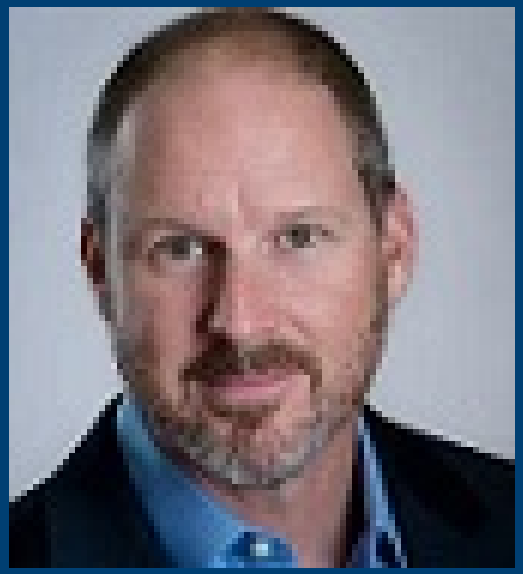
# Research Advisory Committee on Gulf War Veterans' Illnesses

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# Recent Developments in the Identification of PTSD-related Biomarkers

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National Center for PTSD
Posttraumatic Stress Disorder

ADVANCING SCIENCE AND PROMOTING UNDERSTANDING OF TRAUMATIC STRESS

## Collaborators

National Center for PTSD: Mark Logue, Erika Wolf, Danielle Sullivan
Translational Research Center for TBI and Stress Disorders (TRACTS)
Pharmacogenomics Analysis Laboratory, Little Rock VA
Brigham & Women's Hospital, Center for Clinical Spectroscopy: Alex Lin
Psychiatric Genomics Consortium-PTSD Workgroup


## Funding and Other Support

National Institute of Mental Health
National Institute on Aging
VA Clinical Sciences Research and Development Program


## Disclosures

No commercial conflicts of interest to disclose

# National Center for PTSD
# Behavioral Science Division Cohort

Participants: ~750 veterans of various eras and some spouses, apx 200 followed over 10 years
Average current age: ~65
Measures:

- Genotyping: Illumina 2.5M SNP array
- DNA methylation: Illumina 450K and 850K arrays
- Plasma Biomarkers (SIMOA inflammatory and neurology markers, in process)
- Clinical: CAPS & SCID

# Translational Research Center for TBI and Stress Disorders (TRACTS)

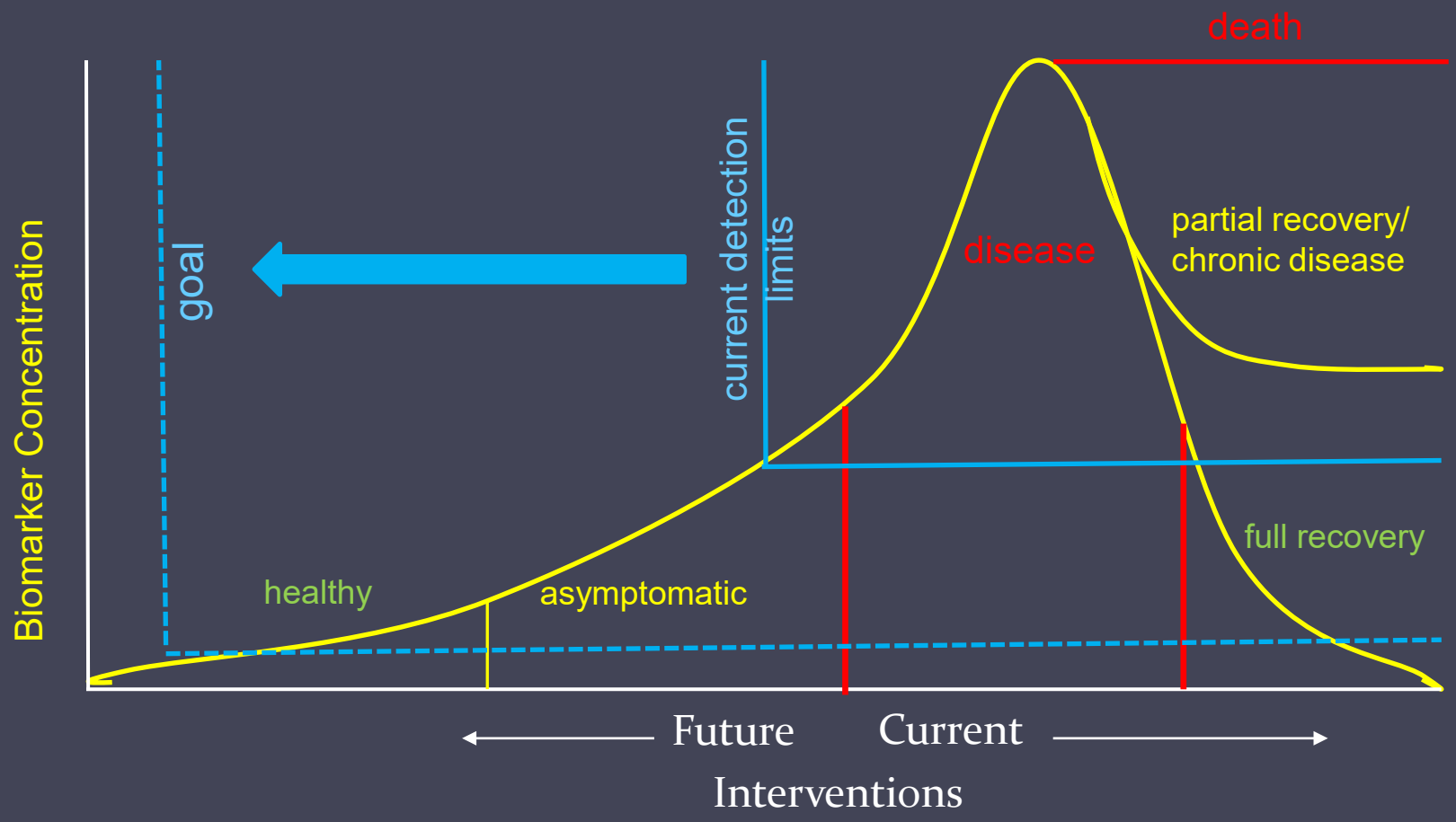
Participants: ~650 U.S. veterans of conflicts in Iraq and/or Afghanistan, apx. 300 with two or more longitudinal assessments at ~2-year intervals
Average current age: ~45
Measures:

- Neuroimaging: Structural Morphology, Diffusion Tensor Imaging, Functional Connectivity
- Genotyping: Illumina 2.5M SNP array
- DNA methylation: Illumina 450K and 850K arrays
- Plasma Biomarkers (SIMOA inflammatory and neurology markers)
- Clinical: CAPS & SCID
- Neurocognitive Battery
- Emphasis on mTBI assessment and analysis

# Biomarker Definition

An objectively measured biological parameter that is used as an Indicator of normal biological processes, pathogenic processes, acute or chronic illness and/or recovery.

# Biomarker Sensitivity and the Disease Continuum



**REVIEW**

# Traumatic stress, oxidative stress and post-traumatic stress disorder: neurodegeneration and the accelerated-aging hypothesis

MW Miller and N Sadeh

Post-traumatic stress disorder (PTSD) is associated with elevated risk for a variety of age-related diseases and neurodegeneration. In this paper, we review evidence relevant to the hypothesis that chronic PTSD constitutes a form of persistent life stress that potentiates oxidative stress (OXS) and accelerates cellular aging. We provide an overview of empirical studies that have examined the effects of psychological stress on OXS, discuss the stress-perpetuating characteristics of PTSD, and then identify mechanisms by which PTSD might promote OXS and accelerated aging. We review studies on OXS-related genes and the role that they may have in moderating the effects of PTSD on neural integrity and conclude with a discussion of directions for future research on antioxidant treatments and biomarkers of accelerated aging in PTSD.

*Molecular Psychiatry* (2014) **19,** 1156–1162; doi:10.1038/mp.2014.111; published online 23 September 2014

REVIEW *(2018) Harvard Review of Psychiatry*, 26, 57-69.

# Oxidative Stress, Inflammation, and Neuroprogression in Chronic PTSD

Mark W. Miller, PhD, Alex P. Lin, PhD, Erika J. Wolf, PhD, and Danielle R. Miller, PhD

**Abstract:** Posttraumatic stress disorder is a serious and often disabling syndrome that develops in response to a traumatic event. Many individuals who initially develop the disorder go on to experience a chronic form of the condition that in some cases can last for many years. Among these patients, psychiatric and medical comorbidities are common, including early onset of age-related conditions such as chronic pain, cardiometabolic disease, neurocognitive disorders, and dementia. The hallmark symptoms of posttraumatic stress—recurrent sensory-memory reexperiencing of the trauma(s)—are associated with concomitant activations of threat- and stress-related neurobiological pathways that occur against a tonic backdrop of sleep disturbance and heightened physiological arousal. Emerging evidence suggests that the molecular consequences of this stress-perpetuating syndrome include elevated systemic levels of oxidative stress and inflammation. In this article we review evidence for the involvement of oxidative stress and inflammation in chronic PTSD and the neurobiological consequences of these processes, including accelerated cellular aging and neuroprogression. Our aim is to update and expand upon previous reviews of this rapidly developing literature and to discuss magnetic resonance spectroscopy as an imaging technology uniquely suited to measuring oxidative stress and inflammatory markers in vivo. Finally, we highlight future directions for research and avenues for the development of novel therapeutics targeting oxidative stress and inflammation in patients with PTSD.

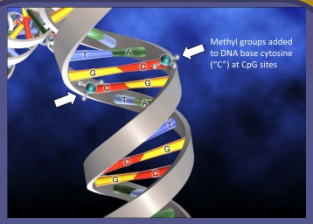
**Keywords:** accelerated aging, inflammation, magnetic resonance spectroscopy, neurodegeneration, neuroprogression, oxidative stress, posttraumatic stress disorder

# Conceptual Framework

*Mechanisms*

*Consequences*

Genetic Factors



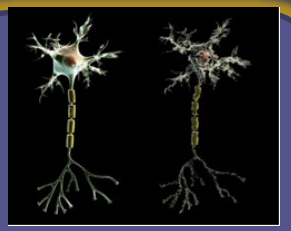

Epigenetic Changes

Accelerated Aging

Trauma

PTSD

Oxidative Stress



Neurodegeneration

Inflammation

Metabolic Syndrome

# What is Oxidative Stress?

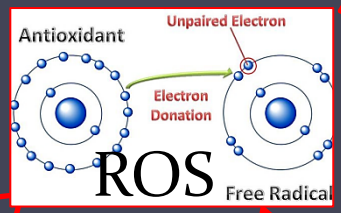
# Oxidative Stress & 12/15LOX

12/15LOX: "the central executioner in an oxidative stress-related neuronal death program" (Pallast et al. 2009)

## Inducers & Pro-oxidants

**Exogenous**
-Pollutants
-UV Radiation

**Endogenous**
-Stress
-Inflammation
-Hypercortisolism
-Sleep Disturbance
(glymphatic system)

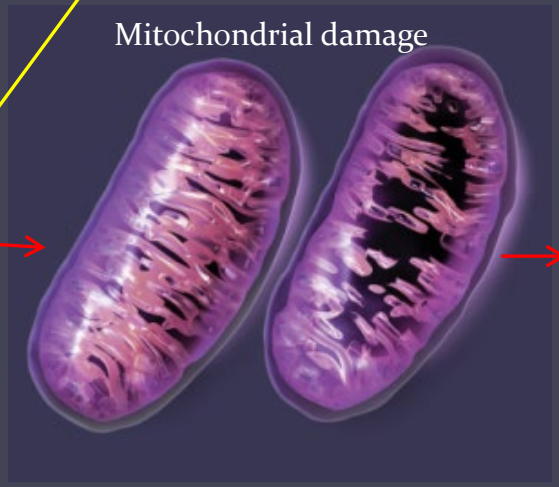


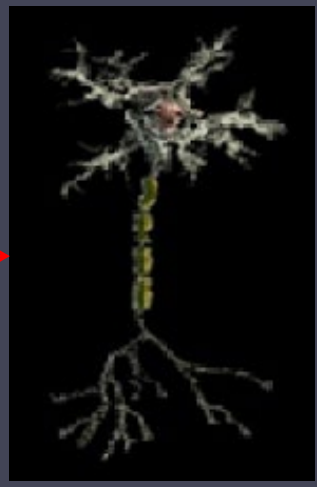

12/15 Lipoxygenase (12/15-LOX)

## Scavengers & Antioxidants

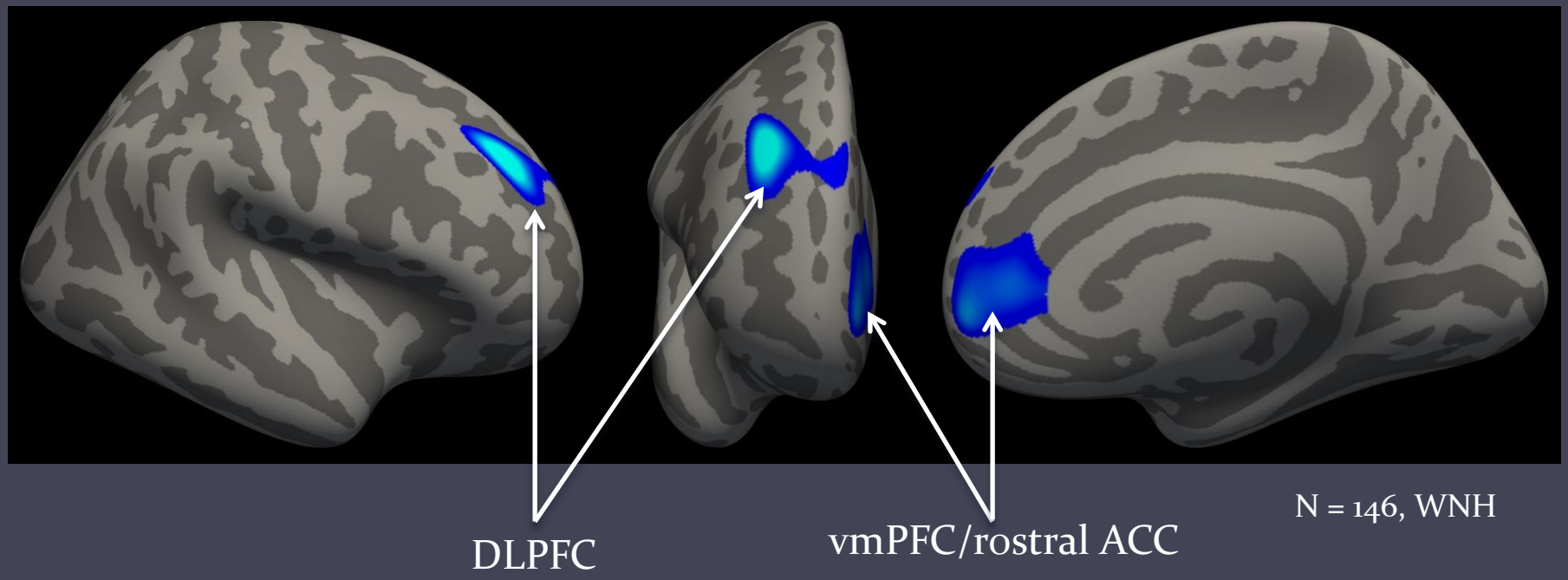
Exogenous Antioxidants (diet)

Endogenous Antioxidants e.g., glutathione



Lateral (left), anterior (middle), and medial (right) views of right hemisphere cortical thickness clusters associated with the rs1042357/rs10852889 x PTSD severity effect.

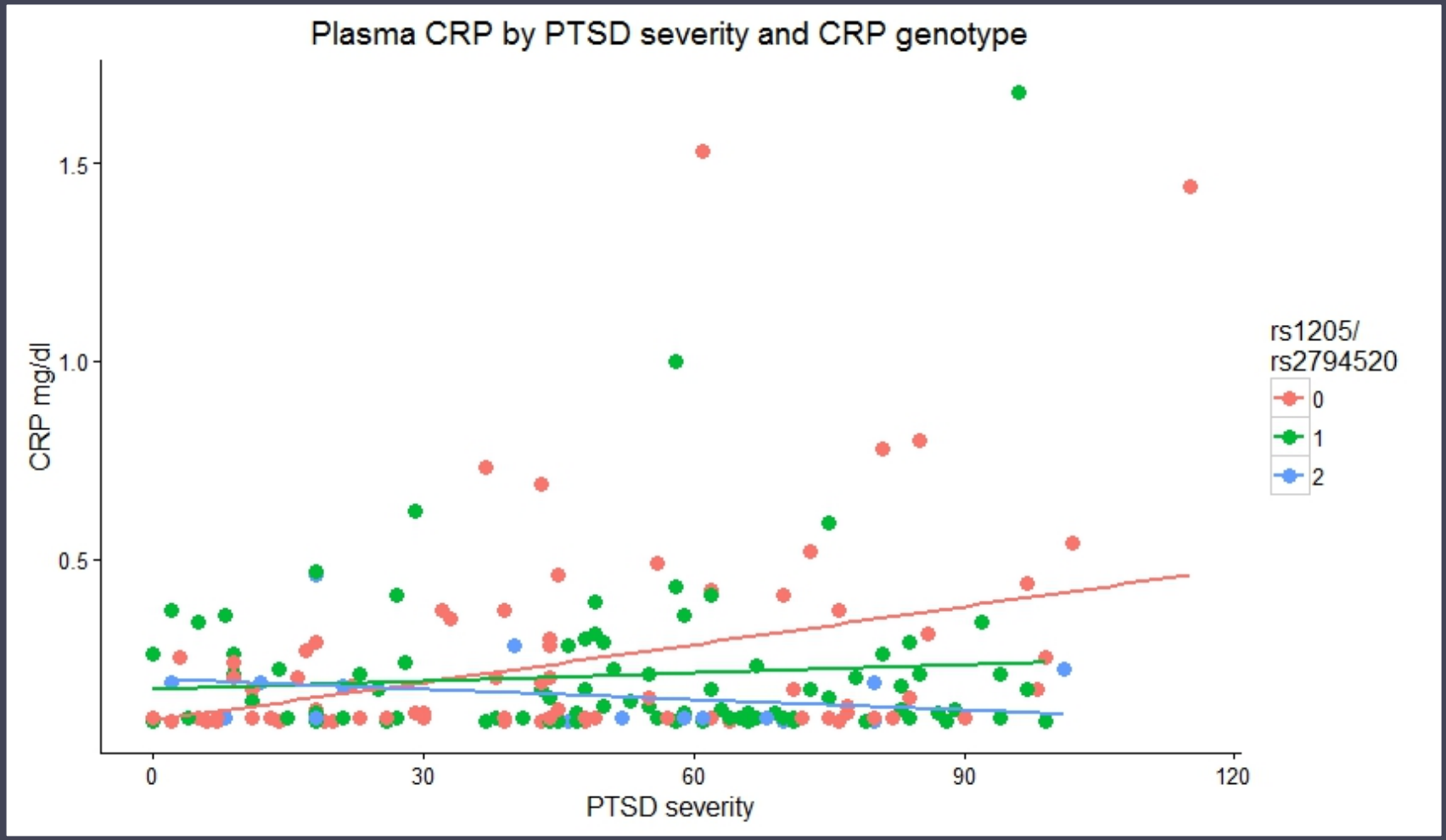



Miller, M.W., Wolf, E.J., Sadeh, N., Logue, M., Spielberg, J., Hayes, J.P., Sperbeck, E., Schichman, S.A. Stone, A., Carter, W.C., Humphries, D.E., Milberg, W., & McGlinchey, R. (2015). A novel locus in the oxidative stress-related gene *ALOX12* moderates the association between PTSD and thickness of the prefrontal cortex. *Psychoneuroendocrinology, 62,* 359–365.

# C-reactive Protein (CRP)

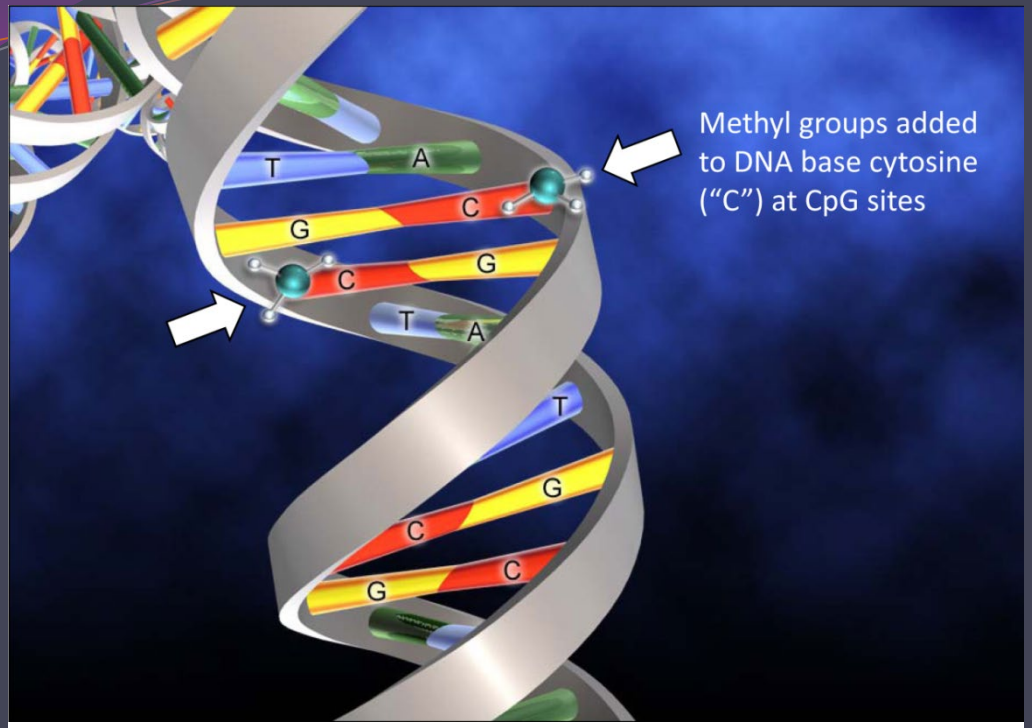
- first identified as a substance in the serum of patients with acute inflammation that reacted with the somatic 'C' carbohydrate antigen of pneumococcus.
- increases during inflammation
- associated with cardiovascular disease and poor recovery post-stroke
- transcribed by the *CRP* gene
- *CRP* polymorphisms are associated with substantial individual differences in baseline CRP levels (e.g., rs3093099 , GG versus TT genotype = 53% increase; rs3091244, AA versus CC = 67% increase; Zacho et al. 2008 )
- associated with DNA methylation in *AIM2* (a key mediator of inflammatory responses)

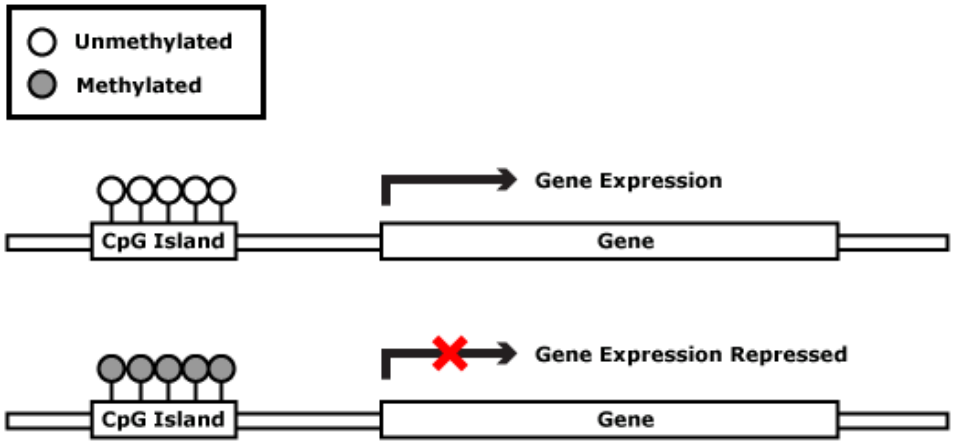
# *CRP SNPs rs1205/rs2794520 Moderate the Association Between PTSD severity and Plasma CRP Levels*




Miller, M.W., Maniates, H., Wolf, E. J., Logue, M.W., Schichman, S.A., Stone, A., Milberg, W., McGlinchey, R. (2018). *CRP* polymorphisms and DNA methylation of the *AIM2* gene influence associations between trauma exposure, PTSD, and C-reactive protein. *Brain, Behavior, and Immunity*, *67*, 194-202.

# DNA Methylation




Modification of DNA caused by enzme-induced addition of methyl groups to nucleobases blocking production of messenger RNA. While there is no change in DNA sequence, methylation prevents translation, and therefore, alters gene expression.

A genome adaption mechanism (i.e., epigenetic).

Logue MW, Miller MW, Wolf EJ, et al. (2020). An epigenome-wide association study of posttraumatic stress disorder in US veterans implicates several new DNA methylation loci. *Clinical Epigenetics*, *12*, 46.

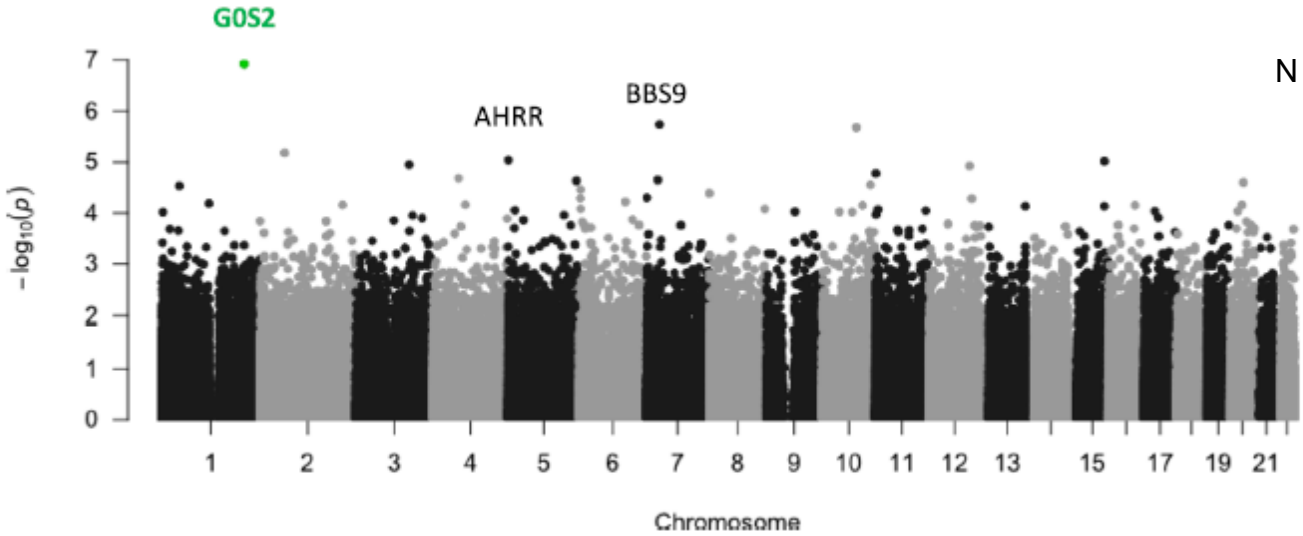


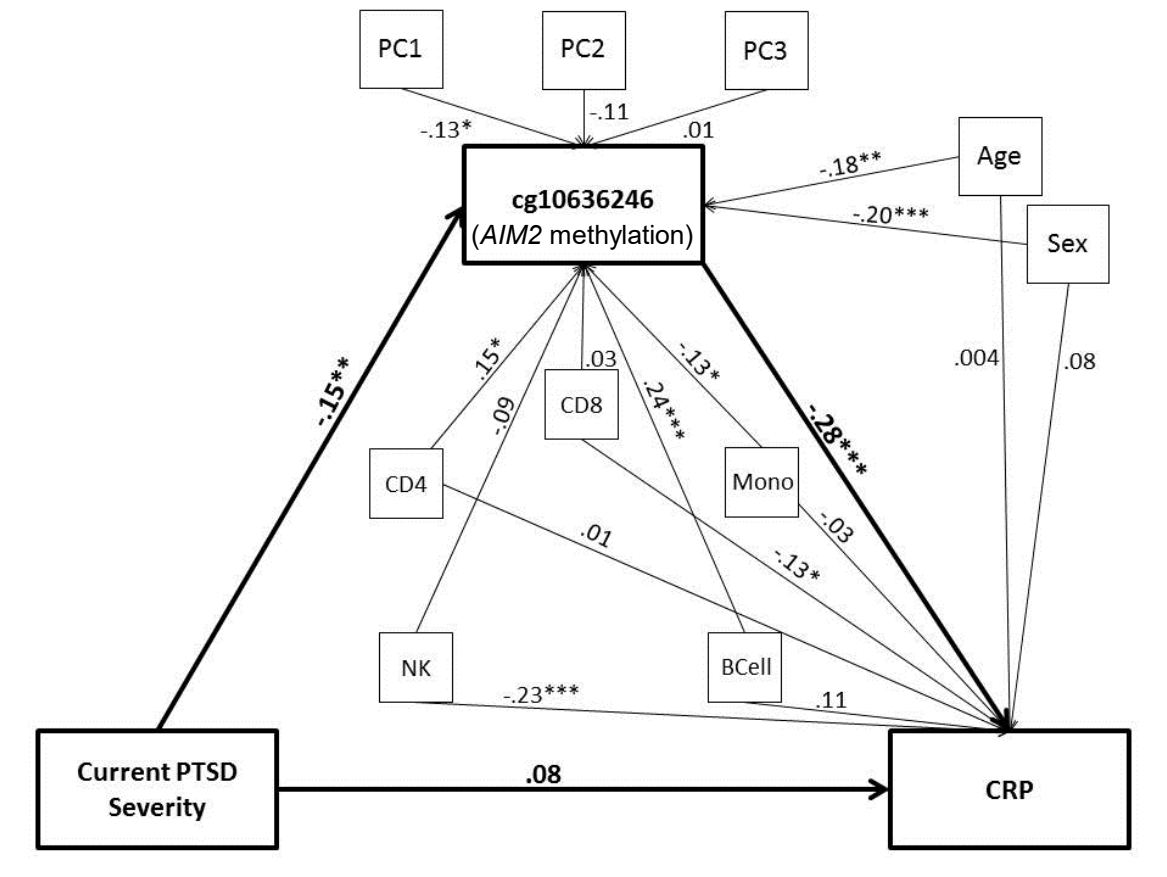

**Fig. 1** Manhattan plot of an epigenome-wide association study of PTSD in US Veterans, the one EWAS significant locus at *G0S2* is highlighted in green

**G0S2:** well-known for its role in regulating lipid metabolism. It has been implicated in mechanisms of obesity, diabetes, aging, and cancer, and linked to gene networks involved in apoptosis, cell communication, and cell death. This effect replicated in a PGC-PTSD EWAS .

**AHRR:** methylation at this locus is one of the strongest and most reliable indicators of smoking in the epigenome but also associated with other phenotypes above and beyond the effects of cigarette smoking, including epigenetic age acceleration and CRP levels. This association with PTSD was previously observed in an independent cohort (Smith et al., 2019).

**BBS9:** no replication

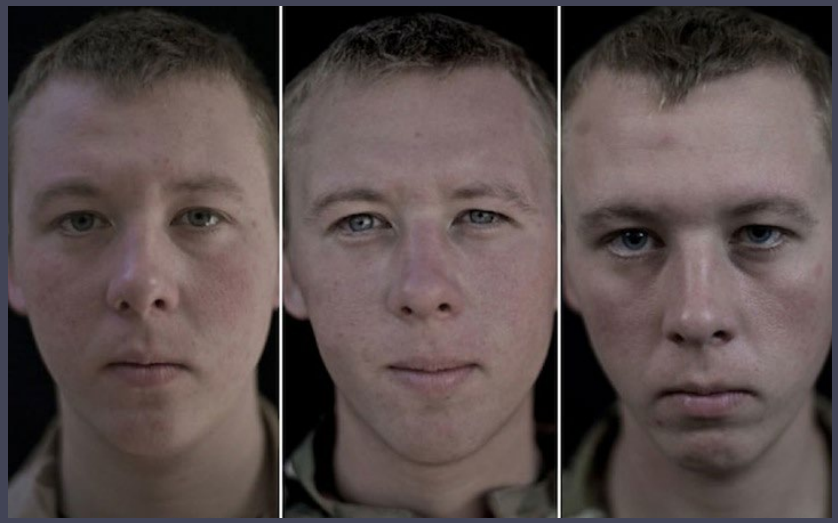
# *AIM2* methylation mediates the association between PTSD and plasma CRP levels



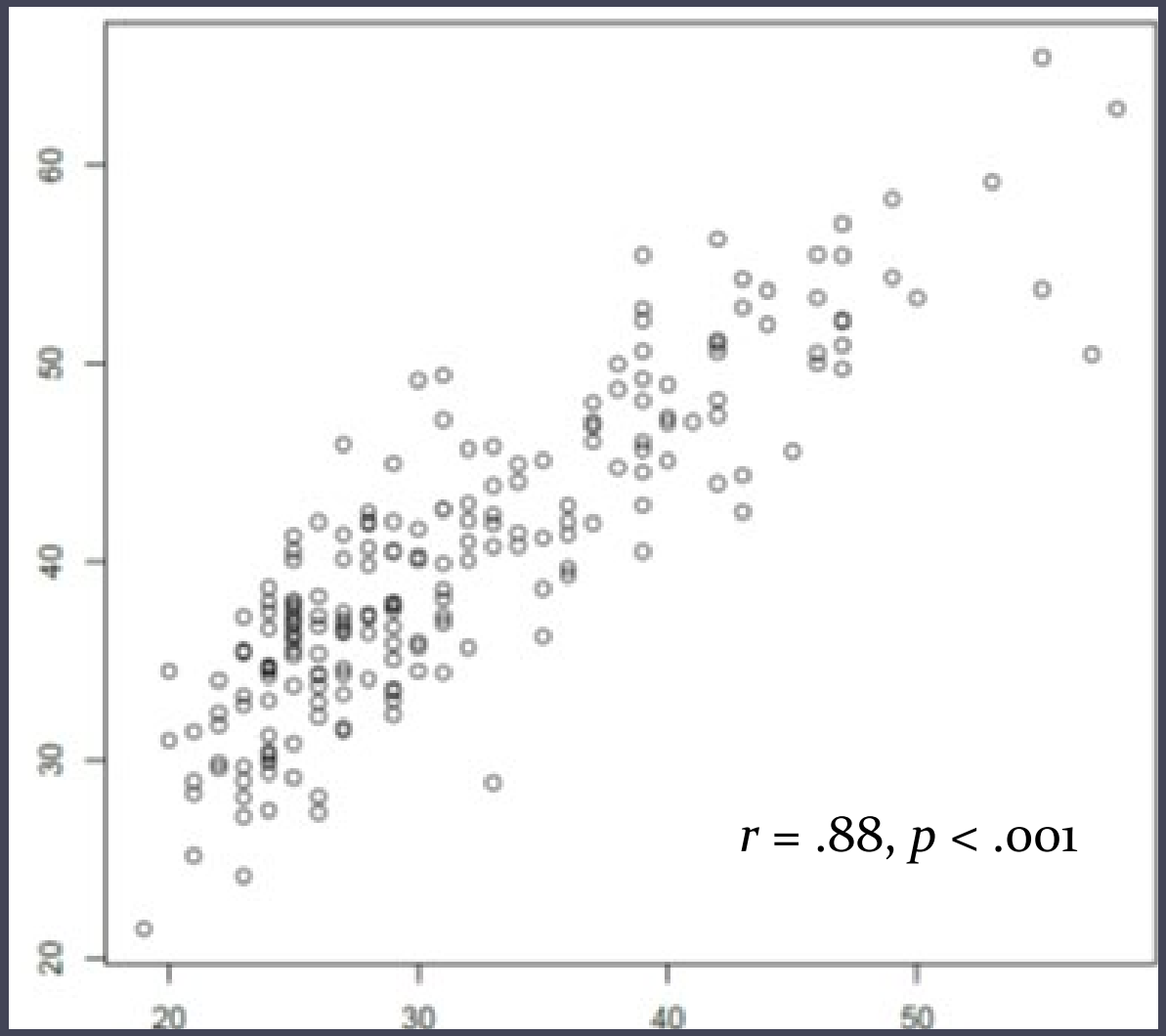

Miller, M.W., Maniates, H., Wolf, E. J., Logue, M.W., Schichman, S.A., Stone, A., Milberg, W., McGlinchey, R. (2018). *CRP* polymorphisms and DNA methylation of the *AIM2* gene influence associations between trauma exposure, PTSD, and C-reactive protein. *Brain, Behavior, and Immunity*, *67*, 194-202.

# PTSD-related Accelerated Aging

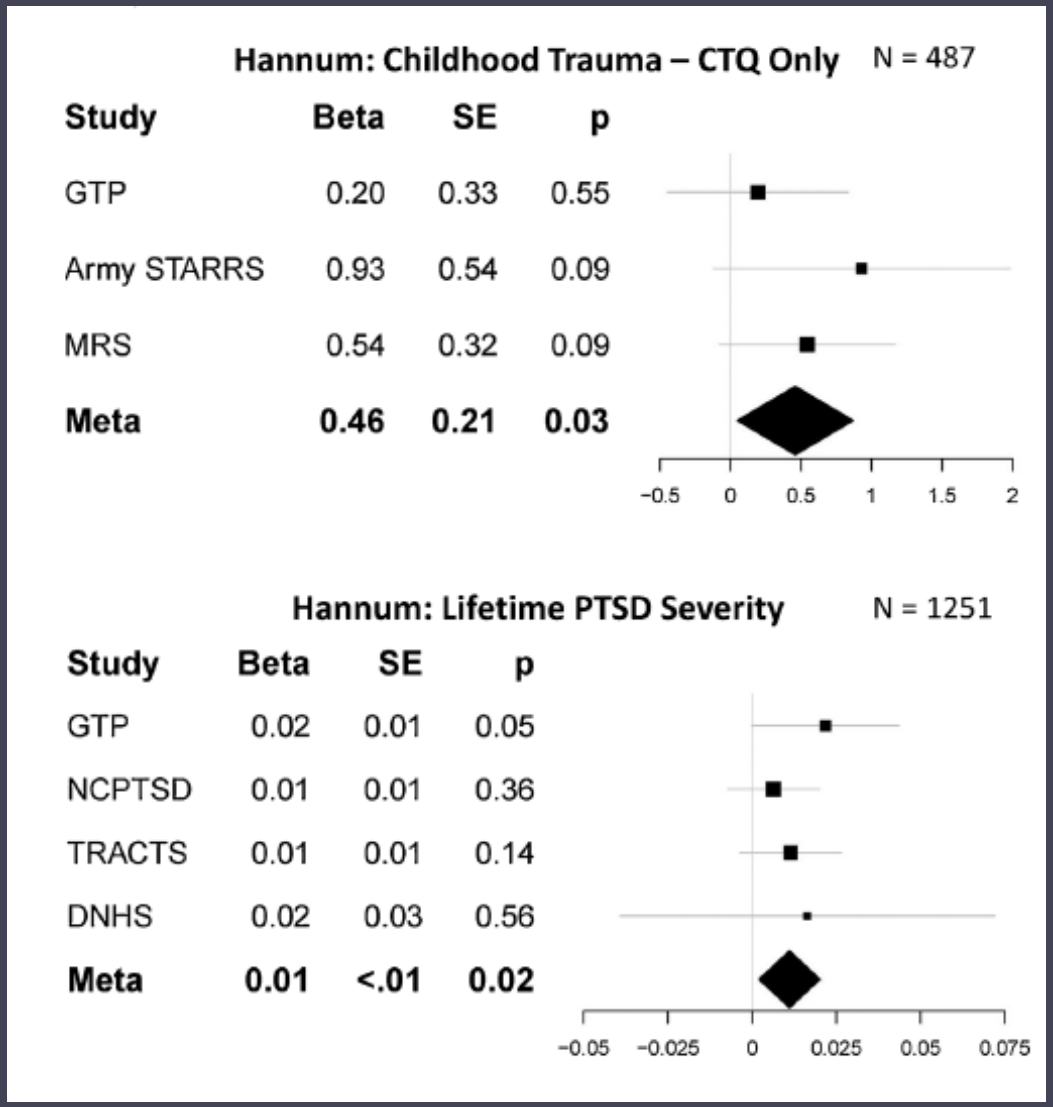
Photographer Lalage Snow photographed Scottish soldiers before they were deployed to Afghanistan, after three months' service, and several days after they returned home.



# Association between DNAm age and Chronological Age



Wolf, E. J.…Miller, M. W…..& Logue, M. W. (2018). Traumatic stress and accelerated DNA methylation age: A meta-analysis. *Psychoneuroendocrinology, 92,* 123-134.



# Metabolic Syndrome (MetS)

- Constellation of Symptoms
  - Obesity
    - Waist-to-hip ratio > 102cm (men)/88cm (women)
  - Elevated blood pressure
    - Systolic ≥ 130 mmHg
    - Diastolic ≥ 85 mmHg
  - Insulin resistance
    - Fasting glucose ≥110 mg/DL
  - Dyslipidemia
    - HDL < 40 mg/dL (men)/50 mg/dL (women)
    - Triglycerides ≥ 150 mg/dL
- Very costly: $80 billion in US (Sullivan et al., 2007)

National Cholesterol Education Program Adult Treatment Panel III

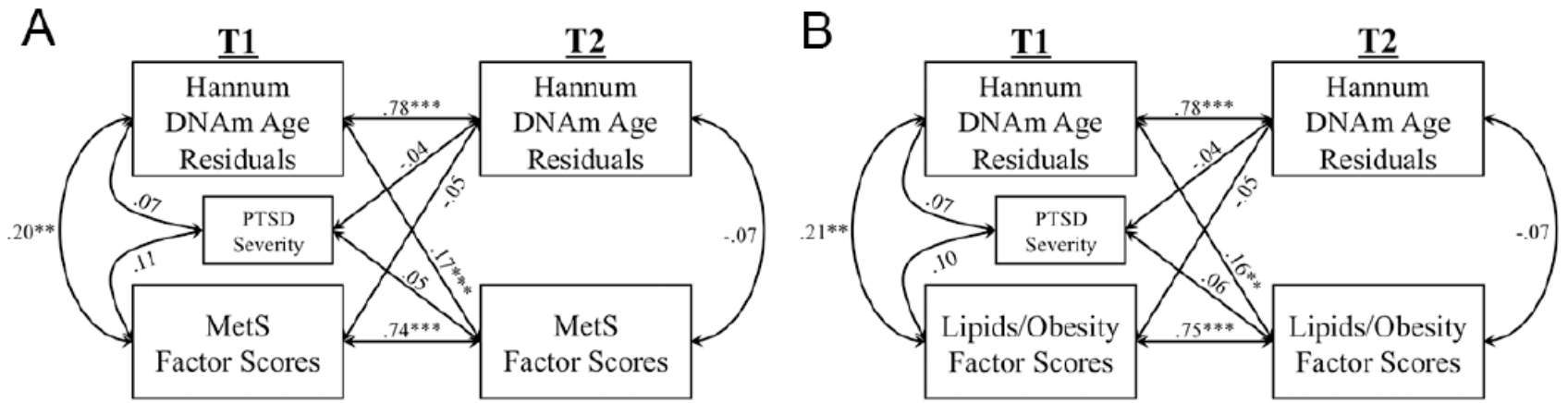



**Figure 1.** The Figure shows the results of cross-lagged models examining longitudinal associations between Hannum DNAm age residuals and metabolic syndrome (MetS) severity factor scores (A), and Lipids/Obesity factor scores (B). Measures of each marker were residualized on age and sex (applicable to A and B). (***$p < 0.005$, **$p < 0.01$, **$p < 0.05$).

Morrison, F.G., Logue, M.W., Guetta, R., Maniates, H., Stone, A., Schichman, S.A. McGlinchey, R.E., Milberg, W.P., Miller, M.W., & Wolf, E.J. (2019). Investigation of bidirectional longitudinal associations between advanced epigenetic age and peripheral biomarkers of inflammation and metabolic syndrome. *Aging*, *11*, 3487-3504.

## PTSD, Metabolic Syndrome, and Reduced Cortical Thickness

Wolf, E.J., Sadeh, N., Leritz, E.C., Logue, M.W., Stoop, T., H. Salat, D.H., McGlinchey, R., Milberg, W. & **Miller, M.W.** (2015). PTSD as a catalyst for the association between metabolic syndrome and reduced cortical thickness. *Biological Psychiatry, 80,* 363–371.

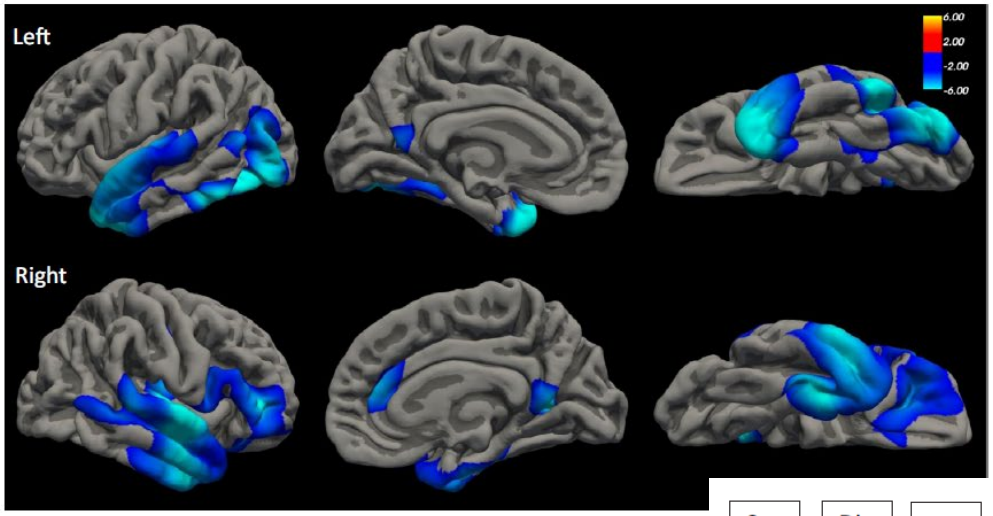
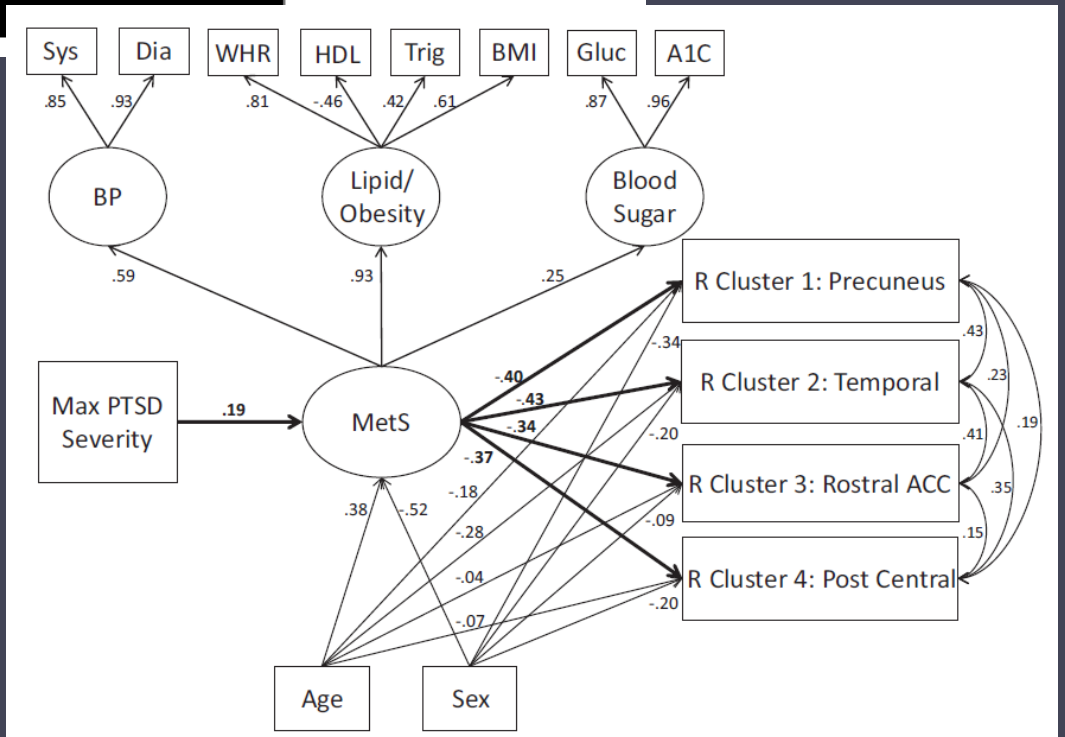
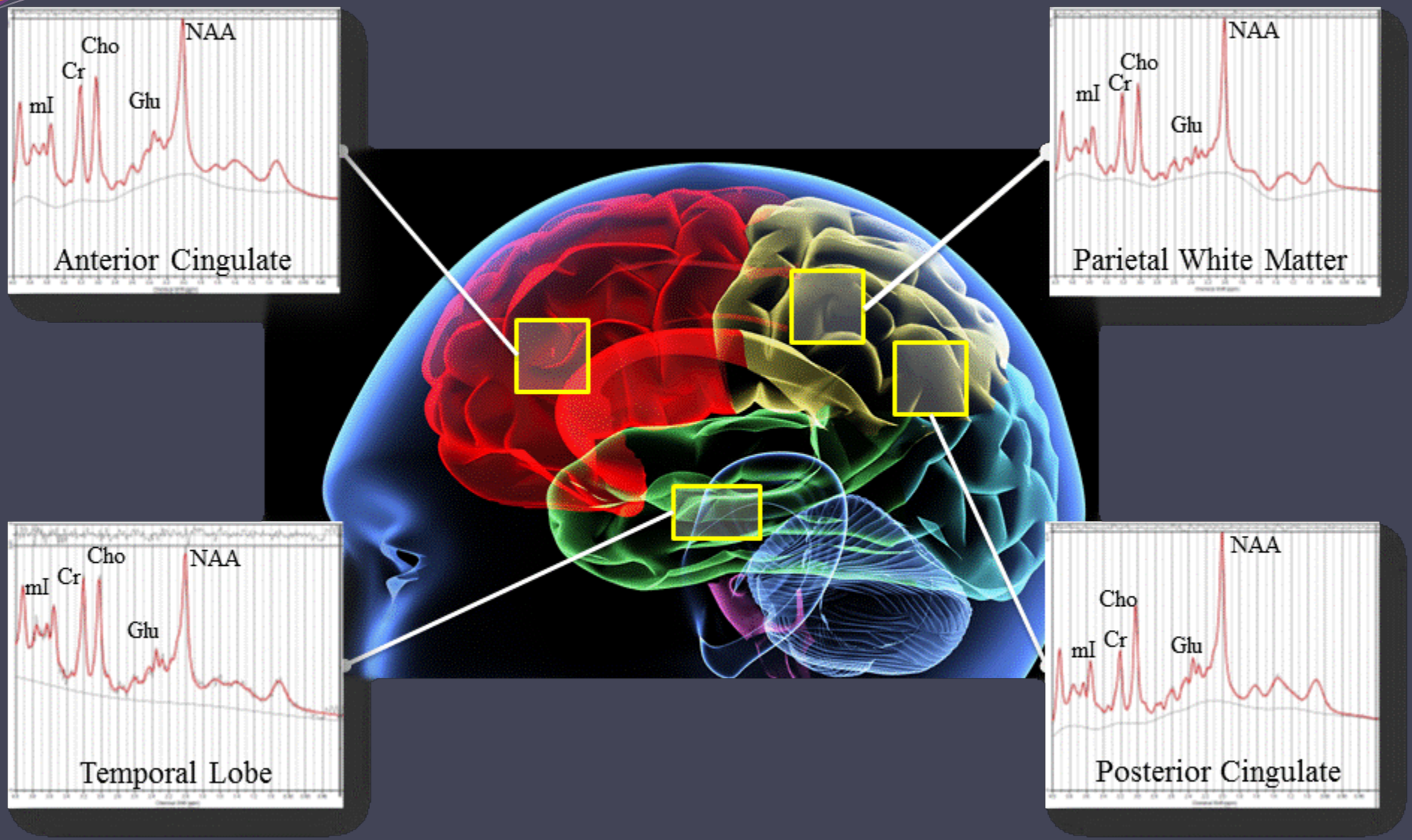



**Figure 1.** Top and bottom panels show the regions of the left and right cortices, respectively, that in whole-brain analysis were associated with metabolic syndrome factor scores, controlling for age and sex. Lateral (left), medial (middle), and ventral (right) slices are shown.

N =346

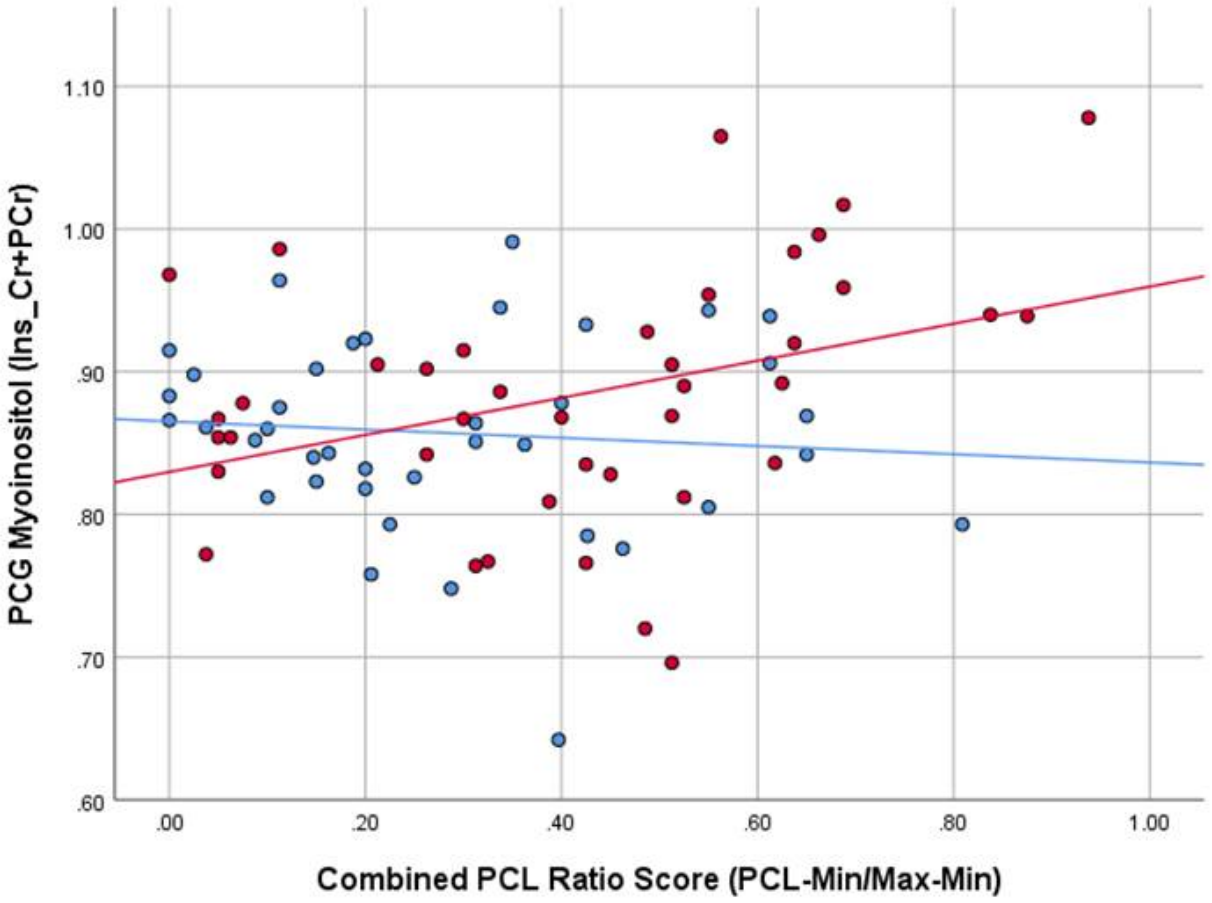


# MR Spectroscopy: The Virtual Biopsy



**Myo-inositol (mI)** is a metabolite present primarily in the inter-cellular solution of glial cells in the brain. When these cells are activated during inflammation, the volume of mI in the cell increases. MRS studies that have found evidence for associations between mI levels and loss of neuronal integrity in a variety of psychiatric and neurodegenerative conditions (for reviews see, Chang et al. 2013; Zahr et al. 2014).

# Myo-inositol levels in the posterior cingulate gyrus as a function of PTSD severity and Age




N=79, p=0.007

*In preparation*

# Conclusions about PTSD Biomarker Research

Panels of relevant biomarkers could aid in PTSD diagnosis and assessment.

There is tension between unbiased discovery- and mechanistic/theory-driven approaches to biomarker discovery.

Blood-based biomarkers may be the most practical solution for biomarker work and technological advances in small molecule assays are opening new avenues for early detection of disease processes and assessing CNS relevant markers in blood.

Many biomarker associations with disease are modified by genetic variation and meaningful associations can be missed if the genetic background of patients are not taken into account.

Biomarkers can inform the development of therapeutics. However, there may always be a disconnect between our diagnostic phenotypes and targets of those therapies.