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Title:
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Date:
2019-05-01

Citation:
El-Osta, A. (2019). Evaluation of genomic methylation indices in juvenile myoclonic epilepsy. *Epilepsia*, 60 (5), pp.e37-e39. <https://doi.org/10.1111/epi.14740>.

Persistent Link:
<https://hdl.handle.net/11343/285790>

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Article type : Commentary

The Evaluation of Differential Methylation Indices in Juvenile Myoclonic Epilepsy

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Data reproducibility is at the cornerstone of scientific research. This is based on the agreement of results using the same methodologies and other definable study indices, however when reproducibility is called into question, science takes a backwards step and the prime focus becomes a reassessment of results and information at multiple angles. In 1960 “Incident at a Corner” a film directed by Alfred Hitchcock opens with a unique scene, intentionally repetitious the scene is shown from three camera angles. The viewpoints of the incident are perfectly clear, objective and straightforward. More importantly the scene shown several times is identical. The director insists that viewers are shown this, repeatedly, **This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/EPL.14740](https://doi.org/10.1111/EPL.14740)**

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three times just to be sure but - with one subtle change, different viewing points. Seemingly uncomplicated at first the reproduced and identical incident is seen by several people in very different ways. The scene by Hitchcock now becomes more than just an introduction of facts to the story it becomes clear that reproducibility is a science and it is clearly - complicated. The very concept of an absolute truth is distorted several ways. Two recent articles published in *Epilepsia* have examined cytosine methylation of the *BRD2* gene in juvenile myoclonic epilepsy in very different ways and with varying conclusions^{1, 2}.

It had been widely assumed that differences in specific genes might explain why some individuals develop certain pathologies. And while genetic determinism remains a major focus of translational research, it is now clear that even with comprehensive sequencing only a proportion of the variability in complex phenotypic traits can be explained by genetic variations using genome wide association studies (GWAS). Except for rare monogenetic disorders, the pathogenesis of disease appears to be because of complex interactions between environmental factors and genetic predisposition. Elucidating the genetic and non-genetic determinants of epilepsy remains one of the definitive aims of contemporary medical research. DNA methylation is a covalent chemical modification of cytosine that can change the activity of genetic elements without sequence change. Some common principles of the modification are (i) mammalian DNA methylation predominantly exists in CG dinucleotides as 5-methylcytosine (5mC), (ii) cytosine methylation exists in CpG islands with the great majority located near promoters and gene bodies to regulate these regions, (iii) methylation of CG-dense promoters inversely regulates gene activity, (iv) 5mC has evolved to effectively repress transposable elements, and (v) gene-body 5mC is highly enriched with actively transcribed genes. The effectiveness of cytosine methylation to precisely coordinate this epigenetic determinant is subject to 5mC-dependent protein readers that contain a highly conserved methyl-binding domain with remarkable specificity.

As for the role of epigenetic control in epilepsy, 5mC is not new to the field, increased cytosine methylation was originally described in human temporal lobe epilepsy (TLE) of the human *Reelin* promoter³. The choice method to detect differential methylation at the time involved bisulfite conversion of DNA followed by Sanger sequencing. Not surprisingly in recent years, 5mC sequencing- and array-based profiling technologies have significantly improved but there is no consensus on the choice nor are there recommendations on the different technologies to investigate cytosine methylation.

Differential DNA methylation (DMR) of CpG Island denoted CpG76 of *BRD2* promoter was associated with the rs3918149 single nucleotide polymorphism (SNP) in a small cohort of Caucasian JME subjects¹. Buffy coats were transformed to establish B-cell lymphoblast cells. DMR assessment involved three pyrosequencing assays designed to distinguish 10 cytosine methylation sites (amplimer set 1 assessed 4 CG sites while amplimer sets 2 and 3 assessed 3 CG sites each) of the CpG76 Island in the *BRD2* promoter. A bimodal distribution for CpG76 was reported for transformed lymphoblasts derived from Caucasian JME subjects (n=23) when compared to unaffected family members (n=23). In a smaller cohort of JME subjects with positive linkage to *BRD2* (n=14) bimodal methylation pattern was detected using the pyrosequencing assays when compared to unaffected family members (n=15). It's unclear from the pyrosequencing results which CG sites are methylated and contribute to the bimodal methylation status of CpG76 Island. DMRs were not observed for non-JME forms of genetic generalized epilepsy (GGE). A separate and much larger study analysed *BRD2* methylation and allelic association in a cohort of 782 European Caucasians including 116 JME subjects, 196 with genetic absence epilepsies (GAE) and 470 German population controls. Infinium Human 450K BeadArrays were used to assess 2 of the 10 reported cytosine methylation sites in CpG76 of the *BRD2* promoter². In contrast to the other report, methylation of *BRD2* was unremarkable with no association with SNP rs3918149 from whole blood. It's clear, cg16801540 and cg07223713 on the Infinium array do not display differential methylation in Caucasian JME's when compared to GAE's and control subjects. Although these studies examine cytosine methylation the similarities end there. Indeed, the challenge here is to reconcile the study differences which remain unexplained. So how does one go about comparing cytosine methylation in epilepsy? In addition to sample size, assay design considerations with respect to profiling technologies are important and this includes the choice of clinical specimens and tissue specificity. This raises the importance of consensus recommendations for cytosine methylation between different laboratories to improve assay accuracy and reliability. This has become even more important in recent years with the development of massive parallel sequencing and high throughput assays such as array-based technologies using CHARM (comprehensive high-throughput relative methylation) technique which uses methylation-sensitive restriction enzymes and the already mentioned Infinium bead array to detect methylated and unmethylated CG sites from bisulfite converted DNA. Not surprisingly, assessment of the success of sodium bisulfite conversion is critical and can be a source of variation. Furthermore, the conversion process

of genomic DNA with sodium bisulfite, leads to deamination of unmethylated cytosines to uracils, while 5-methylcytosine and 5-hydroxymethylcytosine, remain protected from deamination and thus unchanged. Other technologies that rely on the chemical conversion of DNA include whole-genome bisulfite sequencing (WGBS) and reduced representation bisulfite sequencing (RBBS). As is typical for this type of large study, design considerations such as cohort and sample selection including statistical significance for the detection of cytosine methylation need to be adequately powered. One of the major developments in profiling technologies for methylated cytosines is affinity enrichment. This technique uses the highly conserved methyl-binding domain of 5mC-dependent protein readers to enrich for methylated DNA and has been used reliably to examine the functional importance of methylation mediated gene expression using genome-wide computational analyses⁴.

Clearly, the reliable identification of genetic and epigenetic determinants is essential to dissect their role in regulating genes and signaling pathways implicated in epilepsy. While, some reports assessing methylation provide evidence for a role in epilepsy, the exact nature of this epigenetic determinant in other studies remains controversial, ambiguous and even misunderstood. The intent of this editorial is to initiate discussion, explore the technological differences and comment on paradigm recommendations for future cohorts rather than pass judgements. A consideration forward is to establish standard materials and methods to assay methylated cytosine in clinical specimens for the epilepsy field. A major gap in our knowledge are the factors that influence the detection of cytosine methylation in tissues using epigenomic profiling technologies, including how laboratories report on low level and heterogenous cytosine methylation. There are many factors that influence the detection of methylated cytosines and this relates to conflicting definitions of detection and end-point measures; differences in sample preparation methods, clinical definitions and histological/immunohistochemical staining that are often used in characterising epilepsy subtypes. While the combined effect of these factors is clearly important to promote standardization of approaches, tissue-specific controls are recommended as well as calibration of primary detection methods and subsequent validation assays of clinical samples. Guidelines for authors and reviewers on cytosine methylation determination should enhance the quality of research on the role of methylation in clinical epilepsy in the same way consensus classification has been important on the microscopic review of surgical specimens by the International League Against Epilepsy⁵. As for the position of cytosine methylation in juvenile myoclonic epilepsy a promising starting point is the impressive

cohort design by Schulz *et al.*,² which remains the main challenge for most epigenome wide association studies (EWAS). As is typical for this type of large study, the choice of throughput-technology used (in this case 450K array) for cytosine methylation comes down to trade-offs; balancing coverage with resolution, specificity and accuracy and of course benchmarked cost. For these reasons, my enthusiasm for large cohort studies remains undeniably high and with good reason, improved study design and searching for other disease-associated epigenetic variations using genome-wide detection methods to include the integration of EWAS and GWAS datasets should accelerate our understanding of genetic and epigenetic determinants implicated in epilepsy. To achieve this will require cooperation at multiple angles between scientists; geneticists and epigeneticists alike, including cohort registries and the development of unifying consensus recommendations on methods to detect cytosine methylation in epilepsy. Taken together, it seems that how we interpret cytosine methylation is clear however our viewpoint can be complicated. This serves as a useful reminder. Reproducibility, rather than success, is a science; with the same experimental conditions, you get the same experimental results.

‘I confirm that I have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.’

Disclosures None

Acknowledgements

Professor El-Osta is a Senior Research Fellow (1154650) and this work was supported by the National Health and Medical Research Council (1075563).

References

1. Pathak S, Miller J, Morris EC, et al. DNA methylation of the BRD2 promoter is associated with juvenile myoclonic epilepsy in Caucasians. *Epilepsia* 2018;**59**:1011-1019.
2. Schulz H, Ruppert AK, Zara F, et al. No evidence for a BRD2 promoter hypermethylation in blood leukocytes of Europeans with juvenile myoclonic epilepsy. *Epilepsia* 2019.

3. Kobow K, Jeske I, Hildebrandt M, et al. Increased reelin promoter methylation is associated with granule cell dispersion in human temporal lobe epilepsy. *J Neuropathol Exp Neurol* 2009;**68**(4):356-64.
4. Kobow K, Kaspi A, Harikrishnan KN, et al. Deep sequencing reveals increased DNA methylation in chronic rat epilepsy. *Acta Neuropathol* 2013;**126**:741-56.
5. Blumcke I, Thom M, Aronica E, et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia* 2011;**52**:158-74.