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TITLE: Epigenetic Patterns of TBI: DNA Methylation in Serum of OIF/OEF Service Members

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14. ABSTRACT

The purpose of our investigation is to elucidate how patterns of DNA methylation may vary by TBI diagnosis and by severity of the disease. TBI cases with existing serum samples housed at the Department of Defense Serum Repository (DoDSR) will be identified by searching the clinical database of TBI patients at Walter Reed Army Medical Center (WRAMC) for those cases who were diagnosed with a non-penetrating blast TBI (mild, moderate, severe), from February of 2003 to present. Personnel at the DoDSR will then search the DMSS database for those members with at least one serum sample taken before their first deployment and one serum sample taken after their first deployment. We will identify an appropriate control group, frequency matched on various demographics of cases. For each TBI case and control, a serum sample drawn prior to and post first OIF/OEF deployment will be identified. DNA will be extracted from each serum sample, and percent methylated cytosine (%mC) quantified in the promoter regions of the following cytokines: (IL)-1β, IL-6, IL-8, IL-10, and cyclin A1 and D3 and in a global nucleotide element. Comparisons will be made between cases (stratified into mild, moderate, and severe) and controls and between pre- and post-deployments for both cases and controls. We will also look at the levels of cytokines in residual serum not needed for DNA extraction. Since the start of thi study we have been going through the WRAMC IRB process, which has been lengthy, in order to carry out the linkage of case IDs between WRAMC and DoDSR. We have applied for a no-cost extension on the study. This study will help to elucidate the molecular sequelae of brain injury and will fuel novel therapeutic approaches to TBI therapy, particularly since modifications in DNA methylation can potentially be reversed.

15. SUBJECT TERMS
TBI, epigenetics, DNA methylation, cytokines

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Introduction

The molecular mechanisms involved in traumatic brain injury (TBI) are not well characterized. From both human and animal studies, we know that a profound inflammatory response is initiated immediately following TBI and is characterized by the expression of several cytokines with both pro- and anti-inflammatory functions. An epigenetic mechanism, DNA methylation is intrinsically linked to the regulation of gene expression. Thus, the purpose of our investigation is to elucidate how patterns of DNA methylation may vary by TBI diagnosis and by severity of the disease. TBI cases with existing serum samples housed at the Department of Defense Serum Repository (DoDSR) will be identified by searching the clinical database of TBI patients at Walter Reed Army Medical Center (WRAMC) for those cases who were diagnosed with a non-penetrating blast TBI (mild, moderate, severe), from February of 2003 to present. Social security numbers, names, date of diagnosis, and the classification of mild, moderate, or severe for each TBI patient identified will be transferred to the Armed Forces Health Surveillance Center (AFHSC). Personnel at AFHSC will then search the DMSS database for those members with at least one serum sample taken before their first deployment and one serum sample taken after their first deployment. We will identify an appropriate control group consisting of randomly selected service members who were never diagnosed with a TBI at WRAMC and who are frequency matched on various demographics of cases. For each TBI case and each control, a serum sample drawn prior to first OIF/OEF deployment and a sample drawn after that deployment will be identified. DNA will be extracted from each serum sample, and percent methylated cytosine (%mC) will be quantified in the promoter regions of the following cytokines, (IL)-1β, IL-6, IL-8, IL-10, and cyclin A1 and D3 and in a global nucleotide element. Comparisons will be made for patterns of DNA methylation between cases (stratified into mild, moderate, and severe) and controls and between pre- and post-deployments for both cases and controls. We will also look at the levels of cytokines in residual serum not needed for DNA extraction. This study will help to elucidate the molecular sequelae of brain injury and will fuel novel therapeutic approaches to TBI therapy, particularly since modifications in DNA methylation can potentially be reversed.

Body:

From the time that this grant was awarded in September, 2008 until December, 2008, the PI was waiting for IRB approval from USU IRB. During that period, the PI also had many discussions with TBI experts at USU and Walter Reed Army Medical Center (WRAMC), who advised her to revise the methodology of identifying cases. In the original narrative, PI proposed that the Armed Forces Health Surveillance Center (AFHSC) would identify cases with specific ICD-9 codes indicating a TBI. This was considered less than optimal, according to many TBI experts at USU and WRAMC (since there is no specific ICD-9 code for TBI, and combining various other trauma codes would run the risk of lacking specificity), who advised PI to try to identify cases via the clinical database of TBI patients at WRAMC. PI contacted Dr. Louis French at WRAMC, who is the Director, Traumatic Brain Injury Service at the Department of Orthopaedics and Rehabilitation at WRAMC to inquire about the possibility of identifiers being sent from the database he maintains directly to AFHSC, so that personnel at AFHSC could pull serum samples on cases identified via this database. The PI would never have access to any personal identifiers, and all transfer of identifying data between WRAMC and AFHSC would be carried out via double password protected, encrypted methods.

Dr. French agreed to this, and PI contacted Army Contracting Officer Representative, Dr. Tammy Crowder to get permission to proceed with this change in methodology of identifying cases. Since this would significantly improve the quality of the study and since it was not outside the scope of the original grant protocol, Dr. Crowder informed the PI that this would be acceptable (telephone communications with Dr. Tamara Crowder on November 21, 2008. PI subsequently commenced IRB submission to WRAMC. USU IRB informed PI that she would need to get WRAMC IRB approval first and then an expedited review would be carried out after that at USU to update the USU approval. PI submitted protocol to WRAMC on 31 January, 2009 as an exempt study request. WRAMC Department of Clinical Investigation (DCI) then informed PI that the review would have to be expedited, as opposed to exempt, so PI prepared another submission, which was finally submitted on 23 April, 2009 (this submission required a lot of additional work and was very lengthy). The protocol was reviewed by the Clinical Investigation Committee on 02 June, 2009. It was then tabled at this meeting, due to extensive comments by the committee on the protocol. PI has addressed each comment and revised the protocol accordingly, and has re-submitted the protocol for a second CIC review. After passing the CIC, the protocol will be forwarded to the Human Use Committee (HUC) at WRAMC. This is the equivalent of the IRB for that institution.

Because of the lengthy WRAMC IRB process, the PI has not been able to make further progress on this study and is, therefore, in the process of requesting a no cost extension. The following is an updated timeline:

- 01 September, 2009: CIC review of protocol (2nd time)
- Sometime in September, 2009: Forward of protocol to HUC
- 15 October, 2009: HUC review of protocol
- 31 October, 2009: HUC approval of protocol
- 15 November, 2009: USU IRB re-approval of protocol
- 30 November, 2009: Linkage of identifiers between WRAMC and AFHSC
- 15 January, 2010: All serum samples from cases and controls sent to USU from AFHSC
- 15 February, 2010: All DNA extracted from serum samples
- Shortly after that, DNA is sent to Epigendx, lab for DNA methylation measurement
- 15 March, 2010: DNA methylation assays and serum cytokine assays completed
- 15 April, 2010: Analysis of data complete
- Summer, 2010: Manuscript drafted and submitted for publication

Key Research Accomplishments: N/A

Reportable Outcomes: N/A

Conclusions: N/A

References: None

Appendices: N/A

Supporting Data: On 12 August, 2009 Project Manager for DVBIC at WRAMC informed PI that after searching the clinical database of TBI patients at WRAMC, it was found to include 852 blast TBI patients. We anticipate that this will be more than sufficient from which to sample our 150 cases with established inclusion criteria.