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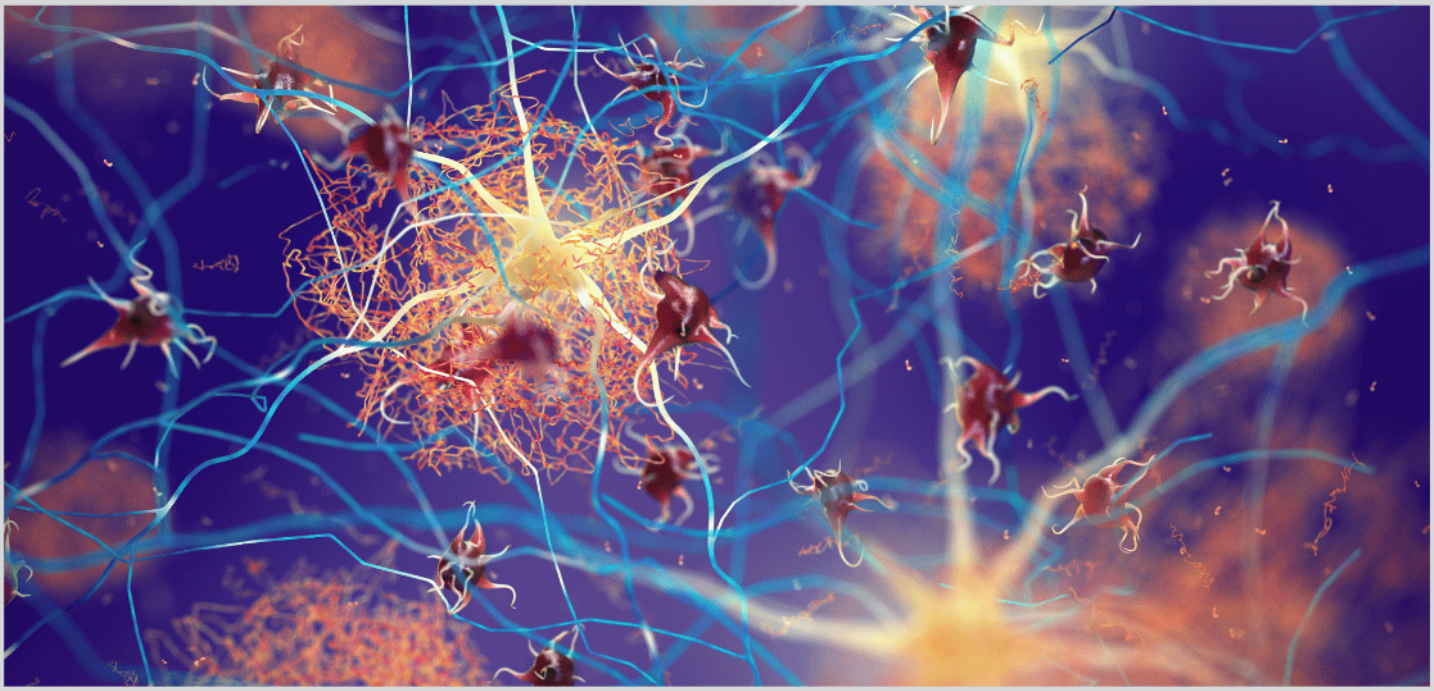
Pathophysiological convergence of epilepsy and Alzheimer's disease



Read about the common pathologies seen with both epilepsy and Alzheimer's disease, and learn more about clinical considerations

UK-NON-23-00089 Date of preparation June 2023.

Epilepsia, 54(1):e16–e19, 2013
doi: 10.1111/j.1528-1167.2012.03673.x

BRIEF COMMUNICATION

Antiepileptic drugs and the fetal epigenome

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Summary

Antiepileptic drugs (AEDs) can lower maternal folate and increase maternal homocysteine levels, which are known to affect the methyl cycle and hence DNA methylation levels. The influence of in utero exposure to AEDs on fetal DNA methylation was investigated. Genome-wide fetal epigenomic profiles were determined using the Infinium 27K BeadArray from Illumina (San Diego, CA, U.S.A.). The Infinium array measures approximately 27,000 CpG loci associated with 14,496 genes at single-nucleotide resolution. Eighteen cord blood samples (nine samples from babies exposed to AEDs and nine controls) from otherwise uncomplicated pregnancies were compared. Unsupervised hierarchic clustering was used to compare the calculated methylation profiles. A clear distinction between the methylation profiles of samples from babies exposed to AEDs in utero compared with controls was detected. These data provide evidence of an epigenetic effect associated with antenatal AED and high-dose folate supplementation during pregnancy. The differences in fetal DNA methylation of those exposed to AEDs shows that a genome-wide effect of methylation is evident. In addition, the epigenetic changes observed appear to be, in this limited sample, independent of extremes of birth weight centiles. These preliminary data highlight possible mechanisms by which AEDs might influence fetal outcomes and the potential of optimizing AED-specific folate supplementation regimens to offset these effects.

KEY WORDS: Methylation, Epigenetics, Epigenomics, Antiepileptics, Infinium.

One in 200 women attending antenatal clinics requires treatment with antiepileptic drugs (AEDs) during pregnancy. Such treatment is associated with an increased risk of major congenital malformations or one of the fetal anticonvulsant syndromes (Cunnington et al., 2011; Tomson et al., 2011). Lamotrigine is associated with the lowest risk of fetal abnormalities and tends to be the most commonly used AED in pregnancy (Cunnington et al., 2011; Tomson et al., 2011). However, depending on seizure control and prepregnancy planning, a significant number of women will start pregnancy with a different type of AED or even multiple AEDs. Furthermore, the risk of congenital malformation associated with commonly used AEDs is not only influenced by type but also dose of AED (Tomson et al., 2011).

DNA methylation (of cytosine bases in the context of CpG dinucleotides) is one of several epigenetic changes that contribute to the regulation of gene expression and maintenance of genome stability, and is considered vital in developmental processes (Suzuki & Bird, 2008). The epigenomic landscape is particularly vulnerable to environmental factors during embryogenesis, since this is the period when DNA methylation patterns required for normal tissue development are established. Indeed, regulation during this highly sensitive period is likely to have significant short- and long-term effects on both the individual and their progeny (Nafee et al., 2008).

Many AEDs are known to lower maternal folate and increase maternal homocysteine levels, and observational studies of periconceptual folic acid supplementation of women taking AEDs suggest that folic acid can lower the risk of fetal abnormalities (Kjaer et al., 2008). Availability of B vitamins (e.g., folic acid and vitamin B_{12}) is important in the methyl donor pathway involving DNA methylation, particularly within the periconceptual period (Sinclair et al., 2007). We hypothesized that AEDs, through their known effects on folate/homocysteine metabolism, will affect gene-specific fetal DNA methylation. We tested this using quantitative interrogation of methylation levels of 27,578 CpG dinucleotide loci associated with 14,496 genes in cord blood in a cohort of babies born to mothers who were known to have epilepsy taking AEDs and prescribed folic acid

Accepted July 30, 2012; Early View publication September 27, 2012.
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supplementation antenatally, compared to a control group of healthy women who had the routine peri-conceptual folic acid supplementation.

Methods

Patients

This series comprised of nine women with epilepsy who were taking AEDs and the recommended folate supplementation antenatally (cases) and nine controls. All study participants had uneventful pregnancies. Of the 9 women taking AEDs, four women were on carbamazepine, three on lamotrigine, and two on polytherapy (one on carbamazepine and valproic acid, and one on lamotrigine and valproic acid, see Table S1). All women with epilepsy who are taking AEDs in our unit are routinely advised to start taking high-dose folic acid (5 mg/day) preconceptually and during pregnancy. Women with pregnancy-related complications were excluded to avoid the potential confounding effect of such conditions on the fetal epigenome. Women who explicitly admitted to not taking their folic acid supplementation were also excluded from this analysis. Demographic data on maternal age at delivery, parity, body mass index, fetal gender, AED use, and folate supplementation during pregnancy were collected verbally and verified against the mother's clinical record. Cord blood samples were collected immediately after delivery. Measured characteristics of the 18 subjects are shown in Table 1. The study was approved by the local research ethics committee, and all women in this series provided written informed consent.

Measurement of folate parameters and CpG methylation

Cord blood folate assays, DNA extraction, and its bisulfite conversion were performed using the methodology we described previously (Fryer et al., 2011). For each CpG, methylation status (Beta-value) was determined with GenomeStudio V2009.1 methylation module 1.1.1 (Illumina, San Diego, CA, U.S.A.). The Beta-value can be interpreted as the proportion of methylation for each CpG site and ranges from 0 (no methylation) to 1.0 (100% methylation of both alleles) (Fryer et al., 2011). Data were normalized using the quantile method using CLC genomics workbench (Katrinebjerg, Aarhus N, Denmark) and then processed as described previously (Fryer et al., 2011).

Table 1. Analysis of biochemical and global methylation parameter values given as median (interquartile range)

	AED- exposed cases	Controls	p-Value (Mann-Whitney)
Birth weight centile (%)	57.7 (57.9)	28.0 (39.15)	0.133
Cord blood serum folate (ng/ml)	20.0 (2.7)	15.4 (5.9)	0.061
Cord blood homocysteine (M)	10.1 (9.8)	13.0 (5.75)	0.832
LINE-1 methylation (%)[a]	71.3 (5.6)	70.2 (4.4)	0.216

[a]Mean percent methylation calculated as described previously (Fryer et al., 2009).

Hierarchic clustering and statistical analysis

We conducted hierarchic clustering of Beta-values from the CpGs of autosome chromosomes (total 21,229 CpG sites). This enables the exploration of complex data without the need for a priori definition of groups that may be biased by experimenter expectations. The most appropriate algorithm for clustering was determined empirically using the ClusterRank software (Clifford et al., 2011). To identify statistically significant differentially methylated sites, normalized data were $\log_2$ transformed and a *t*-test with a false discovery rate (FDR) multiple hypothesis correction was conducted to compare the mean scores between identified clusters.

Results

The unbiased clustering of samples identified a clear distinction between the methylation profiles of cord samples taken from women taking AEDs while pregnant, compared to controls (Fig. 1). The high bootstrap values (generally >90%) demonstrated strong statistical support for each node of the dendrogram. A statistically significant difference in methylation of 662 CpGs was detected between AED and control clusters (FDR <0.05 and mean $\log_2$ group difference

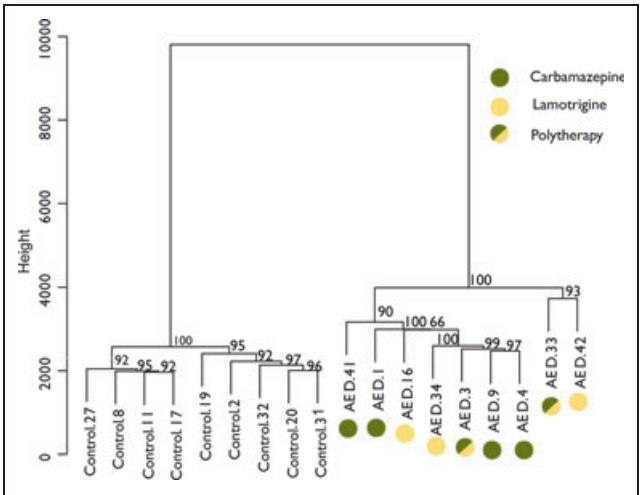

Figure 1.
Hierarchic clustering of normalized Beta-values for all samples. Multiscale bootstrap values for each node determined by re-sampling of 1,000 replicates are shown. Canberra distance and Ward Linkage was determined to provide the greatest cluster separation based on mean silhouette width using the ClusterRank software (Clifford et al., 2011).
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≥1).These CpGs map to 652 individual genes and the majority (95.6%) of these CpGs are located in predicted CpG islands.

Other parameters, namely fetal serum folate, calculated birth weight centile, cord blood homocysteine, and mean long interspersed nuclear element 1 (LINE-1) methylation (a measure of genome-wide methylation), did not show significant differences between the AED and control groups (Mann-Whitney *U* test Table 1). However, with the number of samples analyzed, the power to detect differences is limited. Removal of cord blood samples from infants with extreme birth weights (≤10th [four samples; three control, one AED exposed] and ≥90th [one sample; AED exposed] centiles) did not affect clustering or the conclusions of statistical analyses (data not shown).

Discussion

The results of unsupervised clustering clearly shows differences in genome-wide methylation of cases exposed to AEDs compared to those not. Because all women receiving AEDs in this study also received high-dose folic acid throughout pregnancy we cannot wholly separate the drivers of these epigenetic changes. As we have previously demonstrated that cord blood gene-specific CpG methylation is associated with birth weight centile (Fryer et al., 2011), separate analysis excluding samples from infants with extreme birth weight centiles (≤10% [four samples; three control, one AED exposed] and ≥90% [one sample; AED exposed]) was also conducted and similar clustering on exposure versus nonexposure to AEDs was maintained (data not shown).

The exact mechanism by which AEDs produce their effect on the epigenome is unknown. However, the existing evidence from studies in humans suggests that it is most likely to be linked to the metabolism of folate and its intermediaries. We have previously demonstrated a significant association between methylation and plasma homocysteine levels in cord blood when both global genomic DNA (Fryer et al., 2009) and CpG dinucleotide (Fryer et al., 2011) were analyzed.

AEDs are known to interfere with homocysteine/folate metabolism, albeit through different mechanisms and by varying degrees. Carbamazepine is a potent cytochrome P450 (CYP) inducer. Hepatic CYP induction causes depletion of the cofactors essential for homocysteine metabolism, thereby leading to its accumulation (Verrotti et al., 2000). Lamotrigine, despite its relatively favorable teratogenic profile, has antifolate properties. Rats receiving lamotrigine, in doses equivalent to those received by humans, produced offspring with decreased fetal folate concentrations (Iqbal et al., 2001). However, this trend is not seen in our data and may reflect the current small sample size or simply the fact that high-dose folic acid supplementation attenuates this effect. Valproic acid is a known inhibitor of histone deacetylase, an enzyme known to have an important role in the epigenetic regulation of gene expression and, because of the interrelationship between the chromatin state and DNA methylation, it is suggested that there is an overlapping effect between drugs that target chromatin and those that target DNA methylation (Szyf, 2009).

Irrespective of the exact mechanism underpinning the observed changes, these data provide proof of principle that current regimens for women planning pregnancy while taking AEDs can lead to substantial and relatively predictable epigenetic change. Although this small study cannot separate out the effects of high-dose folic acid supplementation from AED use it does illuminate one mechanism by which AEDs produce their teratogenic and developmental abnormalities. Larger, detailed studies would be an invaluable aid to future policy and the development of individualized recommendations based on type and dose of AEDs and other maternal factors to optimize the best regimen for periconceptual folate supplementation that can ''off set'' antenatal AEDs' unwanted pharmacoepigenetic effects.

Acknowledgments

We thank the study participants and the Childbirth And REproduction for West Midlands North (CARE for WMN) network research midwives (Miss Angela Rooney and Mrs Emma Hubbal) for their invaluable assistance with recruitment as part of the EFFECT-M study, UK Clinical Research Network number (UKCRN ID 6864). We gratefully acknowledge the research grant from the World Cancer Research Fund (2008/15) that supported KEH's salary.

Disclosure

None of the 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.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Biochemical and global methylation parameters.

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