Psychiatry Research 251 (2017) 261–265

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Circulating miRNA associated with posttraumatic stress disorder in a cohort of military combat veterans

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ARTICLE INFO

Keywords:
PTSD
Micro RNA
Biomarker

ABSTRACT

Posttraumatic stress disorder (PTSD) affects many returning combat veterans, but underlying biological mechanisms remain unclear. In order to compare circulating micro RNA (miRNA) of combat veterans with and without PTSD, peripheral blood from 24 subjects was collected following deployment, and isolated miRNA was sequenced. PTSD was associated with 8 differentially expressed miRNA. Pathway analysis shows that PTSD is related to the axon guidance and Wnt signaling pathways, which work together to support neuronal development through regulation of growth cones. PTSD is associated with miRNAs that regulate biological functions including neuronal activities, suggesting that they play a role in PTSD symptomatology.

1. Introduction

Posttraumatic stress disorder (PTSD) is a debilitating anxiety disorder with a disproportionate disease burden on military service members who deploy to combat, with rates between 10–30% (Cohen et al., 2010; Tanielian and Jaycox, 2008). PTSD is triggered by a life-threatening event, such as combat, resulting in the onset of symptoms including intrusion, avoidance, negative alterations in cognition and mood, and alterations in arousal and reactivity ("Trauma- and Stressor-Related Disorders", 2013). Deployment itself can be a period of extreme stress which often includes unsafe living environments and physical injuries, placing individuals at high risk for PTSD. Additionally, military service members in recent conflicts have been at high risk for traumatic brain injuries (TBIs), as well as co-morbid symptoms of depression and sleep disorders along with PTSD (Kang et al., 2015). Mild TBI (mTBI), a subtype of TBI classified by the Department of Defense as a non-penetrating TBI with a loss of consciousness from 0 to 30 min, is by far the most common type of TBI experienced by service members, with mTBIs accounting for 82.4% of DoD reported TBIs in 2015 (2015 DoD TBI Worldwide Numbers, 2016). The cumulative impact of deployment related disorders is estimated to be more than $3 billion annually when considering only reduced productivity and missed work (Zhou et al., 2014). However, our ability to prevent the onset of PTSD and to treat it once symptoms present is limited, based in large part on our minimal understanding of the biological mechanisms related to the onset and maintenance of this disorder. Due to the complex biological nature of PTSD, investigating the molecular networks involved may provide valuable information on the pathogenesis of the disorder and therapeutic targets (Neylan et al., 2014). Although promising biomarkers have been proposed, due to the multifaceted and heterogeneous nature of the disorder, a variety of biomarkers likely reflect separate aspects of mechanisms associated with PTSD (Jergovic et al., 2015; Michopoulos et al., 2015). Thus, the exploration of novel biomarkers can expand our understanding of the biological processes effecting the disorder. Previous biomarker studies indicate the utility of using peripheral blood for approximating biological mechanisms within the central nervous system (Hayashi-Takagi et al., 2014; Kang et al., 2015), which is essential in clinical populations where obtaining cerebral spinal fluid is not plausible.

microRNAs (miRNAs) provides a new opportunity to understand biological underpinnings of PTSD. miRNAs, which are short, noncoding sequences that typically regulate gene expression by suppressing protein coding mRNA, have been demonstrated to be biomarkers for several psychological disorders, including schizophrenia, major depressive disorder, and bipolar disorder (Kichukova et al., 2016; Maffioletti et al., 2016; Schmidt et al., 2015). Within the central nervous system, miRNA are considered to play a major role in gene expression regulation (Kuss and Chen, 2008; Martinetz, 2016); however, the specific role of miRNAs in PTSD remains largely unknown. Previous studies link differential gene expression in the peripheral blood to

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http://dx.doi.org/10.1016/j.psychres.2017.01.081
Received 24 August 2016; Received in revised form 12 December 2016; Accepted 28 January 2017
Available online 31 January 2017
0165-1781/ Published by Elsevier Ireland Ltd.

PTSD in multiple clinical populations, including military and civilians (Glatt et al., 2013; Neylan et al., 2011; Segman et al., 2005; Tylee et al., 2015; Zieker et al., 2007), indicating that altered activity of genes relates to PTSD. Preclinical models of PTSD like behaviors have linked circulating miRNAs to neuronal activity, suggesting circulating miRNA in the periphery provide vital insights into central activity related to PTSD, and may serve as a potential biomarker (Balakathiresan et al., 2014). In an array-based clinical study, 18 dysregulated miRNA in veterans with PTSD have been reported, including miR-125a, which target genes in immune response pathways (Zhou et al., 2014). Two other downregulated miRNA, miR-3130-5p and miR-212, which have previously been implicated in other neurological disorders, were identified in subjects with PTSD and high rates of depression through the use of RNA sequencing (Wingo et al., 2015). By identifying miRNAs with differential expression in subjects with PTSD, necessary insights into gene networks that relate to PTSD symptomatology may be identified, which will ultimately improve our understanding of the biological mechanisms involved in the etiology of PTSD and the necessary insights into pharmacological targets.

To better understand the role of miRNAs in PTSD symptoms, we compared military personnel with and without PTSD using next generation sequencing of total miRNA from blood samples. This approach was selected for its unbiased ability to identify miRNA related to PTSD without being limited to predicted miRNA of interest. We tested the hypothesis that expression of miRNA in peripheral blood of military personnel with PTSD would significantly differ from combat matched controls without PTSD.

2. Methods

2.1. Participants

In this study, a sample of military personnel (24 males, age 33.25 years ± 7.55) from a larger study on sleep (findings previously reported (Mysliwiec et al., 2013)) were evaluated for PTSD. Active duty military personnel who had returned from deployment with Operation Enduring Freedom or Operation Iraqi Freedom within 18 months were eligible for participation. Demographic characteristics analyzed in this study included age, race, education, body mass index (BMI), deployment history, and medication use. Medications that participants were taking at the time of their evaluation were assessed including antidepressants, atypical antipsychotics, benzodiazepines, non-benzodiazepine receptor agonists (NBDRA), prazosin, and narcotics.

2.2. Assessment for PTSD, mTBI, and depression

PTSD symptoms were assessed at two time points using the PTSD Checklist Military Version (PCL-M), which has 17 symptom items that are consistent with the DSM-IV-TR. A score of 50 or higher on the PCL-M is accepted to approximate a clinician assessed diagnosis of PTSD for purposes of informing diagnosis in high risk populations (Betthauser et al., 2012; Forbes et al., 2001). Based on the symptom inventory, subjects were classified into the PTSD (PCL-M > 50) or control group (PCL-M < 50). The Warrior Administered Retrospective Casualty Assessment Tool (WARCAT) was used to determine if a TBI occurred during the most recent deployment. To be included as a control, participants could not have any reported concussion or other head injury within the previous two years. Depression symptoms were assessed using the 16 item Quick Inventory of Depressive Symptomatology (QIDS) questionnaire (Trivedi et al., 2004).

2.3. Blood collection and miRNA sequencing

Blood samples were collected into PAXgene blood RNA tubes and processed with PAXgene blood miRNA kit (Qiagen, Valencia, USA) for miRNA extraction. Quality and quantity of extracted RNA was assessed using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and the Agilent Bioanalyzer 2100 Small RNA assay (Agilent Technologies, Inc., Santa Clara, USA). All samples were enriched for miRNA using standard recommendations for preparation of small RNA libraries protocol. Barcoded libraries were prepared with an input of 1–5 ng of enriched small RNA using the Ion Total RNA-Seq Kit v2 (Life Technologies, Carlsbad, USA). Template-positive Ion PGM™ Ion Sphere™ Particles were prepared for sequencing using Ion PGM™ Template OT2 200 Template Kit v2 with pooled libraries optimized to 14pM. Libraries were sequenced on the Ion Torrent PGM™ using the Ion 316 Chip v2 (Life Technologies, Carlsbad, USA).

2.4. Data analysis

Sequencing reads were generated and initially processed by Ion Torrent Suite software version 4.4.2. Low quality reads and polyclonal sequences were filtered from sequencing data, then raw data was trimmed to 22 base pairs from 5´ end and profiles were generated by aligning sequences to hg19 with BWA; then, we quantified using miRDeep2 v2.0.0.7 and annotated based on miRBase v20. The miRNA-seq data was deposited to GEO under GSE87768. Differential expression analysis was conducted on DESeq2 based on the False Discovery Rate (FDR) by Benjamini and Hochberg multiple testing correction method (Benjamini and Hochberg, 1995). Target prediction and analysis was conducted using the miRWalk2.0 database.

3. Results

3.1. Demographics

There were no significant differences between age, race, education, BMI, number of deployments, or medication use of PTSD and control subjects (Table 1). At baseline, PCL-M scores for control subjects ranged from 17 to 24 (M=20.44, SD=2.24), while scores for PTSD subjects ranged from 52 to 82 (M=64.87, SD=8.62). The only group differences between control and PTSD subjects were mTBI exposure and depression symptoms, with PTSD subjects having higher incidence of mTBI and higher depression symptoms.

3.2. miRNA sequencing

Of the 2578 human mature miRNAs quantified, 8 miRNA sequences were differentially expressed in PTSD participants compared to controls using a corrected threshold of FDR≤.05 (Table 2). These included 4 significantly upregulated miRNA, with a fold change greater

Table 1
Demographics and clinical characteristics.

	PTSD *n*=15	No-PTSD Control *n*=9	x^2/t	P
Age: mean (SD)	31.53 (8.433)	36.11 (4.99)	1.473	0.155
Race: *n* Caucasian (%)	7 (46.7)	5 (55.6)	0.046	0.582
Years of Education: mean (SD)	13.27 (1.53)	13.78 (2.59)	0.611	0.547
BMI: mean (SD)	29.32 (3.98)	31.17 (3.50)	1.152	0.262
Number of Deployments: mean (SD)	2.13 (1.13)	2.56 (1.42)	0.806	0.429
Medication Use: *n* (%)	8 (53.3)	4 (44.4)	0.178	0.5
PCL-M Score: mean (SD)*	64.87 (8.62)	20.44 (2.24)	−18.909	< 0.001
QIDS Score: mean (SD)*	16.67 (2.77)	4.56 (2.96)	−10.11	< 0.001
mTBI: *n* (%)*	8 (53.3)	0 (0)	7.2	0.009