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<b>14. ABSTRACT</b> The study was designed to identify clinically accessible molecular biomarkers of traumatic brain injury (TBI) prior to definitive clinical diagnosis using high throughput microRNA technology. Specifically, these studies will identify, characterize, and validate microRNA biomarker species whose content in peripheral blood mononuclear cells (PBMC) could help to distinguish TBI cases within Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) veteran population. During the past year, we conducted a Biomarker Discovery study using a high-throughput Array chip platform and identified 18 candidate TBI small RNA biomarkers. Moreover, using independent Q-PCR assays, we confirmed that thirteen (13) of these candidate small RNA biomarker species are, indeed, significantly down-regulated in PBMC of TBI compared to non-TBI control veteran cases. Additionally, we use an unsupervised clustering analysis and demonstrated that differential regulations of these small RNA biomarkers in clinically accessible blood cells are capable of dissecting TBI and non-TBI OEF/OIF veteran cases.					
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## **Personalized medicine in veterans with traumatic brain injuries**

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### **Annual Progress Report – Year 3**

#### **Introduction**

Traumatic brain injury (TBI) is a major casualty identified among veterans deployed to the Persian Gulf region in support of Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF). TBI is caused by one or more concussive insult to the head or penetrating head injury that disrupts the normal function of the brain, leading to either transient or chronic impairments in physical, cognitive, emotional, and behavioral functions. TBI in OEF/OIF veterans are largely the result of concussive injuries from blast-producing weaponry. Overall, veterans have lower attention score, although it is not yet known if mild TBI might contribute to this observation. Nevertheless, veterans from prior conflicts exposed to blasts have shown evidence of mild TBI and attention difficulties when compared to similar veterans without blast exposure. Early diagnosis of chronic TBI is important in preventing further progression of symptoms that can disrupt a veteran's life upon return from service overseas. Mild TBI can be difficult to diagnose, and when coupled with psychological illness, can be either misdiagnosed or missed altogether. Traditionally, physicians and scientists have viewed and interpreted diseases at the 'visual' clinical level. With the advent of genomics and proteomics technologies, personalized medicine offers the promise and potential of uncovering the largely 'unseen' details of disease causality, onset, and progression. The proposed studies are to be conducted in collaboration with The War-related Illness and Injury Study Center (WRIISC), Department of Veteran Affairs, New Jersey Health Care System (DVANJHCS), East Orange, NJ and is designed to identify genomic-microRNA fingerprints from clinically assessable blood cell components as independent biological indexes that will allow us to identify unique molecular indices of *Persistent Postconcussive Syndrome* as well as well as other significant injury-related factors associated with mild TBI in OEF/OIF veterans.

#### **Body**

The study was designed to identify clinically accessible molecular biomarkers of TBI injury prior to definitive clinical diagnosis using high throughput microRNA technology. Specifically, these studies will identify, characterize, and validate microRNA biomarker species whose content in peripheral blood mononuclear cells (PBMC) could help to distinguish TBI injury cases within a veteran population following deployment in support of Operation Enduring Freedom (OEF) or Operation Iraqi Freedom (OIF). The overall study is separated into a Biomarker Discovery study to identify candidate microRNA biomarker, and a Biomarker Validation study to validate the sensitivity and specificity of microRNA biomarkers, either

individually or as panels of multiple biomarkers, to correctly identify TBI and control cases in an independent cohort of TBI and non-TBI veterans.

The WRIISC at East Orange, New Jersey received final Department of Defense (DoD) IRB approval to commence recruitment on July 29, 2009. As we have stated in our Year 2 Annual Progress Report, we initiated volunteer recruitment on October 31, 2009. Our inclusion criteria for recruitment are male and females 18-75 years with or without a history of TBI, who have completed a clinical evaluation at the East Orange, New Jersey WRIISC. Cases with inter-current infections of inflammatory-related conditions are excluded. Classification criteria for TBI cases are positive endorsement on the Defense and Veterans Brain Injury Center (DVBIC) criteria confirmation of injury to the head plus subsequent alteration of consciousness, and Repeatable Battery for Neuropsychological Testing (RBANS) score one standard deviation below the norm for age and education. Classification criteria for Control cases are DVBIC confirmation of no injury to the head and RBANS score less than one standard deviation below the norm.

Starting from October 31, 2009 when recruitment was initiated and continued up to the end of Year 2 of our studies on May 10, 2010, we recruited and collected PBMC specimens from 50 cases, comprised of 8 mild TBI (mTBI) and 42 non-TBI cases. In Year 3 (May 11, 2010 to May 31, 2011), we recruited and collected PBMC specimens from an additional 47 cases (14 mTBI and 33 non-TBI cases). Collectively, we have collected and banked PBMC specimens from a total of 97 veteran volunteers to date, among which 22 (22.7%) are classified as mTBI and 75 (77.3%) are classified as non-TBI (Table I). The proportion of veterans classified as mTBI in our recruited cohort is consistent with, and even slightly above, previous prevalence estimates of 12% (Schneiderman et al., *Am J Epidemiol* 167:1446-52, 2008) reported in a cross-sectional survey of 2,235 active duty, guard and reserve OEF/OIF veterans.

	<u>Total</u>	<u>mTBI</u>	<u>Non-TBI control</u>
Years 1-2	50	8	42
Years 3	47	14	33
<b>Total Years 1-3</b>	<b>97</b>	<b>22</b>	<b>75</b>

***Table I: The number of mTBI and non-TBI veteran cases recruited to date for our proposed studies.***

During Year 3, we conducted an interim microRNA Biomarker Discover analysis using 9 mild TBI and 9 matching non-TBI control cases. Demographic information for individual TBI and non-TBI cases is presented in Table II. We note the average age of mTBI and non-TBI cases used in our interim Biomarker Discovery study is, respectively,  $31.6 \pm 7.0$  and  $29.8 \pm 8.2$  years. The interval between the last deployment and recruitment into this study is  $3.9 \pm 2.7$  and  $2.6 \pm 2.1$  years for the mTBI and non-TBI group, respectively. The mTBI group had an average of  $13.3 \pm 1.3$  years of education and the non-TBI group had an average  $13.0 \pm 2.4$  years of education. There is no significant difference in age, deployment interval or years of education between the mTBI and the non-TBI control group (ttest assessments of mTBI versus non-TBI groups: p-

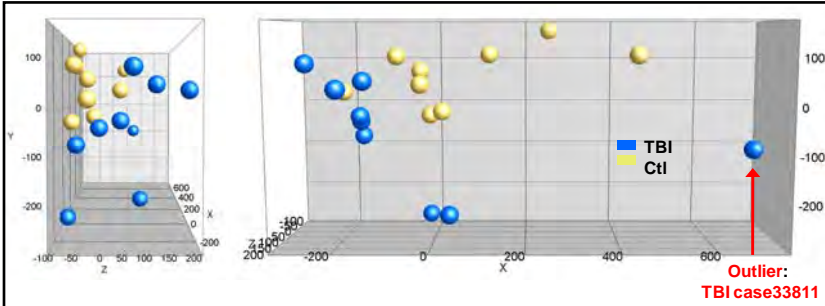
values 0.59 for age, 0.30 for deployment interval, and 0.72 for duration of education). The proportion of males in the mTBI and the non-TBI control group is, respectively 78% and 67%. Lastly, 89% of the TBI veteran cases used in our interim Biomarker Discovery studies are co-morbid with Post-traumatic stress disorders (PTSD), based on a PTSD diagnosis criterion of having a score of 50 or more in the PTSD Checklist – Civilian Version. Thus non-TBI control cases were selected to match for PTSD, with 78% of cases in the non-TBI control group are diagnosed with PTSD.

<u>Case</u>	<u>TBI/ Ctl</u>	<u>Age</u>	<u>Gender</u>	<u>Ethnicity</u>	<u>Interval (yrs) since last deployment</u>	<u>Education (yrs)</u>	<u>Co morbidity PTSD</u>
31529	TBI	38	Male	Black, non- Hispanic	3.0	14	Yes
33297	TBI	41	Male	Native American	4.3	12	Yes
33825	TBI	31	Male	Black, non- Hispanic	3.4	16	Yes
33828	TBI	42	Male	Black, non- Hispanic	0.7	14	Yes
33888	TBI	27	Female	White Hispanic	4.5	14	Yes
33931	TBI	23	Male	White Hispanic	1.2	12	Yes
33947	TBI	25	Female	Black, non- Hispanic	2.8	13	No
33981	TBI	32	Male	White Hispanic	10.2	13	Yes
33811	TBI	27	Male	Black, non- Hispanic	4.8	12	Yes
31705	Non TBI Ctl	27	Male	Black, non- Hispanic	3.4	12	No
33565	Non TBI Ctl	25	Male	White Hispanic	1.1	12	Yes
33578	Non TBI Ctl	30	Female	White Hispanic	4.3	16	Yes
33596	Non TBI Ctl	35	Male	White Hispanic	3.8	16	Yes
33598	Non TBI Ctl	49	Male	White Hispanic	0.7	9	Yes
33821	Non TBI Ctl	26	Female	White Hispanic	0.2	16	No
33834	Non TBI Ctl	24	Male	White Hispanic	2.8	12	Yes
33913	Non TBI Ctl	22	Female	Black, non- Hispanic	6.5	12	Yes
33930	Non TBI Ctl	30	Male	Black, non- Hispanic	1.0	12	Yes

**Table II: Demographic characteristics of mTBI and non-TBI control cases we used in our interim Biomarker Discovery study.** TBI diagnosis is based on positive endorsement on the Defense and Veterans Brain Injury Center (DVBIC) criteria confirmation of injury to the head plus subsequent alteration of consciousness, and Repeatable Battery for Neuropsychological Testing (RBANS) score one standard deviation below the norm for age and education. Non-TBI control classification is based on DVBIC confirmation of no injury to the head and RBANS score less than one standard deviation below the norm. PTSD diagnosis is based on a score of 50 or more in the PTSD Checklist – Civilian Version. Average age: mTBI group, 31.6±7.0 yrs; non-TBI group, 29.8±8.2 yrs. Interval between their last deployment and recruitment into this study: mTBI group, 3.9±2.7 yrs; non-TBI group 2.6±2.1 yrs. Average duration of education: mTBI group, 13.3±1.3 ; non-TBI group, 13.0±2.4 yrs. Percent of male: mTBI group, 78%; non-TBI group, 67%. Percent of cases with co-morbid PTSD: mTBI group, 89%; non-TBI group, 78%.

In our interim microRNA Biomarker Discovery study, we used the Affymetrix Human gene 1.0 ST Array chip as a high-throughput platform to analyze the expression profile of 1500 small RNAs, including microRNA as well as small nucleolar RNA, small cytoplasmic RNA and ribosomal RNA. We detected from our human PBMC specimens a total of 428 small RNA species: 190 microRNAs, 220 small nucleolar RNAs, 8 small cytoplasmic RNAs and 10 ribosomal RNAs. Based on a principal components analysis of all signals detected, we observed

that one TBI case (case #33811) might be an outlier (Fig. 1). Due to this and the fact that the quality of RNA extracted from this case was poor, we decided to remove case #33811 from subsequent statistical analysis.



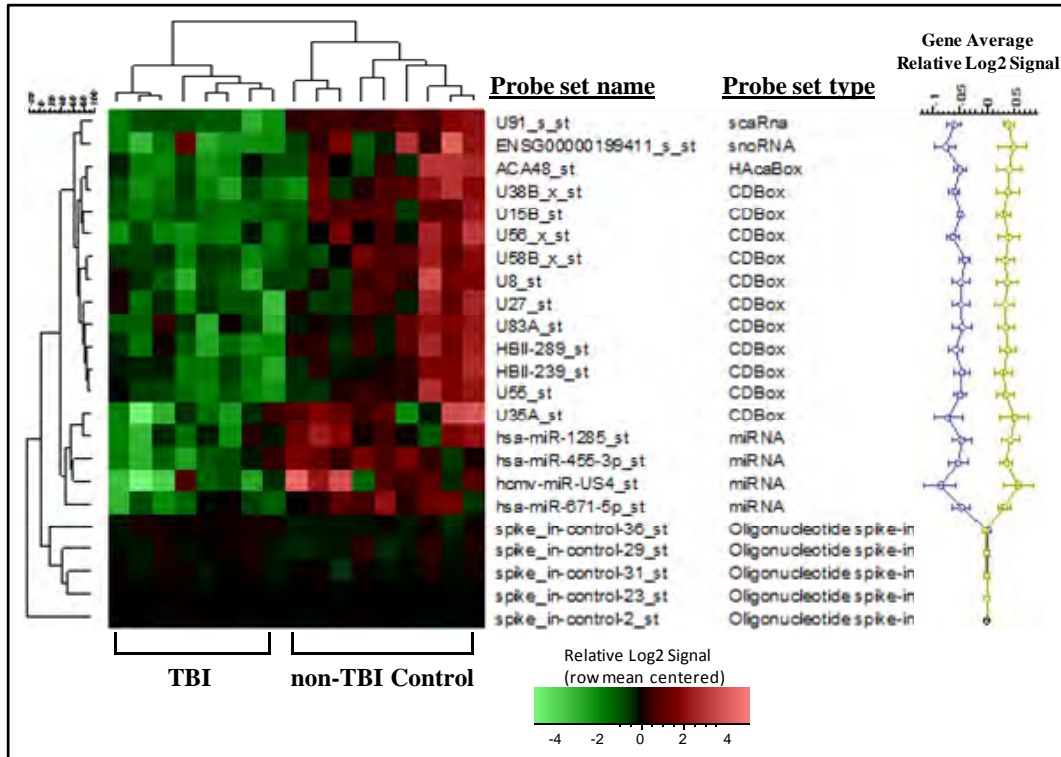
**Figure 1: Principal components analysis of PBMC small RNAs from TBI and control cases.** Small RNA expression profiles for individual cases were assessed by a high-throughput Affymetrix Human gene 1.0 ST Array chip platform, which

detected 428 small RNAs from PBMC specimens. Signals from all small RNA detected for each of the 9 TBI and 9 control case were summarized into single points (represented by balls) plotted on a 3-dimensional plot. Blue and yellow balls represent, respectively, TBI and control cases. The analysis revealed all cases can be clustered into a TBI or a control group, with the exception of TBI case #33811 (indicated by a red arrow), which is plotted far away from the TBI cluster. We concluded case #33811 is an outlier and we removed this case from subsequent statistical analysis.

Statistical analysis to identify candidate TBI biomarkers were conducted using 8 TBI (minus case #33811) and 9 non-TBI control cases. Two criteria were used to identify candidate small RNA biomarkers for TBI: 1) group changes (TBI vs. control groups) must be associated with a magnitude of  $\geq 1.5$ -folds, and 2) group changes must be statically significant with  $p < 0.05$ , based on t-test analysis followed by the application false discovery rate corrections for multi-sampling errors. We identified 18 candidate small RNA biomarkers meeting both criteria that are all significantly down-regulated in TBI versus control cases: 4 microRNAs, 13 small nucleolar RNAs and 1 small cytoplasmic RNA. In an unsupervised clustering analysis using RNA expression data generated from the high-throughput gene chip platform for the 18 candidate small RNA biomarker, we were able to correctly segregate all 17 TBI and control cases analyzed in this interim study (Fig. 2, below).

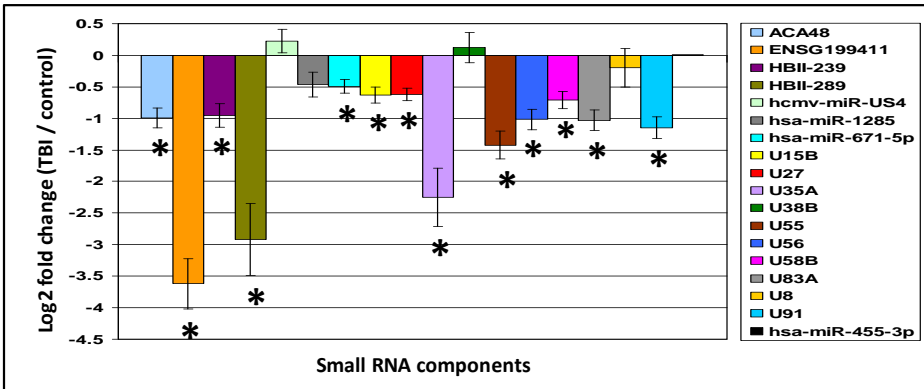
We next used an independent quantitative real time polymerase chain reaction (Q-PCR) procedure to assess the expression of individual candidate small RNA biomarkers in PBMC specimens from the same 9 TBI and 9 non-TBI control cases use used in our high-throughput biomarker discovery studies. We designed a specific Q-PCR primer set for each of the 18 candidate small RNA biomarker and validated the specificity and selectivity of these primer sets for quantitative assessments of their respective small RNA targets (data not shown). Thereafter, we conducted Q-PCR studies and assessed the content of individual candidate small RNA biomarkers in PBMC of TBI compared to non-TBI control cases. Results from our Q-PCR studies confirmed that 13 of the 18 candidate small RNA biomarkers are, indeed, differentially regulated in the PBMC of TBI compared to non-TBI veteran cases (Fig. 3, below). The 13 confirmed small RNA biomarkers include 12 small nucleolar RNA (ACA48, ENSG199411,

HBII-239, HBII-289, U15B, U27, U35A, U55, U56, U58B, U83A, U91) and 1 miRNA (Hsa-miR-671-5p) (Fig. 3). Each of the 13 confirmed small RNA biomarkers are found in significantly lower levels in PBMC specimens from TBI, compared to non-TBI control veteran cases (Fig. 3, below).



**Figure 2: Unsupervised clustering analysis of 18 candidate small RNA TBI biomarker species.** The 18 differentially-regulated small RNAs identified from interim high-throughput Array Chip analysis of 8 TBI and 9 control cases are subjected to unsupervised hierarchical clustering analysis using the UPGMA algorithm with cosine correlation as the similarity metric. Results are present as a heat map (left panel) demonstrating that the panel of 18 small RNA biomarker species is able to correctly segregate TBI from control case. Names for each of the small RNA biomarker species are identified under “Probe Set Name”. Small RNA classes (and subclasses) these 18 differentially-regulated TBI biomarkers belonging to are shown under “Probe Set Type”. Vertical dendrogram (right panel) presents average (+/- SD) signal detections from TBI versus control groups for each of the 18 candidate small RNA biomarkers and confirmed divergent regulations of the biomarkers in PBMC specimens from TBI vs. control groups. Differential regulations of the 18 candidate biomarkers likely reflect true biological effects and not systematic experimental artifact(s) since there is no observable group differences for the detection of spike-in control oligonucleotides in all 17 OIF/OEF veteran cases analyzed (see heat map and vertical dendrogram). Abbreviations: miRNA, microRNA; snoRNA, small nucleolar RNA; C/D Box, the C/D box subclass of small nucleolar RNA; HAc Box, the HAc Box subclass of small nucleolar RNA; scaRNA, small cytoplasmic RNA.



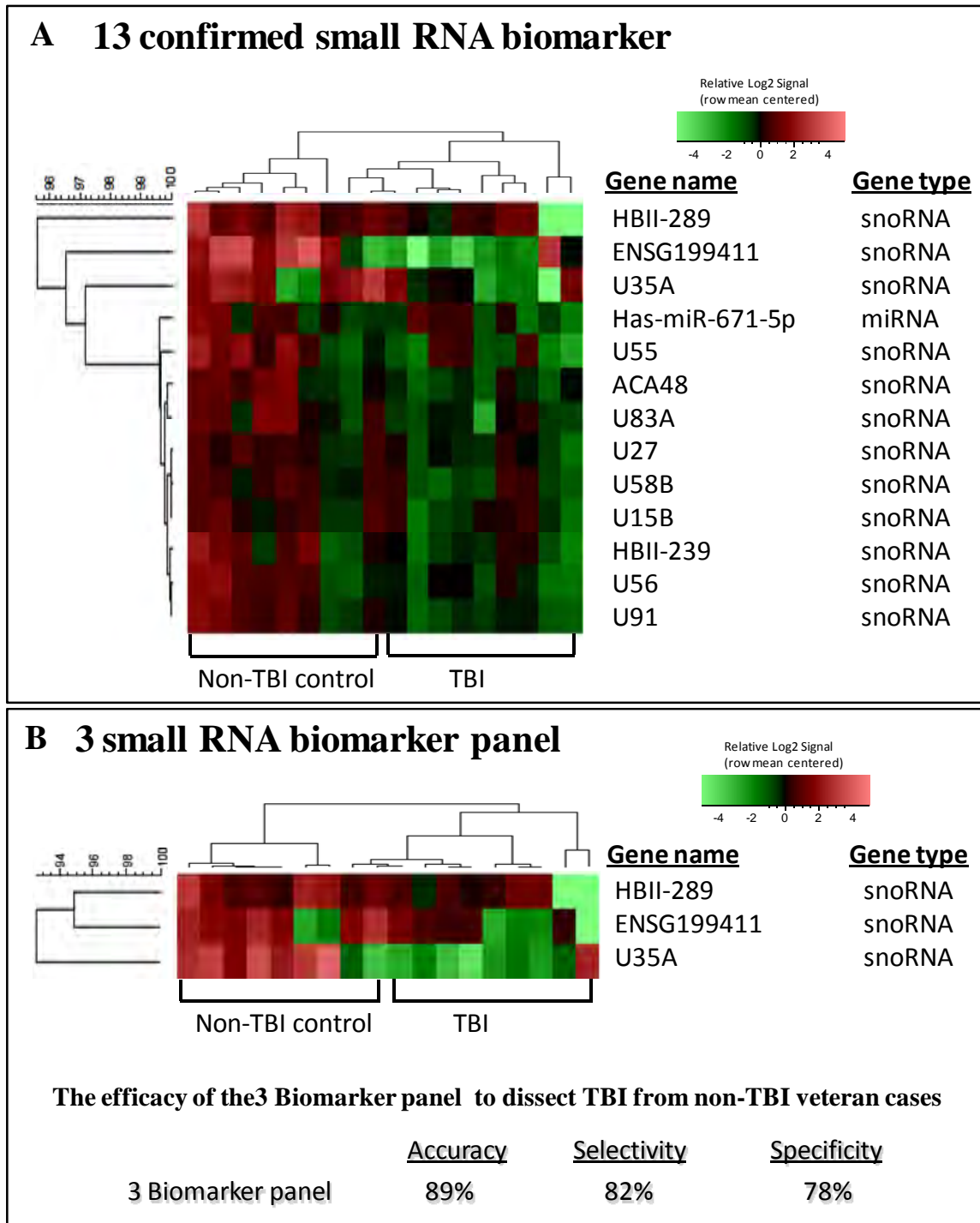


**Figure 3: Independent quantitative real time polymerase chain reaction (Q-PCR) assays confirmed 13 small RNA TBI biomarkers are differentially regulated in PBMC of TBI relative to non-TBI control cases.** PBMC contents for each of the 18 candidate small RNA biomarkers identified by the high-throughput Array Chip platform in Figure 2 were quantitatively assessed using independent Q-PCR assays. The same 9 TBI and 9 non-TBI cases (Table I) we used in our initial high-throughput biomarker discovery assay were assessed in this Q-PCR Biomarker confirmation studies. Bar graphs represent mean small RNA biomarker contents in the TBI group relative to the non-TBI control group; error bars represent standard errors. \* False discovery rate-corrected P-value < 0.05. Q-PCR confirmed 13 small RNA biomarker species are significantly down-regulated in PBMC of TBI compared to non-TBI control veteran cases. These 13 confirmed small RNA biomarkers include 12 small nucleolar RNA (ACA48, ENSG199411, HBII-239, HBII-289, U15B, U27, U35A, U55, U56, U58B, U83A, U91) and 1 miRNA (Has-miR-671-5p) species.

Based on results from our Q-PCR biomarker confirmation studies, we next assessed the value of the 13 confirmed small RNA biomarkers as a criterion to correctly diagnose TBI versus non-TBI veteran cases. Using an unsupervised clustering analysis, we found the 13 confirmed TBI biomarker effectively segregated the cases into correct TBI and non-TBI control groups, with the exception that 2 of the non-TBI cases were incorrectly identified as TBI (Fig. 4A). We continue application of unsupervised clustering analysis to test the efficacy of individual or combination of Q-PCR confirmed biomarkers to correctly segregate TBI and non-TBI control cases. Outcomes from these analyses led to the identification of a 3 small nucleolar biomarker panel, comprised of HBII-289, ENSG199411 and U35A, which is capable of distinguishing TBI from non-TBI veteran cases with 89% accuracy, 82% selectivity and 78% specificity (Fig. 4B).

Collectively, our studies to date have identified a panel of 13 clinically accessible small RNA TBI biomarkers, encompassing 12 small nucleolar RNA and 1 microRNA. Using Q-PCR, we have independently confirmed each of these biomarkers is significantly down-regulated in PBMC specimens from TBI compared to non-TBI control veteran cases. PTSD is commonly co-morbid with TBI in OEF/OIF veterans. We note the majority of TBI in our biomarker study are co-morbid with PTSD and that our non-TBI control cases are selected to match for PTSD diagnosis. Thus our identified panel of 13 small RNA biomarkers likely represents biological indices selective for TBI. Moreover, TBI cases in our biomarker discovery studies were recruited after an average interval of 3.9 years following their last deployment (deployment-to-recruitment

interval: ranging from 0.7 to 10.2 years, with a median interval of 3.4 years) (Table I). Thus, changes in the regulation of these small RNA TBI biomarkers we observed are not acute TBI responses, but likely represent long-term physiological consequences subsequent to TBI.



**Figure 4: The content of small RNA biomarkers in clinically accessible PBMC provides a sensitive and specific criterion for dissecting TBI from non-TBI veteran cases.**

We tested the role of the Q-PCR 13 confirmed small RNA TBI biomarkers as a criterion for distinguishing TBI from non-TBI veteran cases. Biomarker contents in banked PBMC specimens from the same 9 TBI and 9 non-TBI cases (Table I) we used in our biomarker discovery studies were quantified by Q-PCR. (A, B) The efficacy of using biomarker contents from clinically accessible PBMC as a criterion to correctly segregate TBI and non-TBI cases was tested by unsupervised clustering analysis using the UPGMA algorithm with cosine correlation as the similarity metric. Results are presented as heat maps demonstrating the efficacy of using all 13 small RNA biomarkers (A) or using a panel of three small nucleolar RNA biomarkers (B) to correctly segregate TBI from control cases. (B) A three small nucleolar RNA biomarker panel (HBII-289, ENSG199411 and U35A) is capable of distinguishing TBI from non-TBI cases with 89% accuracy, 82% selectivity and 78% specificity. (Accuracy is the percentage of all TBI and non-TBI subjects that are correctly identified; sensitivity is the probability that a case identified as TBI actually is a TBI case; specificity is the probability that a case that a case identified as non-TBI cases is actually a non-TBI case.) Abbreviations: snoRNA, small nucleolar RNA; miRNA, microRNA.

The pathological implications of our observation that select small nucleolar RNA and microRNA are differentially regulated in the PBMC of TBI relative to non-TBI control veteran cases is currently unknown. Small nucleolar RNA and microRNA are members of a family of non-coding RNAs that are involved in many physiological cellular processes and are also known to contribute to molecular alterations in pathologic conditions (Galasso et al., *Genome Med* 2(2):12, 2010). Small nucleolar RNAs are short RNA sequences comprised of ~60-220 nucleotides. They are primarily known for their role as guide molecules for site-specific methylation and pseudouridylation of other RNAs, particularly rRNA, as well as tRNA and small nuclear RNAs. These chemical alterations are required for proper rRNA processing and ribosome functions as well as for proper function of the spliceosome (Holley and Topkara, *Cardiovasc Drug ther* 25(2):151-9, 2011). MicroRNA are short (~22 nucleotides) RNA sequences that binds to complementary sequences on target mRNA, thereby blocking translation or promoting degradation of target mRNA (Holley and Topkara, *Cardiovasc Drug ther* 25(2):151-9, 2011).

Small nucleolar RNA and microRNA are expressed in the brain and both classes of small RNAs have been implicated in neuroplasticity mechanisms and neurological disorders. For example, recent evidence suggests a role for the HBII52 small nucleolar RNA in regulating alternative splicing of the serotonin 2c receptor (Doe et al., *Hum Mol Genet* 18(12):2140-8, 2009), and that patients with autism and Prader-Willi-like characteristics are found to have reduced levels of HBII52 in the brain (Hogart et al., *J Med Genet* 46(2):86-93, 2009). The microRNA miR132 is induced by neuronal activity and neurotrophins in a CREB-dependent manner and plays a role in regulating neuronal morphology and cellular excitability (Lambert et al, *PLoS One* e 5(12):e15182, 2010). Moreover, preclinical evidence in rodent models demonstrated that small RNA expression in the brain is affected by TBI. Redell et al. (*J Neurosci Res* 89(2):212-21, 2011) reported transient elevated expression of the microRNA miRNA-21 in rats following an impact injury to the brain. Using a high-throughput Array Chip platform, Lei et al. (*Brain Res* 1284:191-201, 2009) reported potential aberrant up- or down-expression of 203 miRNA species in the rat cerebral cortex up to 72 hrs following fluid percussion injury to the brain. Redell et al (*J Neurosci Res* 87(6):1435-48, 2009) also identified potential up regulation of

35 and down-regulation of 50 microRNA species in the hippocampus of rats within 72 hrs following an impact TBI to the brain; altered regulations for a smaller subset of 8 (4 up-regulated and 4 down-regulated) microRNA species in the hippocampus were subsequently confirmed by independent Q-PCR.

It is possible that altered regulation of select small nucleolar RNA and microRNA we observed in PBMC of veteran TBI cases might have implications in the central nervous system. Genes relevant to neural circuits, synapses and neural plasticity processes are also expressed in circulating blood cells, such as PBMC. Thus, significant down-regulation of select small RNA biomarkers we observed in PBMC specimens from our veteran TBI population long after their deployment might reflect long-term molecular alterations in the central nervous system contributing to the onset and progression of clinical TBI phenotypes.

Based on outcomes from our Biomarker Discovery studies identifying and confirming 13 small RNA biomarkers from our Biomarker Discovery cohort, we are continuing with our Biomarker validation studies to test the value of these biomarkers to correctly diagnose TBI and non-TBI cases in a new independent cohort of veterans as we have proposed. To date we have 8 TBI and 55 non-TBI control cases available for our Biomarker Validation study (Table III). The number of TBI cases we currently have is not sufficient for our proposed Biomarker Validation studies. As shown in Table III, we require a total 23 TBI and 23 age- gender-matched non-TBI controls for our Biomarker Validation studies. We therefore will need an additional 15 TBI cases for our Biomarker Validation Studies (Table III). Based on our estimation that 25% of the recruits will be identified as TBI, we anticipate we will need to recruit a total of 60 new cases (comprised of 15 TBI and 45 non-TBI control cases) for the Biomarker Validation Cohort before we will have sufficient TBI cases for Biomarker Validation studies (see Table III).

	TBI	Non-TBI control
Cases used in Biomarker Discovery studies	9	9
Cases available for Biomarker Discovery studies	8	55
60 new cases needed to be recruited for Biomarker Validation studies	15	45
Total number of cases that will be available for Biomarker Validation studies	23	100
Total number of cases that will be use for Biomarker Validation studies	23	23

**Table III: Summation of case for our Biomarker Validation studies.** As indicated, we have used 9 TBI and 9 non-TBI cases in our Biomarker Discovery analysis. We currently have 8 TBI and 55 non-TBI control cases for Biomarker Validation studies. We need to recruit 60 new Biomarker Validation cases, which we anticipate will be comprised of 15 TBI and 45 non-TBI control cases. Thus, we will have available 23 TBI and 100 non-TBI cases from

which we will use 23 TBI and 23 age-, gender-matched non-TBI control cases for our final Biomarker Validation studies.

As we approached the end of the project period for our proposed studies, we applied for a one year no-cost extension so we can continue to recruit OIF/OEF veteran cases and complete our proposed Biomarker Validation studies. We received official approval for a one-year no-cost extension on April 13, 2011, allowing us to continue with our proposed studies from May 01, 2011 to April 30, 2012. We are now continuing our recruitment for a final 23 TBI veteran cases. Once we have banked PBMC specimens from our targeted Biomarker Validation cohort of 23 TBI and 23 non-TBI control cases, we will use Q-PCR to quantify PBMC contents for each of the 13 TBI biomarkers we identified from our Biomarker Discovery studies. We will then test the value of TBI biomarkers, either individually or in combinations, to correctly diagnose TBI and non-TBI cases in this new cohort of OEF/OIF veteran as have originally proposed. Since we do not have sufficient funds to complete the final phase of our proposed studies, we have submitted a Plus-Up cost-extension request to the CDMRP for additional funding consideration which is necessary to finalize our proposed Biomarker Validation studies.

Outcomes from our combined Biomarker Discovery and Validation studies will lead to improved TBI detection in OEF/OIF veterans, more sensitive outcome measurement for future clinical trials, a better understanding of the biological mechanisms underlying concussive TBI, and insights into novel therapeutic targets for OEF/OIF veterans with TBI.

### **Key Research Accomplishments**

1. We conducted Biomarker Discovery studies using a high-throughput Array chip platform and identified 18 candidate TBI small RNA biomarkers.
2. Using independent Q-PCR assays, we confirmed that 13 of these candidate small RNA biomarker species are, indeed, significantly down-regulated in PBMC of TBI compared to non-TBI control veteran cases.
3. Using unsupervised clustering, we found that differential regulations of these small RNA biomarkers in clinically accessible blood cells are capable of dissecting TBI and non-TBI OEF/OIF veteran cases.
4. We identified a 3-biomarker panel capable of distinguishing TBI from non-TBI control veteran cases with 89% accuracy, 82% selectivity, and 78% specificity.
5. The majority of TBI in our biomarker study are co-morbid with PTSD and our non-TBI control cases are selected to match for PTSD diagnosis. Thus, our identified panel of 13 small RNA biomarkers likely represents biological indices selective for TBI.

### **Reportable Outcomes**

N/A

### **Conclusion**

Our Biomarker Discovery studies to date has identified panel of small RNA TBI biomarkers whose contents in clinically accessible blood cells provides a criterion for correct diagnosis of TBI from non-TBI veteran cases with high accuracy, specificity and selectivity. The majority of TBI in our biomarker study are co-morbid with PTSD and our non-TBI control cases are selected to match for PTSD diagnosis. Thus our identified panel of 13 small RNA biomarkers likely represents biological indices selective for TBI. As we have proposed in our original study design, our ongoing studies are continuing to validate the values of these biomarker as surrogate

biological indices of TBI in an independent Biomarker Validation study cohort TBI and non-TBI OIF/OEF cases.

**References**

N/A

**Appendices**

N/A