Check for updates

Received: 13 August 2018 | Revised: 7 January 2019 | Accepted: 7 January 2019

DOI: 10.1111/epi.14657

BRIEF COMMUNICATION

Epilepsia

No evidence for a *BRD2* promoter hypermethylation in blood leukocytes of Europeans with juvenile myoclonic epilepsy

Herbert Schulz[1] | **Ann-Kathrin Ruppert**[1] | **Federico Zara**[2] | **Francesca Madia**[2] | **Michele Iacomino**[2] | **Maria S. Vari**[3] | **Ganna Balagura**[3] | **Carlo Minetti**[3] | **Pasquale Striano**[3] | **Amedeo Bianchi**[4] | **Carla Marini**[5] | **Renzo Guerrini**[5] | **Yvonne G. Weber**[6] | **Felicitas Becker**[6,7] | **Holger Lerche**[6] | **Claudia Kapser**[8] | **Christoph J. Schankin**[8,9] | **Wolfram S. Kunz**[10] | **Rikke S. Møller**[11,12] | **Karen L. Oliver**[13] | **Susannah T. Bellows**[13] | **Saul A. Mullen**[13] | **Samuel F. Berkovic**[13] | **Ingrid E. Scheffer**[13,14,15] | **Hande Caglayan**[16,17] | **Ugur Ozbek**[18] | **Per Hoffmann**[19,20,21] | **Sara Schramm**[22] | **Despina Tsortouktzidis**[23] | **Albert J. Becker**[23] | **Thomas Sander**[1]

[1]Cologne Center for Genomics, University of Cologne, Cologne, Germany

[2]Laboratory of Neurogenetics and Neuroscience, G. Gaslini Institute, Genoa, Italy

[3]Pediatric Neurology and Muscular Diseases Unit, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, and Maternal and Child Health, University of Genoa, G. Gaslini Institute, Genoa, Italy

[4]Division of Neurology, Hospital San Donato Arezzo, Arezzo, Italy

[5]Pediatric Neurology and Neurogenetics Unit and Laboratories, A. Meyer Children's Hospital, University of Florence, Florence, Italy

[6]Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Tübingen, Germany

[7]Department of Neurology, University of Ulm, Ulm, Germany

[8]Department of Neurology, Großhadern Hospital, University of Munich, Munich, Germany

[9]Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

[10]Department of Epileptology, Institute of Experimental Epileptology and Cognition Research, University of Bonn Medical Center, Bonn, Germany

[11]Danish Epilepsy Center, Dianalund, Denmark

[12]Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark

[13]Epilepsy Research Centre, Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

[14]Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia

[15]Florey Institute of Neuroscience and Mental Health, Heidelberg, Victoria, Australia

[16]Department of Molecular Biology and Genetics, Boğaziçi University, Istanbul, Turkey

[17]Izmir Biomedicine and Genome Center, Izmir, Turkey

[18]Department of Medical Genetics, Faculty of Medicine, Acibadem University, Istanbul, Turkey

[19]Department of Genomics, Life and Brain Center, Institute of Human Genetics, University of Bonn, Bonn, Germany

[20]Human Genomics Research Group, Department of Biomedicine, University of Basel, Basel, Switzerland

[21]Institute of Medical Genetics and Pathology, University Hospital of Basel, Basel, Switzerland

[22]Institute of Medical Informatics, Biometry, and Epidemiology, University of Duisburg-Essen, Essen, Germany

[23]Translational Epilepsy Research, Department of Neuropathology, University of Bonn Medical Center, Bonn, Germany

© 2019 International League Against Epilepsy

Correspondence
Thomas Sander, Cologne Center for Genomics, University of Cologne, Cologne, Germany.
E-mail: sandert@uni-koeln.de

Funding information
European Union: FP6 Integrated Project EPICURE, grant LSHM-CT-2006-037315 to H.L., and T.S.; FP7 DESIRE, grant 602531 to C.M., R.G., and T.S.; EPITARGET, grant 602102 to A.J.B. and P.H. German Research Foundation (DFG): EUROCORES Program EuroEPINOMICS, grant SA434/5–1 to T.S.; DFG Research Unit FOR2715, grants SA434/6-1 to T.S., WE4896/3-1 and WE4896/4-1 to Y.G.W., LE1030/16-1 to H.L., and BE2078/5-1 to A.J.B. German Federal Ministry of Education and Research: NGFNplus EMINet, grants 01GS08120 to T.S., and 01GS08123 to H.L.; IntenC, grant TUR 09/I10 to T.S. Scientific and Technological Research Council of Turkey: TUBITAK grant 106S027 to H.C. Boğaziçi University Research Fund: grants 05HB104D, 06B107D, and 08HB104D to H.C. National Health and Medical Research Council (Australia): grant to S.A.M., S.F.B., K.L.O., I.E.S., and S.F.B. The Heinz Nixdorf Recall Study is supported by the Heinz Nixdorf Foundation (Germany), the German Ministry of Education and Science, and the DFG (grants SI236/8-1, SI236/9-1, and ER155/6-1).

Summary
Juvenile myoclonic epilepsy (JME) is a common syndrome of genetic generalized epilepsies (GGEs). Linkage and association studies suggest that the gene encoding the bromodomain-containing protein 2 (*BRD2*) may increase risk of JME. The present methylation and association study followed up a recent report highlighting that the *BRD2* promoter CpG island (CpG76) is differentially hypermethylated in lymphoblastoid cells from Caucasian patients with JME compared to patients with other GGE subtypes and unaffected relatives. In contrast, we found a uniform low average percentage of methylation (<4.5%) for 13 CpG76-CpGs in whole blood cells from 782 unrelated European Caucasians, including 116 JME patients, 196 patients with genetic absence epilepsies, and 470 control subjects. We also failed to confirm an allelic association of the *BRD2* promoter single nucleotide polymorphism (SNP) rs3918149 with JME (Armitage trend test, $P = 0.98$), and we did not detect a substantial impact of SNP rs3918149 on CpG76 methylation in either 116 JME patients (methylation quantitative trait loci [meQTL], $P = 0.29$) or 470 German control subjects (meQTL, $P = 0.55$). Our results do not support the previous observation that a high DNA methylation level of the *BRD2* promoter CpG76 island is a prevalent epigenetic motif associated with JME in Caucasians.

KEYWORDS
association analysis, *BRD2*, DNA methylation, genetic generalized epilepsy, juvenile myoclonic epilepsy

1 | INTRODUCTION

Juvenile myoclonic epilepsy (JME) is a common syndrome of genetic generalized epilepsies (GGEs), accounting for 5%-10% of all epilepsies.[1] Genetic factors play a predominant role in the etiology of JME, but the vast majority of the underlying susceptibility genes remain elusive, probably due to an extensive genetic heterogeneity and complex inheritance.[2–4]

Linkage to the chromosomal region 6p21.3 and association studies of positional candidate genes suggest that the gene encoding the bromodomain-containing protein 2 (gene symbol: *BRD2*) may increase risk of JME in Caucasians.[4,5] The reported allelic association of JME with the *BRD2* promoter single nucleotide polymorphism (SNP) rs3918149 and the lack of potentially causative coding mutations suggested that susceptibility of JME could result from a regulatory effect of the promoter SNP on *BRD2* expression.[5] Recently, Pathak and coworkers reported that hypermethylation of a CpG island (CpG76) within the *BRD2* promoter was associated with JME in Caucasians.[6] *BRD2* CpG76 hypermethylation in B-lymphoblastoid cells was found in 12 of 23 Caucasian JME patients, predominantly in 11 of 14 JME patients retrieved from families with positive linkage evidence to the *BRD2* locus. In contrast, the *BRD2* CpG76 methylation state was low in 23 unaffected family members and 16 patients with other (non-JME) GGE syndromes. With regard to the relatively small sample size of the original study and the potential clinical relevance of this prevalent epigenetic biomarker for JME, our present methylation and association analyses explored the validity of a JME-associated DNA hypermethylation of the *BRD2* promoter CpG76 island in Caucasians.

15281167, 2019, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.14657 by Acibadem Mehmet Ali Aydinlar Universitesi, Wiley Online Library on [26/04/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

15281167, 2019, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.14657 by Acibadem Mehmet Ali Aydinlar Universitesi, Wiley Online Library on [26/04/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2 | MATERIALS AND METHODS

2.1 | Study participants

Our multicenter study cohort comprised 782 unrelated European Caucasians (411 females, 371 males; Table S1, Figure S1A and S1B), including 116 JME patients (72 females, 44 males), 196 patients with genetic absence epilepsies (GAEs; 120 females, 76 males; 142 childhood and 53 juvenile absence epilepsies, one unspecified genetic absence epilepsy), and 470 German population controls (219 females, 251 males).[7] Twenty-one JME patients were derived from GGE-multiplex families.[8] Diagnostic classification of GGE syndromes was carried out according to the International League Against Epilepsy guidelines, as described elsewhere.[7] The German control subjects were recruited from the Heinz Nixdorf Recall Study cohort. The study protocol was approved by the local institutional review boards, and all study participants gave informed consent.

2.2 | Methylation analysis and data processing

Individual genomic DNA samples were extracted from whole blood cells. Methylation profiling of sodium bisulfite converted DNA (500 ng) was carried out using the Infinium HumanMethylation450 BeadChip (HM450k array; Illumina). The signal intensities were extracted using GenomeStudio version 2011.1 based on the HumanMethylation450 manifest version 1. The R-package *meffil 1.0.0* was applied for quality control, functional normalization, and surrogate variable analysis (SVA).[9] The CpG methylation level was assessed as methylation percentage (β values). After stringent quality filtering,[9,10] 13 CpGs covering the *BRD2* CpG76 island (chr6:32,935,896-32,936,792, hg19) were chosen for methylation analysis (Table S2). Two CpG76-CpGs (cg16801540, cg07223713) overlap with the original set of 10 CpG76-CpGs (S. Pathak, personal communication).[6] The average CpG76 methylation percentage was determined to infer the methylation state of the *BRD2* promoter CpG76 island.[11] Individual genotypes of the *BRD2* promoter SNP rs3918149 (chr6:32,936,373G>A, hg19) were retrieved from the imputed array-based SNP dataset of the study participants generated for our previous genome-wide association study (GWAS) of common GGE syndromes and genome-wide methylation quantitative trait loci (meQTL) analyses of hippocampal biopsies, respectively.[7,12] The individual sample identity of the SNP and CpG methylation datasets was confirmed based on the genotype concordance of control SNPs assessed by the HM450k and SNP arrays.[7,12]

2.3 | Statistical methods

meQTL analysis and differential methylation analysis was carried out by SVA implemented in *meffil*.[9] The covariates gender and age at sampling were included in the regression model. The Armitage trend test was applied to compare the SNP allele frequencies in case-control cohorts.

3 | RESULTS

3.1 | Exploration of a JME-associated *BRD2* promoter hypermethylation

Our methylation analysis revealed a low individual percentage of methylation for each single CpG76-CpG among all 782 European study participants, including 116 JME patients, 196 patients with genetic absence epilepsies, and 470 German population controls (Figure 1, Table S1). The individual CpG methylation states differed marginally across the 13 investigated CpG76-CpGs (Table S1, Figure S2). Overall, the average methylation percentages of 13 CpG76-CpGs did not exceed 4.5% in the entire study cohort and displayed a unimodal distribution with little interindividual variation (Figure 1). Consistently, the two overlapping CpG76-CpGs displayed uniform individual average methylation percentages below 7% among the 782 study participants (Figure S4, Table S1).

3.2 | Association and meQTL analyses of the *BRD2* SNP rs3918149 for JME

Our association analysis of JME with the *BRD2* promoter SNP rs3918149 obtained similar allele frequencies in 116

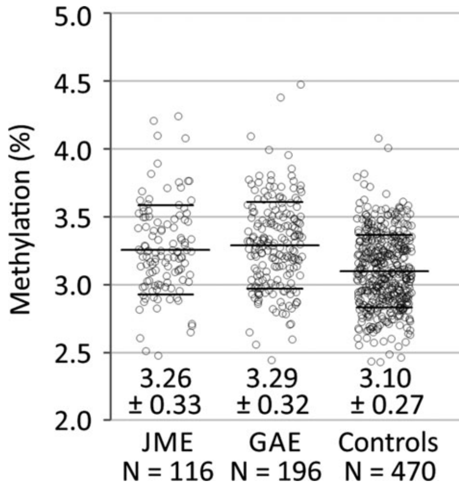


FIGURE 1 Average percentage of methylation of 13 *BRD2* promoter CpG76-CpGs in whole blood cells from 116 European Caucasian patients with juvenile myoclonic epilepsy (JME), 196 European patients with genetic absence epilepsy (GAE), and 470 German population controls. The mean methylation percentage ± SD is given for each study group

European JME patients ($f_A = 0.125$; genotype counts: 1 A/A, 27 A/G, 88 G/G) and 470 German population controls ($f_A = 0.126$; genotype counts: 9 A/A, 100 A/G, 361 G/G; Hardy-Weinberg Equilibrium (HWE), $P = 0.50$; Armitage trend test, $P = 0.983$, two-sided). Likewise, we found no evidence for an association of the SNP rs3918149 with JME in our previous GWAS including 817 European JME patients ($f_A = 0.123$; 11 A/A, 177 A/G, 623 G/G) and 2419 German control subjects ($f_A = 0.113$; 33 A/A, 478 A/G, 1,892 G/G; HWE, $P = 0.65$; Armitage trend test, $P = 0.302$, two-sided; Figure S3).[7] Furthermore, our meQTL analysis provides no evidence for a regulatory *cis*-acting effect of the SNP rs3918149 on the average percentage of CpG76 methylation in 116 JME patients (*cis*-meQTL, $P = 0.285$) as well as 470 German control subjects (*cis*-meQTL, $P = 0.547$; Figure 2). Notably, our previous meQTL analysis of 110 hippocampus biopsies revealed no *cis*-meQTL of SNP rs3918149 and CpG76-CpG methylation.[12] Our SVA did not detect significant differences in the average methylation percentage of the 13 *BRD2* CpG76-CpGs from 116 JME patients compared to 196 GAE patients ($P = 0.637$).

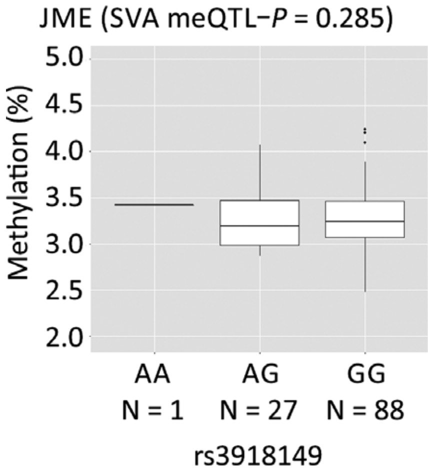
4 | DISCUSSION

In contrast to the previous report,[6] we did not detect *BRD2* CpG76 hypermethylation in whole blood cells from 116 unrelated European Caucasian JME patients, as well as 196 European GAE patients and 470 German control subjects. We found a unimodal distribution of average CpG76 methylation percentage ranging between 2.4% and 4.5% with little interindividual variation. These conflicting results could reflect three critical factors as follows: (1) genetic heterogeneity, (2) cell type–specific differential methylation, and (3) technical variation of the methylation assays.

Despite the predominant genetic etiology of JME, extensive genetic heterogeneity and complex inheritance hinder the molecular genetic dissection of the underlying genetic risk factors.[2,3] Considering that we have applied the same diagnostic criteria for JME and the recruitment scheme,[6,7] the genetic architecture underlying JME should be similar in the original and the present study. Given the high prevalence (>10%) of *BRD2* CpG76 hypermethylation in Caucasian JME patients reported in the original study,[6] it is unlikely ($P < 10^{-5}$) to miss this common epigenetic motif in 116 unrelated European Caucasian JME patients.

Technical variation may be a source for spurious methylation signals. Using accurate experimental procedures, it is unlikely that the technology itself accounts for the contradictory methylation results. Likewise, the different only partially overlapping sets of CpG76-CpGs investigated in the original and the present study should not play a critical role, given a uniform methylation pattern of CpGs within promoter CpG islands.[11] Notably, Pathak et al reported a differential bimodal distribution of the average CpG76 methylation percentages among JME patients (hypermethylated state: 80%-90%, hypomethylated state: 5%-20%).[6] These distinct CpG76 methylation patterns indicate uniform methylation states across the individual CpG76-CpGs. Consequently, the two overlapping CpG76-CpGs should have detected *BRD2* promoter CpG76 hypermethylation in the present study.

Cell type–specific methylation might explain differential methylation of *BRD2* CpG76 in B-lymphoblastoid cells and whole blood leukocytes.[13,14] B-Lymphoblastoid cell lines (LCLs) represent Epstein-Barr virus–immortalized $CD19^+$ B lymphocytes. B Lymphocytes account for a small proportion (4%-10%) of whole blood leukocytes. Therefore, B-lymphocyte–specific hypermethylation may be undetectable in whole blood leukocytes. However, a previous study investigating methylation profiles of flow-sorted blood cell types revealed uniformly low methylation



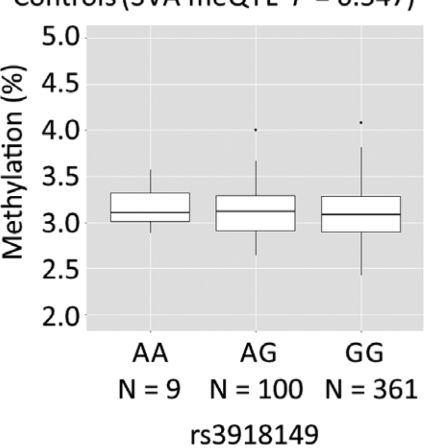


FIGURE 2 *Cis*-methylation quantitative trait loci (meQTL) results of single nucleotide polymorphism rs3918149 genotypes versus average percentage of methylation of 13 *BRD2* CpG76-CpGs in whole blood cells from 116 European juvenile myoclonic epilepsy (JME) patients (left) and 470 German control subjects (right). SVA, surrogate variable analysis

15281167, 2019, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.14657 by Acibadem Mehmet Ali Aydinlar Universitesi, Wiley Online Library on [26/04/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

levels of the CpG76-CpGs with marginal variation across the major blood cell types (Table S2).[15] With regard to the clonality of LCLs[16] and the widespread random clonal-related monoallelic expression/methylation of human autosomal genes,[14,17] differential methylation/expression patterns obtained from virtually monoclonal LCLs should be interpreted with caution.[16] Moreover, multiple random and directed changes in DNA methylation during cell culturing may lead to aberrant methylation signals.[13,14,18]

Given that the association of JME with the *BRD2* promoter SNP rs3918149 and the CpG76 hypermethylation state has been found in the same JME families,[4–6] we examined the hypothesis that the JME-associated SNP rs3918149 exerts a regulatory effect on CpG76 methylation. Unlike the original association, we fail to confirm an allelic association of SNP rs3918149 with JME. Likewise, our methylation analysis does not provide evidence that the SNP rs3918149 affects CpG76 methylation in whole blood leukocytes and in human hippocampus biopsies.[12] Taken together, our present results do not support previous studies[4–6] highlighting the *BRD2* gene as a susceptibility gene for JME.

ACKNOWLEDGMENTS

We thank Dr Pathak and Prof Greenberg for providing supplementary information specifying the pyrosequencing assays of the *BRD2* CpG76-CpGs investigated in their methylation study, and all investigators who contributed to the generation of this dataset.[6] This work was supported by grants from the European Union (FP6 Integrated Project EPICURE, grant LSHM-CT-2006-037315 to H.L., and T.S.; FP7 DESIRE, grant 602531 to C.M., R.G., and T.S.; EPITARGET, grant 602102 to A.J.B. and P.H.), the German Research Foundation (DFG) within the EUROCORES Program EuroEPINOMICS (grant SA434/5–1 to T.S.), the DFG Research Unit FOR 2715 (grants SA434/6-1 to T.S., WE4896/3-1 and WE4896/4-1 to Y.G.W., LE1030/15-1 to H.L., and BE2078/5-1 to A.J.B), the German Federal Ministry of Education and Research (NGFNplus EMINet, grants 01GS08120 to T.S., and 01GS08123 to H.L.; IntenC grant TUR 09/I10 to T.S.), the Scientific and Technological Research Council of Turkey (TUBITAK grant 106S027 to H.C.), the Boğaziçi University Research Fund (grants 05HB104D, 06B107D, and 08HB104D to H.C.); and the National Health and Medical Research Council (Australia; grant to S.A.M., S.F.B., K.L.O., I.E.S., and S.F.B.). Blood samples from the German control subjects were drawn from the Heinz Nixdorf Recall Study (HNR) cohort. The HNR study is supported by the Heinz Nixdorf Foundation (Germany). Additionally, the study is funded by the German Ministry of Education and Science and the DFG (grants SI236/8-1, SI236/9-1, ER155/6-1). The genotyping of the Illumina HumanOmni-1 Quad BeadChips of the HNR subjects was financed by the German Center for Neurodegenerative Disorders, Bonn.

DISCLOSURE

The authors have no conflicts of interest to report. 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.

ORCID

Pasquale Striano https://orcid.org/0000-0002-6065-1476
Samuel F. Berkovic https://orcid.org/0000-0003-4580-841X

REFERENCES

1. Camfield CS, Striano P, Camfield PR. Epidemiology of juvenile myoclonic epilepsy. Epilepsy Behav. 2013;28(suppl 1):S15–7.
2. Delgado-Escueta AV, Koeleman BP, Bailey JN, et al. The quest for juvenile myoclonic epilepsy genes. Epilepsy Behav. 2013;28 (suppl 1):S52–7.
3. Mullen SA, Berkovic SF. Genetic generalized epilepsies. Epilepsia. 2018;59:1148–53.
4. Greenberg DA, Durner M, Keddache M, et al. Reproducibility and complications in gene searches: linkage on chromosome 6, heterogeneity, association, and maternal inheritance in juvenile myoclonic epilepsy. Am J Hum Genet. 2000;66:508–16.
5. Pal DK, Evgrafov OV, Tabares P, et al. BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. Am J Hum Genet. 2003;73:261–70.
6. Pathak S, Miller J, Morris EC, et al. DNA methylation of the BRD2 promoter is associated with juvenile myoclonic epilepsy in Caucasians. Epilepsia. 2018;59:1011–9.
7. EPICURE Consortium, EMINET Consortium, Steffens M, Leu C, Ruppert AK, et al. Genome-wide association analysis of genetic generalized epilepsies implicates susceptibility loci at 1q43, 2p16.1, 2q22.3 and 17q21.32. Hum Mol Genet. 2012;21:5359–72.
8. EPICURE Consortium, Leu C, de Kovel CG, Zara F, et al. Genome-wide linkage meta-analysis identifies susceptibility loci at 2q34 and 13q31.3 for genetic generalized epilepsies. Epilepsia. 2012;53:308–18.
9. Min JL, Hemani G, Davey Smith G, et al. Meffil: efficient normalization and analysis of very large DNA methylation datasets. Bioinformatics. 2018;34:3983–9.
10. Zhou W, Laird PW, Shen H. Comprehensive characterization, annotation and innovative use of Infinium DNA methylation BeadChip probes. Nucleic Acids Res. 2017;45:e22.
11. Long HK, King HW, Patient RK, et al. Protection of CpG islands from DNA methylation is DNA-encoded and evolutionarily conserved. Nucleic Acids Res. 2016;44:6693–706.
12. Schulz H, Ruppert AK, Herms S, et al. Genome-wide mapping of genetic determinants influencing DNA methylation

15281167, 2019, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.14657 by Acibadem Mehmet Ali Aydinlar Universitesi, Wiley Online Library on [26/04/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and gene expression in human hippocampus. Nat Commun. 2017;8:1511.
13. Thompson TM, Sharfi D, Lee M, et al. Comparison of whole-genome DNA methylation patterns in whole blood, saliva, and lymphoblastoid cell lines. Behav Genet. 2013;43:168–76.
14. Onuchic V, Lurie E, Carrero I, et al. Allele-specific epigenome maps reveal sequence-dependent stochastic switching at regulatory loci. Science. 2018;361. pii: eaar3146.
15. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. Genome Biol. 2014;15:R31.
16. Plagnol V, Uz E, Wallace C, et al. Extreme clonality in lymphoblastoid cell lines with implications for allele specific expression analyses. PLoS One. 2008;3:e2966.
17. Gimelbrant A, Hutchinson JN, Thompson BR, Chess A. Widespread monoallelic expression on human autosomes. Science. 2007;318:1136–40.
18. Saferali A, Grundberg E, Berlivet S, et al. Cell culture-induced aberrant methylation of the imprinted IG DMR in human lymphoblastoid cell lines. Epigenetics. 2010;5:50–60.

SUPPORTING INFORMATION

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

How to cite this article: Schulz H, Ruppert A-K, Zara F, et al. No evidence for a *BRD2* promoter hypermethylation in blood leukocytes of Europeans with juvenile myoclonic epilepsy. *Epilepsia*. 2019;60:e31–e36. https://doi.org/10.1111/epi.14657

15281167, 2019, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.14657 by Acibadem Mehmet Ali Aydinlar Universitesi, Wiley Online Library on [26/04/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License