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BRIEF COMMUNICATION

SCN1A pathogenic variants do not have a distinctive blood-derived DNA methylation signature

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Abstract

DNA methylation signatures ("episignatures") can be used as biomarkers of genetic aberrations, clinical phenotypes, and environmental exposures in rare diseases. Episignatures are utilized in molecular diagnostics and can clarify variants of uncertain significance. A growing number of disease genes, including epilepsy genes, exhibit robust and reproducible episignatures. However, whether *SCN1A*, the most prominent epilepsy gene, has one or more episignatures has not yet been determined. We generated genome-wide DNA methylation data and performed episignature analysis on 64 individuals with Dravet syndrome due to pathogenic loss-of-function (LOF) variants in *SCN1A* and seven individuals with early

Christy W. LaFlamme and Karim Karimi contributed equally.

Bekim Sadikovic and Heather C. Mefford jointly supervised the work.

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infantile *SCN1A* developmental and epileptic encephalopathy due to pathogenic gain-of-function (GOF) variants in *SCN1A*, relative to a large reference database of controls and rare disease episignature-positive cohorts. We analyzed all samples with LOF variants together and performed separate analyses for missense, nonsense, and GOF variant cohorts. A reproducible blood-derived episignature was not evident in any of the cohorts using current analytical approaches and reference data.

KEYWORDS
DNA methylation, Dravet syndrome, *SCN1A*

1 | INTRODUCTION

Numerous rare genetic disorders have been linked to unique DNA methylation profiles, known as episignatures.[1,2] Episignatures are patterns of DNA methylation across the genome shared among individuals with pathogenic variants within the same gene, in a specific gene region encoding a functional domain, or in related genes encoding proteins involved in the same protein complex. In recent years, episignatures have emerged as robust and reliable biomarkers, playing a crucial role in diagnosing genetic disorders and reclassifying variants of uncertain significance (VUSs).[2–6] Episignatures can also be used to identify an etiology for individuals without a known genetic diagnosis. Their application in clinical diagnostic laboratories has demonstrated significant utility in providing diagnoses for patients with suspected rare genetic conditions who previously lacked a clear genetic diagnosis, producing an overall 18.7% diagnostic yield.[5,7]

We recently delineated the current diagnostic utility of episignatures in individuals with genetically unresolved developmental and epileptic encephalopathies (DEEs) as 1%.[8] Therefore, mapping episignatures for more genes is a priority to augment their utility across a broader spectrum of disorders, especially the epilepsies.

SCN1A, encoding the sodium channel alpha-1 subunit, is the most implicated gene in epilepsy.[7] Pathogenic loss-of-function (LOF) variants resulting in *SCN1A* haploinsufficiency cause Dravet syndrome (DS).[9] Pathogenic gain-of-function (GOF) variants cause a profound early infantile DEE (EIDEE).[10] The VUS burden of *SCN1A* is especially high, with more than 1230 VUSs in ClinVar.[11] VUSs are primarily missense variants (83%), which are difficult to interpret without functional data. A robust *SCN1A* episignature would help to resolve VUSs, allowing patients with pathogenic variants to benefit from precision therapies, which are currently in development.[12] Identification of an episignature for *SCN1A* could increase the diagnostic yield of episignatures for the epilepsies.[13] Here, we investigated DNA methylation in individuals with pathogenic variants in *SCN1A*, including LOF and GOF pathogenic contexts, and were unable to derive robust and reproducible episignatures for *SCN1A*, emphasizing the importance of alternative strategies for VUS resolution.

2 | MATERIALS AND METHODS

2.1 | Cohorts

We assessed 71 individuals (36 female) harboring *SCN1A* pathogenic variants (Data S1, Figure S1), where 64 of 71 (90%, 35 female) had DS (Mendelian Inheritance in Man [MIM]: 607208) with either nonsense ($n=28$), missense ($n=23$), splice site ($n=11$), or deletion ($n=2$) variants in *SCN1A*. Seven of the 71 total individuals (10%, 1 female) had missense GOF variants in *SCN1A* with EIDEE (MIM: 619317). For DS, methylation testing was performed (1) using all samples and (2) restricting to nonsense or missense cohorts to identify possible variant-type specific signatures. For GOF, all samples were used to train the model. This study was approved by the institutional review board (IRB) of St. Jude Children's Research Hospital (SJCRH). Written informed consent was provided by parents or legal guardians of individuals with DEEs with local IRB approval from SJCRH, the Human Research Ethics Committee of Austin Health (H2007/02961), the Health and Disability Ethics Committee (New Zealand), the University of Washington, and the Western University Research Ethics Board (REB 106302 and 116108).

2.2 | DNA methylation analysis

DNA methylation arrays (Illumina Infinium MethylationEPIC v1 or v2) were processed for bisulfite-converted, peripheral blood-derived DNA according

to the manufacturer's protocol. The data were loaded into R and processed with standard quality control metrics and normalization using the SeSAMe package (version 1.23.3).[14] Matched controls were randomly selected from the EpiSign Knowledge Database (EKD) at the London Health Sciences Center based on age (reported age if available, and if not, calculated), sex, and array type using MatchIt package (version 4.5.1).[15] Analyses were performed on all DS-causing variants ($n = 64$), nonsense variants ($n = 28$), missense variants ($n = 23$), and all GOF variants ($n = 7$). For each analysis, *M*-values (logit transformed β values) were fitted to a multivariate linear regression model using the limma package (version 3.52.4).[16] Batch and cell type composition were incorporated into the model as covariates. The top ~200 probes, representing individual CpG sites, were selected, and the support vector machine (SVM) classification model was constructed using the *SCN1A* training cases, matched controls, 75% of 106 other neurodevelopmental disorders (NDDs) from the EKD, and 75% of the EKD controls. The model was then tested on remaining *SCN1A* samples (if applicable), the remaining 25% of other NDDs, and 25% of EKD controls. More detailed methods are provided in the Supplementary Information.

3 | RESULTS

3.1 | *SCN1A* variants causing DS do not display a robust blood-derived episignature

We performed DNA methylation profiling for all 64 individuals with DS against matched controls, with a final selected probe list of 234 probes (Data S2A, Figure S2A). Both heatmap clustering and multidimensional scaling (MDS) indicated a lack of separation between groups, with the probe set failing to distinguish DS samples from controls (Figure 1A). When incorporated into the SVM classifier, the DS probes lacked sensitivity and specificity tested against 106 NDDs (Figure 1A, bottom). Thus, collectively, these analyses indicate that there is no detectable blood-derived episignature shared among the whole DS cohort.

3.2 | *SCN1A* nonsense and missense variants causing DS do not exhibit identifiable variant type-specific signatures

Because the cohort of all DS samples failed to produce a clear episignature, we tested subsets of the cohort to

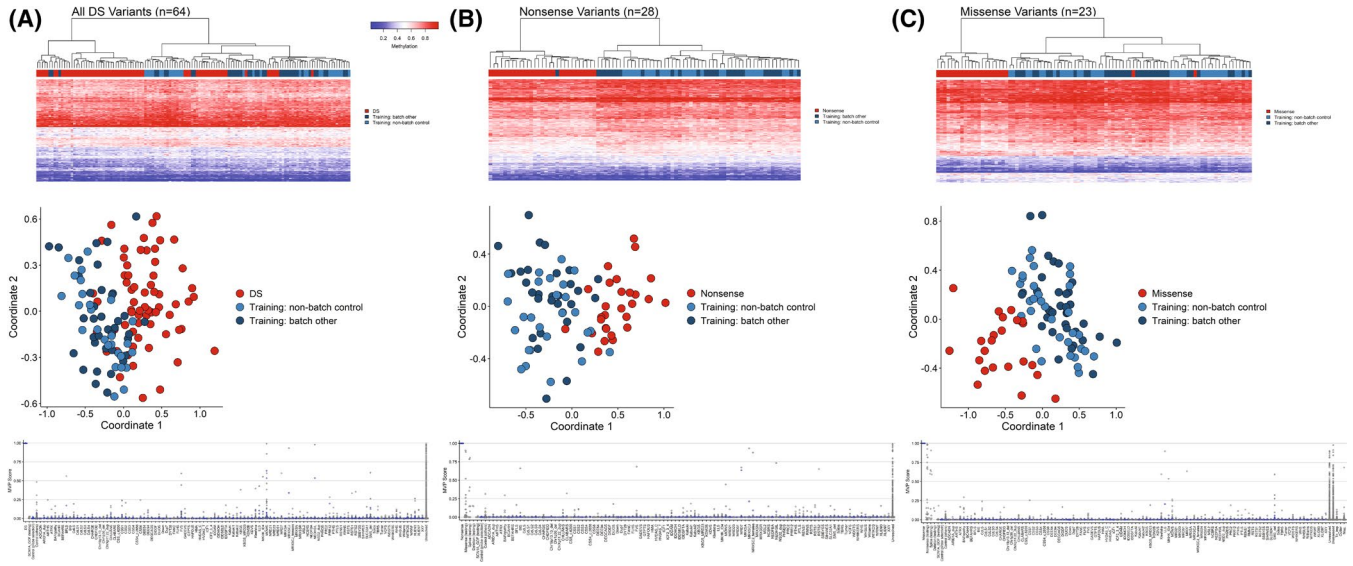

FIGURE 1 DNA methylation profiling of *SCN1A* variants causing Dravet syndrome (DS): (A) all pathogenic *SCN1A* variants ($n = 64$), (B) pathogenic *SCN1A* nonsense variants ($n = 28$), and (C) pathogenic *SCN1A* missense variants ($n = 23$) used to train the model. In each panel, the top plots are heatmaps. No clear clustering was observed between cases (red), controls from different batches (blue), and controls from the shared batches (dark blue). The middle plots are multidimensional scaling. No clear separation was observed between cases (red), controls from different batches (blue), and controls from the shared batches (dark blue). The bottom plots are methylation variant pathogenicity (MVP) plots. MVP scores are presented for all the *SCN1A* cases as well as cases from 106 other neurodevelopmental disorders from the EpiSign Knowledge Database (EKD). The support vector machine (SVM) classifier was trained using *SCN1A* cases ("DS" for all variants, "nonsense," and "missense"), matched controls, 75% of other controls from the EKD, and 75% of other neurodevelopmental disorders from the EKD (blue). The remaining 25% of controls and 25% of other neurodevelopmental disorders from the EKD were used for testing (gray). The model lacked specificity, as many samples from the other disorders also yielded high MVP scores.

investigate the presence of variant type-specific signatures. We performed DNA methylation profiling for 28 individuals with pathogenic nonsense variants in *SCN1A* and 23 individuals with pathogenic missense variants in *SCN1A* against matched controls. For the nonsense group, we derived a final probe set of 257 probes (Data S2B, Figure S2C), and for the missense, we derived 235 probes (Data S2C, Figure S2D). Both heatmaps and MDS plots indicated a lack of clear separation between cases and controls (Figure 1B,C, top and middle). When incorporated into the SVM classifier, both the nonsense and missense probes lacked sensitivity or specificity tested against 106 NDDs (Figure 1B,C, bottom). Collectively, these analyses indicate that there are no variant type-specific signatures for pathogenic nonsense or missense *SCN1A* variants.

3.3 | *SCN1A* GOF variants causing EIDEE lack a clear blood-derived episignature

We also performed analysis on seven *SCN1A* GOF variants associated with EIDEE.[10] This resulted in a final probe set of 226 probes (Table S2D, Figure S2B). Heatmap clustering, MDS, and the SVM classifier all indicate that the *SCN1A* GOF probes do not identify the cases or distinguish them from the other disorders in the EKD (Figure 2). Thus, the *SCN1A* GOF variants do not have a blood-derived episignature.

4 | DISCUSSION

We performed genome-wide DNA methylation profiling on blood-derived DNA from a large cohort of patients with DS or EIDEE associated with LOF or GOF *SCN1A* pathogenic variants, respectively. We used supervised and unsupervised clustering methods to attempt to map episignatures for 64 individuals with DS and seven individuals with EIDEE. Based on the lack of distinguishing probes, sensitivity, and specificity, our analyses indicate that neither LOF variants causing DS (entire cohort or separated into nonsense and missense variants) nor GOF variants causing EIDEE exhibit robust blood-derived episignatures.

Episignatures have predominantly been identified for genes involved in the epigenetic machinery or those that

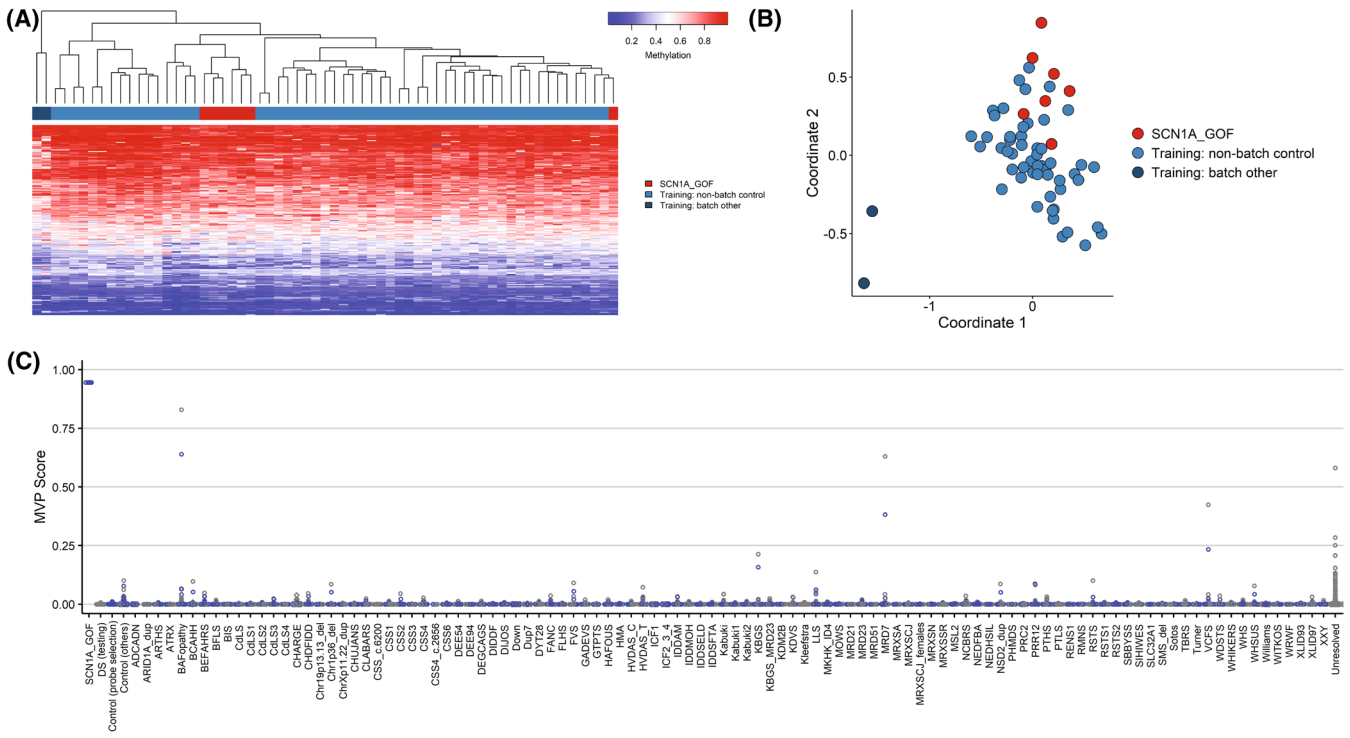

FIGURE 2 DNA methylation profiling of *SCN1A* gain-of-function (GOF) variants causing early infantile encephalopathy. (A) Heatmap. Cases with pathogenic GOF variants in *SCN1A* (red) were clustered with controls from different batches (blue). No robust clustering was observed. (B) Multidimensional scaling plot. *SCN1A* GOF samples (red) did not separate from controls (blue). (C) Methylation variant pathogenicity (MVP) plot. The support vector machine classifier was trained using *SCN1A* GOF cases ($n = 7$), matched controls, 75% of other controls from the EpiSign Knowledge Database (EKD), and 75% of other neurodevelopmental disorders from the EKD (blue). The remaining 25% of controls and 25% of other neurodevelopmental disorders from the EKD were used for testing (gray). The model was not of full specificity, as cases from other disorders also yielded high MVP scores.

function, at least in part, in the nucleus. Furthermore, episignatures are derived in blood DNA, and although ubiquitous expression is not a strict requirement for an episignature, many genes with established episignatures are expressed to some degree in the blood. In contrast, *SCN1A* encodes a subunit of a sodium channel that localizes to the cell membrane and is primarily expressed in the brain, with average transcripts per million in the blood of .000 according to GTEx (V8, dbGaP Accession phs000424.v8.p2, accessed August 20, 2024). Therefore, if an *SCN1A* episignature in blood did exist, it might be more subtle than many of those previously identified. We studied a relatively large cohort of 64 individuals with DS, although analysis of *SCN1A* GOF was restricted to a smaller sample size ($n = 7$) of the considerably rarer phenotype of EIDEE. Thus, more samples would be required to confirm the lack of evidence for an episignature for *SCN1A* GOF cases. In contrast, the episignature for the ubiquitously expressed epilepsy gene *CHD2*, encoding the chromodomain helicase DNA binding protein 2, was derived using only five samples and is a highly robust signature for pathogenic *CHD2* variants.[2]

The lack of probe sets that can distinguish *SCN1A* in all scenarios might be due to current limitations in technology, restricting the identification of all distinct sites. DNA methylation arrays are comprised of a curated set of CpG probes (850 000 for EPIC v1 and 935 000 for EPIC v2), whereas ~28 000 000 CpG sites exist across the human genome.[17] Study of significant changes in DNA methylation for *SCN1A* using whole-genome bisulfite sequencing or long-read sequencing with 5-methylcytosine detection may provide a more complete landscape. Furthermore, it is possible that a DNA methylation signature is present in other tissues, such as affected brain, which we did not address in this study.

Another explanation for the lack of an *SCN1A* episignature is the possibility of interference from other DNA methylation signals. A recent study identified a distinct DNA methylation profile for fetal valproate syndrome caused by antenatal exposure to valproic acid or valproate, which is a commonly prescribed antiseizure medication (ASM).[18] Although none of the participants in our study was exposed to valproic acid in utero, nearly all had been prescribed valproate or other ASMs at some point to treat their seizures, and it is unknown how valproate and other ASMs interact with or modify the presence of genetic episignatures. It will be important to collect data on when patients were exposed to potential chromatin-modifying ASMs like valproate (and other drugs), doses of each drug, and duration of treatment prior to blood collection. Statistical analyses will be challenging but critical to address these important questions. Future studies will need to examine to what extent ASMs and other environmental interactions may be a limiting factor for deriving robust episignatures for other epilepsy-associated genes. Furthermore, it is unknown when, how, and why episignatures are established in the first place, and it is yet to be determined to what extent additional groups of genetic disorders, genes, and environmental influences will exhibit episignatures. Not all genes will have robust episignatures.

In the case of *SCN1A*, efforts to resolve VUSs and classify variants might focus on in vitro high-throughput assays to assess the functional impact of specific variants, machine learning methods, phenomics, or a combination thereof. Although DNA methylation has been an appealing approach for a wealth of genetic conditions, we found that it is currently not a feasible test for the classification of pathogenic *SCN1A* LOF or GOF variants.

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CONFLICT OF INTEREST STATEMENT

B.S. is a shareholder in EpiSign, a company involved in commercialization of EpiSign software. I.E.S. has served on scientific advisory boards for BioMarin, Chiesi, Eisai, Encoded Therapeutics, GlaxoSmithKline, Knopp Biosciences, Nutricia, Takeda Pharmaceuticals, UCB, Xenon Pharmaceuticals, and Longboard Pharmaceuticals; has received speaker honoraria from GlaxoSmithKline, UCB, BioMarin, Biocodex, Chiesi, LivaNova, Nutricia, Zuellig Pharma, Stoke Therapeutics, Eisai, Akumentis, and Praxis; has received funding for travel from UCB, Biocodex, GlaxoSmithKline, BioMarin, Encoded Therapeutics, Stoke Therapeutics, Eisai, and Longboard Pharmaceuticals; has served as an investigator for Anavex Life Sciences, Cerevel Therapeutics, Eisai, Encoded Therapeutics, EpiMinder, Epygenyx, ES-Therapeutics, GW Pharma, Longboard Pharmaceuticals,

Marinus, Neurocrine BioSciences, Ovid Therapeutics, SK Life Science, Takeda Pharmaceuticals, UCB, Ultragenyx, Xenon Pharmaceuticals, Zogenix, and Zynerba; has consulted for Care Beyond Diagnosis, Epilepsy Consortium, Atheneum Partners, Ovid Therapeutics, UCB, Zynerba Pharmaceuticals, BioMarin, Encoded Therapeutics, Biohaven Pharmaceuticals, Stoke Therapeutics, and Praxis; and is a nonexecutive director of Bellberry and a director of the Australian Academy of Health and Medical Sciences. She may accrue future revenue on pending patent WO61/010176 (filed 2008): Therapeutic Compound; has a patent for SCN1A testing held by Bionomics and licensed to various diagnostic companies; and has a patent for a molecular diagnostic/theranostic target for benign familial infantile epilepsy (PRRT2; 2011904493 & 2012900190 and PCT/AU2012/001321; TECH ID: 2012–009). L.G.S. receives funding from the Health Research Council of New Zealand and Cure Kids New Zealand, is a consultant for the Epilepsy Consortium, and has received travel grants from Seqirus and Nutricia. L.G.S. has received research grants and consultancy fees from Zynerba Pharmaceuticals and has served on Takeda and Eisai Pharmaceuticals scientific advisory panels. None of the other authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

Methylation array data for individuals with pathogenic variants in *SCN1A* who have consented to data sharing are made available through GEO (GSE280241). Additional data requests can be directed to H.C.M. Individual genomic, epigenomic, or any other personally identifiable data that have not previously been made publicly available for samples in the EKD are prohibited from deposition in publicly accessible databases due to institutional and ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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