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Neuronal DNA Methylation Profiling of Blast-Related Traumatic Brain Injury

Fatemeh Haghighi,[1–3] Yongchao Ge,[4] Sean Chen,[2] Yurong Xin,[2] Michelle U. Umali,[2] Rita De Gasperi,[3,5,6] Miguel A. Gama Sosa,[3,5,6] Stephen T. Ahlers,[7] and Gregory A. Elder[3,4,5,8]

Abstract

Long-term molecular changes in the brain resulting from blast exposure may be mediated by epigenetic changes, such as deoxyribonucleic acid (DNA) methylation, that regulate gene expression. Aberrant regulation of gene expression is associated with behavioral abnormalities, where DNA methylation bridges environmental signals to sustained changes in gene expression. We assessed DNA methylation changes in the brains of rats exposed to three 74.5 kPa blast overpressure events, conditions that have been associated with long-term anxiogenic manifestations weeks or months following the initial exposures. Rat frontal cortex eight months post-exposure was used for cell sorting of whole brain tissue into neurons and glia. We interrogated DNA methylation profiles in these cells using Expanded Reduced Representation Bisulfite Sequencing. We obtained data for millions of cytosines, showing distinct methylation profiles for neurons and glia and an increase in global methylation in neuronal versus glial cells ($p < 10^{-7}$). We detected DNA methylation perturbations in blast overpressure–exposed animals, compared with sham blast controls, within 458 and 379 genes in neurons and glia, respectively. Differentially methylated neuronal genes showed enrichment in cell death and survival and nervous system development and function, including genes involved in transforming growth factor β and nitric oxide signaling. Functional validation via gene expression analysis of 30 differentially methylated neuronal and glial genes showed a 1.2 fold change in gene expression of the serotonin N-acetyltransferase gene *(Aanat)* in blast animals ($p < 0.05$). These data provide the first genome-based evidence for changes in DNA methylation induced in response to multiple blast overpressure exposures. In particular, increased methylation and decreased gene expression were observed in the *Aanat* gene, which is involved in converting serotonin to the circadian hormone melatonin and is implicated in sleep disturbance and depression associated with traumatic brain injury.

Key words: blast overpressure; DNA methylation; epigenetic; sleep disturbance; traumatic brain injury

Introduction

Traumatic brain injury (TBI) affects 1.7 million Americans each year, according to the Centers for Disease Control and Prevention.[1] The sequelae of TBI can include mood and anxiety disorders, post-traumatic stress disorder (PTSD), heightened suicidality, and diminished cognitive capacity, with deficits in attention and memory. These symptoms can arise years after time of injury, leading to misdiagnosis and lack of proper care and treatment. With an estimated 10% to 20% of returning veterans sustaining TBI, understanding the molecular circuitry of TBI is critically important to the health and productivity of the veteran population.

A number of animal models have been developed to study TBI-related neuropathology.[2] In particular, we have developed an animal model of blast overpressure to explore the pathological mechanisms thought to underlie mild blast injury.[3] To model blast conditions, animals are exposed to different types of blast—from direct exposure to live explosives to controlled blast waves from compressed air generators.[4] In recent years, rodent models have been increasingly utilized.[5–14] Rat and pig research has demonstrated that the main blast wave is transmitted through the skull to the brain with intracranial pressure increasing as the blast wave travels through.[15–17] High-level blast exposure is associated with hemorrhagic lesions, such as intraparenchymal, subdural, and subarachnoid bleeding.[4] Histological effects, including axonal, glial, and

[1]Department of Psychiatry, [6]Research and Development Service, [8]Neurology Service, James J. Peters Department of Veterans Affairs Medical Center, Bronx, New York.
[2]Fishberg Department of Neuroscience, [3]Friedman Brain Institute, [4]Department of Neurology, [5]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, New York.
[7]Department of Neurotrauma, Operational and Undersea Medicine Directorate Naval Medical Research Center, Silver Spring, Maryland.

microglial, were found. Myelin changes also have been observed, sometimes with neuronal apoptosis. A limitation of the animal models to date is the use of blast exposures powerful enough to cause pulmonary and gross intracerebral hemorrhages.[4,18–20] Fewer studies have investigated the effects of lower-strength blasts, which may more closely model the mild TBI exposures more often observed in the current war zones.[10,11,14,21,22] However, even low-level blast pressure waves are transmitted to the brain and exert a variety of biochemical, pathological, and physiological effects in animals, including the induction of chronic behavioral effects.[3,10,14,21–29]

Modulation of gene expression mediated by epigenetic changes, such as deoxyribonucleic acid (DNA) methylation, is one way the brain creates new homeostasis in response to experience, including response to injury.[30] Aberrant regulation of gene expression contributes to long-lasting behavioral abnormalities, where epigenetic modifications serve as a regulatory layer decoding environmental signals into sustained changes in gene expression. Few studies have investigated the role of epigenetic modifications in TBI. Indirect indices of post-TBI changes in DNA methylation, such as relocalization of the enzyme DNMT1 and reduced hippocampal expression of the transcription factor MBD1 have been reported.[31,32] More direct examinations of TBI-associated DNA methylation changes have been scant. Global hypomethylation was found on Day 1 in regions exhibiting pannecrosis and Day 2 in peripannecrosis following injury in a rat TBI model. Further, a sub-population of reactive microglia/macrophages were identified to be a major source of these hypomethylated cells.[33] In a controlled cortical impact model of TBI, Schober and colleagues examined hippocampi from 17 day old rat pups and found increased IGF-1B messenger ribonucleic acid (mRNA) expression associated with increased methylation at P1, as well as increased histone modifications associated with gene activation at P2 and exon5/ESE, along with differential methylation in the exon 5ESE regions.[34] While limited in scope, these studies implicate epigenetic modifications as part of the brain's response to traumatic injury. In the present study, we performed large-scale DNA methylation profiling of blast-related injury using our animal model of blast overpressure and identified DNA methylation perturbations associated with blast-related TBI.[3]

Methods

Animal model of TBI exposure

Eight adult male Long Evans hooded rats (250–350 g; Charles River Laboratories International, Inc., Wilmington, MA) were used as subjects. Rats (10 weeks of age) were exposed to overpressure injury using the Walter Reed Army Institute of Research (WRAIR) shock tube, which simulates air blast exposure under experimental conditions. The shock tube has a 305 mm circular diameter and is a 5.94 m long steel tube divided into a 0.76 m compression chamber that is separated from a 5.18 m expansion chamber. The compression and expansion chambers are separated by polyethylene Mylar™ sheets (Du Pont Co., Wilmington, DE) that control the peak pressure generated. The peak pressure at the end of the expansion chamber was determined by piezoresistive gauges specifically designed for pressure-time (impulse) measurements (Model 102M152, PCB, Piezotronics, Inc., Depew, NY). This apparatus has been used in multiple prior studies to deliver blast overpressure injury to rats.[15,20,23,24] Individual rats were anesthetized using an isoflurane gas anesthesia system consisting of a vaporizer, gas lines and valves, and an activated charcoal scavenging system adapted for use with rodents. Rats were placed into a polycarbonate induction chamber, which was closed and immediately flushed with 5% isoflurane mixture in air for 2 min. Rats were placed into a cone-shaped plastic restraint device and then placed in the shock tube. Movement was further restricted during the blast exposure using 1.5 cm diameter flattened rubber tourniquet tubing. Three tourniquets were spaced evenly to secure the head region, the upper torso, and the lower torso, while the animal was in the plastic restraint cone. The end of each tube was threaded through a toggle and run outside of the exposure cage, where it was tied to firmly affix the animal and prevent movement during the blast overpressure exposure without restricting breathing.

Rats were randomly assigned to sham or blast conditions with the head facing the blast exposure without any body shielding, resulting in full body exposure to the blast wave. Further details of the physical characteristics of the blast wave are described elsewhere.[3] Blast-exposed animals received 74.5 kPa exposures equivalent to 10.8 psi. One exposure per day was administered for three consecutive days. Subjects received blast overpressure or sham exposure at the Naval Medical Research Center and were transferred to the James J. Peters VA Medical Center on the day following the last exposure. Following transfer, the animals were housed at a constant 21–22°C temperature with rooms on a 12:12 h light cycle with lights on at 7 AM. All subjects were individually housed in standard clear plastic cages equipped with Bed-O'Cobs laboratory animal bedding (The Andersons, Maumee, OH) and EnviroDri nesting paper (Sheppard Specialty Papers, Milford, NJ). Access to food and water was ad libitum. The animals studied here represent a subset of those that were part of a previous behavioral study that has been reported elsewhere.[25] Animals were euthanized by CO2 narcosis and the brains were quickly removed, using dissection procedures described previously.[25] Tissue from frontal cortex from animals used in the present study was obtained at eight months following the last blast exposure.

Isolation of neuronal and glial cell populations

Frozen rat tissue from the frontal cortex was used for neuronal nuclei isolation as described by Matevossian and Akbarian[35] with minor modifications. Briefly, approximately 100 mg of postmortem brain tissue was homogenized in 3 mL of Lysis Buffer containing 0.001% 1M Dithiothreitol (Sigma Aldrich, St. Louis, MO) by volume.[35] A nuclear fraction was obtained by ultracentrifugation through a sucrose cushion in an SW40 rotor at 24,500 RPM for 1 h at 4°C. The pellet was resuspended in 100 μL of cold PBS, and immunostaining for FACS was done as described using 1.0 μL of Alexa-Fluor 488 conjugated NeuN antibody (Millipore, Billerica, MA) in 200 μL staining mix (containing 2.5% normal goat serum and 1.25% bovine serum albumin by volume), which was added to 410 μL of 1XPBS. For the unstained control, 5 μL of nuclei from each preparation was added to 300 μL 1XPBS. For the negative control, 5 μL from each preparation was added to 490 μL of 1XPBS and 200 μL of staining mix containing 1.0 μL of Alexa Fluor® 488 conjugated antibody was added. All samples were incubated in the dark for 60 minutes at 4°C with gentle mixing, followed by filtration through a 35 μM cell strainer before FACS. Sorted nuclei were resuspended in 200 μL Resuspension Buffer (100 mM NaCl, 10mM Tris-HCl pH8, 25mM EDTA, 0.5% SDS) with freshly added Proteinase K (final concentration 100 μg/mL) and incubated at 56°C for 30 min. To extract DNA, the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) was used using the protocol supplied by the manufacturer.

DNA methylation profiling via expanded reduced representation bisulfite sequencing (ERRBS)

ERRBS library preparation and sequencing was performed at the Epigenomics Core at Weill Cornell Medical College as described previously.[36,37] This is a modification of the reduced representation bisulfite sequencing approach, which generates single-base resolution DNA methylation bisulfite sequencing libraries that enrich for CpG-dense regions by methylation-insensitive restriction

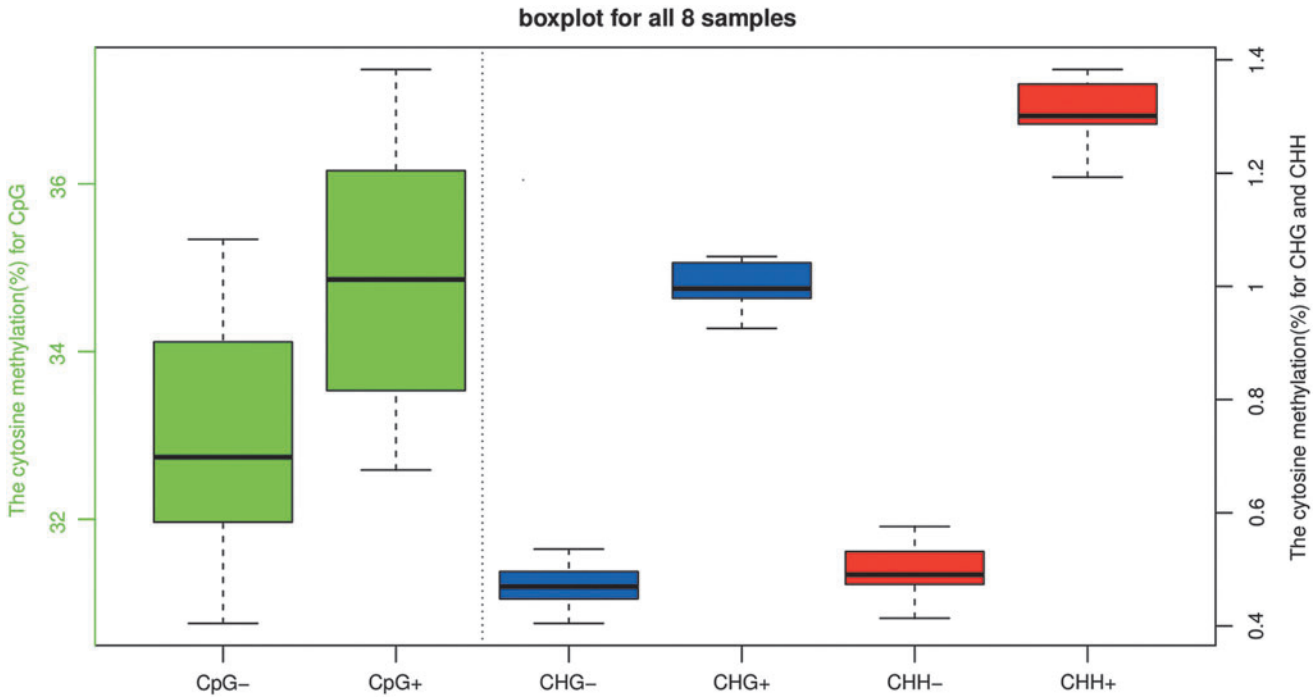

FIG. 1. Global CpG, CHG, and CHH methylation levels in neurons (+) and glia (-), where neurons show elevated DNA methylation in all cytosine contexts, with methylation levels depicted in boxplots. Color image is available online at www.liebertpub.com/neu

digestion.[38] ERRBS is similar to the RRBS method, except longer library fragment lengths of 150–250 bp and 250–400 bp were gel isolated. The libraries were then amplified and sequenced on an Illumina HiSeq2000 per the manufacturer's recommended protocol for 50 bp single end read runs. Image capture, analysis, and base calling were performed using Illumina's CASAVA 1.7 software. For analysis of bisulfite treated sequence reads (with an average bisulfite conversion rate of >99.4% for all samples), reads were filtered from the adapter sequences using FLEXBAR software.[39] Adapter sequence contamination usually occurs towards the 3′ ends of some reads. The adapter matching part of the read was removed if it aligned with the adapter sequence at least 6 bps and had at most a 0.2 mismatch error rate. Reads were aligned to whole genome using the Bismark alignment software with a maximum of two mismatches in a directional manner and only uniquely aligning reads were retained.[40] In order to call a methylation score for a base position, we required that read bases aligning to that position have at least a 20 phred quality score and that the base position should have at least 10×coverage. Only cytosines with these sequencing quality and coverage criteria were retained for subsequent analysis. The percentage of bisulfite converted cytosines (representing unmethylated cytosines) and non-converted cytosines (representing methylated cytosines) were recorded for each cytosine position in CpG, CHG, and CHH contexts (with H corresponding to A, C, or T nucleotides). The sequencing coverage of cytosines in these contexts were 9.1-14% of all 24.7 million CpG sites, 3.2-6.6% of all 113 million CHG sites, and 1.2-2.6% of all 800 million CHH sites in the rat genome. For cytosine residues for which we had at least 10×coverage, there were approximately 5.6-6.0% CpG, 1.5-1.6% CHG, and 0.50-0.55% CHH in the data used for subsequent analyses.

Gene expression analysis via NanoString

We used the NanoString nCounter Elements technology, which provides highly sensitive and reliable expression on a level of single transcript. With this method, it is possible to measure as little as 1.2-fold changes of a single transcript at 20 copies per cell (with $p \le 0.05$). For this technology, digital detection is used on two levels. On the first level, each transcript is detected by a probe bound to a segment of DNA that is attached to a unique string of colored fluorophores (the molecular barcode). Identification of that transcript, therefore, depends only on the order of fluors on the string, rather than on intensities of the fluors. At the second level of digital detection, the number of total transcripts in a sample is quantified by counting the total number of times a particular string of fluors (or barcode) is detected. In contrast to microarrays or polymerase chain reaction (PCR)-based gene expression approaches, NanoString does not depend on synthesis of a complementary DNA strand or PCR amplification, eliminating enzymatic reactions and instead using barcode-labeled probes that anneal directly to mRNAs in solution wherein the hybrid molecule is then immobilized, detected, and counted (www.nanostring.com/elements). For the present study, we ran some of the samples in duplicate and found the data to be highly correlated among all replicates ($r^2 = 0.99$; supplementary Fig. S1; see online supplementary material at www.liebertpub.com).

Data and statistical analysis

For the global methylation analysis to compare the neuron (+) and glia (-) cells, a simple paired t test was applied. For identification of differentially methylated cytosine residues in blast versus sham blast samples, we devised a moderated t test weighted by sequence coverage. For a given cytosine, let $x_1, \cdots, x_4$ be the methylation levels (between zero and one) of the four samples that were given the blast treatment, and $x_5, \cdots, x_8$ for the sham treatment. Let $n_1, \cdots, n_8$ denote the coverages of the cytosine among the corresponding samples. We first computed the variance $s_x^2 = \left(\sum_{i=1}^{4} (x_i - \bar{x}_1)^2 + \sum_{j=5}^{8} (x_j - \bar{x}_2)^2 \right) / 6$, where $\bar{x}_1$ and $\bar{x}_2$ are the sample mean of the methylation levels for the blast and sham groups, respectively. We also computed the variation just due to

the coverage. First, we modified the methylation level x_i by $\tilde{x}_i = (n_i x_i + 0.5)/(n_i + 1)$, which is a Bayesian estimate of the methylation level with a Jeffreys' prior distribution beta (1/2,1/2). From the modified methylation level, a simple estimate of the variance just due to the coverage can be obtained by using the binomial distribution $s_n^2 = \frac{1}{8}\sum_{i=1}^{8} \tilde{x}_i(1-\tilde{x}_i)/(n_i+1)$, which is very close to the variance for the posterior Bayesian distribution if we had started with the prior Beta (1/2,1/2) distribution. The full Bayesian treatment is omitted here for the sake of simplicity. The t-test is computed by $t = (\bar{x}_1 - \bar{x}_2)/\sqrt{\max(s_x^2, s_n^2)/(1/4 + 1/4)}$.

Differentially methylated sites using an a priori selected significance threshold ($p \leq 0.005$) were then mapped to annotated genes in the rat genome via the Homer software[41] to determine overlapping genes and associated regulatory regions. Genes thus identified were then used in gene ontology and pathway analysis.

Gene ontology and pathway analysis

Differentially methylated CpG sites among blast and sham blast controls were mapped to refSeq gene annotations, and these genes were then used for subsequent ontology analyses. The gene lists thus generated were examined to elucidate biological functions and pathways that may be involved in TBI-related neuropathology using QIAGEN's Ingenuity® Pathways Analysis (IPA)®, (QIAGEN, Redwood City) software (www.ingenuity.com), utilizing an unsupervised analysis. Functional analyses for the datasets were run to display the most significant biological functions across the dataset that meet a p value cutoff of 0.05 (corrected for multiple testing via the Benjamini-Hochberg method).[41] For ease of visualization, the top 20 functions are displayed. A second functional analysis on the data was performed that was restricted to CNS-related cell and tissue lines. From these analyses, also, the most significant canonical pathways and their interactions were identified. Data presented from the canonical pathways are representative of the canonical pathway at the cellular level, depicting our differentially methylated genes with other associated genes/proteins, their interactions, and the cellular and metabolic reactions in which the pathway is involved.

Results

For genome-scale DNA methylation profiling of repeated blast exposure, we used the ERRBS method to determine methylation changes associated with blast-related TBI in neurons and glia. The ERRBS method targets regions of the genome enriched for CpG dinucleotides, specifically CpG islands, although proximal cytosine residues in non-CpG contexts also are captured by this method. With these data, we were able to map the methylation profile of

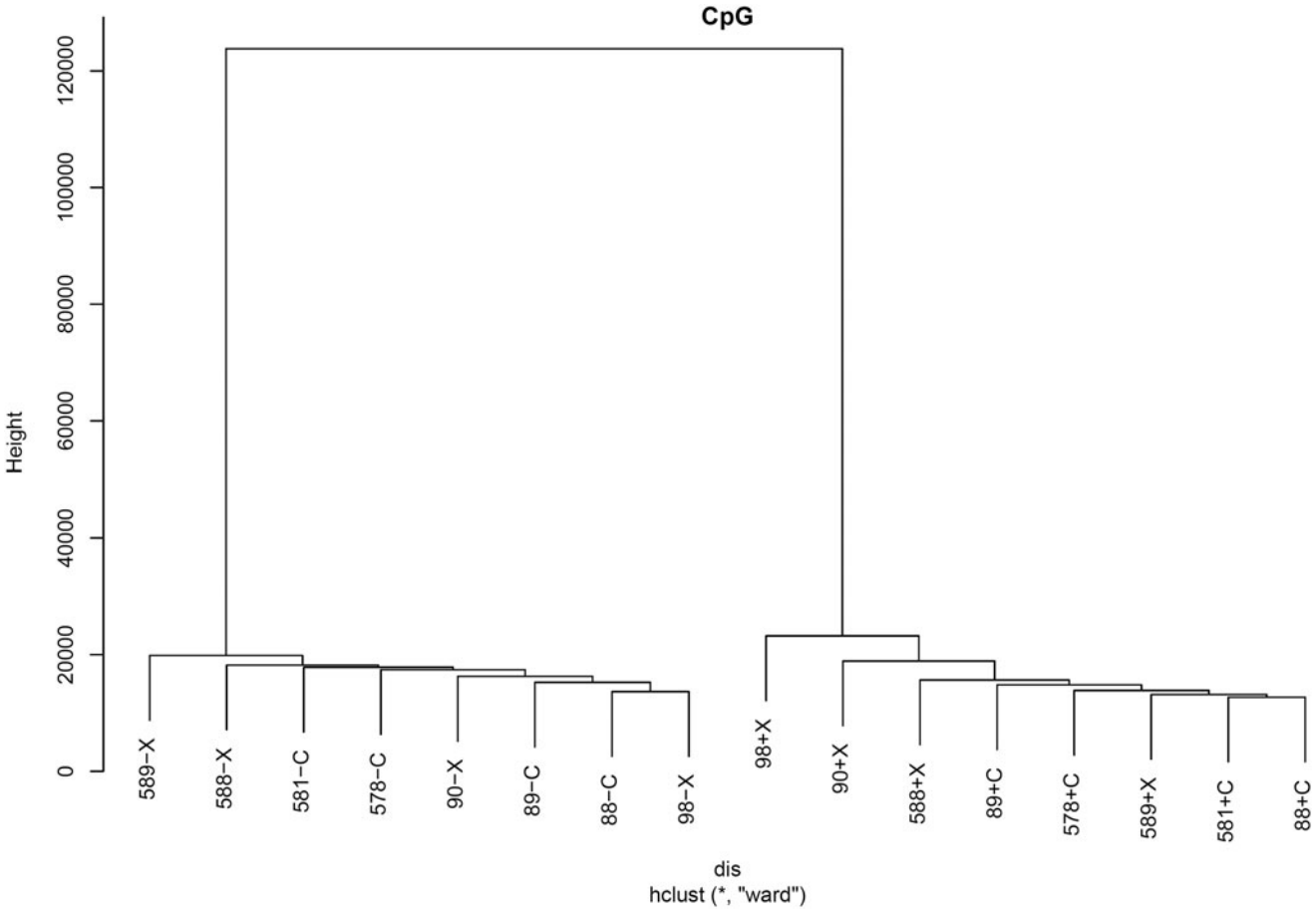

FIG. 2. Unsupervised hierarchical clustering of DNA methylation data shows that neurons (+) and glia (-) have distinct profiles in CpG, ChG, and CHH contexts (data shown for CpG context only). The cytosine residues used for this analysis met the following criteria having: 1) ≥ 10 × sequence coverage; 2) standard deviation of methylation levels across the samples ≥ 10%; and 3) methylation difference between the maximum and the minimum ≥ 10%. The hierarchical clustering is generated by using Ward's minimum variance method on the Manhattan distance matrix. Rats exposed to blast (X) and sham blast (C) are labeled accordingly.

millions of cytosine residues across all samples examined. Examination of DNA methylation distributions within CpG, CHG, and CHH contexts showed that neuronal cells (+) were more methylated than their non-neuronal (-) counterparts (Fig. 1). This difference was highly significant in CHG and CHH contexts ($p \leq 10^{-7}$ and $p \leq 10^{-7}$, respectively), which is also supported in our previously reported findings.[43] To determine whether the genomic regions covered in the present data showed clustering of cytosine residues in various contexts, we examined the pair-wise distances of cytosines in CpG, CHG, and CHH contexts and found relatively little clustering (supplementary Fig. S2; see online supplementary material at www.liebertpub.com). Additionally, we determined whether DNA methylation profiles in these contexts differed within neurons and glia. Interestingly, but not surprisingly, the DNA methylation profiles separated neurons and glia in all cytosine contexts (Fig. 2). These data demonstrate the specificity of the cell sorting results and confirm findings showing that DNA methylation patterns are tissue and cell specific.[44–46]

For discovery of DNA methylation alterations associated with blast injury, we then examined methylation differences between blast and sham blast samples within neurons and glia separately. Comparing the methylation data between the blast and sham blast animals, we found that the animals that have undergone blast exposure show DNA methylation perturbations, compared with sham blast controls (Fig. 3). These data provide the first genome-based evidence for induced changes in DNA methylation in response to blast injury. It also is of note that these changes were seen in animals eight months post-blast exposure, suggesting that these methylation changes are likely long lasting, as are the previously reported behavioral changes relating to anxiety and PTSD-related traits observed within the same cohort of animals exposed to blast.[25] To this end, we performed gene ontology analyses to identify specific genetic pathways that represent clusters of genes with DNA methylation perturbations in blast versus sham blast control animals using Ingenuity Pathway Analysis software. We identified 1807 cytosine sites that mapped to 837 genes in the rat

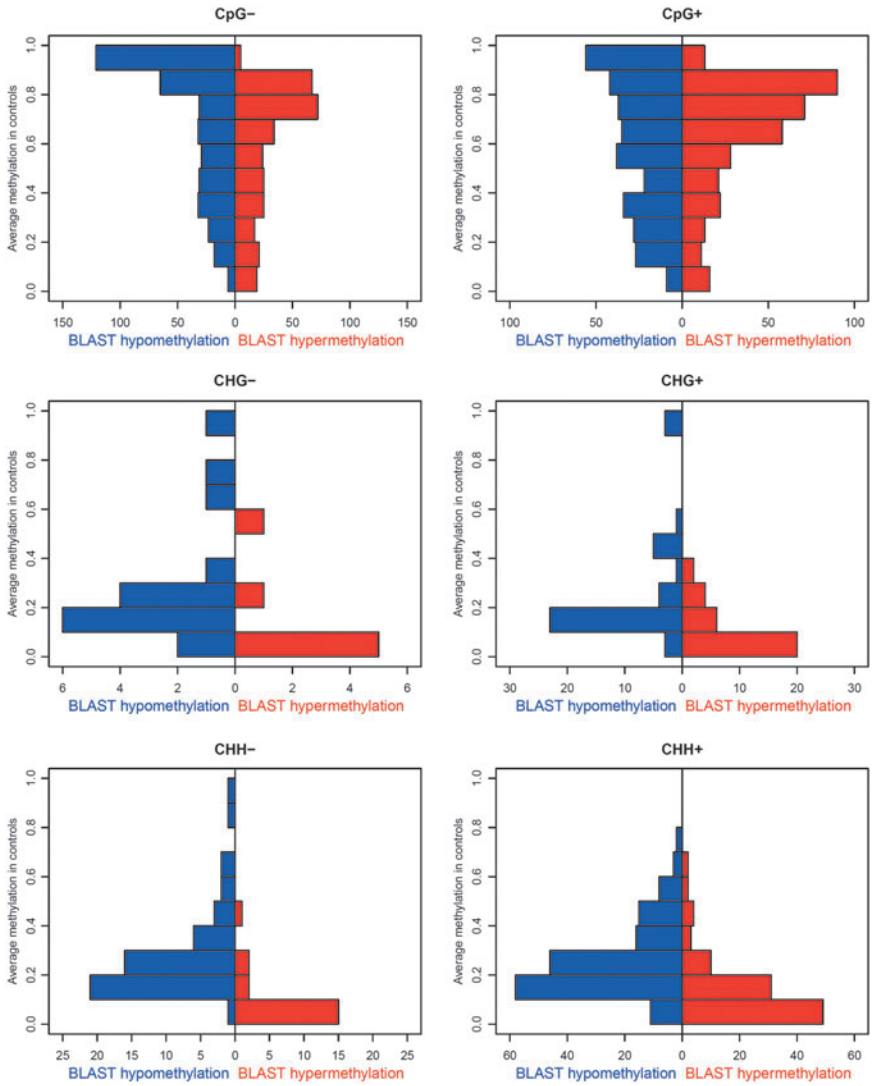

FIG. 3. Bar charts showing the distribution of DNA methylation differences in neurons (+) and glia (-). Gain of methylation is depicted in red (hypermethylation), and loss of methylation is depicted in blue (hypomethylation) comparing blast vs. reference sham blast controls. Distributions of differentially methylated loci are shown separately for various cytosine contexts and cell types in each plot. Color image is available online at www.liebertpub.com/neu

genome. Within these genes, 458 genes were differentially methylated in neurons and 379 in glia. For the neuronal samples, the genes thus identified were significantly enriched in many biological functions, notably, cell death and survival, cell growth and proliferation, and nervous system development (Fig. 4A and supplementary Table S1; see online supplementary material at www.liebertpub.com.). Additionally, 92 of these genes have been previously implicated in neurological disease and 39 in neuropsychiatric disorder (supplementary Table S1). For non-neuronal samples, there was enrichment in cancer, cellular growth and proliferation, as well as nervous system development and function (Fig. 4B and supplementary Table S2; see online supplementary material at www.liebertpub.com).

Further, from these data, we identified many canonical pathways that may be associated with differences in neuronal and glial gene expression between the blast and sham blast groups. In neuronal cells, the transforming growth factor β (TGF- β) signaling pathway ($p < 0.05$) was identified as statistically significant after correction for multiple comparisons (supplementary Table S3; see online supplementary material at www.liebertpub.com), which included a number of genes that showed differential methylation in this dataset among blast and sham blast controls (supplementary Table S3). TGF-β signaling is involved in a variety of functions including cell proliferation, migration, and survival,[47,48] as well as synaptic transmission,[48] and adult neurogenesis.[49] In a mouse model of non-blast TBI, altered expression of members of the TGF-β signaling pathway was reported in brain areas involved in adult neurogenesis.[50] Although not statistically significant, other canonical pathways important to brain function were identified, including nitric oxide, Parkinson's disease, axonal guidance, Huntington's disease, circadian rhythms, and dopamine receptor signaling, as well as serotonin and melatonin synthesis in neurons and glia (supplementary Tables S3 and S4; see online supplementary material at www.liebertpub.com). Genetic pathways and associated genes thus identified from the gene ontology analyses were used to select 30 differentially methylated genes showing both gain and loss of methylation associated with repeated blast exposure (Fig. 5 and supplementary Table S5; see online supplementary material at www.liebertpub.com). Of the genes assayed, 50% showed the expected anticorrelated relationship of DNA methylation and gene expression (Fig. 6). Of note, the *Aanat* gene showed a 1.2 fold

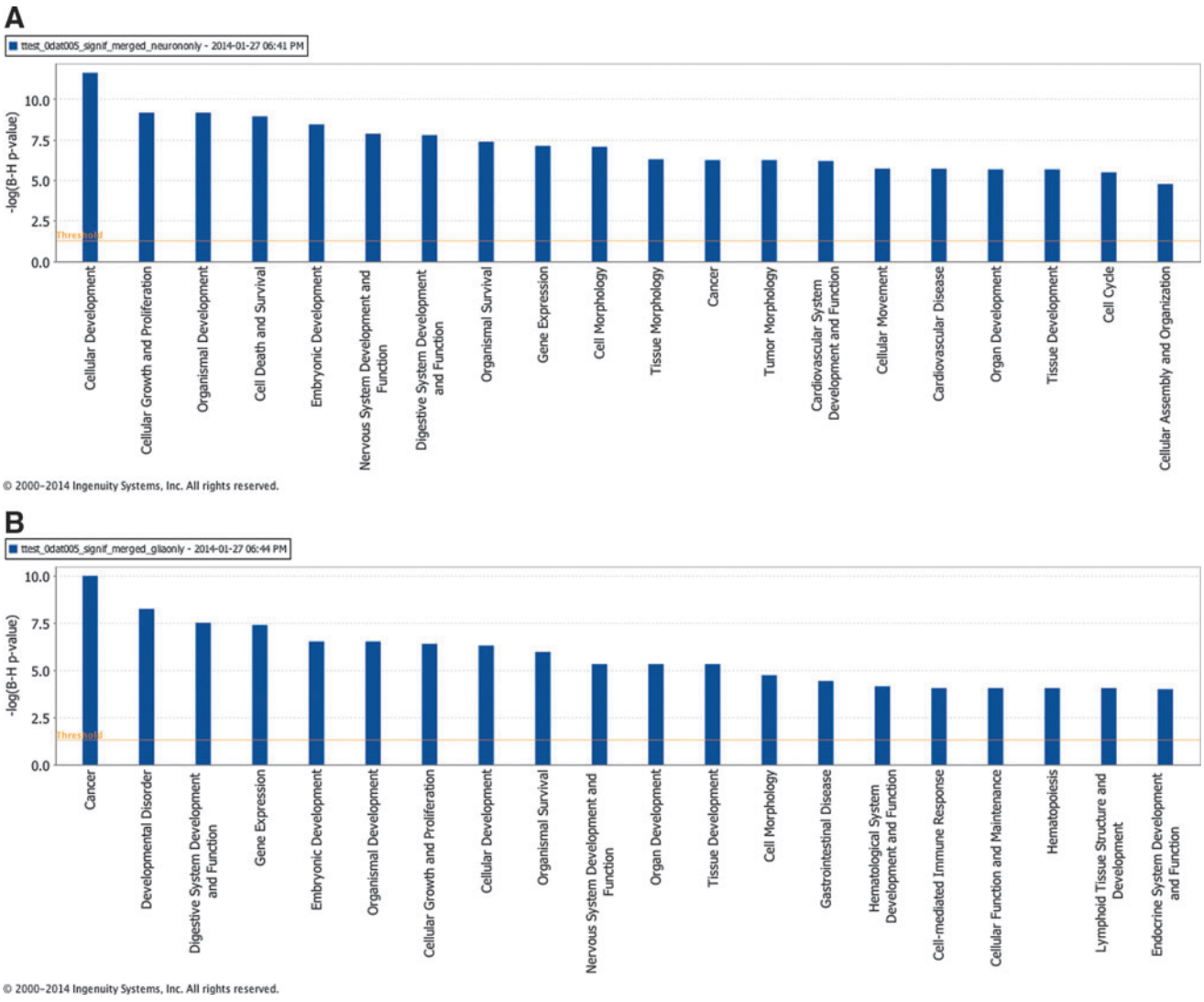

FIG. 4. (A) Top 20 functions – neuron; (B) Top 20 functions – glia. Results are adjusted for multiple testing using the Benjamini-Hochberg procedure ($p \leq 0.05$). Color image is available online at www.liebertpub.com/neu

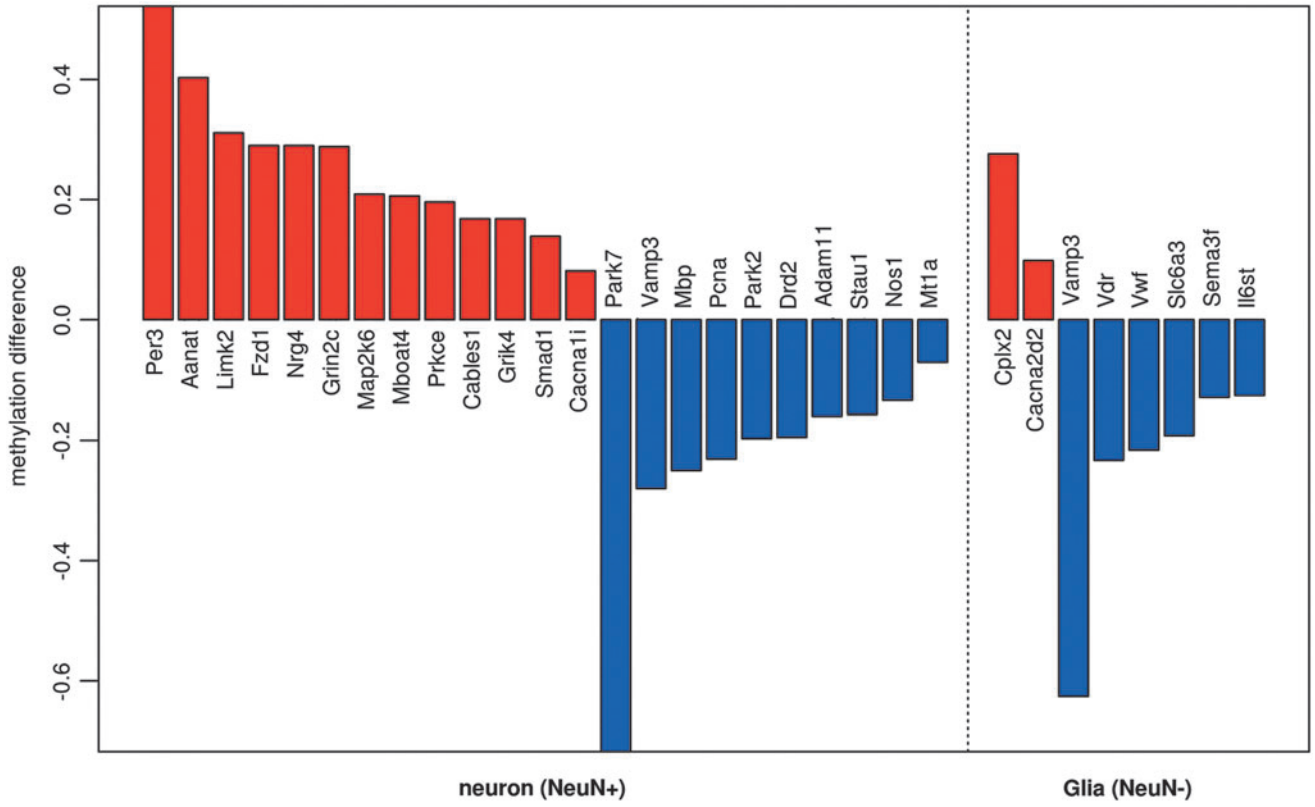

FIG. 5. Bar chart showing gain of in red and loss in blue of methylation in specific genes associated with repeated blast exposure. These genes were also assayed via the NanoString platform to determine association of these DNA methylation changes with changes in gene expression. Color image is available online at www.liebertpub.com/neu

change in gene expression ($p \leq 0.05$), with the expected anti-correlated decrease in gene expression and increase in methylation in blast samples. Although the 1.2-fold change in gene expression is modest, physiologically, such a small perturbation may have noticeable consequences on brain function.[51] Similar fold changes have been reported with gene expression changes associated with blast in human samples.[52] The *Aanat* gene encodes aralkylamine N-acetyltransferase and also is known as serotonin N-acetyltransferase. This enzyme is involved in the conversion of serotonin to the circadian hormone melatonin.[53] This finding is of particular interest given the prevalence of sleep disturbance[54] and depression[4] in individuals with TBI. Reduced salivary melatonin levels also have been reported in TBI patients.[55] Further, there is evidence to support a role for *Aanat* and the melatonin-signaling pathway in susceptibility to major depression.[56] We also identified neuronal DNA methylation differences in blast-exposed animals in several sleep-associated genes, including nitric oxide synthase 1, neuronal (Nos1); interleukin receptor, type 1 (Il1r1); homer homolog 1 (Homer1); cholinergic receptor, nicotinic, alpha 3 (Chrna3); calcium channel, voltage-dependent, N-type, alpha 1B subunit (Cacna1b); and period circadian clock 3 (Per3).

Discussion

We conducted a genome-scale DNA methylation study in a rat model of blast overpressure where long-term behavioral manifestations have been noted many weeks and months after exposure to multiple low-intensity blast overpressure events. We identified cell-specific DNA methylation perturbations in neurons and glia associated with blast exposure. These methylation perturbations appear to be sustained months following blast exposure, suggesting that environmentally-induced DNA methylation alterations attributed to blast injury can persist long term. This study has a number of limitations. One limitation is that these data represent a single time point following blast exposure, and hence it is difficult to know whether these epigenetic perturbations associated with TBI are confounded by DNA methylation changes associated with aging. This is mitigated by comparison of DNA methylation changes in sham blast control animals of the same age and rearing conditions. Future studies that investigate DNA methylation changes over multiple time points across the lifespan will offer greater resolution in identifying stable methylation perturbations associated with blast exposure. Additionally though this is a large-scale DNA methylation profiling study, it does not capture the DNA methylation patterns across the entire genome. Finally, this study is based on a small number of animals and thus warrants replication in a larger cohort.

These data identified DNA methylation alterations in a number of genes and genetic pathways previously implicated in TBI and related neurological and neuropsychiatric disorders. Most notably, the *Aanat* gene, which is involved in sleep regulation, showed increased DNA methylation and a modest decrease in gene expression in the brains of blast exposed animals, compared with those of sham blast controls. In addition to the *Aanat* gene, we identified neuronal DNA methylation differences in blast-exposed animals in several sleep-associated genes. Nos1 has been implicated in the regulation of sleep in the mouse.[57,58] Il1r1 has been implicated in the regulation of non-REM sleep.[59,60] Homer1 is also involved in sleep-wake regulation and has been considered as a marker of sleep

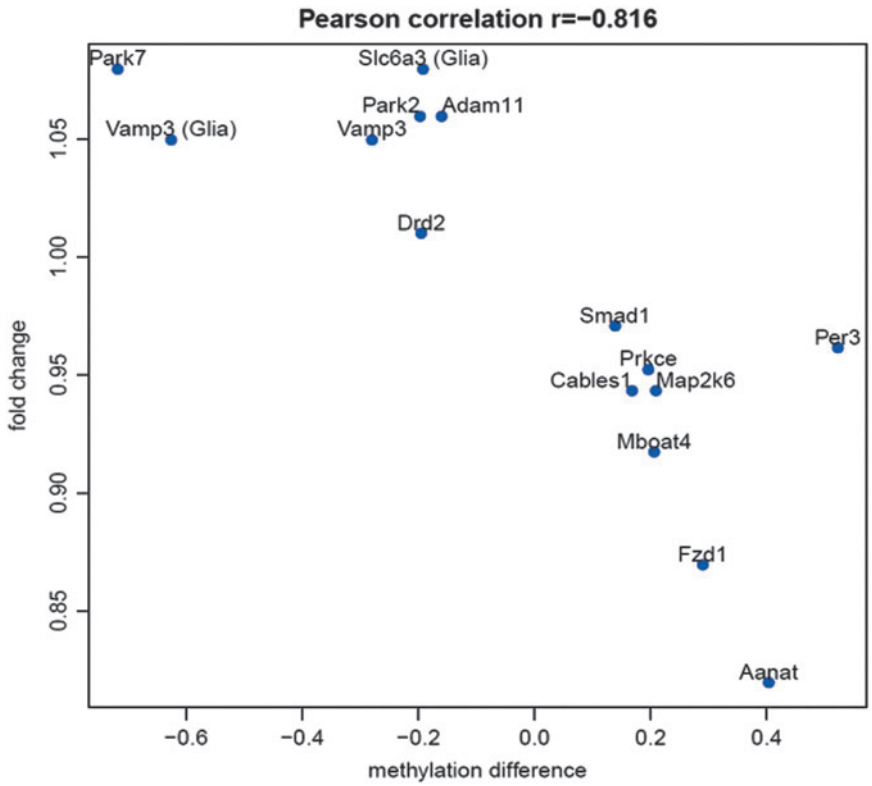

FIG. 6. Functional gene expression validation of DNA methylation differences in blast vs. sham blast controls using NanoString. Shows genes with expected anti-correlated pattern of DNA methylation and gene expression. Color image is available online at www.liebertpub.com/neu

homeostasis[61] and a marker of sleep loss.[62] Chrna3 has been associated with sleeping energy expenditure.[63] Cacna1b has been implicated in rapid-eye movement (REM) sleep consolidation and the timing of intervals between non-REM and wakefulness episodes.[64] In addition, we found increased methylation of the circadian gene Per3, which is associated with diurnal preference and delayed sleep phase disorder.[65]

Sleep disturbances are common following traumatic brain injury[66–71] and occur frequently in veterans who have sustained TBI.[54,66,72,73] A large epidemiological study of 29,640 military members with blast-related TBI showed that sleep disturbances mediate subsequent development of PTSD and depression.[74] Because TBI, PTSD, and depression have overlapping symptoms, particularly symptoms associated with sleep problems, it is difficult to know whether the presence of one condition is a precursor to another. However given the preponderance of sleep disturbances among military and veteran populations following TBI, this may be an early indicator of development of subsequent mental disorders. Although in the present study we did not have data on the sleep behavior of the blast and sham blast animals, the DNA methylation abnormalities in genes involved in sleep regulation coupled with the behavioral abnormalities in anxiety and PTSD-related traits in the blast animals suggest that blast injury induces sustained epigenetic changes in genes associated with sleep regulation, which may contribute to the observed anxiety behaviors. This offers the first molecular evidence linking dysregulation of sleep-related genes with blast exposure. These data will have direct translational relevance to the diagnosis and treatment of individuals with blast-related TBI, providing an objective measure for examining the molecular neurocircuitry of sleep disorders in TBI.

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Author Disclosure Statement

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Address correspondence to:
Fatemeh Haghighi, PhD
Icahn School of Medicine at Mount Sinai
1425 Madison Avenue, Floor 10, Room 10-70D
New York, NY 10029

E-mail: fatemeh.haghighi@mssm.edu