INVITED COMMENTARY

Meeting the Challenge of Systems Biology in Traumatic Brain Injury: Beginning Reverse Translation with Epigenome-Wide Association Studies

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We live in an age of biological wonders. Advancement in the understanding of human disease has accelerated since the sequencing of the human genome in 2001, and the development of the science and methodology of genome-wide association studies (GWAs) [1]. Interest in the genetic underpinning of disease has been fueled by the early successes in the 1990s of eloquent linkage studies, which specifically identified highly penetrant single genes that cause Huntington's disease, myotonic dystrophy, and the spinocerebellar ataxias. A key misinterpretation of the fundamentals of GWAs, primarily used to study complex diseases, is the idea that these association studies will identify single genes with high penetrance, which will be useful to infer causation and calculate risk. Most common, prevalent, complex diseases, such as diabetes and stroke, do not have single genes as risk factors. Conditions caused by trauma are different. Causation is never based on a genetic foundation. However, responses to injury can be associated with specific genes and gene networks, and the value of GWAs in these settings is that they can identify complex biological systems that influence responses to injury [2].

Mapping CpG methylation sites influenced by histone binding has resulted in a new type of genetic association study called epigenome-wide association study (EWAs).

EWAs help to grant insight into how the environment can directly affect gene expression [2]. Differential patterns of methylation reflect differential expression of genes influencing important biological systems and outcomes [3, 4]. Genes affecting response to injury in traumatic brain injury (TBI) can be obtained through EWAs and can potentially help to identify biological system affecting outcomes in TBI [2, 5]. The first successful EWAs completed in smokers identified differential methylation of DNA in smokers and its association with health outcomes [6]. This study was transformative in that it established the analytical pathway leading to gene discovery and the validation of genetic associations with EWAs. This specifically involved an initial discovery cohort, technical validation on a different platform, followed by replication in another cohort [2].

In this month's issue of *Neurocritical Care* in their article entitled "Decreased DNA Methylation of RGMA is Associated with Intracranial Hypertension After Severe Traumatic Brain Injury: an Exploratory Epigenome-wide Association Study," Liu et al. [7] examined the relationship between a composite end point reflecting cerebral edema and intracranial hypertension with CpG binding sites in an EWAs. They obtained genome-wide DNA methylation profiles of DNA extracted from ventricular cerebrospinal and compared it with a composite phenotype combining cerebral edema and intracranial pressure. At 3–4 days post TBI, patients with severe intracranial hypertension had significantly lower levels of methylation at cg22111818. They report a potential association between the RGMA DNA methylation site and their composite end point, suggesting the involvement of

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this biological system as an influencer in TBI outcomes. RGMA encodes a glycosylphosphatidylinositol-anchored glycoprotein expressed predominantly in the developing and adult central nervous system acting as a repulsive guidance cue directing axonal growth in developing neurons. It has been reported to have increased expression in immune cell-related neuroinflammation and neurodegeneration autoimmune encephalitis and brain trauma. Anti-RGMA antibodies have been suggested to promote the repair of the damaged spinal cord and relieve neuropathic pain after spinal cord injury. For the implication of a potential and important biological system, the RGMA-related systems would appear to be a well-supported candidate.

The study is visionary in its attempt to identify a key biological system that may be modifiable in the setting of TBI. They considered many potential biases to include access to a cell type within the cerebral spinal fluid that may be directly modifiable by the disease phenotype. They sampled cerebral spinal fluid on multiple days. They created a new composite phenotype. They used an unorthodox statistical approach called “repulsive guidance” and incorporated a permutation analysis to establish a significant permutation of $p = 4.20 \times 10^{-8}$.

However, the study has some very serious flaws. This study did not use a combination of a discovery cohort with technical validation on a different platform followed by replication for confirmation in another cohort, as is the standard in EWAs. Most EWAs and GWAs include large populations in the 1000 s–10,000 s. This study included 89 patients. The sample size precludes testing for issues such as linkage and population stratification, as would be standard in these studies. The study has not undergone replication in another independent population, as would be required for a verification. Although this study maybe exploratory in nature, the experiment in its present form is incomplete and needs further validation in future work. Presently, the role of RGMA as a therapeutic target is not strengthened or diminished by these reported findings. Its use as a biomarker for disease and predictor of potential recovery also remain unclear, and presently its detection has no clinical impact. This study does, however, suggest that the expensive and larger EWAs in TBI may yield important and valuable results in the future.

Source of support

This work received no funding.

Conflict of interest

The author declares no conflicts of interest.

Ethical approval/informed consent

I have followed adherence to ethical guidelines and indicate ethical approvals (institutional review board) and the use of informed consent, as appropriate.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 29 December 2021 Accepted: 4 January 2022

Published: 27 January 2022

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