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Assessing the Role of Cortisol in Anxiety, Major Depression, and Neuroticism: A Mendelian Randomization Study Using *SERPINA6/SERPINA1* Variants

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ABSTRACT

BACKGROUND: Previous evidence informed by the toxic stress model suggests that higher cortisol causes anxiety and major depression, but clinical success is lacking. To clarify the role of cortisol, we used Mendelian randomization to estimate its associations with anxiety, major depression, and neuroticism, leveraging the largest available genome-wide association studies including from the Psychiatric Genomics Consortium, the UK Biobank, and FinnGen.

METHODS: After meta-analyzing 2 genome-wide association studies on morning plasma cortisol (n = 32,981), we selected single nucleotide polymorphisms (SNPs) at $p < 5 \times 10^{-8}$ and $r^2 < 0.3$ in the *SERPINA6/SERPINA1* gene region encoding proteins that influence cortisol bioavailability. We applied these SNPs to summary genetic associations with the outcomes considered (n = 17,310–449,484), and systolic blood pressure as a positive outcome, using inverse-variance weighted meta-analysis accounting for correlation. Sensitivity analyses addressing SNP correlation and confounding by childhood maltreatment and follow-up analyses using only SNPs that colocalized with *SERPINA6* expression were conducted.

RESULTS: Cortisol was associated with anxiety (pooled odds ratio [OR] 1.16 per cortisol z score; 95% CI, 1.04 to 1.31), but not major depression (pooled OR 1.02, 95% CI, 0.95 to 1.10) or neuroticism (β −0.025; 95% CI, −0.071 to 0.022). Sensitivity analyses yielded similar estimates. Cortisol was positively associated with systolic blood pressure, as expected. Using rs9989237 and rs2736898, selected using colocalization, cortisol was associated with anxiety in the UK Biobank (OR 1.32; 95% CI, 1.01 to 1.74) but not with major depression in FinnGen (OR 1.14; 95% CI, 0.95 to 1.37).

CONCLUSIONS: Cortisol was associated with anxiety and may be a potential target for prevention. Other targets may be more relevant to major depression and neuroticism.

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Globally, anxiety and major depression are among the most prevalent mental disorders (1), and neuroticism is a major risk factor for both (2). Neuroticism is typically considered to be a core personality trait (3) and has been described as a tendency toward emotional instability in reaction to stressful situations (4). Anxiety and major depression are highly disabling and have risen substantially in incidence and prevalence in recent years as a likely consequence of increasing environmental stress (5). While reducing stress can effectively prevent mental disorders (6), stress reduction on a population level remains highly challenging, if not impossible (7). As such, insights into the neurobiology of mental disorders that are seemingly affected by high levels of stress may inform molecular targets of prevention. Cortisol is a steroid hormone synthesized from cholesterol in the adrenal gland and is widely known as the "stress hormone" because its level is closely and positively correlated with perceived stress (8). Cortisol, regulated by the hypothalamus-pituitary-adrenal (HPA) axis (9), exhibits a circadian rhythm and typically peaks in the morning.

Chronic activation of the HPA axis resulting in persistently high levels of circulating cortisol is a hallmark of toxic stress response (10) and has frequently been associated with anxiety and major depression, but observational evidence remains inconclusive (11). Childhood maltreatment, which increases the risk of mental disorders (12) and has been associated with both cortisol blunting (13) and hyperactivity (14), may confound the observed effects of cortisol toward or away from the null, respectively. Previous randomized controlled trials (RCTs), which are free of confounding by design, did not find CRF1 (corticotropin-releasing factor receptor 1) antagonists, an HPA-targeting medication, to have an effect on either anxiety or depression (15,16). However, it was unclear whether the tested compounds reduced cortisol sufficiently (17). Conversely, RCTs of other HPA-targeting medications, such as V1b (vasopressin 1B) receptor antagonists, and mifepristone, a glucocorticoid receptor antagonist, have shown favorable effects on major depression (18). Given the mixed findings from observational studies and RCTs (19), the use of a

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quasi-experimental approach, such as Mendelian randomization (MR) (20), may help clarify the role of cortisol.

Using genetic variants as instrumental variables (IVs) for cortisol (21) to mimic the deconfounding property of RCTs, previous MR studies did not clearly show an association between cortisol and major depression or neuroticism (22,23). However, in one of the studies, the analysis did not account for the correlation between IVs (22), making the estimates difficult to interpret. Furthermore, a recent systematic review showed that very few MR studies on anxiety have used standardized criteria for diagnosis (24). Therefore, the role of cortisol in anxiety remains to be elucidated.

In this MR study, to clarify the role of cortisol, and by implication the HPA axis in anxiety and major depression, and to overcome some of the challenges in previous observational and MR studies, we used an extended set of genetic variants from the *SERPINA6/SERPINA1* gene region that encode proteins that influence cortisol bioavailability as IVs for cortisol. We pooled the largest available genome-wide association studies (GWASs) to assess the associations of morning plasma cortisol with anxiety and major depression. We also included neuroticism because of its strong link with anxiety and major depression. We used systolic blood pressure (SBP) as the positive control outcome because exogenous cortisol increases SBP (25).

METHODS AND MATERIALS

Study Design

This is a 2-sample MR study based on the largest available GWASs on anxiety, major depression, and neuroticism (Figure 1). MR relies on the 3 IV assumptions (26) (Figure 2). First, the IV is associated with the exposure of interest. Second, the IV is independent of confounders of exposure and outcome. Third, the IV is associated with the outcome only through affecting the exposure. In this MR study, we used genetic variants from the *SERPINA6/SERPINA1* gene region as IVs. The *SERPINA6* and *SERPINA1* genes encode corticosteroid-binding globulin and α_1-antitrypsin, respectively. Both proteins have well-established biological functions that influence cortisol bioavailability (27). To strengthen the validity of the results, we conducted sensitivity analyses addressing correlation of the IVs and potential confounding by childhood maltreatment. Additionally, we performed follow-up analyses using only IVs that showed evidence of colocalization with *SERPINA6* expression. This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines for MR (Table S1).

Data Source for Cortisol

To maximize power to detect associations with the outcomes considered, we obtained and combined summary genetic associations with morning plasma cortisol from the CORNET (Cortisol Network) consortium (28) and the Leipzig Research Centre for Civilization Diseases LIFE-Adult (95% of analyzed samples obtained between 7:00 and 10:30 AM) and LIFE-Heart studies (95% of analyzed samples obtained between 6:00 and 11:45 AM) (29), using fixed-effects inverse-variance weighted (IVW) meta-analysis (30), which resulted in a total of up to 32,981 participants. We only included studies that reported the use of morning samples to minimize measurement error given the diurnal and ultradian nature of cortisol secretion. The CORNET consortium, with a pooled mean age (SD) of 53.3 (11.2) years and 36.3% men, was a GWAS meta-analysis of 25,314 participants of European ancestry from 17 European population-based cohorts adjusted for age, sex, and genetic principal components (PCs) for population structure. The LIFE-Adult and LIFE-Heart studies, with a pooled mean age (SD) of 59.9 (12.0) years and 55.3% men, included 7667 participants of European ancestry from Leipzig, Germany. The GWAS adjusted for age, sex, and body mass index (BMI). The combined genetic associations with cortisol *z* scores (per pooled SD or ~179 nmol/L) were checked for potential effect size inflation from confounding. We used the linkage disequilibrium (LD) score regression intercept, where a value substantially different from unity indicates the presence of confounding by population stratification (31).

Genetic Instrument for Cortisol

Because similar to previous GWASs, only genetic variants in the *SERPINA6/SERPINA1* gene region (chromosome 14: 94,768,859–94,843,565) (28,32) were associated with morning plasma cortisol at genome-wide significance ($p < 5 \times 10^{-8}$), consistent with previous MR studies, we selected single nucleotide polymorphisms (SNPs) associated with cortisol in low ($r^2 < 0.3$) LD (28,33). The *SERPINA6/SERPINA1* SNPs have been shown to affect cortisol levels throughout the day (34), which makes them suitable IVs for long-term cortisol exposure.

Data Sources for Anxiety, Major Depression, and Neuroticism

We obtained summary genetic associations with anxiety, major depression, and neuroticism from a GWAS of participants of European ancestry mainly from the PGC (Psychiatric Genomics Consortium) (35), an international consortium of genetic studies of psychiatric disorders; the UK Biobank (36), an ongoing cohort study of ~500,000 adults from Great Britain recruited at ages 40 to 69 years; and FinnGen (37), an expanding cohort study intended to recruit 500,000 participants from Finland. Here, we used FinnGen's ninth public data release (data freeze 9), which consists up to 377,277 participants. Details of the included studies, including source population, sample size, overlap of participants, case and control definitions, and covariate adjustment, are detailed in Table S2.

Anxiety

We obtained summary genetic associations with anxiety disorders from 4 major data sources. The ANGST (Anxiety NeuroGenetics Study) Consortium included 3695 cases and 13,615 controls (38). The UK Biobank included 25,453 cases and 58,113 controls (39). Both the ANGST Consortium and UK Biobank defined cases based on self-report lifetime diagnosis and DSM-based diagnostic assessment. The iPSYCH Cohort, part of the The Lundbeck Foundation Initiative for Integrative Psychiatric Research, included 4584 cases and 19,225

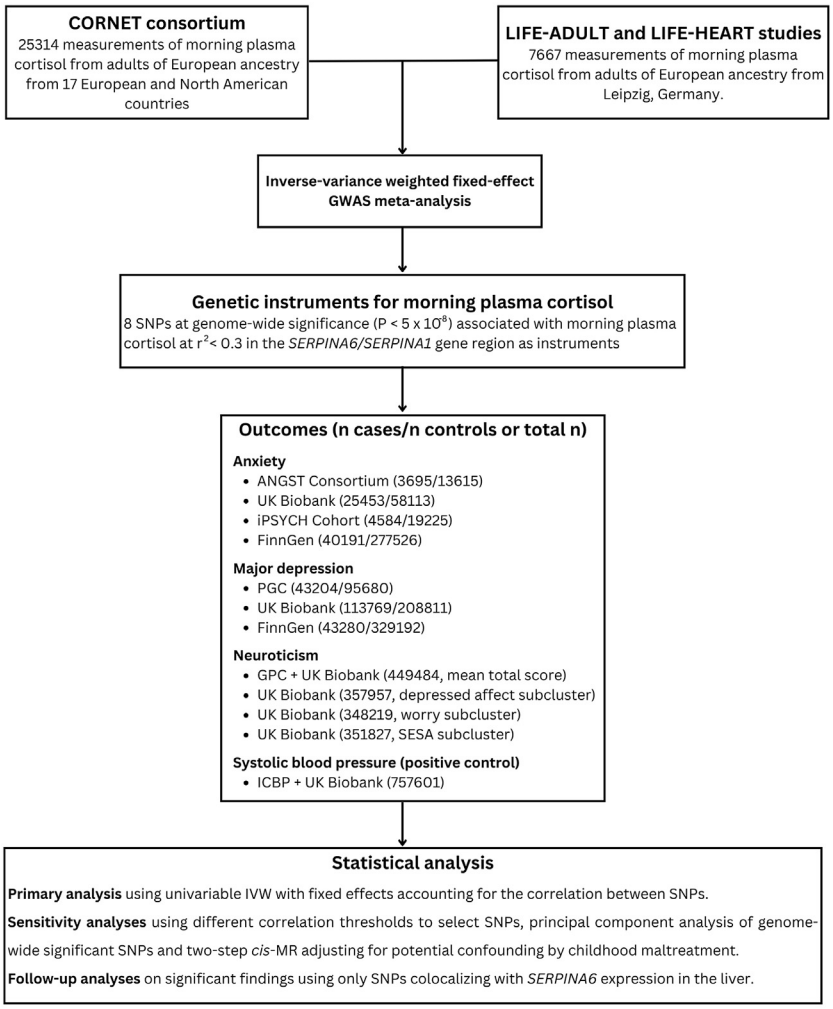

Figure 1. Study design of the current Mendelian randomization study on morning plasma cortisol and anxiety, major depression, and neuroticism. ANGST, Anxiety NeuroGenetics Study; CORNET, Cortisol Network; GPC, Genetics of Personality Consortium; GWAS, genome-wide association study; ICBP, International Consortium for Blood Pressure; LIFE, Leipzig Research Centre for Civilization Diseases; PGC, Psychiatric Genomics Consortium; SESA, sensitivity to environmental stress; SNP, single nucleotide polymorphism.

controls (40). FinnGen included 40,191 cases and 277,526 controls (37). The iPSYCH Cohort and FinnGen defined cases based on linked medical records. In the ANGST Consortium, the UK Biobank, and FinnGen, the GWASs all adjusted for age, sex, and some genetic PCs, but in the iPSYCH Cohort, the GWAS only adjusted for genetic PCs.

Major Depression

We obtained summary genetic associations with major depression from 3 major data sources including the PGC (43,204 cases and 95,680 controls) (41), the UK Biobank (113,769 cases and 208,811 controls) (42), and FinnGen (43,280 cases and 329,192 controls) (37). All 3 studies defined cases based on medical records, and in the PGC and the UK Biobank, cases were also defined using DSM- or ICD-based diagnostic assessment and history of help seeking for mental health difficulties, respectively. All 3 GWASs adjusted for genetic PCs, and in the UK Biobank and FinnGen, other covariates such as age and sex were also included. In the UK Biobank, for statistical power, we selected the broadest case definition available for major depression due to its high genetic correlation with probable depression (r_g = 0.871, p = 4.18 × 10^{-67}) and ICD-coded major depressive disorder (r_g = 0.863, p = 5.03 × 10^{-80}). Furthermore, the broad depression phenotype was not correlated with the anxiety factor score (r_g = 0.052 ± 0.11) (42), which makes bias via association with anxiety unlikely.

Neuroticism Overall and Subclusters

We obtained summary genetic associations with the neuroticism mean total score (in z scores) from a GWAS meta-analysis of the GPC (Genetics of Personality Consortium) and the UK Biobank that involved a total of 449,484 participants (43). Both GWASs adjusted for age and sex, and in the UK Biobank, genetic PCs, genotyping array, and Townsend deprivation index were also adjusted for. Given the heterogeneity between the neuroticism items, we also obtained summary genetic associations with the total scores (in z scores) of

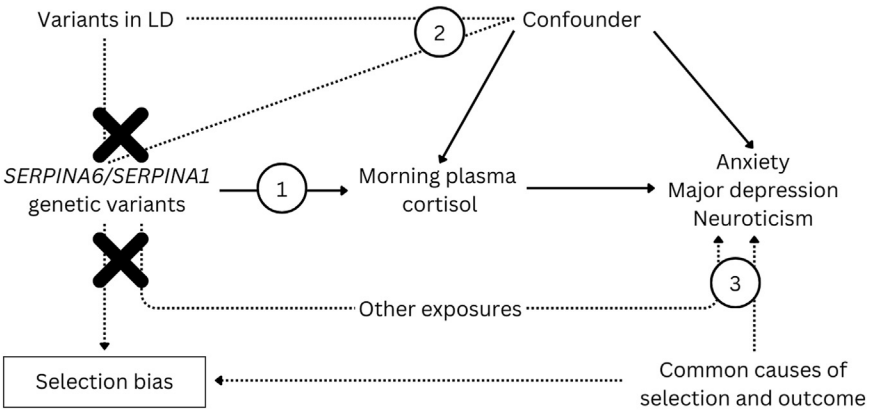

Figure 2. Directed acyclic graph illustrating the instrumental variable assumptions in Mendelian randomization and potential biasing pathways (in dashed lines). LD, linkage disequilibrium.

3 neuroticism subclusters from a GWAS of the UK Biobank, including depressed affect (357,957 participants) (44), worry (348,219 participants) (44), and sensitivity to environmental stress (351,827 participants) (45), which adjusted for the same covariates as the GWAS of the UK Biobank neuroticism mean total score.

Data Source for SBP

We obtained summary genetic associations with SBP (n = 757,601) in mm Hg from a GWAS meta-analysis of the UK Biobank (36) and the International Consortium for Blood Pressure (46), which adjusted for age, age^2, sex, and BMI.

Statistical Analysis

Instrument strength was calculated using the F statistic approximated by the squared SNP-cortisol association divided by the variance of the association (47). An F statistic <10 indicates weak instruments, which bias the MR estimates toward the observational direction, where there is overlap of participants for exposure and outcome studies, and toward the null, where there is no overlap (48). Power calculations were conducted based on the proportion of phenotypic variance explained by the IVs (49), using the more conservative estimate of 0.5% taken from previous findings for the *SERPINA6/SERPINA1* locus (32) given the weak LD of the selected SNPs. SNPs for cortisol and outcomes were aligned on the same effect alleles. SNPs available for cortisol but not the outcomes and palindromic SNPs (A/T and G/C) with ambiguous effect allele frequency (0.42–0.58), which are difficult to align, were replaced by LD proxies (r^2 = 1.0) where available (50).

To estimate the associations of cortisol with the outcomes considered, we used the IVW method with fixed effects to combine the SNP-specific Wald estimates, allowing for the correlation between SNPs in the weights. Where applicable, to increase precision, we meta-analyzed with fixed effects the estimates for the same outcome to give an overall estimate. Given the fact that we used SNPs from the same gene region, standard sensitivity analyses for MR, such as weighted median (51), which assumes that the majority of SNPs are valid IVs, and MR-Egger (52), which requires invalid IVs to not be associated with the outcome of interest via the same pleiotropic paths, are not suitable in this setting. To check whether the estimates were sensitive to the SNP correlation threshold ($r^2 < 0.3$) (53), we repeated the analysis using $r^2 < 0.1$ and 0.5 as thresholds. We also applied the IVW-PC method to all genome-wide significant SNPs in the *SERPINA6/SERPINA1* gene region (54) to further address the correlation between SNPs. The IVW-PC method uses PC analysis to identify uncorrelated linear combinations of SNPs that explain the largest proportion of variance in cortisol. Compared to selecting SNPs based on an r^2 cutoff, the MR estimates obtained from the IVW-PC method is less sensitive to differences in the SNP correlation matrix. To check whether the estimates could be confounded by LD with childhood adversity affecting steroid hormone exposure (55), we applied two-step *cis*-MR (TSCMR) to adjust for childhood adversity (56), using summary genetic associations with childhood adversity indicated by maltreatment from a GWAS meta-analysis of 185,414 individuals (57) that measured emotional, sexual, and physical abuse and emotional and physical neglect. TSCMR extends the 2-step MR approach used for mediation analysis (58). Specifically, TSCMR similarly uses the product and difference of coefficients methods, leveraging the SNP-confounder and confounder-outcome associations to adjust for potential bias via the confounder in the SNP-outcome associations. TSCMR differs from conventional MR methods used for estimating independent effects, such as multivariable MR, which typically uses uncorrelated IVs (59).

Where significant associations were found, we first applied leave-one-out analyses to identify possible outlier SNPs with large effects. Then, to further rule out potential bias from LD with unmeasured confounders that violated the IV assumptions, we repeated the primary analysis using SNPs with evidence of colocalization with *SERPINA6* expression in the liver (60), given that SNPs in this specific region influence cortisol levels by affecting *SERPINA6* expression (28). We applied approximate Bayes factor colocalization analysis (61) to summary genetic associations with morning plasma cortisol and gene expression of *SERPINA6* in the GTEx (Genotype-Tissue Expression) project version 8 (n = 208) (62). We selected the SNP with the strongest posterior probability of colocalization within 100 kilobases of the *SERPINA6/SERPINA1* gene region. We also included rs2736898, which was identified in a previous colocalization analysis using the

Stockholm-Tartu Atherosclerosis Reverse Networks Engineering Task study (28).

We used a Bonferroni-corrected threshold of .017 (.05/3) for statistical significance given the 3 main outcomes considered, namely anxiety, major depression, and neuroticism. We used METAL software (http://genome.sph.umich.edu/wiki/METAL) to meta-analyze the GWAS estimates for cortisol, the R packages TwoSampleMR and MendelianRandomization to obtain MR estimates, TwoStepCisMR to obtain adjusted SNP-outcome associations, coloc to obtain colocalization estimates, LDlinkR to obtain LD proxies, and metafor to meta-analyze MR estimates. All statistical analyses were conducted using R (version 4.2.2, The R Foundation for Statistical Computing).

Ethics

This study only used publicly available data, so specific ethical approval was not required. Ethical approval for the studies included can be found in the original publications.

RESULTS

From the combined CORNET and LIFE studies, there were 8 SNPs (Table S3) at $p < 5 \times 10^{-8}$ and $r^2 < 0.3$ with a mean F statistic of 58.1 (32.2–114.4). All the SNPs or their proxies (rs11620763 replaced by rs7141205) were available. Power calculations showed that at a Bonferroni-corrected alpha level (5%/3), this study had 80% power to detect per morning plasma cortisol z score, a positive/negative association with anxiety, with an odds ratio (OR) of 1.19/0.81 and 1.12/0.88 for major depression in the studies combined and a β of ±0.07 to 0.08 for neuroticism total and by subcluster.

As shown in Figure 3, cortisol was positively associated with anxiety in the UK Biobank (OR 1.27; 95% CI, 1.03 to 1.57) and when meta-analyzed with the other 3 studies (OR 1.16; 95% CI, 1.04 to 1.31), although no significant associations were observed in the ANGST Consortium (OR 1.18; 95% CI, 0.70 to 1.98), iPSYCH Cohort (OR 0.89; 95% CI, 0.55 to 1.42), or FinnGen (OR 1.14; 95% CI, 0.98 to 1.33). Cortisol was

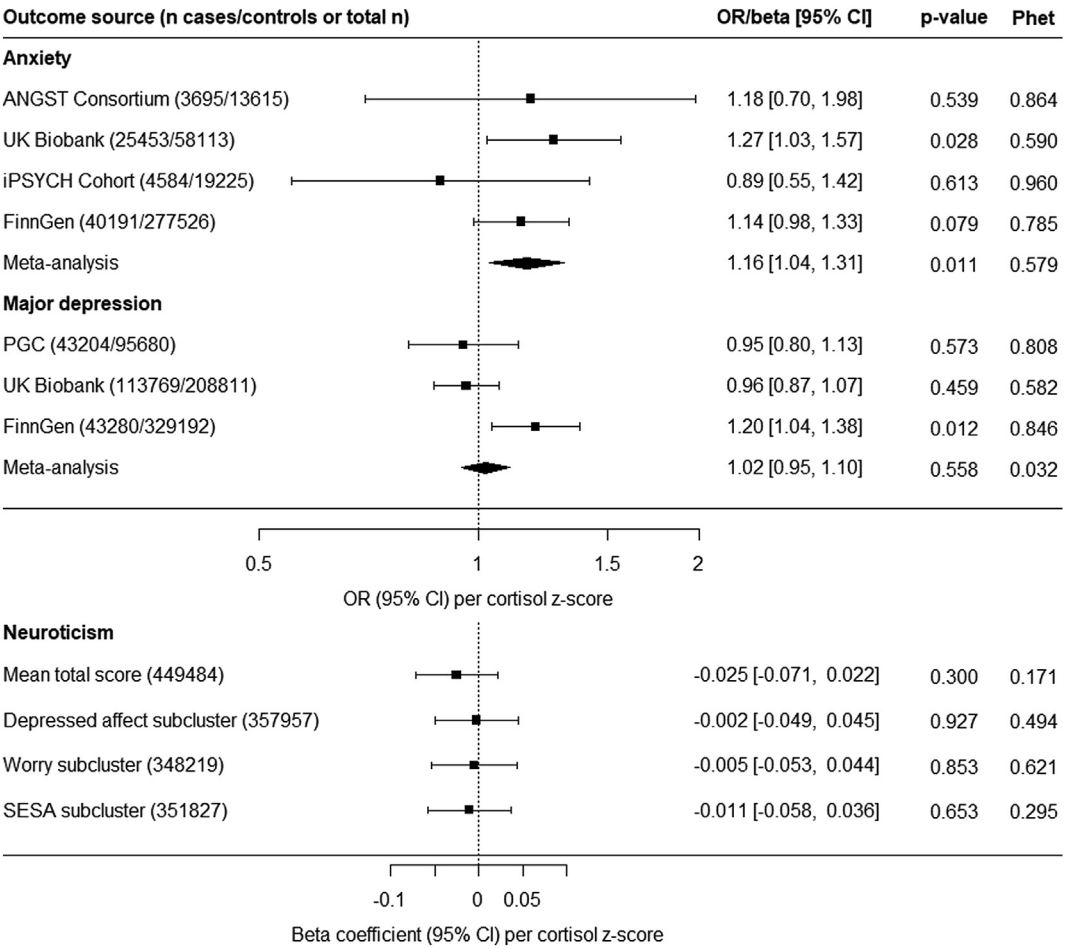

Figure 3. Mendelian randomization estimates of the association of morning plasma cortisol with anxiety, major depression, and neuroticism using genetic variants from the *SERPINA6/SERPINA1* gene region as instruments. *p* Values for heterogeneity for individual (between single nucleotide polymorphisms) and combined (between studies) Mendelian randomization estimates were obtained using Cochrane's *Q* test. ANGST, Anxiety NeuroGenetics Study; OR, odds ratio; PGC, Psychiatric Genomics Consortium; Phet, *p* values for heterogeneity; SESA, sensitivity to environmental stress.

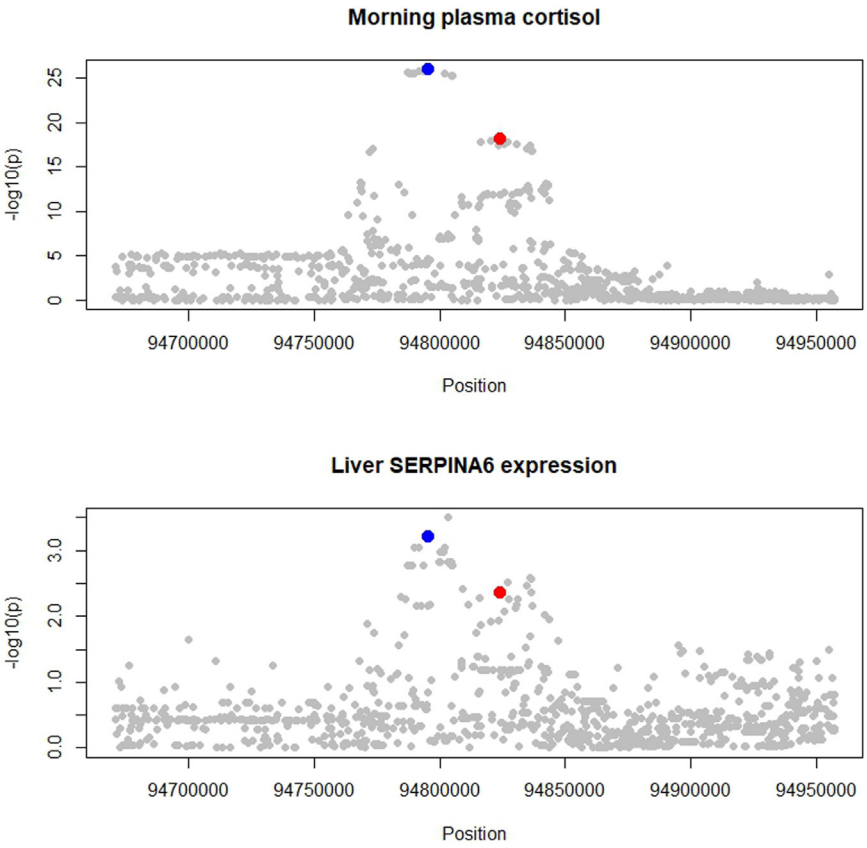

Figure 4. Regional association plots of genetic associations with morning plasma cortisol and liver *SERPINA6* expression highlighting rs9989237 (blue) and rs2736898 (red).

positively associated with major depression (OR 1.20; 95% CI, 1.04 to 1.38) in FinnGen, but not in other studies or when meta-analyzed with the other 2 studies (OR 1.02; 95% CI, 0.95 to 1.10). Cortisol was not associated with neuroticism overall (β −0.025 *z* score; 95% CI, −0.071 to 0.022) or any of its subclusters. Repeating the analysis using different r^2 values to select SNPs and the IVW-PC method (Table S4) gave similar estimates. Adjusting for childhood maltreatment (Tables S5, S6) also gave similar estimates. Cortisol was positively associated with SBP (Table S7) in the primary (β 0.660 mm Hg; 95% CI, 0.051 to 1.270) as well as the sensitivity analyses.

Leave-one-out analyses (Table S8) did not show any specific SNPs driving the associations of cortisol with anxiety in the UK Biobank or major depression in FinnGen. The regional association plots (Figure 4) showed well-defined peaks within the same region for morning plasma cortisol and *SERPINA6* expression. Colocalization analysis in the GTEx project suggested moderate evidence (59.2%) of the 2 traits sharing 1 causal SNP, with rs9989237 being the lead SNP (Table S9). Using only rs9989237 and rs2736898, at reduced power, cortisol was nominally associated with anxiety (OR 1.32; 95% CI, 1.01 to 1.74) in the UK Biobank but not major depression (OR 1.14; 95% CI, 0.95 to 1.37) in FinnGen (Figure 5).

DISCUSSION

Consistent with the toxic stress model (10), which suggests that elevated cortisol contributes to the development of mental disorders, we found that morning plasma cortisol was positively associated with the risk of anxiety. Our findings also clarify that, contrary to one previous MR study (22) but consistent with another MR study (23), RCTs of CRF1 antagonists (15) and previous observational studies on personality (63), cortisol was not associated with major depression or neuroticism. Therefore, our findings suggest that elevated cortisol, and by implication overactivation of the HPA axis, primarily contributes to anxiety but not major depression and does not play a role in neuroticism.

Compared to previous MR studies that have examined the role of cortisol in mental disorders (22,23), our study has several strengths. First, we accounted for the correlation between SNPs, thereby minimizing the possibility of false positives. Second, we pooled the largest available studies to minimize the possibility of false negatives. Additionally, in addition to major depression, we included anxiety in our study and clarified the association of cortisol with anxiety. Notably, cortisol was associated with anxiety overall, but not in ANGST or iPSYCH, possibly due to the relatively smaller sample size. However, in iPSYCH, the lack of adjustment for age or sex may have resulted in confounding from population stratification (64). Our findings are also broadly consistent with previous prospective cohort studies of adolescents, which showed a positive association of cortisol with symptoms of anxiety (65) but not major depression (66). However, our findings are not entirely consistent with previous RCTs of CRF1 antagonists,

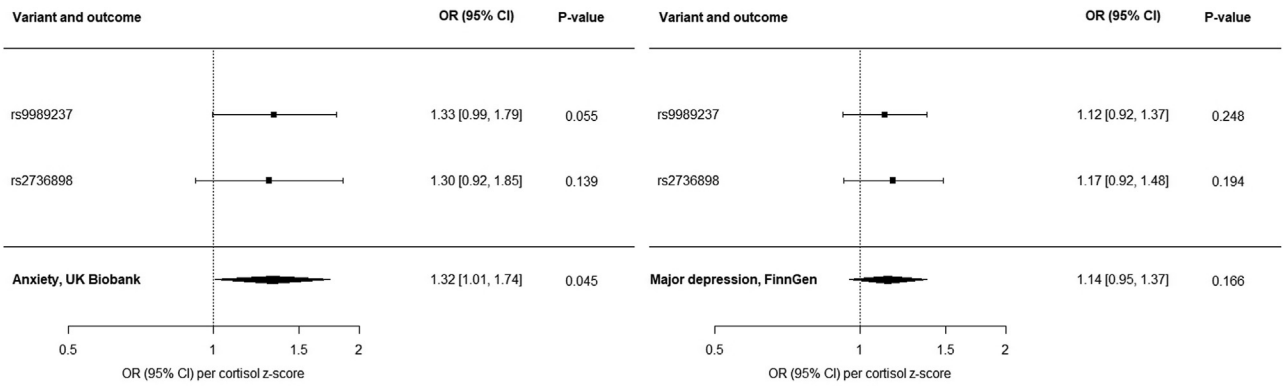

Figure 5. Mendelian randomization estimates of the association of cortisol with anxiety in the UK Biobank (p for heterogeneity = .921) and major depression in FinnGen (p for heterogeneity = .804) using rs9989237 and rs2736898. OR, odds ratio.

which did not find an association of CRF1 inhibition with anxiety (16), or with RCTs of mifepristone and V1b receptor antagonists, which showed favorable effects on major depression. Depletion of CRF1 signaling in mice led to reduced symptoms related to anxiety and depression (67), and elevated cortisol was observed in patients with comorbid major depression and anxiety, but not in patients with major depression alone (68). Mifepristone may act via antiandrogen pathways to reduce depression (69). Animal studies suggest that it is likely that V1b receptor depletion does not reduce aggression via lowering androgen (70). It has been suggested that the effect of cortisol on mental disorders could be mediated by inflammation (71). However, given the anti-inflammatory properties of α_1-antitrypsin (72) and the negative associations of morning plasma cortisol with proinflammatory cytokines (73), the mediating role of inflammation may not fully explain the positive associations of cortisol with anxiety. Alternatively, cortisol suppresses androgens (74), which may in turn increase depressive symptoms (75). Additional research is warranted to better understand the mediating mechanisms, as well as the possibility of sex-specific effects (76).

Despite the MR design, which minimizes bias from environmental confounding, some limitations of the current study require attention. First, the MR estimates would be invalid if the IV assumptions were violated, and in the current study, we implemented several measures to ensure that this was not the case. We exclusively used SNPs from the *SERPINA6/SERPINA1* gene region, which has well-established biological relevance to cortisol bioavailability (27). Furthermore, the selected SNPs were associated with morning plasma cortisol at genome-wide significance, and we used SBP as a positive control outcome for validation. In addition to the extensive sensitivity analyses addressing correlation between SNPs and potential confounding by childhood maltreatment, in the follow-up analysis, we leveraged colocalization to select SNPs for further validation, so it is unlikely that the positive findings with anxiety were driven by LD with unmeasured confounding. There was little sample overlap between exposure and outcome overall, except for ANGST at 39%. However, the F statistics were well above the rule-of-thumb value of 10 for all SNPs, with a mean of 58.1, indicating that bias was at most 0.67% for ANGST and was unlikely for outcomes where there was no sample overlap (48). In the LIFE studies, the GWAS adjusted for BMI, potentially introducing collider bias via the common causes of cortisol and BMI (77). However, using only SNPs from the *SERPINA6/SERPIN1* gene region minimized this possibility. Second, we did not consider subtypes of anxiety and depression despite the known heterogeneity (78), primarily to avoid false negatives given the much smaller sample sizes of the subtypes (20). Furthermore, the overarching aim of this study was to examine the role of cortisol in the most prevalent mental disorders, namely anxiety and major depression, meaning that the use of broadly defined outcomes is necessary. Similarly, given the overarching aim of this study, we did not conduct a reverse MR, which addresses the question of whether differences in liability to mental disorders influence morning plasma cortisol. For the same reason, a colocalization analysis of morning plasma cortisol and mental disorders was not conducted because this addresses the question of whether a specific SNP may causally influence both cortisol and mental disorders irrespective of the direction of causality (61). Third, we assessed the lifetime but not the age-specific risk of anxiety and major depression, and as such our findings do not clarify whether a critical window of risk (20), for example adolescence (79), exists for exposure to higher cortisol. However, the use of lifetime diagnoses encompasses cases diagnosed at younger ages, which avoids selection bias (80). Future studies of younger participants are warranted to clarify the possibility of a critical window. Fourth, this study used a 2-sample MR design, meaning that only linear relationships could be assessed. Although we cannot preclude the possibility of a threshold effect for cortisol, very low or very high levels of cortisol are indicators of poor health (81), which can be a source of confounding in conventional observational studies but is less likely in MR studies. Nevertheless, the assessment of nonlinear effects is possible but will require that the exposure be measured in the outcome population (82), which is not often available. Fifth, using TSCMR, we ruled out the possibility of confounding by LD with childhood maltreatment, but whether the MR estimates could be modified by childhood maltreatment, which remains a possibility (83), warrants further elucidation and will have profound implications for the role and importance of childhood and adolescent well-being. Sixth, some cases of anxiety and major depression

were based on self-report, so the possibility of bias due to misclassification exists (84) but is likely small given that most cases were ascertained based on DSM/ICD criteria. Seventh, cortisol secretion generally follows a diurnal and ultradian pattern but is highly variable between individuals (85). Random variation in morning plasma cortisol could bias the genetic estimates toward null. Replication using other cortisol measurements, such as hair cortisol, which is more stable over time (86), is warranted. Eighth, this MR study is based on GWASs of participants of European ancestry, mostly adjusted for but not stratified by sex. Replication by sex in other populations, which have different presentations of anxiety and depressive symptoms (87), is warranted.

From a public health and clinical perspective, stress is increasingly recognized as a serious public health issue that influences both physical and mental health (88). The current findings provide additional evidence that cortisol plays a causal role in mental health, particularly in anxiety. Our findings also support ongoing efforts aimed at targeting the cortisol pathway to alleviate anxiety and redirect focus toward other pathways for major depression (89).

Conclusions

Morning plasma cortisol, instrumented by SNPs from the *SERPINA6*/*SERPINA1* gene region, was positively associated with anxiety but not major depression or neuroticism. Consistent with the toxic stress model, our findings suggest that cortisol can be targeted to reduce the risk of anxiety, while other pathways may be more relevant to major depression and neuroticism.

ACKNOWLEDGMENTS AND DISCLOSURES

IIC and AMSW conceptualized the study and interpreted the results. IIC conducted the formal analyses and wrote the first draft, with critical feedback and revisions from AMSW.

We thank the study participants and researchers for making the genome-wide association studies summary data used in this study available.

Data sources for cortisol, anxiety, major depression, neuroticism, and systolic blood pressure can be found in Table S2. The analysis code and combined summary statistics for cortisol are available from the corresponding author upon request.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

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