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59th Medical Wing

The epigenetic changes of adverse childhood experience, combat exposure, and post-traumatic stress disorder in active duty service members.

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Disclosures

The opinions expressed in this presentation are solely those of the author and do not represent an endorsement by, or the views of, the United States Air Force, the United States Army, the Department of Defense, or the United States Government.

Air Force Medical Support Agency - Intramural Research Development Test & Evaluation FY14-15 Funds

Introduction

- Since 9/11 1.64 million soldiers deployed
- 50% experienced traumatic events
  - Friends killed
  - Seeing dead/ injured non-combatants
  - Witnessing accidents leading to individual's death
  - Smelling decomposing bodies
- Of subset, 300,000 are projected to meet diagnosis criteria of PTSD*
  - Direct and indirect cost of care is $25,757 per individuals in the first two years of deployment
  - Increased risk of psychiatric comorbidities, domestic violence, family strain, and homelessness

PTSD is Complexity

Combat PTSD is complex likely involving multiple risk factors and multiple pathophysiologies.

Risk factors:

- Adult traumatic even (combat)
- Childhood adversity
- Genetic risk factors (SLC6A4)
- Environment x Genetics (Multiple SNPs)

Pathophysiologies

- Methylations changes
- Autonomic pathologies

Goal of Research

To understand the complex interaction of experience, genetics, epigenetics in active duty soldiers with PTSD.

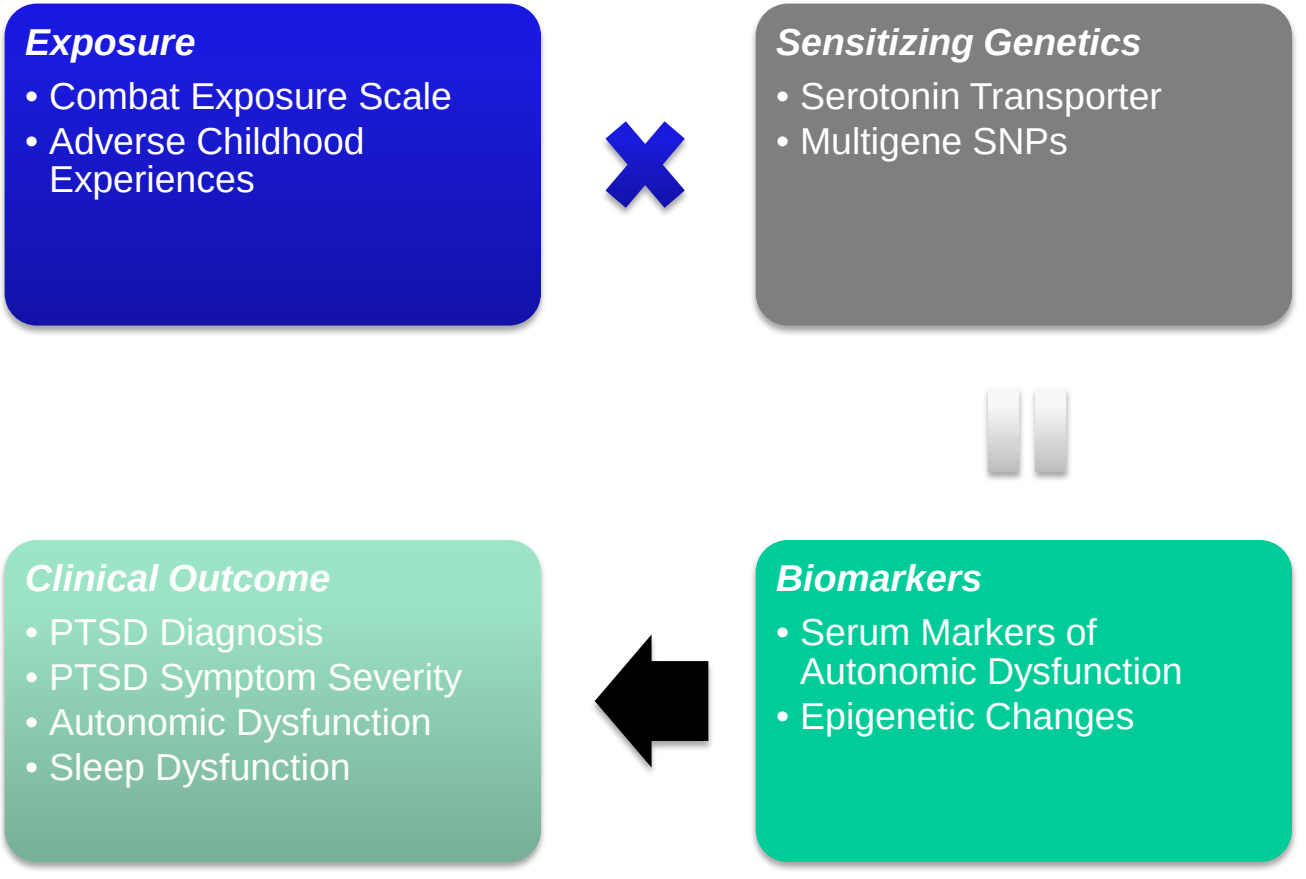
If we know risk factors, can we intervene efficiently to reduce symptoms?

If we know the pathophysiology, can we develop biomarkers?

If we know pathophysiology, can be build targeted treatments?

Building the ACES framework

Build a recruitment infrastructure to test available hypotheses in active duty service members.



Building the ACES framework

Recruitment

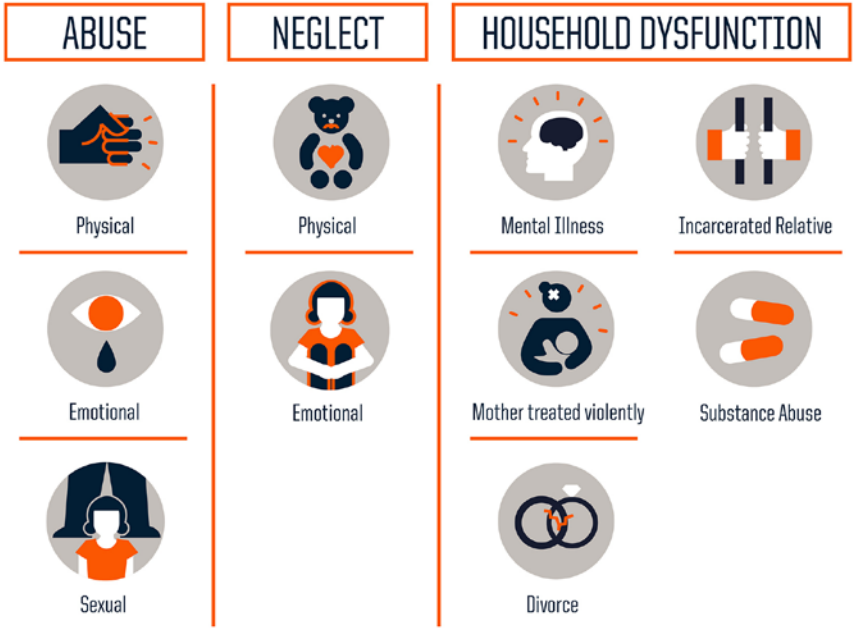
- Prospective case-control study
  - 70 patients with PTSD and 210 patients w/o PTSD
- Inclusion criteria
  - Active duty
  - Deployed for greater than 3 months
  - “Seen combat”
- Exclusion criteria
  - Known history of structural brain damage as demonstrated by imaging (CT head / MRI)
  - History TBI resulting in loss of consciousness
  - Pre-deployment diagnosis of PTSD or anxiety disorder
  - Pre-deployment prescription of a selective serotonin reuptake inhibitors (SSRI), serotonin and norepinephrine reuptake inhibitors (SNRI), or tricyclic antidepressants (TCA).

Building the ACES framework

Measuring experiential risk factors:

Adverse Childhood Experiences

Combat Exposure Scale



Source: Centers for Disease Control and Prevention
Credit: Robert Wood Johnson Foundation

CES

Please circle the number above the answer that best describes your experience

1) Did you ever go on combat patrols or have other dangerous duty?

1	2	3	4	5
No	1-3X	4-12x	13-50x	51+times

2) Were you ever under enemy fire?

1	2	3	4	5
Never	<1 month	1-3 months	4-6 months	7 mos or more

3) Were you ever surrounded by the enemy?

1	2	3	4	5
No	1-2X	3-12x	13-25x	26+times

4) What percentage of the soldiers in your unit were killed (KIA), wounded or missing in action (MIA)?

1	2	3	4	5
None	1-25%	26-50%	51-75%	76% or more

5) How often did you fire rounds at the enemy?

1	2	3	4	5
Never	1-2X	3-12x	13-50x	51 or more

6) How often did you see someone hit by incoming or outgoing rounds?

1	2	3	4	5
Never	1-2X	3-12x	13-50x	51 or more

7) How often were you in danger of being injured or killed (i.e., being pinned down, overrun, ambushed, near miss, etc.)?

1	2	3	4	5
Never	1-2X	3-12x	13-50x	51 or more

Building the ACES framework

Testing genetic risk factors – Collection of whole blood, PCR, exome sequencing) at CAMD

Presence of “L” or “S” of SLC6A4 transporter

Cumulative presence of sensitizing SNPs (diagnosis vs autonomic outcomes)

Gene	SNP	Risk Genotype
ADCYAP1 R1	Rs2267735	CC
COMT	Rs4680	GG
CRHR1	Rs7209436	CC
DBH	Rs1611115	CC
DRD2	Rs6277	TT
FAAH	Rs324420	CC
FKBP5	Rs1360780	TT
NPY	Rs16147	GG
NTRK2	Rs1867283	GG
PCLO	Rs2522833	AA
TPH2	Rs4570625	AA
TPH2	Rs1386494	CC
DAT1	Rs40184	CC
DRD2	Rs1800497	TT

Gene	SNP/CpG
SLC6A2	rs2242446
SLC6A2	rs5564
SLC6A2	rs5569
SLC6A2	rs7194256
ACE	rs4311
ACE	rs4305

Building the ACES framework

Testing Biomarkers – Pyrosequencing – CRD Methylation

Gene	Function
BDNF	protein that promotes nerve growth and synaptic plasticity
NR3C1	glucocorticoid receptor which binds cortisol and other glucocorticoids
MAN2C1	T-cell regulation and overall immune function
TLR8	neurogenesis in developing brains and participates in inflammatory processes along with suppression of neurite outgrowth in adults
SLC6A4	monoamine transporter protein that transports serotonin from the synaptic cleft to the presynaptic neuron
IL-18 (interleukin 18)	pro-inflammatory cytokine and induces interferon activity within the brain
SKA2	cellular mitotic activity and interacts with the glucocorticoid receptor

Building the ACES framework

Testing outcomes –

- PTSD diagnosis (Gold standard for diagnosis)
- PCL-M (numerical weight of PTSD symptoms)
- Compass – 31 (questionnaire for autonomic instability)

Results- Exposure, Genetics, and Outcomes

Warrior Medics – Mission Ready – Patient Focused

Characteristic	No PTSD (n = 210)	PTSD (n = 64)	p
Age, Mean (SD)	36.8 (6.9)	38.1 (6.5)	0.17
Length of Deployment, Median (IQR)	17.0 (11.0-24.0)	21.0 (11.5-32.5)	0.06
Gender, n (%)			
Female	29 (13.8)	17 (26.6)	0.02*
Male	181 (86.2)	47 (73.4)	
Race, n (%)			
Caucasian	107 (51.0)	18 (28.1)	0.01*
African American	40 (19.1)	19 (29.7)	
Hispanic	36 (17.1)	18 (28.1)	
Others	27 (12.9)	9 (14.1)	
Education, n (%)			
High School	44 (21.0)	12 (18.8)	0.23
2-Year College	72 (34.3)	23 (35.9)	
4-Year College	42 (20.0)	20 (31.3)	
Masters	49 (23.3)	8 (12.5)	
Doctorate	3 (1.4)	1 (1.6)	
Marital Status, n (%)			
Not married	40 (19.1)	16 (25.0)	0.31
Married	169 (80.9)	48 (75.0)	
Number of Children			
None	38 (18.1)	11 (17.2)	0.86
1-2	103 (49.1)	30 (46.9)	
3-4	59 (28.1)	21 (32.8)	
More than 4	10 (4.8)	2 (3.1)	
Tobacco Use			
No	144 (68.6)	42 (65.6)	0.76
Yes	65 (31.0)	22 (34.4)	
Unknown	1 (0.5)	0 (0.0)	
Alcohol Use			
None	73 (34.8)	21 (32.8)	0.17
1-3	98 (46.7)	24 (37.5)	
4-6	30 (14.3)	14 (21.9)	
7 and more	9 (4.3)	4 (6.3)	
Unknown	0 (0.0)	1 (1.6)	
Treatment at Behavioral Health			
No	154 (73.3)	3 (4.7)	0.0001*
Yes	56 (26.7)	61 (95.3)	

Results – Exposure, Genetics, and Outcomes

Pearson Correlation coefficients for exposure and outcome variables

	PTSD	PCL-M	CES	ACES
PTSD	1.00			
PCL-M	0.6734 (<0.0001)	1.00		
CES	0.2627 (<0.0001)	0.3057 (<0.0001)	1.0	
ACES	0.14747 (<0.0001)	0.2309 (0.001)	-0.0427 (0.4713)	1.0

Significant Genetic Outcomes

Rs7209436	PTSD (0)	PTSD (1)	Odds Ratio (95% CI)	p
2	63 (29.7%)	26 (42.6%)	1.00	
1	100 (47.2%)	20 (32.8%)	0.47 (0.24 – 0.92)	0.03
0	49 (23.1%)	15 (24.6%)	0.67 (0.31 – 1.42)	0.29
Total	212	61		

No significant interactions between experience and genetics tested

Results- Methylation Analysis

	PTSD (cases)	No PTSD (controls)
	70	100
Gender		
Male	53	87
Female	17	13
Age (mean)	38.7	35.1
Ethnicity		
Caucasian	21	50
African American	21	22
Hispanic/Latino	17	20
Asian	4	4
Other	7	4
Anti-depressant use*	50 (p< 0.0001)	21
ACES score (mean)	2.8 (p=0.0015)	1.7
Combat Exposure Scale [0-41] (mean)	17.5 (p <0.0001)	11.3
PCL-M score [0-85] (mean)	56.9 (p <0.0001)	27.8

Results – Methylation Analysis

Significant methylation differences found on all methylation sites for BDNF and NR3C1 (hypomethylation at all sites) and one site of MAN2C1.

Significance remained in BDNF (8/9 sites) and NR3C1 after controlling for antidepressant use

BDNF 08							
	Subject	n	Mean	STD Deviation	dF	F stat (t-value)	p-value
Pos 1	Control	100	6.0731	1.92967	151	2.5	0.013
	PTSD	70	5.3276	1.88788			
Pos 2	Control	100	6.2551	2.14882	158	2.9	0.004
	PTSD	70	5.3281	1.92692			
Pos 3	Control	100	4.1285	1.70582	164	3.8	0
	PTSD	70	3.2133	1.40401			
Pos 4	Control	100	5.3835	1.80351	155	3.3	0.001
	PTSD	70	4.5024	1.67951			

Methylation in all genes (except IL-18) were lower in PTSD group

Conclusions

- Understanding associations and outcomes is complicated
- Experiential risk for PTSD is significantly associated with combat exposure AND adverse childhood experiences.
- Risk of active duty service members linked to a polymorphism attributed to increased baseline cortisol levels and cortisol response

Conclusions

- Diagnosis of PTSD had alterations of epigenetics in genes associated with
  - BDNF:
    - highest concentrations in the amygdala, hippocampus, and prefrontal cortex
    - Mixed findings in previous studies
  - N3RC1:
    - Glucocorticoid receptor
    - Decreased methylation linked with early life adversity as well as depression, anxiety and substance abuse

- Recent prospective epigenome (including BDNF and NR3C1) showed decreased methylation with PTSD symptoms in 9/11 World Trade Center first responders
- longitudinal analysis of active duty military personnel post-deployment with decreased methylation levels in PTSD subjects

Next Steps?

- Link genetics and methylation studies for further pathophysiologic understanding
- Study physiologic parameters (heart rate variability) as function of genetics and methylation patterns
- Prospective study to determine if genetics can predict early treatment success.

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- Clinical Research Division
- Center for Advanced Molecular Detection
- 59th MDW/ST

Questions?

Warrior Medics – Mission Ready – Patient Focused


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