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Genomic Perspectives on PTSD

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Understanding the molecular basis of individual differences in susceptibility to post-traumatic stress disorder (PTSD) will facilitate the development of diagnostics and personalized therapeutics for this chronic, devastating mental disorder. The differential risk determining those who do versus those who do not develop PTSD is multi-factorial. It is part genetic, with 20-40% risk heritability for PTSD following trauma; and in part depends on past personal history, including adult and childhood trauma and psychological factors which may differentially mediate fear and emotion regulation. Recent large genome-wide association studies (GWAS) confirmed significant heritability in both sexes and identified reliable PTSD-associated loci (Gelernter et al, 2019; Stein et al, 2021). GWASs for PTSD have revealed genetic correlations with other psychiatric conditions such as major depressive disorder (MDD) and anxiety disorders. Further, cortical brain regions are enriched for genomic risk loci. Among the most significant PTSD-GWAS loci is Crh17q21.3 in which transcriptomic fine mapping prioritized the gene *CRHR1* (corticotropin-releasing hormone receptor 1), an important regulatory protein in the stress response.

Brain molecular phenotypes, like gene expression (GE) and DNA methylation (DNAm), can be used to translate psychiatric GWAS loci to cell type-specific mechanisms and identify causal disease pathways (Sekar et al., 2016). PTSD likely arises from differences at various levels of gene regulation and diverse brain cell types that converge on specific pathways (e.g., glucocorticoid and GABAergic signaling) harboring clinical significance. Multi-omics analysis provides the resolution needed to understand brain molecular mechanisms of neuropsychiatric disorders. The gene-regulatory landscape of the brain is highly dynamic in health and disease, coordinating many biological processes across distinct cell types. Complex psychiatric disorders such as PTSD result from differences at various levels of regulation (epigenomic, transcriptomic and proteomic) and converge on specific biological pathways with clinical significance. We are beginning to understand more about the neurobiology of PTSD and the molecular mechanisms associated with it, however, many critical questions remain.

The first major studies of the postmortem PTSD brain examined the changes in the transcriptome. As the output of the DNA and transcript by which protein molecules are generated, the transcriptome is a popular read out of the differences in diseased tissue. By using massively parallel RNA sequencing, these early studies examined changes in the transcriptome across several cortical and subcortical regions (e.g. the amygdala). Specifically, two of these studies identified vulnerabilities in a group of inhibitory neurons (somatostatin and cortistatin positive) and discovered GABAergic signaling deficits centered on the interneuron-specific transporter *ELFN1* (Girgenti et al., 2021; Jaffe et al., 2022). *ELFN1* transcript was significantly downregulated and

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Continued from cover

associated with PTSD by transcriptomic imputation with the largest PTSD GWAS from the Million Veteran Program (Stein et al., 2019).

Additionally, there is considerable evidence for the role of microglia in many neural processes including the stress response. A role for microglial dysfunction in PTSD has been suspected based on blood transcriptomic work that has identified inflammatory and immune-related gene dysregulation (Passos et al., 2015). These initial transcriptomic studies also identified dysregulation of microglia in the central nervous system (CNS) PTSD transcriptome. The immune gene *UBA-7* was identified by transcriptome-wide association studies as associating with PTSD and is a significant transcriptomic key driver in females with PTSD (Girgenti et al., 2021). Another study within the National Center for PTSD using a partially overlapping dataset (PTSD and neurotypical controls) also identified numerous inflammatory DEGs across comparisons, specifically the cytokine *IL-1B* (Logue et al., 2021).

Approximately 10% of women will develop PTSD in their lifetime, and women are twice as likely as men to develop PTSD after a traumatic event. Neurobiological evidence suggests structural differences between the brains of males versus females with PTSD. Further, women experience more severe, debilitating, and persistent symptoms pointing to possible sex-specific alterations occurring in the neurocircuitry and brain structure of women with PTSD. Remarkably, recent transcriptomic analysis has revealed that sex accounted for almost half of the total explained variance (48.9%) in gene expression in the PTSD brain. Interestingly, this effect is not driven by genes on the sex chromosomes; when these genes are removed sex still has the greatest effect on variance overall. These studies have also identified numerous sex-specific differentially expressed transcripts as well, suggesting a fundamental role of sex in shaping gene expression patterns of the PTSD brain.

Importantly, all three of these studies included a psychiatric control group (major depressive disorder) as greater than 50% of patients diagnosed with PTSD are comorbid for depression and PTSD and MDD share >60% genetic overlap. Surprisingly, these bulk tissue transcriptomic studies found moderate to low overlap with MDD, inconsistent with the high degree of genetic overlap and comorbidity. Further, the transcriptomic correlation between PTSD, MDD, and an independent MDD profile from the PsychENCODE consortium (Gandal et al., 2018) revealed no significant correlation between the transcriptomic patterns of the two disorders strengthening the cases for different molecular pathologies at play.

PTSD is among the most likely of psychiatric disorders to be understood from the perspective of environmental influences interacting with genetic vulnerability because diagnosis requires a specific, highly traumatizing, fear-evoking experience. Methylation of DNA (specifically at cytosine nucleotides) is a critical, epigenetic regulator of genome architecture, gene expression, and cell function. Epigenetic changes evoked by stress are thus encoded into the genome and can serve as a link between the genetic architecture and the response (i.e., gene expression). In this way, epigenetic changes are a path through which traumatic stress and other environmental exposures influence PTSD vulnerability and resilience and converge mechanistically with the underlying risk for PTSD. Elucidating this interplay is thus fundamental to advancing our understanding of the etiology and pathophysiology of PTSD.

The few studies that have examined the epigenetic regulation of gene expression in PTSD have focused primarily on peripheral tissues such as blood. The first comprehensive examination of the DNA methylome in PTSD was performed by the Williamson and Girgenti Labs at the National PTSD Brain Bank Intramural program (Li et al., 2023). They examined 5.1M DNA methylation (DNAm) sites from 171 donors including neurotypicals, PTSD, and MDD cases across six areas implicated in the fear circuitry of the brain (within the amygdala and hippocampus). Approximately 30% of differentially DNAm sites were present near risk loci for PTSD. To identify potential therapeutic intersections for PTSD, the authors found significant methylation changes in the *MAD1L1, ELFN1,* and *WNT5A* genes in PTSD patients who responded to the rapid-acting antidepressant ketamine. Previous trials for ketamine effectiveness in treating PTSD have yielded mixed results. The machine learning algorithms employed in this study were the first to identify an actual responder group using epigenetic signals. These findings clearly implicated DNA methylation as a molecular mechanism underlying the changes associated with the PTSD brain circuitry.

Gene expression is characterized by two molecular species: RNA (transcription) and protein (translation) in sequential order. Proteins are downstream of RNA and closer to cellular expression output, and therefore possibly offer a more accurate physiological representation of normal versus diseased tissue phenotypes. Besides representing the final 'gene expression' stage that furthers understanding of disease mechanisms, protein characterization also offers the advantage of direct drug targeting in comparison to RNA, which still needs to be translated into proteins. Moreover, published studies report poor correlations between 'RNA' target levels and corresponding 'protein' target abundances probably due to different regulatory mechanisms (transcription versus translation), turnover rates (mRNA and protein), and 'alternatively spliced' transcript versus protein products derived from the same genes. PTSD is a unique psychiatric disorder where the epigenetic influence is integral to its diagnosis, making the epigenetics and genetics extremely relevant to disease mechanisms, which could ultimately translate into alterations in protein abundances and protein post-translational modifications (PTMs).

The first study to examine the role of changes in the proteomic composition of the PTSD brain found dysregulation in several critical genes including SLC32A1, a GABAergic vesicle transporter (Wang et al., 2023). GABAergic cell signaling was previously implicated in PTSD by transcriptomic and epigenetic findings, further strengthening the evidence for PTSD vulnerability in this cell type. In addition to these findings, the authors identified enrichment of Alzheimer's disease risk genes in PTSD proteomic modules suggesting converging molecular alterations between the two disorders. This is particularly important as patients with PTSD are twice as likely to develop dementia and PTSD is a strong risk factor for Alzheimer's disease development.

Bulk genomic methods described previously have uncovered global and sex-specific molecular signatures for PTSD. However, cell type-specificity in these analyses is inferred. Unlike most other organs of the body, the human brain is composed of a myriad number of different cell types- all of which may be contributing to PTSD etiology in different ways. While accurately measuring expression levels from homogenate/bulk tissue or pools of cells is well established, homogenate human tissue contains multiple

cell types and their relative populations may differ between samples based on several factors, including dissection, tissue quality, and diagnosis. Single-cell sequencing is an appealing and potentially critical approach to the discovery of the cell biology of PTSD. However, accurately measuring expression levels in individual cells is difficult, particularly in postmortem brain tissue, and not yet a standard procedure. The first single-cell RNA-sequencing study in PTSD showed excitatory and inhibitory neuron transcriptomic changes in the dorsolateral prefrontal cortex (Chatzinakos et al., 2023). These genes were mostly associated with immune, mitochondrial and glucocorticoid signaling. While an exciting finding, this study is somewhat underpowered and future studies in larger cohorts will add the groundwork laid by this work.

Postmortem brain genomics has done a fantastic job in helping us identify genes involved in the etiology of PTSD. The next step is to translate these findings into biological mechanisms for therapeutic intervention. Induced pluripotent stem cells (iPSCs) derived from donors in our brain bank hold the promise of modeling "stress in a dish". This work captures both the genetic and environmental contributors of maladaptive stress response. One recent study examined the activation of glucocorticoid receptors in iPSC-derived neurons using a specific GC-agonist (hydrocortisone) (Seah et al., 2022). They found that transcriptomic changes positively correlated with PTSD molecular signatures in the postmortem brain. This study demonstrated the relevance of the *in vitro* stress paradigm and set the stage for future research steps connecting PTSD-associated genetic and epigenetic factors with brain function.

Conclusion

Whole genome profiling across molecular layers of the postmortem brain provides information to identify not only specific genes that are highly regulated, but also networks of genes that are altered, hub genes that influence many other gene sets and pathways, and further higher-order information. From these data, we gain significant insight into the neurobiological mechanisms affected by PTSD and identify specific therapeutic targets for drug development. Future work using iPSC models of traumatic stress will provide us with validation and a complete functional output of the dysregulated mechanisms of PTSD. This work is contributing importantly to our understanding of this devastating disease and opening new avenues to develop therapies and preventative measures.

FEATURED ARTICLES

Chatzinakos, C., Pernia, C. D., Morrison, F. G., Iatrou, A., McCullough, K. M., Schuler, H., Snijders, C., Bajaj, T., DiPietro, C. P., Soliva Estruch, M., Gassen, N. C., Anastasopoulos, C., Bharadwaj, R. A., Bowlby, B. C., Hartmann, J., Maihofer, A. X., Nievergelt, C. M., Ressler, N. M., Wolf, E. J., Traumatic Stress Brain Research Group, … Daskalakis, N. P. (2023). **Single-nucleus transcriptome profiling of dorsolateral prefrontal cortex: Mechanistic roles for neuronal gene expression, including the 17q21.31 locus, in PTSD stress response.** *The American Journal of Psychiatry, 180*(10), 739–754. doi:10.1176/appi.ajp.20220478 *Objective:* Multidisciplinary studies of posttraumatic stress disorder (PTSD) and major depressive disorder (MDD) implicate the dorsolateral prefrontal cortex (DLPFC) in disease risk and pathophysiology. Postmortem brain studies have relied on bulk-tissue RNA sequencing (RNA-seq), but single-cell RNA-seq is needed to dissect cell-type-specific mechanisms. The authors conducted the first single-nucleus RNA-seq postmortem brain study in PTSD to elucidate disease transcriptomic pathology with cell-type-specific resolution. *Method:* Profiling of 32 DLPFC samples from 11 individuals with PTSD, 10 with MDD, and 11 control subjects was conducted (~415K nuclei; >13K cells per sample). A replication sample included 15 DLPFC samples (~160K nuclei; >11K cells per sample). *Results:* Differential gene expression analyses identified significant single-nucleus RNA-seq differentially expressed genes (snDEGs) in excitatory (EX) and inhibitory (IN) neurons and astrocytes, but not in other cell types or bulk tissue. MDD samples had more false discovery rate-corrected significant snDEGs, and PTSD samples had a greater replication rate. In EX and IN neurons, biological pathways that were differentially enriched in PTSD compared with MDD included glucocorticoid signaling. Furthermore, glucocorticoid signaling in induced pluripotent stem cell (iPSC)-derived cortical neurons demonstrated greater relevance in PTSD and opposite direction of regulation compared with MDD, especially in EX neurons. Many snDEGs were from the 17q21.31 locus and are particularly interesting given causal roles in disease pathogenesis and DLPFC-based neuroimaging (PTSD: ARL17B, LINC02210-CRHR1, and LRRC37A2; MDD: LRRC37A and LRP4), while others were regulated by glucocorticoids in iPSC-derived neurons (PTSD: SLC16A6, TAF1C; MDD: CDH3). *Conclusions:* The study findings point to cell-type-specific mechanisms of brain stress response in PTSD and MDD, highlighting the importance of examining cell-type-specific gene expression and indicating promising novel biomarkers and therapeutic targets.

Gandal, M. J., Haney, J. R., Parikshak, N. N., Leppa, V., Ramaswami, G., Hartl, C., Schork, A. J., Appadurai, V., Buil, A., Werge, T. M., Liu, C., White, K. P., CommonMind Consortium, PsychENCODE Consortium, iPSYCH-BROAD Working Group, Horvath, S., & Geschwind, D. H. (2018). **Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap.** *Science (New York, N.Y.), 359*(6376), 693–697. doi:10.1126/science.aad6469 The predisposition to neuropsychiatric disease involves a complex, polygenic, and pleiotropic genetic architecture. However, little is known about how genetic variants impart brain dysfunction or pathology. We used transcriptomic profiling as a quantitative readout of molecular brain-based phenotypes across five major psychiatric disorders-autism, schizophrenia, bipolar disorder, depression, and alcoholism-compared with matched controls. We identified patterns of shared and distinct gene-expression perturbations across these conditions. The degree of sharing of transcriptional dysregulation is related to polygenic (single-nucleotide polymorphism-based) overlap across disorders, suggesting a substantial causal genetic component. This comprehensive systems-level view of the neurobiological architecture of major neuropsychiatric illness demonstrates pathways of molecular convergence and specificity.

Gelernter, J., Sun, N., Polimanti, R., Pietrzak, R., Levey, D. F., Bryois, J., Lu, Q., Hu, Y., Li, B., Radhakrishnan, K., Aslan, M., Cheung, K. H., Li, Y., Rajeevan, N., Sayward, F., Harrington, K., Chen, Q., Cho, K., Pyarajan, S., Sullivan, P. F., … Department of Veterans Affairs Cooperative Studies Program (#575B) and Million Veteran Program. (2019). **Genome-wide association study of post-traumatic stress disorder reexperiencing symptoms in >165,000 US veterans.** *Nature Neuroscience, 22*(9), 1394–1401.

doi:10.1038/s41593-019-0447-7 Post-traumatic stress disorder (PTSD) is a major problem among military veterans and civilians alike, yet its pathophysiology remains poorly understood. We performed a genome-wide association study and bioinformatic analyses, which included 146,660 European Americans and 19,983 African Americans in the US Million Veteran Program, to identify genetic risk factors relevant to intrusive reexperiencing of trauma, which is the most characteristic symptom cluster of PTSD. In European Americans, eight distinct significant regions were identified. Three regions had values of $P < 5 \times 10\text{-}10$: CAMKV; chromosome 17 closest to KANSL1, but within a large high linkage disequilibrium region that also includes CRHR1; and TCF4. Associations were enriched with respect to the transcriptomic profiles of striatal medium spiny neurons. No significant associations were observed in the African American cohort of the sample. Results in European Americans were replicated in the UK Biobank data. These results provide new insights into the biology of PTSD in a well-powered genome-wide association study.

Girgenti, M. J., Wang, J., Ji, D., Cruz, D. A., Traumatic Stress Brain Research Group, Stein, M. B., Gelernter, J., Young, K. A., Huber, B. R., Williamson, D. E., Friedman, M. J., Krystal, J. H., Zhao, H., & Duman, R. S. (2021). **Transcriptomic organization of the human brain in post-traumatic stress disorder.** *Nature Neuroscience, 24*(1), 24–33. doi:10.1038/s41593-020-00748-7 Despite extensive study of the neurobiological correlates of post-traumatic stress disorder (PTSD), little is known about its molecular determinants. Here, differential gene expression and network analyses of four prefrontal cortex subregions from postmortem tissue of people with PTSD demonstrate extensive remodeling of the transcriptomic landscape. A highly connected downregulated set of interneuron transcripts is present in the most significant gene network associated with PTSD. Integration of this dataset with genotype data from the largest PTSD genome-wide association study identified the interneuron synaptic gene ELFN1 as conferring significant genetic liability for PTSD. We also identified marked transcriptomic sexual dimorphism that could contribute to higher rates of PTSD in women. Comparison with a matched major depressive disorder cohort revealed significant divergence between the molecular profiles of individuals with PTSD and major depressive disorder despite their high comorbidity. Our analysis provides convergent systems-level evidence of genomic networks within the prefrontal cortex that contribute to the pathophysiology of PTSD in humans.

Jaffe, A. E., Tao, R., Page, S. C., Maynard, K. R., Pattie, E. A., Nguyen, C. V., Deep-Soboslay, A., Bharadwaj, R., Young, K. A., Friedman, M. J., Williamson, D. E., Traumatic Stress Brain Research Group, Shin, J. H., Hyde, T. M., Martinowich, K., & Kleinman, J. E. (2022). **Decoding shared versus divergent transcriptomic signatures across cortico-amygdala circuitry in PTSD and depressive disorders.** *The American Journal of Psychiatry, 179*(9), 673–686. doi:10.1176/appi.ajp.21020162 *Objective:* Posttraumatic stress disorder (PTSD) is a debilitating neuropsychiatric disease that is highly comorbid with major depressive disorder (MDD) and bipolar disorder. The overlap in symptoms is hypothesized to stem from partially shared genetics and underlying neurobiological mechanisms. To delineate conservation between transcriptional patterns across PTSD and MDD, the authors examined gene expression in the human cortex and amygdala in these disorders. *Methods:* RNA sequencing was performed in the postmortem brain of two prefrontal cortex regions and two amygdala regions from donors diagnosed with PTSD (*N*=107) or MDD (*N*=109) as well as from neurotypical donors (*N*=109). *Results:* The authors identified a limited number of differentially expressed genes (DEGs) specific to PTSD, with nearly all mapping to cortical versus amygdala regions. PTSD-specific DEGs were enriched in gene sets associated with downregulated immune-related pathways and microglia as well as with subpopulations of GABAergic inhibitory neurons. While a greater number of DEGs associated with MDD were identified, most overlapped with PTSD, and only a few were MDD specific. The authors used weighted gene coexpression network analysis as an orthogonal approach to confirm the observed cellular and molecular associations. *Conclusions:* These findings provide supporting evidence for involvement of decreased immune signaling and neuroinflammation in MDD and PTSD pathophysiology, and extend evidence that GABAergic neurons have functional significance in PTSD.

Li, H., Wang, J., Cruz, D. A., Modliszewski, J. L., Corcoran, D. L., Martínez-Magaña, J. J., Montalvo-Ortiz, J. L., Roache, J. D., Averill, L. A., Young-McCaughan, S., Shiroma, P. R., Traumatic Stress Brain Research Group, Lewis, D. A., Glausier, J., Holtzheimer, P., Friedman, M. J., Zhang, J., Peterson, A. L., Abdallah, C. G., Zhang, X., … Girgenti, M. J. (2023). **Functional annotation of the human PTSD methylome identifies tissue-specific epigenetic variation across subcortical brain regions.** *medRxiv* 2023.04.18.23288704. doi:10.1101/2023.04.18.23288704 Post-traumatic stress disorder is a mental disorder that may occur in the aftermath of severe psychological trauma. We examined 1,065,750 DNA methylation (DNAm) sites from 171 donors including neurotypicals, PTSD, and major depressive disorder cases across six areas implicated in the fear circuitry of the brain. We found significant differential methylation for PTSD near 195 genes and utilizing cross-region modeling, identified 6,641 candidate genes. Approximately 26% of differentially methylated CpGs were present near risk loci for PTSD. To identify potential therapeutic intersections for PTSD, we found significant methylation changes in the MAD1L1, ELFN1, and WNT5A genes in ketamine responders. Finally, to better understand the unique biology of PTSD, we analyzed matching methylation data for a cohort of MDD donors with no known history of trauma or PTSD. Our results implicate DNAm as an epigenetic mechanism underlying the molecular changes associated with the subcortical fear circuitry of the PTSD brain.

Logue, M. W., Zhou, Z., Morrison, F. G., Wolf, E. J., Daskalakis, N. P., Chatzinakos, C., Georgiadis, F., Labadorf, A. T., Girgenti, M. J., Young, K. A., Williamson, D. E., Zhao, X., Grenier, J. G., Traumatic Stress Brain Research Group, Huber, B. R., & Miller, M. W. (2021). **Gene expression in the dorsolateral and ventromedial prefrontal cortices implicates immune-related gene networks in PTSD.** *Neurobiology of Stress, 15,* 100398. doi:10.1016/j.ynstr.2021.100398 Studies evaluating neuroimaging, genetically predicted gene expression, and pre-clinical genetic models of PTSD, have identified PTSD-related abnormalities in the prefrontal cortex (PFC) of the brain, particularly in dorsolateral and ventromedial PFC (dlPFC and vmPFC). In this study, RNA sequencing was used to examine

gene expression in the dlPFC and vmPFC using tissue from the VA National PTSD Brain Bank in donors with histories of PTSD with or without depression (dlPFC *n*=38, vmPFC *n*=35), depression cases without PTSD (*n*=32), and psychopathology-free controls (dlPFC *n*=24, vmPFC *n*=20). Analyses compared PTSD cases to controls. Follow-up analyses contrasted depression cases to controls. Twenty-one genes were differentially expressed in PTSD after strict multiple testing correction. PTSD-associated genes with roles in learning and memory (FOS, NR4A1), immune regulation (CFH, KPNA1) and myelination (MBP, MOBP, ERMN) were identified. PTSD-associated genes partially overlapped depression-associated genes. Co-expression network analyses identified PTSD-associated networks enriched for immune-related genes across the two brain regions. However, the immune-related genes and association patterns were distinct. The immune gene IL1B was significantly associated with PTSD in candidate-gene analysis and was an upstream regulator of PTSD-associated genes in both regions. There was evidence of replication of dlPFC associations in an independent cohort from a recent study, and a strong correlation between the dlPFC PTSD effect sizes for significant genes in the two studies (r=0.66, p < 2.2 × 10-16). In conclusion, this study identified several novel PTSD-associated genes and brain region specific PTSD-associated immune-related networks.

Passos, I. C., Vasconcelos-Moreno, M. P., Costa, L. G., Kunz, M., Brietzke, E., Quevedo, J., Salum, G., Magalhães, P. V., Kapczinski, F., & Kauer-Sant'Anna, M. (2015). **Inflammatory markers in post-traumatic stress disorder: A systematic review, meta-analysis, and meta-regression.** *The Lancet. Psychiatry, 2*(11), 1002–1012. doi:10.1016/S2215-0366(15)00309-0 *Background:* Studies investigating inflammatory markers in post-traumatic stress disorder (PTSD) have yielded mixed results. The aim of our study was to compare concentrations of inflammatory markers in patients with PTSD compared with healthy controls. *Methods:* We did a meta-analysis and meta-regression of studies comparing inflammatory markers between patients with PTSD and healthy controls by searching PubMed, Embase, Scopus, Web of Science, and PsycINFO for articles published between Jan 1, 1960, and April 7, 2015. From eligible studies (ie, cross-sectional studies or baseline data from longitudinal studies of peripheral blood cytokine concentrations that compared adults with PTSD with healthy controls), we extracted outcomes of interest, such as mean and SD of peripheral blood cytokines, the time of day blood was collected, whether the study allowed patients with comorbid major depressive disorder in the PTSD group, whether patients were medication free, and severity of PTSD symptoms. We undertook meta-analyses whenever values of inflammatory markers were available in two or more studies. A random-effects model with restricted maximum-likelihood estimator was used to synthesise the effect size (assessed by standardised mean difference [SMD]) across studies. *Findings:* 8057 abstracts were identified and 20 studies were included. Interleukin 6 (SMD 0.88; p=0.0003), interleukin 1β (SMD 1.42; p=0.045), and interferon γ (SMD 0.49; p=0.002) levels were higher in the PTSD group than in healthy controls. Subgroup meta-analysis of patients who were not given medication showed higher tumour necrosis factor α (TNFα; SMD 0.69, 95% CI 0.35-1.02; p<0.0001) in the PTSD group than the control group in addition to the aforementioned cytokines. TNFα (SMD 1.32, 0.13-2.50; p=0.003), interleukin 1β (SMD 2.35, 0.01-4.68; p=0.048), and interleukin 6 (SMD 1.75, 0.97-2.53; p<0.0001) levels remained increased in the PTSD group in a subgroup meta-analysis of studies that excluded comorbid major depressive disorder. Illness duration was positively associated with interleukin 1β levels (b=0.33, p<0.0001) and severity with interleukin 6 (b=0.02, p=0.042). A model composed of several variables-presence of comorbid major depressive disorder, use of psychotropic medications, assay used, and time of day blood was collected-explained the large amount of heterogeneity between interleukin 1β, interleukin 6, and C-reactive protein studies. Egger's linear regression test revealed a potential publication bias for interleukin 1β. Additionally, for most inflammatory markers, study heterogeneity was reported to be high (I(2)>75%). *Interpretation:* PTSD is associated with increased interleukin 6, interleukin 1β, TNFα, and interferon γ levels. This information might be useful for consideration of chronic low-grade inflammation as a potential target or biomarker in PTSD treatment. Use of psychotropic medication and presence of comorbid major depressive disorder were important moderators that might explain the inconsistency between results of previous studies. Our search strategy used a range of databases and we made exhaustive effort to acquire data by contacting the authors. Notably, high levels of between-study heterogeneity were recorded for most cytokine variables measured in our analysis. However, meta-regression analysis could explain a large amount of this heterogeneity.

Seah, C., Breen, M. S., Rusielewicz, T., Bader, H. N., Xu, C., Hunter, C. J., McCarthy, B., Deans, P. J. M., Chattopadhyay, M., Goldberg, J., Desarnaud, F., Makotkine, I., Flory, J. D., Bierer, L. M., Staniskyte, M., NYSCF Global Stem Cell Array® Team, Noggle, S. A., Huckins, L. M., Paull, D., Brennand, K. J., … Yehuda, R. (2022). **Modeling gene × environment interactions in PTSD using human neurons reveals diagnosis-specific glucocorticoid-induced gene expression.** *Nature Neuroscience, 25*(11), 1434–1445. doi:10.1038/s41593-022-01161-y Post-traumatic stress disorder (PTSD) can develop following severe trauma, but the extent to which genetic and environmental risk factors contribute to individual clinical outcomes is unknown. Here, we compared transcriptional responses to hydrocortisone exposure in human induced pluripotent stem cell (hiPSC)-derived glutamatergic neurons and peripheral blood mononuclear cells (PBMCs) from combat veterans with PTSD (n=19 hiPSC and n=20 PBMC donors) and controls (n=20 hiPSC and n=20 PBMC donors). In neurons only, we observed diagnosis-specific glucocorticoid-induced changes in gene expression corresponding with PTSD-specific transcriptomic patterns found in human postmortem brains. We observed glucocorticoid hypersensitivity in PTSD neurons, and identified genes that contribute to this PTSD-dependent glucocorticoid response. We find evidence of a coregulated network of transcription factors that mediates glucocorticoid hyper-responsivity in PTSD. These findings suggest that induced neurons represent a platform for examining the molecular mechanisms underlying PTSD, identifying biomarkers of stress response, and conducting drug screening to identify new therapeutics.

Sekar, A., Bialas, A. R., de Rivera, H., Davis, A., Hammond, T. R., Kamitaki, N., Tooley, K., Presumey, J., Baum, M., Van Doren, V., Genovese, G., Rose, S. A., Handsaker, R. E., Schizophrenia

Working Group of the Psychiatric Genomics Consortium, Daly, M. J., Carroll, M. C., Stevens, B., & McCarroll, S. A. (2016). **Schizophrenia risk from complex variation of complement component 4.** *Nature, 530,* 177–183. doi:10.1038/nature16549 Schizophrenia is a heritable brain illness with unknown pathogenic mechanisms. Schizophrenia's strongest genetic association at a population level involves variation in the major histocompatibility complex (MHC) locus, but the genes and molecular mechanisms accounting for this have been challenging to identify. Here we show that this association arises in part from many structurally diverse alleles of the complement component 4 (C4) genes. We found that these alleles generated widely varying levels of C4A and C4B expression in the brain, with each common C4 allele associating with schizophrenia in proportion to its tendency to generate greater expression of C4A. Human C4 protein localized to neuronal synapses, dendrites, axons, and cell bodies. In mice, C4 mediated synapse elimination during postnatal development. These results implicate excessive complement activity in the development of schizophrenia and may help explain the reduced numbers of synapses in the brains of individuals with schizophrenia.

Stein, M. B., Levey, D. F., Cheng, Z., Wendt, F. R., Harrington, K., Cho, K., Quaden, R., Radhakrishnan, K., Girgenti, M. J., Ho, Y-L. A., Posner, D., PTSD Working Group of the Psychiatric Genomics Consortium (PGC), Traumatic Stress Brain Research Study Group, VA Million Veteran Program, VA Cooperative Studies Program, Aslan, M., Duman, R. S., Zhao, H., Polimanti, R., Concato, J., & Gelernter, J. (2019). **Genomic Characterization of Posttraumatic Stress Disorder in a Large US Military Veteran Sample.** *bioRxiv* 764001. doi:10.1101/764001 Individuals vary in their liability to develop Posttraumatic Stress Disorder (PTSD), the symptoms of which are highly heterogeneous, following exposure to life-threatening trauma. Understanding genetic factors that contribute to the biology of PTSD is critical for refining diagnosis and developing new treatments. Using genetic data from more than 250,000 participants in the Million Veteran Program, genomewide association analyses were conducted using a validated electronic health record-based algorithmically-defined PTSD diagnosis phenotype (48,221 cases and 217,223 controls), and PTSD quantitative symptom phenotypes (212,007 individuals). We identified several genome-wide significant loci in the case-control analyses, and numerous such loci in the quantitative trait analyses, including some (e.g., MAD1L1; TCF4; CRHR1) that were associated with multiple symptom sub-domains and total symptom score, and others that were more specific to certain symptom sub-domains (e.g., CAMKV to re-experiencing; SOX6 to hyperarousal). Genetic correlations between all pairs of symptom sub-domains and their total were very high (rg 0.93 – 0.98) supporting validity of the PTSD diagnostic construct. We also demonstrate strong shared heritability with a range of traits, show that heritability persists when conditioned on other major psychiatric disorders, present independent replication results, provide support for one of the implicated genes in postmortem brain of individuals with PTSD, and use this information to identify potential drug repositioning candidates. These results point to the utility of genetics to inform and validate the biological coherence of the PTSD syndrome despite considerable heterogeneity at the symptom level, and to provide new directions for treatment development.

Stein, M. B., Levey, D. F., Cheng, Z., Wendt, F. R., Harrington, K., Pathak, G. A., Cho, K., Quaden, R., Radhakrishnan, K., Girgenti, M. J., Ho, Y. A., Posner, D., Aslan, M., Duman, R. S., Zhao, H., Department of Veterans Affairs Cooperative Studies Program (no. 575B), VA Million Veteran Program, Polimanti, R., Concato, J., & Gelernter, J. (2021). **Genome-wide association analyses of post-traumatic stress disorder and its symptom subdomains in the Million Veteran Program.** *Nature Genetics, 53*(2), 174–184. doi:10.1038/s41588-020-00767-x We conducted genome-wide association analyses of over 250,000 participants of European (EUR) and African (AFR) ancestry from the Million Veteran Program using electronic health record-validated post-traumatic stress disorder (PTSD) diagnosis and quantitative symptom phenotypes. Applying genome-wide multiple testing correction, we identified three significant loci in European case-control analyses and 15 loci in quantitative symptom analyses. Genomic structural equation modeling indicated tight coherence of a PTSD symptom factor that shares genetic variance with a distinct internalizing (mood-anxiety-neuroticism) factor. Partitioned heritability indicated enrichment in several cortical and subcortical regions, and imputed genetically regulated gene expression in these regions was used to identify potential drug repositioning candidates. These results validate the biological coherence of the PTSD syndrome, inform its relationship to comorbid anxiety and depressive disorders and provide new considerations for treatment.

Wang, J., Li, H., Wilson, R., Wang, W., Lam, T. T., Traumatic Stress Brain Research Group, Lewis, D. A., Glausier, J., Holtzheimer, P. H., Friedman, M. J., Williams, K. R., Picciotto, M. R., Nairn, A. C., Krystal, J. H., Duman, R. S., Zhao, H., & Girgenti, M. J. (2023). **A Proteome-wide, Multi-Omics Analysis Implicates Novel Protein Dysregulation in Post-Traumatic Stress Disorder.** *medRxiv* 2023.05.05.23289589 doi:10.1101/2023.05.05.23289589 Post-traumatic stress disorder (PTSD) is a common and disabling psychiatric disorder. Here we present findings from the first proteome-wide study of the postmortem PTSD brain. We performed tandem mass spectrometry on large cohort of donors (*N*=66) in two prefrontal cortical areas and found differentially expressed proteins and co-expression modules disturbed in PTSD. Integrative analysis pointed to hsa-mir-589 as a regulatory miRNA responsible for disruptions in neuronal protein networks for PTSD, including the GABA vesicular transporter, SLC32A1. In addition, we identified significant enrichment of risk genes for Alzheimer's Disease (*N*= 94,403), major depression (*N*=807,553), and schizophrenia (*N*=35,802) within PTSD co-expression protein modules, suggesting shared molecular pathology. Our findings highlight the altered proteomic landscape of postmortem PTSD brain and provide a novel framework for future studies integrating proteomic profiling with transcriptomics in postmortem human brain tissue.