTL; meSNP, methylation SNP; SNP, single nucleotide polymorphism.

CpGs overlapped with our GR-response meCpG sites (Figure 4B), suggesting that while GR-response mechanisms exhibit correlation at both DNAm and gene expression levels, only a small fraction (4%) are under genetic influence. Interestingly, a larger proportion (9.7%) of GR-response eQTM CpGs overlapped with GR-response DMP CpGs (1388 CpGs) than the minimal overlap with GR-response meCpGs. It is noteworthy that we identified merely 1307 eQTMs at baseline (consisting of 1126 eQTM CpGs and 512 eQTM transcripts) (Tables S5 and S7).

Integration of GR-Response me/eQTLs and GR-Response eQTMs via Multiomics Network Analysis: Capturing Immune-Related Pathways

We conducted a physical position overlap analysis to identify common GC-induced QTL loci. Limited overlap was observed between GR-response meQTLs, eQTLs, and eQTMs (Figure 4A; Supplement). The *CLEC4C* gene demonstrated multiple omics associations and significant eQTMs correlations both at baseline and after GR stimulation (Figure 4B, C). However, when adjusting for the me/eSNP effect, the baseline correlation disappeared (Figure 4C). This highlights the importance of multiomic analysis for identifying true associations. Consequently, we generated a multiomic GR network (7193 nodes and 30,332 edges) reflecting the complex interplay between transcripts, SNPs, CpGs, and biological factors (Supplement and Table S8).

We further examined GR-response network trios (Figure 5A), which are GR-response meQTLs that also act as GR-response eQTLs or GR-response eQTMs, thus forming SNP-CpG-transcript combinations or trios. Our analysis revealed 552 eQTMs and 297 eQTLs (Figure 5B and Table S9). Among these, we discovered 613 GR-response trio genes enriched in immune-related pathways (Figure 5C) and significant overlap with dexamethasone-regulated genes in the mouse brain (OR = 1.8, p value = 1.4×10^{-6}) (Figure 5D, E; Table S10; Supplement) (31). These findings highlight the pivotal role of these trio genes in stress impacts on the brain and their critical influence in mediating GC effects on brain function and behavior.

A key hub within this network was identified as a single CpG site (cg09614808) in the *APP* gene, connected with 164 genes across all 22 autosomes. Additional notable hubs included cg09614808 in the *YWHAZ* gene and cg17764313 in the *MCM2* gene, connected to 14 and 25 genes, respectively.

The GR-Response SNP-Methylation-Messenger RNA Trios Are Relevant to Psychiatric-Related Diseases

Given the established impact of stress on various health conditions, particularly inflammation, autoimmune disorders, and psychiatric traits, we investigated the functional relevance of GR-response trio variants, i.e., 1) variants influencing both DNAm (GR-response meQTLs) and gene expression (GR-response eQTLs) following GR stimulation or 2) variants associated with GR-response meCpGs that also exhibit regulatory effects on gene expression upon GR stimulation (GR-response eQTMs) in our network analysis.

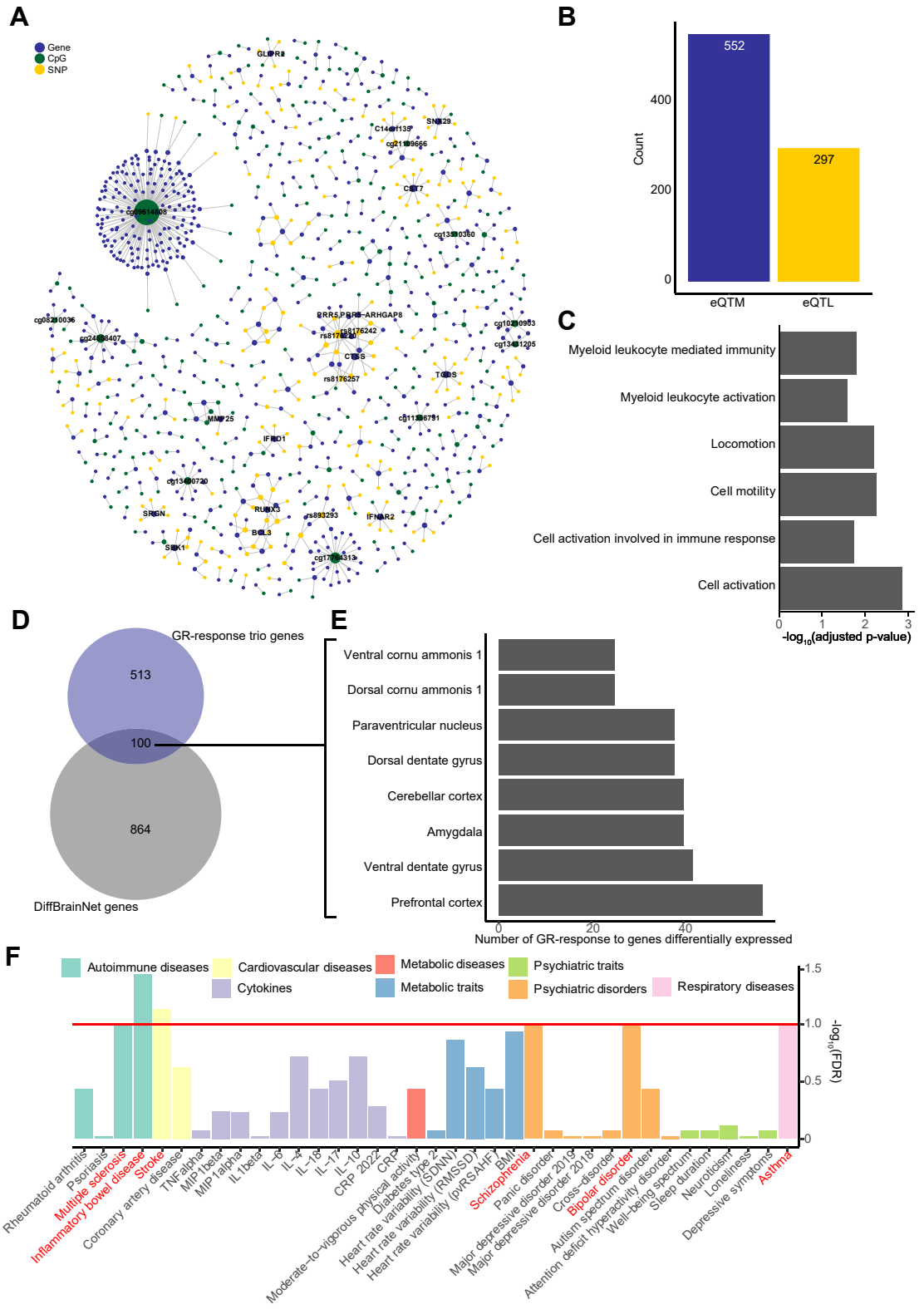
We identified 7591 GR-response trio SNPs (including 321 LD-independent SNPs) (Table S10), enriched in diverse traits (FDR $\leq$ 10%), including psychiatric and autoimmune diseases (detailed in the Supplement). Enrichments were calculated over nontrio GR-response meSNPs and included bipolar disorder (OR = 1.36), schizophrenia (OR = 1.38), multiple sclerosis (OR = 1.45), asthma (OR = 1.32), stroke (OR = 1.51), and inflammatory bowel disease (OR = 1.77) (Figure 5F).

To explore causal pathways, we conducted a mediation analysis focusing on 172 GR-response trio SNPs associated with at least one of the 6 enriched diseases (Table S12). DNAm was found to mediate gene expression for 26.3% of these SNPs ($p < .05$), with a strong average causal mediation effect in 89.7% of cases. However, 35.3% of the SNPs also exerted a direct effect on gene expression independent of DNAm (see the Supplement for additional details).

To further understand the clinical relevance of the identified 172 GR-response trio SNPs, we performed partitioned LD score regression analysis using the 6 GWASs with significant GR-response trio SNP enrichment. This analysis revealed a modest absolute contribution of GR-trio SNPs to overall heritability (0.21%–1.51%, depending on phenotype) (Table S12). However, considering the limited number of SNPs (n = 172), the relative heritability (SNP-based heritability divided by the number of SNPs) demonstrated a higher information content per SNP within these GWASs for GR-response trio SNPs than for GR-response meQTLs and baseline QTL SNPs (Figure S2). This finding underscores the potential disease relevance of GR-response trio SNPs and warrants further investigation.

DISCUSSION

In this study, we used advanced network analysis and multilevel GR-response data to explore 1) the influence of genetic variants on DNAm patterns in response to GC exposure, 2) the consequent effects of these changes on gene expression when induced by GCs, and 3) the associated genetic risk for various medical diseases and traits. While recent investigations have begun exploring multiomics data in various fields [for example (32–35)], none have yet integrated data that reflect dynamic changes following a disease-relevant stimulus. Here, we leveraged such data with repeated measures before and after GC stimulation in 199 individuals, integrating across genetic, DNAm, and gene expression data. Our network model incorporated 88,585 GR-response meSNPs and 3772 GR-response meCpGs and approximately 12,000 transcripts. Our analysis highlights not only the dynamic changes in DNAm following GR activation but also the different levels of regulations with distinct effects of genetic variants on changes in GC-induced gene expression and DNAm. Additionally, GR-response meQTLs were distinct from baseline meQTLs, suggesting that some QTLs are only revealed through exposures, including exposure to GCs. This finding underscores the importance of challenge experiments to fully understand the role of associated variants identified in GWASs. In fact, focusing on GR-response trio variants from our multiomics model, we showed that genetic variants that alter the molecular impact of GCs in immune cells are enriched for variants associated with risk for psychiatric disorders, autoimmune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Psychiatric traits
Cytokines
Metabolic traits
Psychiatric disorders
Respiratory diseases
1.5
1.0
0.5
0
-log10(FDR)
Rheumatoid arthritis
Psoriasis
Multiple sclerosis
Inflammatory bowel disease
Stroke
Coronary artery disease
TNFalpha
MIP1beta
MIP1alpha
IL1beta
IL-6
IL-4
IL-18
IL-17
IL-10
CRP 2022
CRP
Diabetes type 2
Moderate-to-vigorous physical activity
Heart rate variability (SDNN)
Heart rate variability (RMSSD)
Heart rate variability (pNN50)
BMI
Schizophrenia
Panic disorder
Major depressive disorder 2019
Major depressive disorder 2018
Cross-disorder
Bipolar disorder
Autism spectrum disorder
Attention deficit hyperactivity disorder
Well-being spectrum
Sleep duration
Neuroticism
Loneliness
Depressive symptoms
Asthma

disorders, respiratory diseases, and cardiovascular disease, even over nontrio GR-response meQTL SNPs. This supports the observation that chronic stress, partly mediated by GC exposure, is a shared risk factor across psychiatric and medical diseases. Our findings suggest that the genetic variants that moderate the effects of GCs may underlie some of the genetic correlations that have been observed across these traits [for example (36–38)].

In our study, we identified 3280 CpG sites displaying significant differential DNAm (DMPs) after GR activation. Most DMPs showed hypomethylation (76.4%) and were mainly located in open sea regions. These findings are consistent with existing reports suggesting that GR binding can lead to active DNA demethylation through DNA repair mechanisms (15). Furthermore, it is consistent with recent insight from Isbel *et al.* (16), which indicates similar mechanisms for local epigenetic changes following transcription factor binding in general.

While we discovered a substantial number of GR-response meQTLs (n = 104,828), 99.7% of them showed no change in average DNAm across all genotypes but only exhibited significant DNAm changes upon GR activation in an allele-specific manner (Figure S1). These GR-response meQTLs exhibited distinct patterns and mechanisms compared with previously identified GR-response eQTLs (9), with <1% overlap at the SNP level. Discrepant levels of overlap, ranging from substantial (39) to <5% (40), have previously been reported, likely highlighting the contextual nature of the interaction between meQTLs and eQTLs.

The cell-type specificity of baseline DNAm, even within blood cell types, is well established (41). In contrast to this pattern, we found in the current study that only a smaller subset of GR-response meQTLs displayed such specificity. This suggests that epigenetic modifications related to stress may have shared genetic regulation across a diverse range of immune cell types and possibly even beyond the immune system. Moreover, the transcripts within the GR-response trio genes from our network analysis were responsive to GCs in various mouse brain regions (31). This observation aligns well with previous findings indicating that GR-responsive sequences, as identified in eQTL and massively parallel reporter assays, are enriched in cross-tissue enhancers (8,10). Consistent with these findings, the shared genetic associations that we observed within the GR-response trio SNPs encompass a range of psychiatric and medical disorders and suggest a potential role in moderating stress effects across multiple systems. Notably, the GR-response trio genes were significantly enriched in immune-related pathways as well as brain-related pathways including neurotransmitter signaling (Figure 5C). This raises the intriguing possibility that these SNPs may influence stress-induced changes in the immune system, which in turn could impact brain function both directly as well as indirectly via cytokine signaling within inflammatory pathways. Furthermore, our mediation analysis highlights DNAm as a potential regulatory layer that links these genetic variants to altered gene expression. Our study focused on peripheral immune cells, so it is important to acknowledge that additional relationships may exist in other tissues.

The GR-response trio SNPs may also contribute to disease heritability beyond other QTLs. Although partitioning heritability analysis revealed a modest absolute contribution of GR-response trio SNPs to overall heritability, their relative heritability per SNP suggests a potentially greater impact on disease risk than other QTL SNPs. This highlights the importance of investigating response QTLs with disease-relevant stimuli to better understand genetic disease risk. Future studies should explore such response QTLs in tissues relevant to the disorders of interest.

While our study contributes significant insights, there are limitations that should be considered. Our sample included 199 individuals, limiting the power to detect smaller effect sizes or *trans*-effects. Future studies should use single-cell resolution methods to explore cellular heterogeneity, as highlighted in the human brain cell atlas (42). Additionally, investigating different exposure lengths and timings of GR stimulation may provide further insights into the temporal dynamics of the molecular response. Furthermore, additional experiments exploring different layers of epigenetic regulation are needed to investigate the role of DNAm in regulatory elements and its impact on gene expression upon GR stimulation. While our multiomic network analysis considers *trans*-effects, it is important to note that our GR-response meQTL analysis primarily focuses on *cis*-meQTLs. Therefore, network nodes with many potential *trans*-effects, such as the CpG site within *APP*, warrant cautious interpretation and replication studies. In our study, we identified that the GC-induced methylation changes of this CpG site, residing within the *APP* gene, was associated with changes in gene expression of many genes (n = 164) but not with *APP* itself, reducing the likelihood of direct

Figure 5. GR-response trios: **(A)** The GR-response trio network is composed of methylation QTLs acting as both eQTLs and eQTMs, forming interactions between SNPs, CpG sites, and transcripts. The network is color coded, with SNPs in yellow, genes in blue, and CpG sites in green. Node size represents the number of connections that a node has (node degree). Nodes with a node degree >5 are labeled by their name. **(B)** A barplot illustrating the distribution of eQTM and eQTL relationships within the GR-response trio network. **(C)** The GR-response trio genes displayed enrichment in immune-related Gene Ontology pathways such as myeloid leucocyte–mediated immunity, cell activation involved in immune response, and myeloid leucocyte activation, as well as pathways related to cell activation, cell motility, and locomotion. **(D)** Venn diagram displaying the overlap between GR-response trio genes and genes differentially regulated in the mouse brain following dexamethasone administration. Of the overlapping genes, 59% were differentially regulated in the mouse prefrontal cortex following dexamethasone. **(E)** A bar plot depicting the distribution of the overlapping genes across various brain regions. **(F)** Enrichment of nominal genome-wide association study associations for the GR-response trio SNPs across various genome-wide association study datasets, including psychiatric disorders, psychiatric traits, cytokines, metabolic markers, autoimmune diseases, and asthma. The y-axis represents the enrichment p value compared with nontrio GR-response methylation SNPs, with significant enrichment indicated by red labels. Bars are color coded according to their disease or trait class. BMI, body mass index; CRP, C-reactive protein; eQTL, expression quantitative trait locus; eQTM, expression quantitative trait methylation; FDR, false discovery rate; GR, glucocorticoid receptor; IL, interleukin; MIP, macrophage inflammatory protein; pvRSAHF, peak-valley respiratory sinus arrhythmia; RMSSD, root mean square of successive difference; SDNN, standard deviation of normal-to-normal interbeat interval; SNP, single nucleotide polymorphism; TNF, tumor necrosis factor.

associations with neurodegenerative disease or other disorders that *APP* has been implicated in.

Conclusions

By generating multiomic networks of the regulation of the molecular response to GC in immune cells, we identified shared genetic influences of stress-moderating variants on a range of diseases—from psychiatric to medical—that have previously been reported to be affected by chronic stress and GC exposure. SNPs moderating the response to GC on the level of DNAm and gene expression seem to be of particular interest because they are enriched for GWAS variants and may explain a relatively larger fraction of heritability than other QTL SNPs. If this is extrapolated to QTLs that use additional relevant stimuli in additional tissues, response QTLs may end up explaining large fractions of disease heritability. By pinpointing such key multilevel dynamic regulators, we enhance the pathomechanistic understanding of how stress can serve as a transdiagnostic risk factor.

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JK-A conducted the computational analysis, generated data visualizations, and drafted the initial manuscript. EBB contributed intellectually, conducted substantial manuscript revisions, and provided critical feedback as well as funding. JK-A and EBB collaboratively conceptualized, designed, and supervised the study. AH was responsible for the quality control of the methylation data. All authors had the opportunity to review, offer feedback on, and approve the final manuscript.

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The computational code developed for this study has been made available on GitHub (https://github.com/jArloth/GR-meQTLs/), and the DNAm data are accessible in the GEO repository (GEO: GSE249113).

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ARTICLE INFORMATION

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REFERENCES

1. Seiler A, Fagundes CP, Christian LM (2020): The impact of everyday stressors on the immune system and health. In: Choukèr A, editor. Stress Challenges and Immunity in Space. Springer: Cham, 71–92.
2. Brown DW, Anda RF, Tiemeier H, Felitti VJ, Edwards VJ, Croft JB, Giles WH (2009): Adverse childhood experiences and the risk of premature mortality. Am J Prev Med 37:389–396.
3. Bjørndal LD, Kendler KS, Reichborn-Kjennerud T, Ystrom E (2023): Stressful life events increase the risk of major depressive episodes: A population-based twin study. Psychol Med 53:5194–5202.
4. Williams DM (2018): Clinical Pharmacology of corticosteroids. Respir Care 63:655–670.
5. Quatrini L, Ugolini S (2021): New insights into the cell- and tissue-specificity of glucocorticoid actions. Cell Mol Immunol 18:269–278.
6. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, *et al.* (2019): Chronic inflammation in the etiology of disease across the life span. Nat Med 25:1822–1832.
7. Klengel T, Binder EB (2015): Epigenetics of stress-related psychiatric disorders and gene × environment interactions. Neuron 86:1343–1357.
8. Penner-Goeke S, Bothe M, Rek N, Kreitmaier P, Pöhlchen D, Kühnel A, *et al.* (2023): High-throughput screening of glucocorticoid-induced enhancer activity reveals mechanisms of stress-related psychiatric disorders. Proc Natl Acad Sci U S A 120:e2305773120.
9. Moore SR, Halldorsdottir T, Martins J, Lucae S, Müller-Myhsok B, Müller NS, *et al.* (2021): Sex differences in the genetic regulation of the blood transcriptome response to glucocorticoid receptor activation. Transl Psychiatry 11:632.
10. Arloth J, Bogdan R, Weber P, Frishman G, Menke A, Wagner KV, *et al.* (2015): Genetic differences in the immediate transcriptome response to stress predict risk-related brain function and psychiatric disorders. Neuron 86:1189–1202.
11. Elbau IG, Brücklmeier B, Uhr M, Arloth J, Czamara D, Spoormaker VI, *et al.* (2018): The brain's hemodynamic response function rapidly changes under acute psychosocial stress in association with genetic and endocrine stress response markers. Proc Natl Acad Sci U S A 115:E10206–E10215.
12. Bartlett AA, Lapp HE, Hunter RG (2019): Epigenetic mechanisms of the glucocorticoid receptor. Trends Endocrinol Metab 30:807–818.
13. Provençal N, Arloth J, Cattaneo A, Anacker C, Cattane N, Wiechmann T, *et al.* (2020): Glucocorticoid exposure during hippocampal neurogenesis primes future stress response by inducing changes in DNA methylation. Proc Natl Acad Sci U S A 117:23280–23285.
14. Wiechmann T, Röh S, Sauer S, Czamara D, Arloth J, Ködel M, *et al.* (2019): Identification of dynamic glucocorticoid-induced methylation changes at the FKBP5 locus. Clin Epigenetics 11:83.
15. Kress C, Thomassin H, Grange T (2006): Active cytosine demethylation triggered by a nuclear receptor involves DNA strand breaks. Proc Natl Acad Sci U S A 103:11112–11117.
16. Isbel L, Grand RS, Schübeler D (2022): Generating specificity in genome regulation through transcription factor sensitivity to chromatin. Nat Rev Genet 23:728–740.
17. Martin EM, Fry RC (2018): Environmental influences on the epigenome: Exposure- associated DNA methylation in human populations. Annu Rev Public Health 39:309–333.
18. Hawe JS, Wilson R, Schmid KT, Zhou L, Lakshmanan LN, Lehne BC, *et al.* (2022): Genetic variation influencing DNA methylation provides insights into molecular mechanisms regulating genomic function. Nat Genet 54:18–29.
19. Oliva M, Demanelis K, Lu Y, Chernoff M, Jasmine F, Ahsan H, *et al.* (2023): DNA methylation QTL mapping across diverse human tissues provides molecular links between genetic variation and complex traits. Nat Genet 55:112–122.
20. Chen L, Ge B, Casale FP, Vasquez L, Kwan T, Garrido-Martín D, *et al.* (2016): Genetic drivers of epigenetic and transcriptional variation in human immune cells. Cell 167:1398–1414.e24.
21. Bergstedt J, Azzou SAK, Tsuo K, Jaquaniello A, Urrutia A, Rotival M, *et al.* (2022): The immune factors driving DNA methylation variation in human blood. Nat Commun 13:5895.
22. Kreitmaier P, Katsoula G, Zeggini E (2023): Insights from multi-omics integration in complex disease primary tissues. Trends Genet 39:46–58.
23. Zhu Y, Strachan E, Fowler E, Bacus T, Roy-Byrne P, Zhao J (2019): Genome-wide profiling of DNA methylome and transcriptome in

peripheral blood monocytes for major depression: A monozygotic Discordant Twin Study. Transl Psychiatry 9:215.
24. Chen C, Zhang C, Cheng L, Reilly JL, Bishop JR, Sweeney JA, *et al.* (2014): Correlation between DNA methylation and gene expression in the brains of patients with bipolar disorder and schizophrenia. Bipolar Disord 16:790–799.
25. Shabalin AA (2012): Matrix eQTL: Ultra fast eQTL analysis via large matrix operations. Bioinformatics 28:1353–1358.
26. Yu G, Wang L-G, He Q-Y (2015): ChIPseeker: An R/Bioconductor package for ChIP peak annotation, comparison and visualization. Bioinformatics 31:2382–2383.
27. Ernst J, Kellis M (2010): Discovery and characterization of chromatin states for systematic annotation of the human genome. Nat Biotechnol 28:817–825.
28. Road map Epigenomics Consortium, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, *et al.* (2015): Integrative analysis of 111 reference human epigenomes. Nature 518:317–330.
29. Ogris C, Hu Y, Arloth J, Müller NS (2021): Versatile knowledge guided network inference method for prioritizing key regulatory factors in multi-omics data. Sci Rep 11:6806.
30. Watanabe K, Taskesen E, van Bochoven A, Posthuma D (2017): Functional mapping and annotation of genetic associations with FUMA. Nat Commun 8:1826.
31. Gerstner N, Krontira AC, Cruceanu C, Roeh S, Pütz B, Sauer S, *et al.* (2022): DiffBrainNet: Differential analyses add new insights into the response to glucocorticoids at the level of genes, networks and brain regions. Neurobiol Stress 21:100496.
32. Pineda-Cirera L, Cabana-Domínguez J, Lee PH, Fernàndez-Castillo N, Cormand B (2022): Identification of genetic variants influencing methylation in brain with pleiotropic effects on psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry 113:110454.
33. Korologou-Linden R, Leyden GM, Relton CL, Richmond RC, Richardson TG (2023): Multi-omics analyses of cognitive traits and psychiatric disorders highlight brain-dependent mechanisms. Hum Mol Genet 32:885–896.
34. Lin D, Chen J, Duan K, Perrone-Bizzozero N, Sui J, Calhoun V, Liu J (2021): Network modules linking expression and methylation in prefrontal cortex of schizophrenia. Epigenetics 16:876–893.
35. Kamali Z, Keaton JM, Haghjooy Javanmard S, International Consortium Of Blood Pressure, Million Veteran Program, eQTLGen Consortium, *et al.* (2022): Large-Scale Multi-Omics Studies Provide New Insights into Blood Pressure Regulation. Int J Mol Sci 23:7557.
36. Seligowski AV, Misganaw B, Duffy LA, Ressler KJ, Guffanti G (2022): Leveraging large-scale genetics of PTSD and cardiovascular disease to demonstrate robust shared risk and improve risk prediction accuracy. Am J Psychiatry 179:814–823.
37. Baltramonaityte V, Pingault J-B, Cecil CAM, Choudhary P, Järvelin M-R, Penninx BWJH, *et al.* (2023): A multivariate genome-wide association study of psycho-cardiometabolic multimorbidity. PLoS Genet 19: e1010508.
38. Zeng Y, Suo C, Yao S, Lu D, Larsson H, D'Onofrio BM, *et al.* (2023): Genetic associations between stress-related disorders and autoimmune disease. Am J Psychiatry 180:294–304.
39. Pierce BL, Tong L, Argos M, Demanelis K, Jasmine F, Rakibuz-Zaman M, *et al.* (2018): Co-occurring expression and methylation QTLs allow detection of common causal variants and shared biological mechanisms. Nat Commun 9:804.
40. Gibbs JR, van der Brug MP, Hernandez DG, Traynor BJ, Nalls MA, Lai S-L, *et al.* (2010): Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. PLoS Genet 6: e1000952.
41. Loyfer N, Magenheim J, Peretz A, Cann G, Bredno J, Klochendler A, *et al.* (2023): A DNA methylation atlas of normal human cell types. Nature 613:355–364.
42. Maroso M (2023): A quest into the human brain. Science 382:166–167.