COMMENT & RESPONSE

Misinterpretation of Study Data

To the Editor The recent Wilson et al study\(^1\) reported that next-generation sequencing served as a diagnostic tool for neurological infections. It also posited that data from our group\(^2,3\) reflected a contamination of the molecular biological reagents and not the presence of bacteria in surgically resected and autopsied brains. We take issue with this latter conclusion because it is based on several mistaken interpretations of our data.

Unlike Wilson et al’s contention that we applied deep sequencing to RNA that was derived from cerebrospinal fluid, we sequenced only brain-derived RNA. Given the results presented by Wilson et al that demonstrated a noise reduction with RNA doping (using RNA concentrations lower than the input brain-derived RNA in our studies), our findings are unlikely to be nonspecific/chromatin. We also discarded reagents that showed evidence of contamination before verifying our deep sequencing results by conventional Sanger sequencing of reverse-transcription polymerase chain reaction-derived cloned amplicons.

Wilson et al indicate that we did not include water or otherwise RNA-negative samples. However, it does not acknowledge that we subtracted from analysis any nonhuman hits that were common across unconnected libraries in a manner similar to their background subtraction approach to eliminate sequences that might have been introduced during the library construction steps.

Our deep and conventional (bacterial RNA) sequencing of brains indicated that the bacterial profiles differed between clinical groups in our analyses and also in the analyses of our data within Wilson et al,\(^1\) which is an improbable outcome if our findings were solely due to contamination given that the samples were prepared concurrently with the same reagents. The likelihood of our deep and conventional sequencing results being contamination is also offset by our concurrent detection of bacterial RNA by in situ hybridization and bacterial protein immunoreactivity in brain that were selectively detected in glial cells in nonuniform and nonrandom distribution, largely in white matter, that were similar to other groups.\(^4\)

Although we did not assume any causal relationship between bacterial detection in brain, the presence of bacterial RNA, DNA, and proteins in the human (and nonhuman primate) brain tissues has the capacity to influence nervous system functions that have yet to be determined, as proposed by other groups.\(^5\) Thus it is essential that readers appreciate that the criticism of our published studies on human brain tissue is not based on an accurate and complete analysis of the techniques and data. Thank you for considering our comments.

Christopher Power, MD
Jon D. Laman, PhD
William Branton, MSc

Author Affiliations: Department of Medicine (Neurology), University of Alberta, Edmonton, Alberta, Canada (Power, Branton); Department of Neuroscience, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands (Laman).

Published Online: November 26, 2018. doi:10.1001/jamaneurol.2018.3668

Conflict of Interest Disclosures: None reported.


In Reply We thank Power and colleagues for their comments regarding our recent article\(^1\) on metagenomic next-generation sequencing (mNGS). In our article, we expressed concern that their recent reports of a brain microbiome may be confounded by environmental microbial—contaminating sequences in their mNGS data sets.\(^2,3\)

We make no conclusions about the source of the potential contaminating sequences in Power and colleagues’ mNGS data. Environmental contaminants may arise from molecular biological reagents,\(^4,5\) the laboratory where the sample was acquired, its storage container, and/or from laboratory surfaces and personnel present where the mNGS samples were prepared. We did not contend the authors performed mNGS on RNA from cerebrospinal fluid. We stated in eFigure 2D in the article’s Supplement that we analyzed “publicly available data from...brain specimens” and labeled the figure panel accordingly. In addition, we compared the authors’ data with (“no-template”) water controls because cerebrospinal fluid is not an appropriate comparator for a study that asserts that there is a central nervous system microbiome.

The RNA doping experiment showed that for very low-input (ie, picogram) nucleic acid samples, adding the RNA of a known sequence substantially reduces (but does not eliminate) the relative abundance of contaminating sequences. This merely establishes the relative mass of reagent contaminants, not that contaminants are absent from high-input nucleic acid samples, including brain tissue.

We commend the authors for discarding obviously contaminated reagents. However, mNGS reveals the presence of...

Letters
nucleic acid species at levels that are undetectable by conventional spectroscopic methods. Thus, it is essential to perform mNGS on no-template controls regardless of whether nucleic acid can be detected postextraction.

Power et al state that they subtracted “any nonhuman hits that were common across unconnected libraries in a manner similar to our background subtraction approach.” We did not subtract common sequences, but rather their rank was lowered in our z score–based algorithm to indicate that the organism’s level of abundance was typical for controls.

The variance in the relative abundance of the 4 bacterial classes that were identified in our water controls was generally wider than in the brain mNGS data sets from Power et al. Even within batched mNGS data sets, wide sample-to-sample variation is common because of sample-to-sample variability in contamination levels that arise from nonreagent sources, sample-to-sample variation in input amounts of nucleic acid (see RNA doping experiments), and differences in sample handling during mNGS library preparation.

We commend Power et al for detecting bacterial RNA by in situ hybridization and assessing for bacterial protein immuno-reactivity. However, the former experiments are subject to some of the same potential pitfalls as mNGS, and the latter were not specific for the variety of bacterial taxa on which the claim of a multiple sclerosis brain dysbiosis was made. Given the overlap between the microbiota of laboratory-grade reagents generated by our mNGS protocol with the microbiota identified by Power et al in autopsy brain tissue, more experiments are required to provide definitive evidence that the organisms reported by Power and colleagues truly represent resident brain microbiota.

Michael R. Wilson, MD, MAS
Brian D. O’Donovan, MS
Joseph L. DeRisi, PhD

Author Affiliations: Weill Institute for Neurosciences, University of California, San Francisco (Wilson); Department of Neurology, University of California, San Francisco (Wilson); Department of Biochemistry and Biophysics, University of California, San Francisco (O’Donovan, DeRisi); Chan Zuckerberg Biohub, San Francisco, California (DeRisi).

Corresponding Author: Michael R. Wilson, MD, MAS, Weill Institute for Neurosciences, Department of Neurology, University of California, San Francisco, 675 Nelson Rising Ln, NS212A, Campus Box 3206, San Francisco, CA 94158 (michael.wilson@ucsf.edu).

Published Online: November 26, 2018. doi:10.1001/jamaneurol.2018.3671

Conflict of Interest Disclosures: Dr Wilson receives grant support from the Sandler Foundation, the William K. Bower, Jr Foundation, the Rachleff Foundation, and the National Institute of Neurological Disorders and Stroke (grant K08NS096167). No other disclosures are reported.


Overlooked Implications of Disturbed Sleep in Traumatic Brain Injury

To the Editor A recent propensity-matched cohort study of more than 350 000 veterans with and without traumatic brain injury (TBI) by Barnes et al1 found that mild TBI was associated with more than a 2-fold increase in the risk of dementia, yielding implications for long-term neurodegenerative consequences following TBI. This longitudinal study allowed for the power to detect associations and to adjust for a range of potential confounders, including other medical and psychiatric comorbidities. While we applaud the authors and feel the findings are important, we note a discrepancy in their report of comorbidities, specifically “sleep disorders” (ie, sleep apnea, insomnia, hypersomnia, parasomnia, and circadian rhythm disorders).

The authors report less than a 4% incidence of any sleep disorder in the TBI and the matched cohort, noting that those with a TBI had a lower rate of diagnosed sleep disorders (3.6%) than those without a TBI (3.9%). In light of extensive literature on TBI and sleep disturbances, this number is low and highlights the fact that sleep disorders are underdiagnosed in veterans. Two meta-analyses reported marked subjective and objective sleep disturbances following TBI with a prevalence of more than 50%.2,3 Further, an interim analysis of the Million Veteran Program, a large and comparable cohort with that examined in Barnes et al, found a prevalence of sleep apnea alone of nearly 25%.4

Sleep disorders are not only highly prevalent following TBI but also have profound implications for subsequent neurodegeneration. Sleep enhances lymphatic flow in the brain, increasing the clearance of interstitial β-amyloid species.5 Thus, it is possible that it is not just TBI, but also the synergistic combination of TBI and disturbed sleep (frequently comorbid with TBI) that contributes to the increased risk of dementia. Therefore, the link between the high prevalence of sleep disorders and TBI is extremely relevant to dementia and requires further study. As the appropriate treatment of sleep disorders can only improve TBI-associated outcomes and mitigate the neurodegenerative processes, we advocate for sleep-focused interventions in patients with TBI and sleep disturbances.

Miranda M. Lim, MD, PhD
Vincent Mysliwiec, MD

Author Affiliations: Veterans Affairs Portland Health Care System, Oregon Health & Science University, Portland (Lim); San Antonio Military Health System, Department of Sleep Medicine, JBSA Lackland, Texas (Mysliwiec).

Corresponding Authors: Miranda M. Lim, MD, PhD, Veterans Affairs Portland Health Care System, Oregon Health & Science University, 3710 SW US Veterans Hospital Rd, Mail Code P3-ROD2, Portland, OR 97239 (miranda.lim@ohsu.edu); Vincent Mysliwiec, MD, 2200 Bergquist Dr, Ste 1, JBSA-Lackland, TX 78236 (vincent.mysliwiec.mil@mail.mil).

Published Online: December 3, 2018. doi:10.1001/jamaneurol.2018.3738

Conflict of Interest Disclosures: None reported.

Editorial Note: This letter was shown to the corresponding author of the original article, who declined to reply on behalf of the authors.

Disclaimer: The content of this article does not represent the views of the US Department of Veterans Affairs or the US government.

Additional Contributions: We gratefully acknowledge support from the Veterans Affairs Biomedical Laboratory Research & Development Career Development Award (IK2 BX002712 [M.M.L.]).