Identification of the Key Genes Involved in Posttraumatic Stress Disorder: Evidence from Human Blood and Postmortem Tissue Studies

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Disclosure

I have no actual or potential conflict of interest to declare.
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Overview of the Presentation

• Define PTSD
• Impact of prolonged combat efforts in Iraq and Afghanistan on risk for PTSD among soldiers following September 11, 2001 and the ensuing impact on public mental health
• Describe a blood-based genomic study to identify biomarkers and gene pathways underlying risk to develop PTSD, predictors of PTSD treatment response, and detection of PTSD diagnosis
• Review our efforts to identify the transcriptomic landscape of PTSD in selected brain regions using human postmortem tissue
• Discuss next steps and future research
DSM-5** Criteria for PTSD

• **Exposure to actual or threatened death, serious injury, or sexual violence in one or more of the following ways**
  - Directly experiencing the traumatic event(s); Witnessing in person the event(s) as it occurred to others; Learning that a close family member or close friend experienced an actual or threatened violent or accidental death; Experiencing repeated or extreme exposure to distressing details of an event (e.g. police officer repeated hearing details about child sexual abuse)

• **Presence of intrusion symptoms associated with traumatic event**
  - Recurrent, involuntary and intrusive memories, dreams and dissociative reactions (flashbacks)
  - Intense physiological and psychological distress in response to cues that symbolize or resemble an aspect of the traumatic event

• **Persistent avoidance of stimuli associated with the trauma**
  - Avoidance of people, places, conversations, activities, objects, or situations that bring up traumatic memories

• **Negative alterations in cognitions and mood associated the traumatic event(s)**
  - Inability to recall an important aspect of the trauma
  - Markedly diminished interest or participation in significant activities
  - Feeling of detachment from others etc.

• **Marked alterations in arousal and reactivity**
  - Difficulty falling or staying asleep, reckless or self-destructive behavior, irritability, difficulty concentrating, hypervigilance, exaggerated startle response

• Additional criteria require one-month duration of symptoms and functional impairment

** American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders (5th ed.)
Epidemiology of PTSD

• U.S. National Comorbidity Survey Replication (2001-2003) ¹
  • 6.8% lifetime prevalence among adult Americans
    - 3.6% in men
    - 9.7% in women
  • 3.5% current past year prevalence among adult Americans
    - 1.8% in men
    - 5.2% in women

• National Vietnam Veterans Readjustment Study ²
  • Lifetime prevalence
    - 30.9% in men
    - 26.9% in women
  • Current past year prevalence
    - 15.2% in men
    - 8.1% in women

² Kulka et al., 1990
Epidemiology of PTSD (continued)

• Operation Enduring Freedom (OEF) / Operation Iraqi Freedom (OIF)
  • 30% of all troops experiencing significant psychiatric distress and upwards of 20% with clinically significant PTSD symptoms (Hoge et al., 2006)

29% of all newly enrolled veterans from OEF/OIF between 2002-2012 met diagnostic criteria for PTSD (Congressional Research Service, 2013)
NRAP called for the creation of the **Consortium to Alleviate PTSD** (CAP) with funding from the DoD and VA

“... accelerate discovery of underlying mechanisms and rapidly translate this understanding into actionable tools for prevention, early diagnosis, and better treatment.”
Consortium to Alleviate PTSD (CAP)

CAP is a joint effort of the DoD STRONG STAR Consortium (UT Health San Antonio) and the VA National Center for PTSD (Jamaica Plain and West Haven VA)

https://patriot.uthscsa.edu/strongstar/cap.asp
Overview of CAP Structure

Cores
• Administrative (Peterson, Keane)
• Assessment (Litz)
• Data & Statistics (Mintz)
• Biomarkers & Genomics (Williamson, Xu)

Key Projects
• Posttraumatic Stress & Headache (McGeary)
• Ketamine RCT (Krystal)
• Neurobiological Predictors in Prolonged Exposure (Rauch)
• Doxazosin RCT for Comorbid PTSD and Alcohol Use Disorders (Back)
• RCT for Sleep and PTSD (Taylor)
• Intensive Outpatient Treatment for PTSD (Peterson)
• irTMS for PTSD (Fox)
• Genetic and Epigenetic Biomarkers of PTSD Diagnosis and Prognosis (Williamson et al.)
CAP-Genetic and Epigenetic Biomarkers of PTSD Diagnosis and Prognosis

• **Prognostic** of Risk to Develop PTSD: – indicate who will develop PTSD after trauma exposure
Pre-/Post-Deployment Study Examining Genetic and Environmental Predictors of Combat-Related PTSD (Williamson PI)
Pre-/Post-Deployment Sample

• 4,112 soldiers assessed pre-deployment
  • DNA/RNA samples collected pre-deployment
  • GWAS run using Illumina PsychArray DNA Analysis BeadChip - (~700,000 SNPs)

• 2,215 soldiers reassessed post-deployment
  • DNA/RNA samples collected pre-deployment

• Combat exposure and deployment environment assessed

• Growth mixture models were used to identify trajectory groups based on PTSD symptoms across the deployment cycle
Psychiatric Genetic Consortium - PTSD

Study included over 30,000 PTSD cases and 170,000 controls from 60 ancestrally diverse studies from Europe, Africa and the Americas

<table>
<thead>
<tr>
<th>Group</th>
<th>GWAS hit lead variant</th>
<th>predicted genes in risk locus</th>
<th>Chromatin state analysis (Roadmap Epigenomics) in neuronal cell lines/tissues</th>
<th>eQTL</th>
</tr>
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<td>European ancestry</td>
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<tr>
<td>All</td>
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<td>ZDHHC14(upstream of TSS)</td>
<td>Transcriptional active chromatin at TSS</td>
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<tr>
<td>All</td>
<td>rs9364611</td>
<td>PARK2(intronic)</td>
<td>Overall quiescent, some enhancer function</td>
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<td>ZNF813</td>
<td>Weak transcription</td>
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<td>African ancestry</td>
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<tr>
<td>All</td>
<td>rs115539978</td>
<td>LINC02335, MIR5007, TUC338</td>
<td>Overall silenced chromatin (score of 15), some SNPs map to loci with weak transcription or enhancer function</td>
<td>None</td>
</tr>
</tbody>
</table>

Nievergelt et al., Nat Commun, 2019
Pre-/Post-Deployment Sample

• 4,112 soldiers assessed pre-deployment
  • DNA/RNA samples collected pre-deployment
  • GWAS run using Illumina PsychArray DNA Analysis BeadChip - (~700,000 SNPs)

• 2,215 soldiers reassessed post-deployment
  • DNA/RNA samples re-collected post-deployment

• Combat exposure and deployment environment assessed

• Growth mixture models were used to identify **trajectory groups**
  based on PTSD symptoms across the deployment cycle
Symptom Trajectories Over the Deployment Cycle

Fluctuating = 15.2%
Chronic = 8.3%
Susceptible = 10.1%
Resilient = 66.4%
Propensity Analysis Used for Matching

Propensity Matching: All Subjects

Propensity Matching: 102 case/controls matched
Prognostic Biomarkers of Risk for PTSD

**Genomic Assays**
- Infinium Multi-Ethnic Global-8 Kit 1.7M SNPs
- Infinium MethylationEPIC 850k BeadChip to assess DNA methylation
- NextGen sequencing for micro RNA detection
- NextGen sequencing total RNAseq to examine gene expression

**Biomarker detection**
- 10-fold cross validation using LASSO to detect best fitting genomic biomarker set identifying risk derived from pre-deployment samples
Biomarker Panel of PTSD Risk

AUC - 0.91
Sens. - 0.86
Spec. - 0.84
8 CpG sites, 38 mRNA
**Prognostic Biomarkers of Risk for PTSD**

**Genomic Assays**
- Infinium Multi-Ethnic Global-8 Kit 1.7M SNPs
- Infinium MethylationEPIC 850k BeadChip to assess DNA methylation
- NextGen sequencing for micro RNA detection
- NextGen sequencing total RNA-seq to examine gene expression

**Biomarker detection**
- 10-fold cross validation using LASSO to detect best fitting genomic biomarker set identifying risk derived from pre-deployment samples

**Transcriptomic Analyses**
- Examining differentially expressed genes (adjusting for covariates)
- Gene Set Enrichment Analyses (GSEA) to identify significant pathways
- Weighted Gene Correlation Network Analyses (WGCNA) to identify significant modules of co-expressed genes and intersection of modules with GSEA pathways
### Gene Set Enrichment Analysis (GSEA) – Hallmark Pathways

#### Risk to Develop PTSD

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Adjusted P</th>
<th>NES</th>
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<tbody>
<tr>
<td>1 HALLMARK_INTERFERON_ALPHA_RESPONSE</td>
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</table>
WGCNA Module Convergence with GSEA Pathways
CAP-Genetic and Epigenetic Alterations as Biomarkers for PTSD Diagnosis and Prognosis

- **Prognostic of Risk to Develop PTSD**: – indicate who will develop PTSD after trauma exposure

- **Prognostic of Treatment Response**: – indicate who will respond to treatment
Symptom Trajectories Across Treatment

N=702 active-duty Soldiers treated for PTSD at Ft. Hood in STRONG STAR trials

Random Intercept
Random slopes

Piecewise linear model, knot at ~100 days

Red – Prediction
Black – Raw data

Model indicates treatment response during the first 100 days, but no change afterwards.

End of Treatment
Reliable Change Index\textsuperscript{1} Classification of Treatment Response

\textsuperscript{1} Jacobson & Truax, 1991
Biomarker Panel of Treatment Response

- AUC: 0.97
- Sens.: 0.89
- Spec.: 0.93
- 51 CpG sites, 8 mRNA
### Gene Set Enrichment Analysis (GSEA) – Hallmark Pathways

#### Treatment Response

<table>
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Pathways highlighted in red overlap with PTSD risk pathways
WGCNA Module Convergence with GSEA Pathways

Cluster Dendrogram

Height

Module colors

0.6
0.7
0.8
0.9
1.0

PreTreatment

Hypergeometric test for module/Hallmark pathway overlap

Heme Metab.

IFNg

IFNa

−log10(pval)
CAP- Genetic and Epigenetic Alterations as Biomarkers for PTSD Diagnosis and Prognosis

- **Prognostic** of Risk to Develop PTSD: – indicate who will develop PTSD after trauma exposure
- **Prognostic** of Treatment Response: – indicate who will respond to treatment
- **Diagnostic**: - presence of PTSD
Diagnostic Biomarkers of PTSD

• PTSD cases currently meeting DSM-IV criteria post-deployment (n=100)
  • New-onset PTSD following recent combat exposure

• Combat trauma exposed controls free of lifetime PTSD and other comorbid neuropsychiatric conditions (e.g. depression) (n=100)
PTSD Diagnostic Biomarker Panel

AUC - 0.80
Sens. - 0.68
Spec. - 0.78
69 CpG sites
### Gene Set Enrichment Analysis (GSEA) – Hallmark Pathways

#### Diagnostic

<table>
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Pathways highlighted in red overlap with PTSD risk pathways.
WGCNA Module Convergence with GSEA Pathways

Cluster Dendrogram

Module colors

Height

Module colors

HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_HYPOXIA
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_MITOTIC_SPINDLE
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_DNA_REPAIR
HALLMARK_G2M_CHECKPOINT
HALLMARK_APOPTOSIS
HALLMARK_NOTCH_SIGNALING
HALLMARK_ADIPOGENESIS
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_ANDROGEN_RESPONSE
HALLMARK_MYOGENESIS
HALLMARK_PROTEIN_SECRETION
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_APICAL_JUNCTION
HALLMARK_APICAL_SURFACE
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_COMPLEMENT
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_MTORC1_SIGNALING
HALLMARK_E2F_TARGETS
HALLMARK_MYC_TARGETS_V1
HALLMARK_MYC_TARGETS_V2
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_XENOBIOTIC_METABOLISM
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_GLUCOLYSIS
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY
HALLMARK_P53_PATHWAY
HALLMARK_UV_RESPONSE_UP
HALLMARK_UV_RESPONSE_DN
HALLMARK_ANGIOGENESIS
HALLMARK_HEME_METABOLISM
HALLMARK_COAGULATION
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_PEROXISOME
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_SPERMATOGENESIS
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_PANCREAS_BETA_CELLS

Hypergeometric test for module/Hallmark pathway overlap

CaseControl_PostTreat

Hypergeometric test for module/Hallmark pathway overlap

Log(pval)

0
20
40
60
−log10(pval)
• In addition to forming the Consortium to Alleviate PTSD …

• NRAP also called for the creation of a PTSD brain bank to advance mechanistic discoveries

• VA - National Posttraumatic Stress Disorder Brain Bank (NPSDBB)
  - Jamaica Plain VA – Boston (McKee et al.)
  - Durham VA – Durham (Cruz et al.)

https://www.research.va.gov/programs/tissue_banking/PTSD/

Frontal Lobe Circuitry in PTSD

**Conditioned fear extinction circuitry.** Fear conditioning is mediated in the basolateral amygdala where nociceptive information is integrated with auditory and visual sensory information, as well as contextual information from the hippocampus. Fear conditioned responses are enacted via efferents to the hypothalamus and brain stem. Extinction of fear conditioning involves new learning in the vmPFC (shaded area), including cingulate (Brodmann area BA25, BA32) and orbitofrontal cortices (BA10 and BA11), and projections of the vmPFC to the amygdala to curtail the fear conditioned output of the amygdala.

**Mood circuitry.** The lateral orbital frontal cortex (OFC, shaded area) receives limbic input from the amygdala and hippocampus that impart emotional and contextual importance and relays information via dorsal anterior cingulate cortex (dACC, BA24) to the subgenual anterior cingulate cortex (sgACC, BA25), a major output node of the mood network that influences the autonomic system via the hypothalamus and brain stem and the reward system via the ventral striatum.

Selemon, Cruz, Young & Williamson, Chronic Stress, 2019
NPSDBB Intramural Transcriptomics Project

- PTSD (n=52), MDD (n=45), and control subjects (n=46)
- Selected frontal lobe brain regions
  - dorsal anterior cingulate cortex (dACC, BA24)
  - subgenual anterior cingulate cortex (sgACC, BA25)
  - medial orbital frontal cortex (mOFC, BA10)
  - dorsolateral prefrontal cortex (dlPFC, BA9/BA46)
- RNA-seq of gray matter
Brain Transcriptomic Organization of Human Posttraumatic Stress Disorder

Disruption of Interferon Signaling - mOFC

WGCNA Key Drivers Implicate Interneurons

Girgenti et al., Nature Medicine – under review (https://www.biorxiv.org/content/10.1101/2020.01.27.921403v1)
Transcriptome-Wide Association Study (TWAS)

Girgenti et al., Nature Medicine – under review (https://www.biorxiv.org/content/10.1101/2020.01.27.921403v1)
Implication of Findings

• Convergence from blood-based studies on downregulation of the innate and adaptive immune system for PTSD risk, predictors of treatment response and for diagnosis

• First-of-its-kind transcriptomic study on brain regions regulating fear response identify downregulation of GABA signaling in inhibitory neurons
Interferon Signaling

Overlay: DPLM_POOLED TT_main - 2020-03-13 09:58 AM, Expr Log Ratio
LETTER

Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour

Anthony J. Filiano1,2, Yang Xu3, Nicholas J. Tustison4, Rachel L. Marsh1,2, Wendy Baker1,2, Igor Smirnov1,2, Christopher C. Overall1,2, Sachin P. Gadani1,2,5,6, Stephen D. Turner7, Zhiping Weng8, Sayeda Najamussahar Peerzade3, Hao Chen8, Kevin S. Lee1,2,5,9, Michael M. Scott5,10, Mark P. Beenhakker5,10, Vladimir Litvak3* & Jonathan Kipnis1,2,5,6*

Immune dysfunction is commonly associated with several neurological and mental disorders. Although the mechanisms by which peripheral immunity may influence neuronal function are largely unknown, recent findings implicate meningeal immunity influencing behaviour, such as spatial learning and memory1. Here we show that meningeal immunity is also critical for social behaviour; mice deficient in adaptive immunity exhibit social amnesia and develop age-related motor, or olfactory deficits (Extended Data Fig. 1b–j). We confirmed that SCID mice have social deficits by analysing social interactions in a home cage (Extended Data Fig. 1k). To test whether social deficits were reversible, we repopulated 4-week-old SCID mice with wild-type lymphocytes (Extended Data Fig. 1l–n) and measured social behaviour 4 weeks after transfer. SCID mice repopulated with lymphocytes, unlike those injected with the vehicle, showed social preference indistinguishable from that of wild-type mice.
IFNγ Supports Proper Neural Connectivity and Social Behavior

IFNg Regulation of Inhibitory Neurons

Next Steps

• Externally validate biomarkers of PTSD risk and treatment response in active duty, veteran, and civilian populations
• Single-cell RNA-seq to identify cell-specific expression differences in both blood and brain studies
• Preclinical models examining fear conditioning extinction in IFNG−/− mice
• Expand brain regions to include subcortical regions including the amygdala and hippocampus (snRNA-seq, snATAC-seq, and methyl-seq)
• Generating fibroblasts and reprogramming to iPSCs for 2D and 3D cell culture mechanistic studies
Generating Fibroblasts and Reprogramming to iPSCs from Dura

Fibroblasts

Day 0

Day 7

Day 15

Day 21

iPSCs using Epi5

Day 23 images

Day 27 images

5X

10X

Ramamani Arumugam, PhD
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