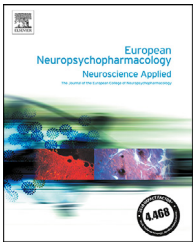
European Neuropsychopharmacology (2019) 29, 1354–1364

www.elsevier.com/locate/euroneuro



Multivariate genome-wide analysis of stress-related quantitative phenotypes

Dick Schijven[a,b,c], Elbert Geuze[a,d], Christiaan H. Vinkers[e,f], Sara L. Pulit[b,g], Remmelt R. Schür[a], Marie Malgaz[a], Erwin Bekema[a,b,c], Jelena Medic[a,b,c], Kendrick E. van der Kust[a], Jan H. Veldink[b], Marco P. Boks[a], Eric Vermetten[a,d,h,i], Jurjen J. Luykx[a,c,j,*]

[a] *Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht University, the Netherlands*
[b] *Department of Neurology and Neurosurgery, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht University, the Netherlands*
[c] *Department of Translational Neuroscience, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht University, the Netherlands*
[d] *Research Centre, Military Mental Healthcare, Ministry of Defense, Utrecht, the Netherlands*
[e] *Department of Psychiatry, Amsterdam UMC (location VUmc) / GGZ InGeest, Amsterdam, the Netherlands*
[f] *Department of Anatomy and Neurosciences, Amsterdam UMC (location VUmc), Amsterdam, the Netherlands*
[g] *Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, the Netherlands*
[h] *Department of Psychiatry, Leiden University Medical Center, Leiden, the Netherlands*
[i] *Arq Psychotrauma Expert Group, Diemen, the Netherlands*
[j] *GGNet, Apeldoorn, the Netherlands*

Received 28 March 2019; received in revised form 11 July 2019; accepted 20 September 2019

KEYWORDS
GWAS;
Stress;
Deployment;

Abstract
Exposure to traumatic stress increases the odds of developing a broad range of psychiatric conditions. Genetic studies targeting multiple stress-related quantitative phenotypes may shed light on mechanisms underlying vulnerability to psychopathology in the aftermath of stressful events. We applied a multivariate genome-wide association study (GWAS) to a unique military

* Corresponding author at: Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, Heidelberglaan 100, 3508 GA Utrecht, PO Box 85500, the Netherlands. Tel.: +31 88 75 68638.
E-mail address: J.Luykx@umcutrecht.nl (J.J. Luykx).

https://doi.org/10.1016/j.euroneuro.2019.09.012

Quantitative phenotype;
Hormone;
Questionnaire

cohort ($N = 583$) in which we measured biochemical and behavioral phenotypes. The availability of pre- and post-deployment measurements allowed to capture changes in these phenotypes in response to stress. For genome-wide significant loci, we performed functional annotation, phenome-wide analysis and quasi-replication in PTSD case-control GWASs. We discovered one genetic variant reaching genome-wide significant association, surviving permutation and sensitivity analyses (rs10100651, $p = 9.9 \times 10^{-9}$). Functional annotation prioritized the genes *INTS8* and *TP53INP1*. A phenome-wide scan revealed a significant association of these same genes with sleeping problems, hypertension and subjective well-being. Finally, a targeted lookup revealed nominally significant association of rs10100651 in a PTSD case-control GWAS in the UK Biobank ($p = 0.02$). We provide comprehensive evidence from multiple resources hinting at a role of the highlighted genetic variant in the human stress response, marking the power of multivariate genome-wide analysis of quantitative measures in stress research. Future genetic and functional studies can target this locus to further assess its effects on stress mediation and its possible role in psychopathology or resilience.

1. Introduction

Military personnel report increased stress-related mental health symptoms after deployment, including mostly post-traumatic stress, depression, fatigue and anxiety (Lapierre et al., 2007; Reijnen et al., 2015b). Unambiguous clinical diagnosis of psychopathology following trauma is cumbersome, as supported by shifting diagnostic classification criteria of post-traumatic stress-disorder (PTSD) and moderate to low inter-rater reliability in establishing a diagnosis of trauma-related disorders (American Psychiatric Association, 2013; 2000; Regier et al., 2013). In studies investigating genetic mechanisms of stress-related disorders, imprecise diagnostic ascertainment for case-control status may be circumvented by targeting phenotypes that correspond to more specific behavioral and biological mechanisms underlying psychopathology. Overall, such phenotypes are reliably quantifiable, lie closer to disease biology than clinical diagnoses, and generally show less measurement variability (Flint et al., 2014). In addition, novel tools now allow for the integration of multiple layers of such phenotypic data into multivariate genome-wide analyses, increasing power relative to univariate analyses (Porter and O'Reilly, 2017).

To find novel genetic loci associated with modulation of stress and psychopathology following trauma, we performed a multivariate genome-wide analysis of stress-related phenotypes (both biochemical and behavioral) in a military cohort. The collection of an extensive and unique set of pre- and post-deployment phenotypic measurements allowed us to carry out unprecedented multivariate analyses (Fig. 1) in a reverse regression framework (Magi et al., 2017), capturing the effect of deployment stress on these phenotypes. We report a genome-wide significant locus, perform several sensitivity analyses to show the robustness of the locus, assess the functional consequences this variant exerts on surrounding genes, and perform a phenome-wide analysis, highlighting association of functionally prioritized genes with other stress-related phenotypes. Finally, we discuss the potential future gains that may result from analyzing quantitative phenotypes in GWASs of stress-related phenotypes.

2. Experimental procedures

2.1. Participants and procedures

The data used for this study is part of an ongoing longitudinal study on stress-related mental health symptoms in Dutch military personnel, in which individuals were deployed to Afghanistan during a four-month period between 2005 and 2008. Approximately one month prior to deployment and one month after deployment, blood samples were collected and individuals completed questionnaires at the army base (Fig. 1) (Reijnen et al., 2015b). Military personnel volunteering to take part in the study received verbal and written descriptions of the study and provided informed consent. The study was approved by the Institutional Review Board of the University Medical Center Utrecht (Utrecht, the Netherlands). We adhered to the ethical statements described in the declaration of Helsinki (World Medical Association, 2013).

2.2. Phenotype collection and measurements

All blood plasma samples were collected between 8 AM and 11:30 AM (Reijnen et al., 2015a). Plasma concentrations of dehydroepiandrosterone sulfate (DHEA-S), a metabolite of the anti-glucocorticoid hormone DHEA (van Zuiden et al., 2017), were measured using an electrochemiluminescence immunoassay on the Modular E170 (Roche Diagnostics GmbH, Mannheim, Germany). The lower limit of detection was 0.05 μmol/l with an inter-assay variation $< 4.4\%$ in the range of 1.7-12 μmol/l. Other measurements included cortisol, the most important human glucocorticoid (Geuze et al., 2012); gamma-aminobutyric acid (GABA), a modulator of hypothalamic-pituitary-adrenal (HPA)-axis activity (Schür et al., 2016); neuropeptide Y (NPY), a peptide neurotransmitter and modulator of the stress response (Reijnen et al., 2018); testosterone, a product of the hypothalamic-pituitary-gonadal (HPG)-axis and involved in psychosocial and behavioral phenotypes (Reijnen et al., 2015a); and sex hormone-binding globulin (SHBG), a protein produced by the liver regulating bioavailable testosterone (Reijnen et al., 2015a). These were quantified in plasma as described in the referenced manuscripts.

Behavioral phenotypes were measured using surveys and clinically-implemented symptom checklists. We assessed subject mental health using the Dutch revised Symptom Checklist (SCL-90-R) (Arrindell and Ettema, 2003), evaluated symptoms of PTSD using the Dutch Self-Rating Inventory for PTSD (SRIP) (Hovens et al., 2002; Hovens 2000), measured fatigue using the

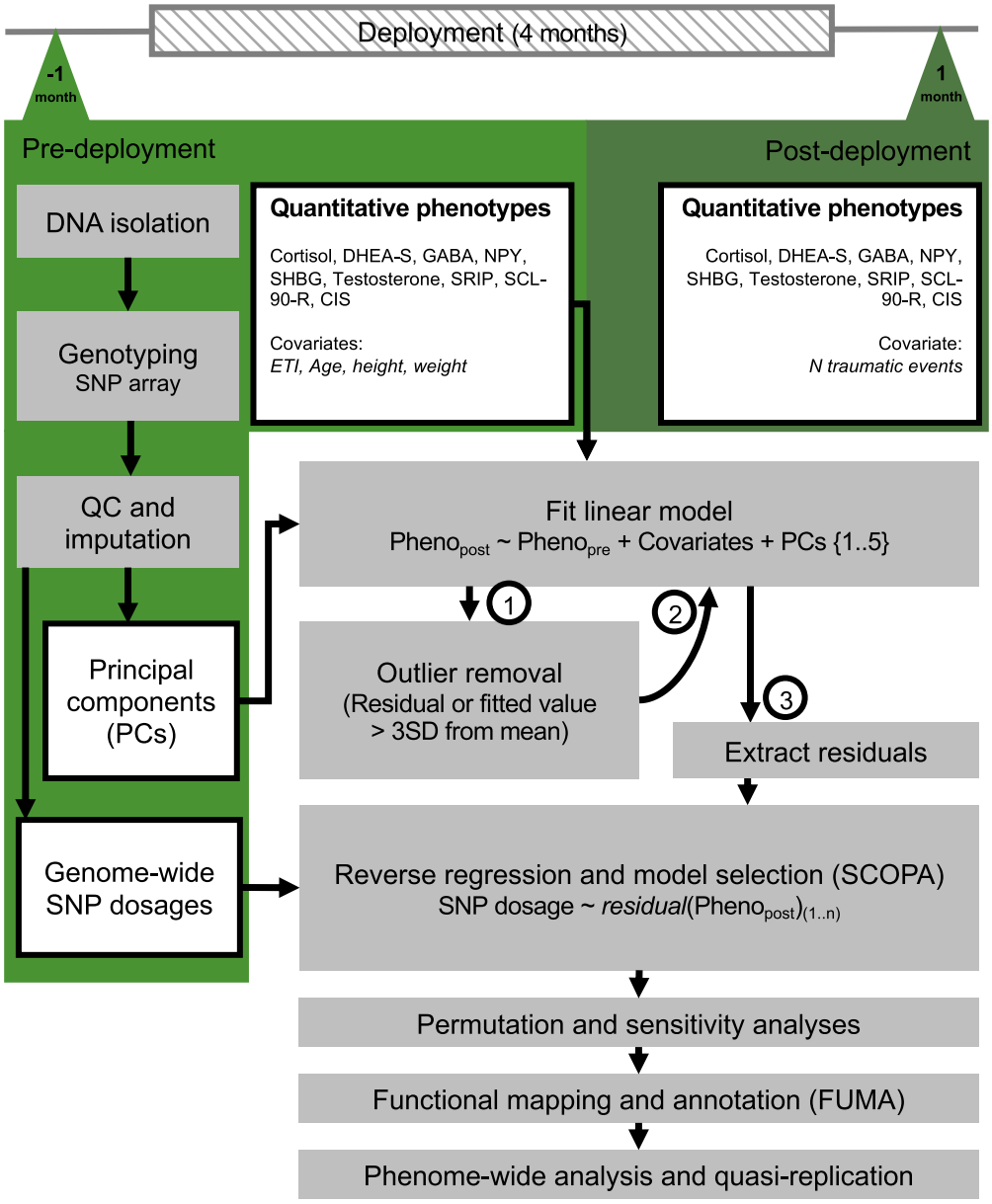


Fig. 1 Analysis overview. Data on quantitative phenotypes were collected one month before (pre-deployment) and one month after (post-deployment) a 4-month deployment period (phenotypes in *italic* were used as covariates). Additionally, DNA was isolated from whole blood and genotyped on a SNP array after which quality control (QC) and imputation procedures were completed. Principal components and SNP dosages were extracted from imputed data. Residuals were calculated for each quantitative phenotype by fitting a linear model of the post-deployment measurement against the pre-deployment measurement, covariate phenotypes and the first five genetic PCs. Outliers in residuals or fitted values were removed. SNP dosage data and residuals were combined in reverse regression analysis using Software for Correlated Phenotype Analysis (SCOPA) and we followed up genome-wide significant hits with permutations, sensitivity analyses, functional annotation, phenome-wide analysis and quasi-replication in PTSD case-control GWASs.
Abbreviations: DHEA-S, Dehydroepiandrosterone sulfate; GABA, Gamma-aminobutyric acid; NPY, Neuropeptide Y, SHBG, Sex hormone-binding globulin; SRIP, Self-Rating Inventory PTSD; SCL-90-R, Dutch version of the Revised 90-item symptom checklist; CIS, Checklist Individual Strength; ETI, Early Trauma Inventory; SD, Standard Deviation; PC, Principal Component.

Checklist Individual Strength (CIS) (Vercoulen et al., 1999), gathered data on combat-related stressors and potentially traumatic experiences using a 19-item checklist (Reijnen et al., 2015b), and finally assessed exposure to trauma before the age of 18 using the Early Trauma Inventory (ETI) (Supplementary Table 1) (Bremner et al., 2007).

2.3. Genotyping, quality control, imputation and calculation of genetic relationship matrix

DNA from a total of 1015 individuals was isolated from whole blood at University Medical Center Utrecht (Utrecht, the Netherlands) and shipped to IoPPN Genomics & Biomarker Core

Facility of King's College London for genotyping on the Illumina Human Omniexpress-24 v1.1 platform (Illumina, San Diego, CA, USA). This platform contains a total number of 713,040 genetic markers. Genotypes were called using Illumina GenomeStudio software without any filtering presets. Subsequently, we applied quality control (QC) to the genotype data using PLINK version 1.90b3z (22-11-2015; www.cog-genomics.org/plink/1.9, Supplementary Table 2) (Chang et al., 2015). We removed samples with a genotype call rate < 0.95, discordant genetic sex and reported sex in phenotype data, and an excess heterozygosity rate (heterozygosity $>$ 3SD from the mean) or excess homozygosity (heterozygosity $<$ 3SD from the mean). Additionally, we identified pairs of individuals with a relatedness coefficient (PLINK PIHAT) > 0.1 and randomly removed one individual from the pair. Next, we performed SNP-level QC: we removed non-autosomal genetic markers, SNPs with a call rate < 0.95, SNPs out of Hardy-Weinberg Equilibrium (HWE, p-value $< 1 \times 10^{-6}$) and SNPs with a strand-ambiguous A/T or C/G genotype. In preparation for imputation, we removed SNPs that were not present in the reference panel for imputation or had a difference in minor allele frequency (MAF) > 0.15 compared to the reference panel.

We imputed the genetic data using minimac3 in a 500 kilobase window as implemented on the Michigan Imputation Server (Das et al., 2016), with the Haplotype Reference Consortium (HRC) release 1.1 as a reference panel (McCarthy et al., 2016), and the Eagle2 algorithm for phasing (Loh et al., 2016). We applied additional quality control (Supplementary Table 3) to the imputed data (post-imputation QC): we removed SNPs with a MAF < 0.05, imputation $R^2 < 0.3$ and a MAF difference from the reference panel > 0.15. We used genotype dosages in our analyses, unless stated otherwise.

We calculated a genetic relationship matrix (GRM) using Genome-wide Complex Trait Analysis (GCTA) software (version 1.24.4) (Yang et al., 2011), and subsequently derived 100 principal components (PCs) from this GRM. The first five PCs were used as covariates in analyses to capture genetic variation due to population stratification.

2.4. Multi-phenotype association analysis

Prior to the association analysis, we excluded individuals with non-male sex (due to the low number of females in the cohort and sex-dependent differences in hormone levels), missing data for analysis covariates and without a completed pre- and post-deployment measurement for at least one quantitative phenotype (cortisol, DHEA-S, GABA, NPY, testosterone, SHBG; and total scores for SRIP, SCL-90-R and CIS). To assess pre-post deployment differences in these measurements we applied a two-sided paired t-test. Additionally, we calculated correlatons between the phenotypes at pre- and post-deployment measurements. To capture genetic association with the effect of deployment stress, we fitted post-deployment measurements on pre-deployment values of the included phenotypes using R version 3.3.3 (www.r-project.org). We additionally included age, ETI total score, number of experienced potentially traumatic events during deployment and five PCs based on the genetic data as covariates in the regression model and additionally included pre-deployment height and weight as covariates for the biochemical phenotypes. We removed samples when the residual or fitted value deviated $>$ 3 SD from the mean, and then fitted the linear model again for the remaining individuals. We derived the variance in post-deployment measurements explained by pre-deployment measurements, covariates and residuals in the models through dividing the sum of squares of each predictor by the total sum of squares obtained through Analysis of Variance (ANOVA) on each linear model. Residuals were extracted from the final models and used as input in the multivariate genome-wide association analysis in Software for Correlated Phenotype Analysis (SCOPA) (Magi et al., 2017). In SCOPA, we applied reverse regression for each SNP with genotype dosage as the dependent variable and post-deployment phenotype residuals as the independent variables. Additionally, Bayesian Information Criterion (BIC) for optimal model selection was applied to variants reaching the established stringent threshold for genome-wide significance ($p < 5 \times 10^{-8}$), where the combination of phenotypes with the lowest BIC resulted in the optimal model.

For genome-wide significant hits, we ran an additional association analysis in a $\pm$2 Mb locus around the top variant testing all SNPs with the optimal model (lowest BIC) for the genome-wide significant variant.

2.5. Permutation and sensitivity analyses of genome-wide significant hits

We performed several sensitivity analyses on lead SNPs of loci that reached genome-wide significance in the main multivariate analysis. First, we performed $n = \frac{1}{p}$ permutations of sample labels and subsequent SCOPA regression using the optimal model for the corresponding genome-wide significant variant, where p is the nominal association p-value of the variant. We calculated an empirical p-value for each top variant using $p_{empirical} = \frac{s+1}{n+1}$, where s is the number of times the permuted p-value was lower than the nominal p-value and n the total number of permutations performed. Second, we performed the same analysis pipeline using log-transformed questionnaire data to reduce skewness in the distribution of residuals. Finally, we substituted the questionnaire total scores with the scores of the subscales, as these pinpoint more specific phenotypic domains, and repeated the analysis pipeline. For each of the sensitivity analyses, we applied the same threshold for genome-wide significance ($p < 5 \times 10^{-8}$).

2.6. Functional annotation, phenome-wide analysis and quasi-replication

We used Functional Mapping and Annotation (FUMA) for annotation of genome-wide significant loci with expression quantitative trait locus (eQTL) effects and chromatin interactions (Watanabe et al., 2017).

The unique longitudinal design and phenotype collection in the cohort precluded us from finding a dataset with identical phenotypes to replicate genome-wide significant loci. To assess the role of genome-wide significant loci and functionally prioritized genes in other phenotypes, we first performed a hypothesis-generating phenome-wide scan using the GWAS atlas (atlas.ctglab.nl) (Watanabe et al., 2018). The GWAS atlas contains SNP- and gene-level association results of publicly available GWASs and UK Biobank traits with at least 50,000 individuals and more than 10,000 cases, totaling 2824 unique phenotypes (accessed 29-11-2018). Second, to test how our findings reflect genetic risk for PTSD, the main diagnosis related to trauma-induced psychopathology, we performed a quasi-replication of any genome-wide significant loci in the European subset of the largest published case-control GWAS meta-analysis on PTSD (2424 cases; 7113 controls) (Duncan et al., 2018), and in a case-control GWAS of self-reported PTSD from the UK Biobank (266 cases; 360,875 controls; accessed August 2018) (Neale, 2018; Sudlow et al., 2015). The first dataset was also included in the phenome-wide analysis, but the latter was not due to the low number ($<$ 10,000) of cases. From the UK Biobank PTSD GWAS summary statistics, we first removed SNPs with an expected minor allele count < 25 or a minor allele frequency < 0.05 as these are likely to inflate the number of false positives given the low number of cases and disbalance in case-control ratio.

Table 1 Descriptive statistics of male individuals after application of exclusion criteria.

	N	Pre-deployment			Post-deployment			Paired *t*-test p (t-statistic)
		Mean [range]	SD	Median	Mean [range]	SD	Median	
General statistics (covariates) (total $N = 583$)[a]								
Age at start of deployment (years)	583	29.27 [18-60]	9.40	26	-	-	-	
ETI total score	583	3.46 [0-17]	3.01	3	-	-	-	
Potential traumatic events during deployment	583	-	-	-	4.55 [0-14]	3.23	4	
Height at start of deployment (cm)	575	183 [165-202]	6.53	183	-	-	-	
Weight at start of deployment (kg)	556	83 [58-130]	10	83	-	-	-	
Biochemical data (total $N = 556$)[b]								
Cortisol (nmol/l)	524	439.83 [122-1412]	141.35	433.5	449.30 [143-917]	135.99	443.5	0.22 (−1.24)
DHEA-S (μmol/l)	520	10.25 [1.48-22.12]	3.49	9.99	10.33 [1.92-25.97]	3.84	10.02	0.38 (−0.87)
GABA (nmol/l)	521	120.08 [70-201]	21.93	118	124.74 [75-217]	23.08	121	4.4×10^{-9} (−5.97)
NPY (ng/ml)	522	41.40 [15-236.9]	17.55	38.05	43.15 [10.8-160.5]	18.29	40.5	0.016 (−2.43)
SHBG (nmol/l)	520	19.07 [7-58]	6.39	18	19.80 [4-50]	6.54	19	2.3×10^{-5} (−4.27)
Testosterone (nmol/l)	520	17.87 [0.65-39]	5.95	18	19.16 [4.9-280]	13.61	18	0.030 (−2.18)
Questionnaire data (total $N = 583$)[a]								
SRIP total score	431	26.87 [22-57]	4.98	25	27.92 [22-57]	6.08	26	5.7×10^{-5} (−4.06)
SCL-90-R total score	496	102.40 [90-209]	14.57	98	103.30 [90-194.65]	17.40	97	0.15 (−1.46)
CIS total score	519	45.91 [20-101]	17.52	43	49.32 [20-111]	21.45	44	5.2×10^{-5} (−4.08)

Abbreviations: N, number of individuals with complete data; SD, Standard Deviation; ETI, Early Trauma Inventory; DHEA-S, Dehydroepiandrosterone sulfate; GABA, Gamma-aminobutyric acid; NPY, Neuropeptide Y; SHBG, Sex hormone-binding globulin; SRIP, Self-Rating Inventory PTSD; SCL-90-R, Dutch version of the Revised 90-item symptom checklist; CIS, Checklist individual strength.

[a] After exclusion of individuals with missing data on Age, ETI and potential traumatic event count.

[b] After exclusion of individuals with missing data on Age, ETI, potential traumatic event count, pre-deployment height and pre-deployment weight.Sample size column may not add up to total sample size per data category due to missing values.

3. Results

3.1. Descriptive statistics

From our initial study population after genotype QC ($n = 963$), we excluded the following samples: non-male sex ($n = 86$), missing reported age ($n = 5$), missing reported number of traumatic events during deployment ($n = 292$), missing reported ETI total score ($n = 91$), missing reported pre-deployment height ($n = 47$) and missing reported pre-deployment weight ($n = 88$), the latter two being only relevant for biochemical phenotypes.

The mean pre-deployment age of the included participants was 29.27 (SD 9.40) with an exposure to a mean of 4.55 (SD 3.23) potentially traumatic events during deployment. Mean concentrations of GABA, NPY, SHBG, testosterone and mean total scores for SRIP and CIS were significantly higher after deployment (Table 1). After calculating residuals of post-deployment measurements and removal of outliers, 560 individuals with at least one complete pre- and post-deployment measurement of a single phenotype remained. 293 participants had non-missing data for all phenotypes (Supplementary Fig. 1), in which we detected a significantly positive correlation between: behavioral questionnaires; DHEA-S, testosterone and SHBG; and DHEA-S and cortisol. In addition, we detected a significantly negative correlation between NPY and SHBG (Supplementary Fig. 2). Most of the variance in the post-deployment measurements was explained by the pre-deployment measurements, whereas the number of experienced potentially traumatic events had a significant effect on the total score after deployment of all psychiatric questionnaires. The variance explained by residuals used as input in the multivariate GWAS ranged from 23.33% to 92.34% (Supplementary Table 4).

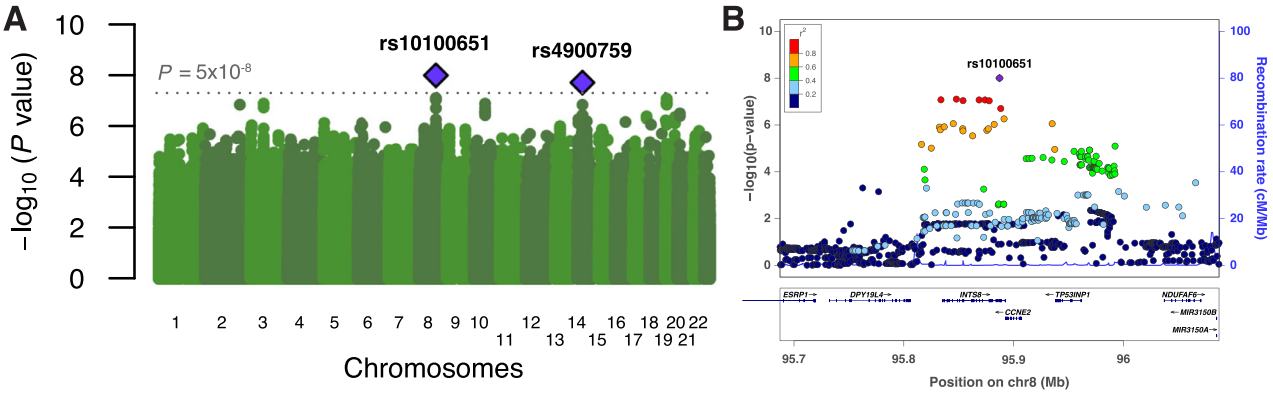


Fig. 2 Multivariate GWAS result. A) Manhattan plot showing all tested variants with their relative position across the chromosomes (*x*-axis) and their association with post-deployment quantitative phenotype residuals at their optimal model as selected through BIC in SCOPA (*y*-axis). Genome-wide significance threshold (dotted line) is $p < 5 \times 10^{-8}$. Diamonds indicate variants reaching genome-wide significance. B) Regional association plot of rs10100651 on chromosome 8. All SNPs in this plot were tested with the optimal model for lead SNP rs10100651 (including DHEA-S, GABA, NPY, SHBG, SRIP and SCL-90-R). Colors indicate LD (R^2) in the European population of surrounding variants relative to rs10100651.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2 Statistics of genome-wide significant hits in multivariate GWAS.

SNP	Effect (other) allele	HWE	MAF	R^2	*N*	null Log Likelihood	Likelihood Ratio	*P*-value	BIC	Model	P_{perm}
rs10100651	C (G)	0.30	0.46	1.00	307	−51.37	48.39	9.9×10^{-9}	94.44	DHEA-S GABA NPY SHBG SRIP SCL-90-R	2.0×10^{-8}
rs4900759	T (C)	0.23	0.26	1.00	381	−1.25	35.55	1.9×10^{-8}	−15.22	SRIP SCL-90-R	3.8×10^{-8}

Abbreviations: SNP, Single-nucleotide polymorphism; HWE, *p*-value of Hardy-Weinberg Equilibrium test; MAF, Minor allele frequency; R^2, Imputation INFO score; *N*, sample size of the model; BIC, Bayesian Information Criterion; P_{perm}, Empirical *p*-value calculated from permutation analysis; DHEA-S, Dehydroepiandrosterone sulfate; GABA, Gamma-aminobutyric acid; NPY, Neuropeptide Y, SHBG, Sex hormone-binding globulin; SRIP, Self-Rating Inventory PTSD; SCL-90-R, Dutch version of the Revised 90-item symptom checklist.

3.2. Multivariate association testing

After quality control and imputation of genetic variants, the dataset comprised > 5 million variants (Supplementary Table 2, Supplementary Table 3). Samples were primarily of European descent, with the vast majority (± 91%) lying < 6 SD from the HapMap3 European populations along PCs one and two (Supplementary Fig. 3). The first five genetic PCs, reflecting population-specific genetic differences and included as covariates in the analyses, explained a minor and mostly non-significant proportion of the variance in the post-deployment phenotypes (Supplementary Table 4).

Multiple-phenotype reverse regression analysis revealed two SNPs passing the threshold for genome-wide significance ($p < 5 \times 10^{-8}$) under optimal models composed of several phenotypes (Fig. 2A, Table 2). The first SNP mapped to an intron of the Integrator Complex Subunit 8 gene (*INTS8*) on chromosome 8 (rs10100651, $p = 9.9 \times 10^{-9}$) in a regression model including DHEA-S, GABA, NPY, SHBG, SCL-90-R and SRIP data (BIC = 94.44) (Table 2, Supplementary Table 5). DHEA-S was the strongest contributor to the association (beta = 0.12, se = 0.023) (Supplementary Table 6, Supplementary Figure 4). This SNP had a G/C genotype with a MAF of 0.46 (C-allele). To ensure no strand inconsistencies influenced association at this G/C variant with high MAF, we calculated haplotype frequencies including surrounding variants in high linkage disequilibrium (LD $R^2 > 0.7$) with rs10100651, of which one variant was directly genotyped and the others were imputed, using HaploView software (version 4.2) (Supplementary Table 7) (Barrett et al., 2005). We found five different haplotypes, the most common of which were comprised of either only major alleles (0.51) for all SNPs, or minor alleles for all SNPs (0.39). We also calculated haplotypes in the European subset of 1000 Genomes Phase 3 and compared them to haplotype frequencies in our cohort (The 1000 Genomes Project Consortium, 2015). None of the identified haplotypes in our data had > 7% frequency difference when compared to haplotype frequencies in 1000

Genomes. Overall, these results suggested no strand inconsistencies at this locus in our dataset.

The second SNP we discovered in the multi-phenotype regression was an intronic variant of the MAM Domain Containing Glycosylphosphatidylinositol Anchor 2 gene (*MDGA2*) on chromosome 14 (rs4900759, $p = 1.9 \times 10^{-8}$). The optimal model included total scores of SRIP and SCL-90-R questionnaires as phenotypes (BIC = −15.22) (Table 2, Supplementary Table 5), with SRIP being the strongest contributor to the association (beta = −0.06, se = 0.009) (Supplementary Table 6).

To assess statistical credibility of the discovered loci, we performed permutation of sample labels followed by reverse regression in SCOPA with the corresponding optimal model for the top SNPs of the discovered loci. Permutations at rs10100651 resulted in an empirical *p*-value of $p = 2.0 \times 10^{-8}$. Permutation analysis for rs4900759 resulted in an empirical *p*-value of $p = 3.8 \times 10^{-8}$ (Table 2). To reduce skewness in the residuals of questionnaire data (Supplementary Fig. 1), we then log-transformed questionnaire total scores, re-calculated residuals and reran the analysis pipeline for the two genome-wide significant hits. The association signal at rs10100651 remained significant at $p = 2.9 \times 10^{-8}$, with the optimal model identified through BIC remaining similar to the main analysis (DHEA-S, GABA, NPY, SHBG, SCL-90-R and SRIP), whereas the association at rs4900759 dropped below significance ($p = 1.5 \times 10^{-5}$) and the model differed from the optimal model in the main analysis (GABA, NPY, SCL-90-R, SRIP) (Supplementary Table 6).

As a final sensitivity analysis, questionnaire total scores were replaced by subscale scores and the analysis pipeline for the genome-wide significant SNPs was rerun. For rs10100651, the *p*-value had further decreased ($p = 3.2 \times 10^{-9}$) with an optimal model composed of the same biochemical phenotypes as in the original analysis (DHEA-S, GABA, NPY and SHBG). Subscales of the same questionnaires as in the primary analysis were present in the optimal model (SRIP avoidance, SRIP re-experiencing and SCL-90-R sleeping problems). For rs4900759, again the *p*-value increased to non-significance ($p = 1.6 \times 10^{-6}$) with a different optimal model (Supplementary Table 6). Therefore, all these analyses confirmed the robustness of the chromosome 8 locus (rs10100651) to permutations and phenotype rescaling, while diminishing the evidence for the signal at chromosome 14 (rs4900759).

For rs10100651, we analyzed all variants in a ±2 Mb window surrounding this SNP using the optimal model from the primary analysis to highlight association and LD structure at this locus (Fig. 2B).

3.3. Functional annotation, phenome-wide analysis and quasi-replication

Functional annotation of rs10100651 using eQTL and chromatin interaction data implemented in FUMA revealed both significant eQTL- and chromatin interaction of (the genomic region including) rs10100651 on two surrounding genes (*INTS8; TP53INP1,* Tumor Protein P53 Inducible Nuclear Protein 1) and either eQTL or chromatin interaction effects on other genes (Supplementary Table 8, Supplementary Table 9, Supplementary Fig. 5).

Hypothesis-free phenome-wide analysis of the functionally prioritized genes *INTS8* and *TP53INP1* was performed using the GWAS atlas. Bonferroni correction of the *p*-value threshold for significance was applied to two genes and 2824 unique traits assessed ($p = 8.9 \times 10^{-6}$). The analysis revealed significant association of these genes with multiple phenotypes (Fig. 3, Supplementary Table 10, Supplementary Table 11), including: insomnia (*TP53INP1*, $p = 6.7 \times 10^{-8}$) and sleeplessness (*TP53INP1*, $p = 3.3 \times 10^{-6}$), subjective well-being and general happiness (*TP53INP1*, $p = 7.8 \times 10^{-7}$), hypertension (*TP53INP1*, $p = 1.3 \times 10^{-7}$; *INTS8*, $p = 3.42 \times 10^{-7}$) and blood pressure (*INTS8*, $p = 3.3 \times 10^{-8}$). A specific scan of rs10100651 revealed no phenotypes passing the significance threshold, and this variant was only present in 52 GWAS results.

We additionally performed a targeted lookup of rs10100651 in existing PTSD case-control GWASs. rs10100651 (MAF = 0.48, C-allele) passed SNP filtering for low MAF and minor allele count in the UK Biobank self-reported PTSD GWAS data (Supplementary Fig. 6A). This SNP showed nominally significant association and directional consistency with DHEA-S and SRIP total score in our cohort (C-allele, beta = 1.5×10^{-4}, se = 6.4×10^{-5}, $p = 0.02$) (Supplementary Fig. 6B). The SNP was contained in a locus with rs12550765 as a lead SNP (LD with rs10100651 $R^2 = 0.47$ in the British population, $R^2 = 0.34$ in the European population, association $p = 6.7 \times 10^{-4}$), a SNP also showing nominally significant association in our multi-phenotype GWAS ($p = 2.1 \times 10^{-3}$) when tested with the same set of phenotypes as the optimal model for rs10100651. In the largest PTSD case-control GWAS meta-analysis, association at rs10100651 did not reach significance ($p = 0.18$ in a meta-analysis limited to male subjects of European ancestry). The lack of evidence for the discovered association at rs4900759 on chromosome 14 was further confirmed by non-replication in the UK Biobank GWAS on self-reported PTSD status ($p = 0.39$) and the largest PTSD case-control GWAS meta-analysis ($p = 0.86$).

4. Discussion

By performing a multivariate GWAS on stress-related longitudinally collected phenotypes, we discovered one locus reaching genome-wide significance. The association at this locus remained significant after permutation and sensitivity analyses, the lead SNP exerted functional effects on surrounding genes that were in turn associated with multiple stress-related phenotypes, and the locus quasi-replicated in a case-control GWAS of self-reported PTSD. From a general perspective, this study shows the future potential of multivariate GWAS approaches in psychiatric genetics.

The reverse regression approach as implemented in SCOPA allowed us to use genotype dosages to account for possible imputation uncertainty of SNPs and to dissect the association at genome-wide significant SNPs to obtain the optimal regression model and contribution of each phenotype to the association using BIC (Magi et al., 2017). The optimal regression model at the credible locus (rs10100651) included four biochemical phenotypes (DHEA-S, GABA, NPY, SHBG) and two behavioral questionnaires (SRIP and SCL-

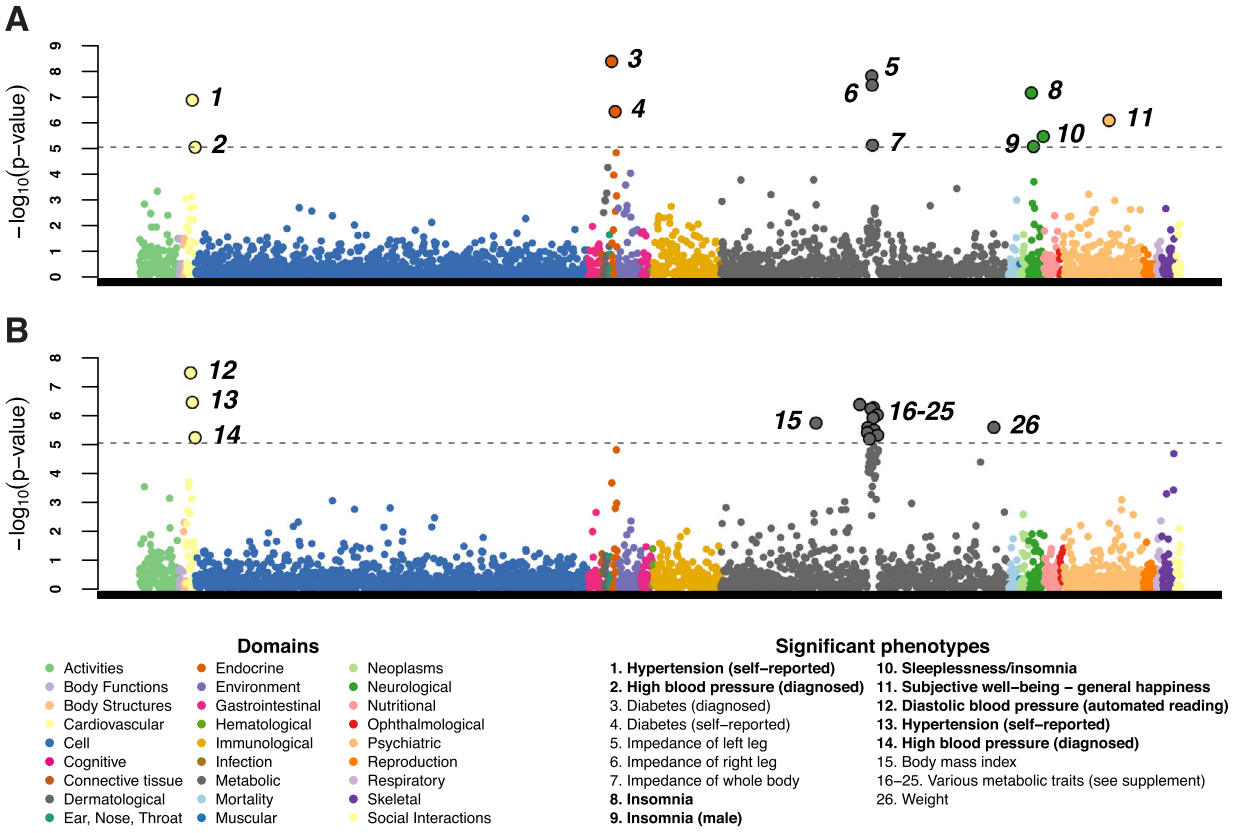


Fig. 3 Phenome-wide analysis of *TP53INP1* and *INTS8*. Association -$\log_{10}$-converted *p*-values (*y*-axis) of A) *TP53INP1* and B) *INTS8* are shown for 2824 phenotypes across 27 domains (indexed on the *x*-axis). Phenotypes reaching significance ($p < 8.9 \times 10^{-6}$, dashed line) are numbered and correspond to the significant phenotypes listed (stress-related phenotypes are highlighted). Association results for all phenotypes reaching significance are shown in Supplementary Tables 10 and 11. The results of this analysis were obtained from the GWAS atlas (atlas.ctglab.nl) (Watanabe et al., 2018).

90-R) with the strongest additive genetic effects at DHEA-S (positive), SHBG (negative) and GABA (negative; Supplementary Fig. 4). This combination of associations reflects genotype-dependent differences in the change of these hormone levels throughout deployment and a possible effect of this locus on stress responsiveness. DHEA has antioxidant effects and is a possible neuroprotective compound in stressed individuals (Bastianetto et al., 1999; Kimonides et al., 1998; Russo et al., 2012). Moreover, increased DHEA and DHEA-S associate with symptom improvement in veterans suffering from PTSD and in trauma-exposed controls compared to non-trauma exposed controls (van Zuiden et al., 2017; Yehuda et al., 2006). The role of SHBG in stress is largely unknown, although it is well-established that SHBG regulates the amount of bioavailable testosterone. The close association between SHBG and testosterone is shown by the positive correlation in post-deployment residuals in our cohort (Supplementary Fig. 2). The negative direction of effect at rs10100651 might indicate less sensitivity to changes in testosterone levels at this variant, which has been associated with lower post-traumatic stress symptoms after deployment. In contrast, individuals with larger pre-post deployment changes of testosterone report higher levels of PTSD symptoms (Reijnen et al., 2015a). Although increased GABA was not found to be a strong predictor of psychopathology right after deployment in a previous study in this cohort, an increase of GABA one month and six months post-deployment is associated with more mental health problems, suggesting stronger increase in GABA levels as a risk factor for post-trauma psychopathology (Schür et al., 2016). The negative effect direction at the identified locus hints at a protective role for the effect allele in stress-related GABA regulation. The role of GABA in stress-induced psychopathology is not completely clear, as illustrated by lower plasma GABA levels in individuals who meet criteria for PTSD immediately after deployment (Vaiva et al., 2006). Overall, the effect directions for these hormones at this SNP are in agreement: the positive effect at deployment-induced changes in DHEA-S (a stress-protective factor) and the negative effects at SHBG (in relation to testosterone a stress-inducing factor when levels strongly change) and GABA (previously reported to be a stress-inducing factor upon strong increase) show the possible relevance of this variant as a factor involved in stress and resilience. Furthermore, different effect directions at GABA and DHEA-S are of interest as DHEA-S is an antagonist of the $GABA_A$ receptor, suggesting opposing functions in the maintenance of the inhibitory-excitatory balance with regard to stress for these hormones (Pitman et al., 2012).

The lead SNP, rs10100651, showed significant eQTL effects and/or chromatin interactions with surrounding genes and in particular *INTS8* and *TP53INP1* (Supplementary

Table 8, Supplementary Table 9). *INTS8* has been linked to human brain development (Oegema et al., 2017), and *TP53INP1* operates as an antioxidant upon exposure to stress (Saadi et al., 2015). Phenome-wide analysis highlighted the SNP-based association of these genes with several phenotypes that closely relate to stress. First, combat deployment has been shown to be a risk factor for hypertension in military personnel and there is strong evidence for hypertension and increased cardiovascular disease risk in PTSD (Edmondson and Känel, 2017; Granado et al., 2009). Second, sleeping problems are one of the symptoms defining the diagnostic classification criteria for PTSD (American Psychiatric Association, 2013), and a subtle post-deployment increase has previously been reported in this cohort (Reijnen et al., 2015b). Third, subjective wellbeing is negatively correlated to symptoms of PTSD and depression and increases upon treatment of these symptoms (Berle et al., 2018). Of note, eQTLs and chromatin interactions are ubiquitous throughout the genome, so the functional annotations of the discovered SNP do not necessarily highlight the causal genes involved in these phenotypes.

Although the highlighted locus is likely broadly associated to stress, we assessed its association to PTSD, which is the main clinical diagnosis regarding post-trauma psychopathology. In the UK Biobank case-control GWAS on self-reported PTSD (Sudlow et al., 2015), rs10100651 reached nominal significance (Supplementary Fig. 6), but the locus did not show association in the largest published meta-analysis. The replication in one out of two datasets might reflect a difference in definition of PTSD susceptibility between the replication sets: In the cohorts included in the PGC meta-analysis, PTSD case-control status was ascertained using DSM-IV based methods (questionnaires and clinical interviews) (Duncan et al., 2018), while in the UK Biobank GWAS a self-reported case-control status independent of DSM criteria was used (which is more line with the self-reported longitudinal data collected here).

The number of studies employing a quantitative phenotype approach in stress-related health conditions has so far been limited (Almli et al., 2015; Morey et al., 2017). Although ours is the first to implement longitudinally measured stress-related phenotypes into a multivariate GWAS, enhancing power over single-phenotype (case-control) GWAS (Magi et al., 2017; Porter and O'Reilly, 2017), our study also has limitations. First, our sample size was small which is in part due to data incompleteness for several participants. This a common problem in longitudinal studies with extensive phenotype collection. Due to this small sample size, we also lacked power to estimate SNP-based heritability of included phenotypes in our GWAS (Visscher et al., 2014). Although for some of the included phenotypes the SNP-based heritability has been reported for baseline levels (Neumann et al., 2017; Prins et al., 2017), the heritability of change in these phenotypes as response to stress has not been captured so far and thus remains unavailable. Second, other well-established stress-related phenotypes, such as brain imaging parameters, were not available in our dataset but may in the future add an extra layer of information to these multiple phenotype genetic studies (Schmidt et al., 2015). Additional covariates of interest, such as extensive data on substance abuse and diet, which are potential modulators of psychiatric distress and blood hormone levels, respectively, were either not recorded in this cohort or had a high level of missing data. Additionally, we were unable to correct for possible confounding of civilian trauma exposure during adulthood (prior to deployment), as such data were not collected. Third, our analysis included only males. Given the variance in plasma hormone concentrations between males and females, this association might be specific to males. Finally, we lacked a replication cohort with similar phenotypes, precluding generalization of our findings to other (military) cohorts.

In conclusion, we have demonstrated the potential of targeting multiple quantitative phenotypes underlying stress sensitivity and highlight a credible genetic locus that may play a role in the modulation of the stress response upon exposure to trauma and/or resulting psychopathology. This locus may be targeted in future downstream analyses, including fine-mapping and analyses of additional epigenomic annotations. As our understanding of the biological substrates involved in stress-related psychopathology increases, so do large-scale initiatives to collect genetic and quantitative phenotype data on these conditions (Liberzon, 2018; Nievergelt et al., 2018). Future studies using multivariate GWAS approaches may further refine heritability and genetic risk factors underlying susceptibility to adverse effects of traumatic stress.

Role of the funding source

This study was funded by the Dutch Ministry of Defense.

Contributors

Authors D.S., E.G., C.H.V, S.L.P, R.R.S, J.H.V., M.P.B., E.V. and J.J.L designed the study and analysis protocols. Authors M.M., E.B. and J.M. performed experimental procedures in the lab. Author D.S. and K.E.K. undertook statistical analyses. Author D.S. wrote the initial draft of the manuscript. All authors critically reviewed the manuscript and have approved the final version.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank the Psychiatric Genomics Consortium for publicly sharing summary statistics of their PTSD GWAS meta-analysis. We thank the UK Biobank and the Neale lab for generating and sharing PTSD GWAS results. We thank Dr. Caroline Nievergelt and Adam Maihofer for their cooperation regarding the replication of our findings in the PGC cohort.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2019.09.012.

References

Almli, L.M., Stevens, J.S., Smith, A.K., Kilaru, V., Meng, Q., Flory, J., Abu-Amara, D., Hammamieh, R., Yang, R., Mercer, K.B., Binder, E.B., Bradley, B., Hamilton, S., Jett, M., Yehuda, R., Marmar, C.R., Ressler, K.J., 2015. A genome-wide identified risk variant for ptsd is a methylation quantitative trait locus and confers decreased cortical activation to fearful faces. Am. J. Med. Genet. B Neuropsychiatr. Genet. 168B, 327–336. doi:10.1002/ajmg.b.32315.

American Psychiatric Association, 2000. Diagnostic and Statistical Manual of Mental Disorders, 4 ed. Washington, DC.

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, 5 ed. Washington, D.C.

Arrindell, W.A., Ettema, J., 2003. Revised Manual for a Multidimensional Indicator of Psychopathology In: Herziene handleiding Bij Een Multidimensionele Psychopathologie Indicator. Swets Zeitlinger, Lisse, the Netherlands.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of ld and haplotype maps. Bioinformatics 21, 263–265. doi:10.1093/bioinformatics/bth457.

Bastianetto, S., Ramassamy, C., Poirier, J., Quirion, R., 1999. Dehydroepiandrosterone (DHEA) protects hippocampal cells from oxidative stress-induced damage. Brain Res. Mol. Brain Res. 66, 35–41.

Berle, D., Hilbrink, D., Russell-Williams, C., Kiely, R., Hardaker, L., Garwood, N., Gilchrist, A., Steel, Z., 2018. Personal wellbeing in posttraumatic stress disorder (PTSD): association with ptsd symptoms during and following treatment. BMC Psychol. 6, 594. doi:10.1186/s40359-018-0219-2.

Bremner, J.D., Bolus, R., Mayer, E.A., 2007. Psychometric properties of the early trauma inventory-self report. J. Nerv. Ment. Dis. 195, 211–218. doi:10.1097/01.nmd.0000243824.84651.6c.

Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., Lee, J.J., 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 4, 7. doi:10.1186/s13742-015-0047-8.

Das, S., Forer, L., Schonherr, S., Sidore, C., Locke, A.E., Kwong, A., Vrieze, S.I., Chew, E.Y., Levy, S., McGue, M., Schlessinger, D., Stambolian, D., Loh, P.R., Iacono, W.G., Swaroop, A., Scott, L.J., Cucca, F., Kronenberg, F., Boehnke, M., Abecasis, G.R., Fuchsberger, C., 2016. Next-generation genotype imputation service and methods. Nat. Genet. 48, 1284–1287. doi:10.1038/ng.3656.

Duncan, L.E., Ratanatharathorn, A., Aiello, A.E., Almli, L.M., Amstadter, A.B., Ashley-Koch, A.E., Baker, D.G., Beckham, J.C., Bierut, L.J., Bisson, J., Bradley, B., Chen, C.Y., Dalvie, S., Farrer, L.A., Galea, S., Garrett, M.E., Gelernter, J.E., Guffanti, G., Hauser, M.A., Johnson, E.O., Kessler, R.C., Kimbrel, N.A., King, A., Koen, N., Kranzler, H.R., Logue, M.W., Maihofer, A.X., Martin, A.R., Miller, M.W., Morey, R.A., Nugent, N.R., Rice, J.P., Ripke, S., Roberts, A.L., Saccone, N.L., Smoller, J.W., Stein, D.J., Stein, M.B., Sumner, J.A., Uddin, M., Ursano, R.J., Wildman, D.E., Yehuda, R., Zhao, H., Daly, M.J., Liberzon, I., Ressler, K.J., Nievergelt, C.M., Koenen, K.C., 2018. Largest gwas of ptsd (N=20 070) yields genetic overlap with schizophrenia and sex differences in heritability. Mol. Psychiatry 23, 666–673. doi:10.1038/mp.2017.77.

Edmondson, D., Känel, von, R., 2017. Post-traumatic stress disorder and cardiovascular disease. Lancet Psychiatry 4, 320–329. doi:10.1016/S2215-0366(16)30377-7.

Flint, J., Timpson, N., Munafò, M., 2014. Assessing the utility of intermediate phenotypes for genetic mapping of psychiatric disease. Trends Neurosci. 37, 733–741. doi:10.1016/j.tins.2014.08.007.

Geuze, E., van Wingen, G.A., van Zuiden, M., Rademaker, A.R., Vermetten, E., Kavelaars, A., Fernández, G., Heijnen, C.J., 2012. Glucocorticoid receptor number predicts increase in amygdala activity after severe stress. Psychoneuroendocrinology 37, 1837–1844. doi:10.1016/j.psyneuen.2012.03.017.

Granado, N.S., Smith, T.C., Swanson, G.M., Harris, R.B., Shahar, E., Smith, B., Boyko, E.J., Wells, T.S., Ryan, M.A.K.Millennium Cohort Study Team, 2009. Newly reported hypertension after military combat deployment in a large population-based study. Hypertension 54, 966–973. doi:10.1161/HYPERTENSIONAHA.109.132555.

Hovens, J.E., Bramsen, I., van der Ploeg, H.M., 2000. Self-report Measure for PTSD symptoms: SRIP Manual In: De Zelfinventarisatielijst Post traumatische Stressstoornis: ZIL Handleiding. Swets Zeitlinger, Lisse, the Netherlands.

Hovens, J.E., Bramsen, I., van der Ploeg, H.M., 2002. Self-rating inventory for posttraumatic stress disorder: review of the psychometric properties of a new brief dutch screening instrument. Percept. Mot Skills 94, 996–1008. doi:10.2466/pms.2002.94.3.996.

Kimonides, V.G., Khatibi, N.H., Svendsen, C.N., Sofroniew, M.V., Herbert, J., 1998. Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Proc. Natl. Acad. Sci. U S A 95, 1852–1857. doi:10.1073/pnas.95.4.1852.

Lapierre, C.B., Schwegler, A.F., Labauve, B.J., 2007. Posttraumatic stress and depression symptoms in soldiers returning from combat operations in iraq and afghanistan. J. Trauma Stress 20, 933–943. doi:10.1002/jts.20278.

Liberzon, I., 2018. Searching for intermediate phenotypes in posttraumatic stress disorder. Biol. Psychiatry 83, 797–799. doi:10.1016/j.biopsych.2017.06.005.

Loh, P.R., Danecek, P., Palamara, P.F., Fuchsberger, C., Y, A.R., H, K.F., Schoenherr, S., Forer, L., McCarthy, S., Abecasis, G.R., Durbin, R., A, L.P., 2016. Reference-based phasing using the haplotype reference consortium panel. Nat. Genet. 48, 1443–1448. doi:10.1038/ng.3679.

Magi, R., Suleimanov, Y.V., Clarke, G.M., Kaakinen, M., Fischer, K., Prokopenko, I., Morris, A.P., 2017. SCOPA and META-SCOPA: software for the analysis and aggregation of genome-wide association studies of multiple correlated phenotypes. BMC Bioinform. 18, 25. doi:10.1186/s12859-016-1437-3.

McCarthy, S., Das, S., Kretzschmar, W., Delaneau, O., Wood, A.R., Teumer, A., Kang, H.M., Fuchsberger, C., Danecek, P., Sharp, K., Luo, Y., Sidore, C., Kwong, A., Timpson, N., Koskinen, S., Vrieze, S., Scott, L.J., Zhang, H., Mahajan, A., Veldink, J., Peters, U., Pato, C., van Duijn, C.M., Gillies, C.E., Gandin, I., Mezzavilla, M., Gilly, A., Cocca, M., Traglia, M., Angius, A., Barrett, J.C., Boomsma, D., Branham, K., Breen, G., Brummett, C.M., Busonero, F., Campbell, H., Chan, A., Chen, S., Chew, E., Collins, F.S., Corbin, L.J., Smith, G.D., Dedoussis, G., Dorr, M., Farmaki, A.E., Ferrucci, L., Forer, L., Fraser, R.M., Gabriel, S., Levy, S., Groop, L., Harrison, T., Hattersley, A., Holmen, O.L., Hveem, K., Kretzler, M., Lee, J.C., McGue, M., Meitinger, T., Melzer, D., Min, J.L., Mohlke, K.L., Vincent, J.B., Nauck, M., Nickerson, D., Palotie, A., Pato, M., Pirastu, N., McInnis, M., Richards, J.B., Sala, C., Salomaa, V., Schlessinger, D., Schoenherr, S., Slagboom, P.E., Small, K., Spector, T., Stambolian, D., Tuke, M., Tuomilehto, J., Van den Berg, L.H., Van Rheenen, W., Volker, U., Wijmenga, C., Toniolo, D., Zeggini, E., Gasparini, P., Sampson, M.G., Wilson, J.F., Frayling, T., de Bakker, P.I., Swertz, M.A., McCarroll, S., Kooperberg, C., Dekker, A., Altshuler, D., Willer, C., Iacono, W., Ripatti, S., Soranzo, N., Walter, K., Swaroop, A., Cucca, F., Anderson, C.A., Myers, R.M., Boehnke, M., McCarthy, M.I., Durbin, R., 2016. A reference panel of 64,976 haplotypes for genotype imputation. Nat. Genet. 48, 1279–1283. doi:10.1038/ng.3643.

Morey, R.A., Davis, S.L., Garrett, M.E., Haswell, C.C., Marx, C.E., Beckham, J.C., McCarthy, G., Hauser, M.A., Ashley-Koch, A.E.Mid-Atlantic MIRECC Workgroup, 2017. Genome-wide association study of subcortical brain volume in ptsd cases

and trauma-exposed controls. Transl. Psychiatry 7, 1265. doi:10.1038/s41398-017-0021-6.

Neale, B.M., 2018. http://www.nealelab.is/uk-biobank/[WWW Document]. URL http://www.nealelab.is/uk-biobank/ (accessed 8.20.18).

Neumann, A., Direk, N., Crawford, A.A., Mirza, S., Adams, H., Bolton, J., Hayward, C., Strachan, D.P., Payne, E.K., Smith, J.A., Milaneschi, Y., Penninx, B., Hottenga, J.J., de Geus, E., Oldehinkel, A.J., van der Most, P.J., de Rijke, Y., Walker, B.R., Tiemeier, H., 2017. The low single nucleotide polymorphism heritability of plasma and saliva cortisol levels. Psychoneuroendocrinology 85, 88–95. doi:10.1016/j.psyneuen.2017.08.011.

Nievergelt, C.M., Ashley-Koch, A.E., Dalvie, S., Hauser, M.A., Morey, R.A., Smith, A.K., Uddin, M., 2018. Genomic approaches to posttraumatic stress disorder: the psychiatric genomic consortium initiative. Biol. Psychiatry 83, 831–839. doi:10.1016/j.biopsych.2018.01.020.

Oegema, R., Baillat, D., Schot, R., van Unen, L.M., Brooks, A., Kia, S.K., Hoogeboom, A.J.M., Xia, Z., Li, W., Cesaroni, M., Lequin, M.H., van Slegtenhorst, M., Dobyns, W.B., de Coo, I.F.M., Verheijen, F.W., Kremer, A., van der Spek, P.J., Heijsman, D., Wagner, E.J., Fornerod, M., Mancini, G.M.S., 2017. Human mutations in integrator complex subunits link transcriptome integrity to brain development. PLoS Genet. 13, e1006809. doi:10.1371/journal.pgen.1006809.

Pitman, R.K., Rasmusson, A.M., Koenen, K.C., Shin, L.M., Orr, S.P., Gilbertson, M.W., Milad, M.R., Liberzon, I., 2012. Biological studies of post-traumatic stress disorder. Nat. Rev. Neurosci. 13, 769–787. doi:10.1038/nrn3339.

Porter, H.F., O'Reilly, P.F., 2017. Multivariate simulation framework reveals performance of multi-trait gwas methods. Sci. Rep. 7, 38837. doi:10.1038/srep38837.

Prins, B.P., Kuchenbaecker, K.B., Bao, Y., Smart, M., Zabaneh, D., Fatemifar, G., Luan, J., Wareham, N.J., Scott, R.A., Perry, J.R.B., Langenberg, C., Benzeval, M., Kumari, M., Zeggini, E., 2017. Genome-wide analysis of health-related biomarkers in the uk household longitudinal study reveals novel associations. Sci. Rep. 7, 11008. doi:10.1038/s41598-017-10812-1.

Regier, D.A., Narrow, W.E., Clarke, D.E., Kraemer, H.C., Kuramoto, S.J., Kuhl, E.A., Kupfer, D.J., 2013. DSM-5 field trials in the united states and canada, part II: test-Retest reliability of selected categorical diagnoses. Am. J. Psychiatry 170, 59–70. doi:10.1176/appi.ajp.2012.12070999.

Reijnen, A., Geuze, E., Eekhout, I., Maihofer, A.X., Nievergelt, C.M., Baker, D.G., Vermetten, E., 2018. Biological profiling of plasma neuropeptide y in relation to posttraumatic stress symptoms in two combat cohorts. Biol. Psychol. 134, 72–79. doi:10.1016/j.biopsycho.2018.02.008.

Reijnen, A., Geuze, E., Vermetten, E., 2015a. The effect of deployment to a combat zone on testosterone levels and the association with the development of posttraumatic stress symptoms: a longitudinal prospective dutch military cohort study. Psychoneuroendocrinology 51, 525–533. doi:10.1016/j.psyneuen.2014.07.017.

Reijnen, A., Rademaker, A.R., Vermetten, E., Geuze, E., 2015b. Prevalence of mental health symptoms in dutch military personnel returning from deployment to afghanistan: a 2-year longitudinal analysis. Eur. Psychiatry 30, 341–346. doi:10.1016/j.eurpsy.2014.05.003.

Russo, S.J., Murrough, J.W., Han, M.-.H., Charney, D.S., Nestler, E.J., 2012. Neurobiology of resilience. Nat. Neurosci. 15, 1475–1484. doi:10.1038/nn.3234.

Saadi, H., Seillier, M., Carrier, A., 2015. The stress protein TP53INP1 plays a tumor suppressive role by regulating metabolic homeostasis. Biochimie 118, 44–50. doi:10.1016/j.biochi.2015.07.024.

Schmidt, U., Willmund, G.-.D., Holsboer, F., Wotjak, C.T., Gallinat, J., Kowalski, J.T., Zimmermann, P., 2015. Searching for non-genetic molecular and imaging ptsd risk and resilience markers: systematic review of literature and design of the german armed forces ptsd biomarker study. Psychoneuroendocrinology 51, 444–458. doi:10.1016/j.psyneuen.2014.08.020.

Schür, R.R., Boks, M.P., Geuze, E., Prinsen, H.C., Verhoeven-Duif, N.M., Joëls, M., Kahn, R.S., Vermetten, E., Vinkers, C.H., 2016. Development of psychopathology in deployed armed forces in relation to plasma gaba levels. Psychoneuroendocrinology 73, 263–270. doi:10.1016/j.psyneuen.2016.08.014.

Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M., Liu, B., Matthews, P., Ong, G., Pell, J., Silman, A., Young, A., Sprosen, T., Peakman, T., Collins, R., 2015. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 12, e1001779. doi:10.1371/journal.pmed.1001779.

The 1000 Genomes Project Consortium, 2015. A global reference for human genetic variation. Nature 526, 68–74. doi:10.1038/nature15393.

Vaiva, G., Boss, V., Ducrocq, F., Fontaine, M., Devos, P., Brunet, A., Laffargue, P., Goudemand, M., Thomas, P., 2006. Relationship between posttrauma gaba plasma levels and ptsd at 1-year follow-up. Am. J. Psychiatry 163, 1446–1448. doi:10.1176/ajp.2006.163.8.1446.

van Zuiden, M., Haverkort, S.Q., Tan, Z., Daams, J., Lok, A., Olff, M., 2017. DHEA and dhea-s levels in posttraumatic stress disorder: a meta-analytic review. Psychoneuroendocrinology 84, 76–82. doi:10.1016/j.psyneuen.2017.06.010.

Vercoulen, J., Alberts, M., Bleijenberg, G., 1999. De checklist individuele spankracht (CIS). Gedragstherapie 32, 131–136.

Visscher, P.M., Hemani, G., Vinkhuyzen, A.A.E., Chen, G.-.B., Lee, S.H., Wray, N.R., Goddard, M.E., Yang, J., 2014. Statistical power to detect genetic (co)variance of complex traits using snp data in unrelated samples. PLoS Genet. 10, e1004269. doi:10.1371/journal.pgen.1004269.

Watanabe, K., Stringer, S., Frei, O., Umićević Mirkov, M., Polderman, T.J.C., van der Sluis, S., Andreassen, O.A., Neale, B.M., Posthuma, D., 2018. A global view of pleiotropy and genetic architecture in complex traits. bioRxiv doi:10.1101/500090.

Watanabe, K., Taskesen, E., van Bochoven, A., Posthuma, D., 2017. Functional mapping and annotation of genetic associations with fuma. Nat. Commun. 8, 1826. doi:10.1038/s41467-017-01261-5.

World Medical Association, 2013. World medical association declaration of helsinki. JAMA 310, 2191–2194. doi:10.1001/jama.2013.281053.

Yang, J., Lee, S.H., Goddard, M.E., Visscher, P.M., 2011. GCTA: a tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88, 76–82. doi:10.1016/j.ajhg.2010.11.011.

Yehuda, R., Brand, S.R., Golier, J.A., Yang, R.K., 2006. Clinical correlates of dhea associated with post-traumatic stress disorder. Acta Psychiatr. Scand. 114, 187–193. doi:10.1111/j.1600-0447.2006.00801.x.