Analysis of Blood DNA Methylation in Early-Life Seizures

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Epigenome-Wide Association Study of Seizures in Childhood and Adolescence

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The occurrence of seizures in childhood is often associated with neurodevelopmental impairments and school underachievement. Common genetic variants associated with epilepsy have been identified, and epigenetic mechanisms have also been suggested to play a role. In this study, we analyzed the association of genome-wide blood DNA methylation with the occurrence of seizures in ~800 children from the Avon Longitudinal Study of Parents and Children, United Kingdom, at birth (cord blood), during childhood, and adolescence (peripheral blood). We also analyzed the association between the lifetime occurrence of any seizures before age 13 with blood DNA methylation levels. We sought replication of the findings in the Generation R Study and explored causality using Mendelian randomization, that is, using genetic variants as proxies. The results showed 5 CpG sites which were associated cross-sectionally with seizures either in childhood or adolescence (1%-5% absolute methylation difference at pFDR < 0.05), although the evidence of replication in an independent study was weak. One of these sites was located in the brain-derived neurotrophic factor gene, which is highly expressed in the brain, and showed high correspondence with brain methylation levels. The Mendelian randomization analyses suggested that seizures might be causal for changes in methylation rather than vice versa. In conclusion, we show a suggestive link between seizures and blood DNA methylation while at the same time exploring the limitations of conducting such study.

Commentary

Epigenetic mechanisms are histone and DNA modifications that affect gene activity without changing the DNA sequence and have been identified as critical mediators of large-scale gene transcription programs in the brain. Disruptions in any of these epigenetic processes contribute to aberrant neuronal activity in epilepsy.[1,2] DNA methylation is a well-studied epigenetic modification involved in the silencing of gene transcription and chromatin plasticity in epilepsy.[2-9] Two major forms of DNA methylation have been studied in the brain, DNA methylation at 5-methylcytosine (5-mC) mediated by DNA methyltransferases and the partial oxidation of 5-mC by the ten-eleven translocation family of dioxygenases to 5-hydroxymethylcytosine (5-hmC).[2] Both 5-mC and 5-hmC DNA methylation forms are dynamically controlled and considered to be stable epigenetic marks required for gene transcription homeostasis. Hence, an imbalance in DNA methylation, that is, hypo or hypermethylation, can alter gene networks and influence synaptic plasticity,[10] which may contribute to early-life seizures.[11]

Febrile seizures occur in 2% to 4% of children younger than 6 years in Europe and the United States, with peak incidence at 18 months of age. Childhood seizures can result in cellular and molecular changes that are potential risk factors for the development of epilepsy, in conjunction with a high association with neurodevelopmental conditions and learning and behavioral disabilities.[12,13] Over the years, genome-wide association epilepsy studies have focused on the identification of common genetic variants linked to epilepsy etiology in hopes of developing novel preventive strategies for therapeutic intervention.[14,15] However, epigenome-wide association studies (EWAS) have proven to be an additional factor to consider in the identification of high-risk gene loci in epilepsy.

In this study, Caramaschi and colleagues employed a combination of investigative techniques to determine whether blood DNA methylation tracked with the occurrence of seizures in childhood and adolescence. Using a prospective birth cohort study based in the United Kingdom, the Avon Longitudinal Study of Parents and Children (ALSPAC), Caramaschi and colleagues examined the epigenome-wide association of DNA methylation in the peripheral blood of children. To analyze seizures within the chosen cohorts from ALSPAC and Generation R cohorts, questionnaires were assigned to the parents of epileptic children near the time of seizure episodes to decrease recollection biases. Modeled DNA methylation blood sampling from ALSPAC cohorts included mother–child pairs

at several time points, which allowed age-specific cross-sectional analyses. Blood samples included the following: blood methylation, seizure status, age, sex, birth weight (only used for infants), gestational age, maternal prenatal smoking, maternal education, nucleated red blood cells, granulocytes, monocytes, natural killer cells, B cells + CD4(+)T cells, CD8(+)T cells, SV1 + . . . + SV15.

Regarding bioinformatic analyses, Caramaschi and colleagues collected meticulous information from obstetric records and models for DNA methylation were adjusted with specific analytical methods. Replication analyses were performed in multiple cohorts, exhausting numerous statistical analyses. Additionally, the authors used false discovery rate (FDR) correction for seizures that scored below the threshold to look at DNA methylation at specific cytosines followed by guanine residues (CpG) sites. In a second replication analysis, the authors used the Infinium HumanMethylation450 (HM450) BeadChip for assessment of DNA methylation in peripheral blood from children. A final cohort affected with or without seizures was analyzed with probes against the brain-derived neurotrophic factor (*BDNF*) gene on Generation R on EWAS FDR-corrected methylation sites. The Mendelian randomization method was used to examine EWAS results for CpG sites to determine a possible causal relationship between DNA methylation and seizure/epilepsy.

Blood DNA methylation analyses revealed similar changes in blood and brain DNA methylation levels at the *BDNF* gene. Caramaschi and colleagues speculate that *BDNF* DNA methylation in blood samples might be linked to epigenetic changes in brain regions impacted by seizure/epilepsy episodes. The authors observed hypermethylation at the promoter and within introns of the *BDNF* gene, which correlated with seizures with the Mendelian randomization analysis. However, changes in *BDNF* mRNA levels in blood were not supported by their Mendelian randomization analysis of seizures method. Hence, the authors concluded that this unexpected finding was because *BDNF* mRNA levels in blood might be very low. Furthermore, when the authors analyzed DNA methylation in cross-tissue blood–brain concordance in 3 independent databases at 5 CpG sites that passed FDR correction in ALSPAC, the assumption that brain DNA methylation reflected changes in blood DNA methylation was not replicated, as this association was not observed in other human nor animal studies. Interestingly, the *MACROD2* gene had promising associations with seizure-induced DNA methylation represented at a CpG site within the *MACROD2* gene region, which had the strongest blood–brain correlation in the temporal cortex with low correlation in other brain regions. Analysis of *MACROD2* DNA methylation was not explored in the blood–brain concordance studies from the ALSPAC database.

There are several limitations to the study that may explain the more negative results and lack of replication. First, while it is appreciated that the authors used serum assays as a less invasive, more practical method for analysis of DNA methylation, whole blood preparations may not necessarily reflect all DNA methylation changes occurring in the brain. However, blood-derived exosome preparations may prove to be more reflective of these epigenetic changes. Exosomes are vesicle containing protein, DNA, and RNA that are secreted from cells and taken up by distant cells to affect cellular function and behavior.[16] Another limitation of the study is that the authors did not consider the antiepileptic drugs (AEDs) regimen of epileptic patients which could have modified DNA methylation levels and influenced findings. Additionally, consideration of the medical history of the parents with genetic epilepsy and how their AEDs regimen could have impacted DNA methylation mechanisms in the offspring. Another major limitation of the study is that the DNA methylation detection strategy employed in the study does not distinguish between the 2 forms of DNA methylation, 5-mC and 5-hmC individually, but rather detects accumulative changes in both 5-mC and 5-hmC levels. Thus, future studies should consider analysis of 5-mC or 5-hmC DNA methylation markers individually in peripheral blood and in brain regions of epileptic patients across the different age groups. This could have greatly reduced variability and identified whether 5-mC, 5-hmC, or both DNA methylation forms were altered in blood samples. Finally, transcriptional states of the pathogenic variants in genes encoding for epigenetic enzymes that modify DNA and histones, control splicing, remodel chromatin, or modulate enhancers should be evaluated in epilepsy and/or seizures.

In conclusion, this study faced many challenges in conducting these EWAS, which no doubt, limited any definitive conclusions on whether seizures and blood DNA methylation changes correlated with childhood and adolescence epilepsy. Nonetheless, a major strength of the study that remains is the developmental assessment of DNA methylation changes in blood and brain across several ages, suggesting that the positive brain–blood correlations may have been developmentally influenced. Therefore, future studies should consider epigenetic factors that are developmentally regulated by seizures and in epilepsy.

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