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Deciphering the role of epigenetics in self-limited epilepsy with centrotemporal spikes

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ABSTRACT

Objective: The aetiology of self-limited epilepsy with centro-temporal spikes (SECTS) remains controversial and a strong genetic basis has long been presumed. The discordant monozygotic twin (MZ) model controls for shared genetic and environmental factors, enabling focus on the potential role of the non-shared environment.
Methods: DNA methylation data was acquired from DNA extracted from three discordant MZ twin pairs, from both new born blood spots before epilepsy onset, and blood samples taken after epilepsy onset. An epigenome-wide analysis was performed, using the Illumina Infinium EPIC array. Differentially methylated regions (DMR) were identified using the *bumphunter* package in R. Comparative analyses were undertaken at the two different time points as well as a combined analysis independent of time.
Results: Many of the top DMR-associated genes have previously been described in neurodevelopmental disorders. The *LYPD8* gene was associated with a top-ranked DMR both at birth and across the two time points.
Conclusion: We have demonstrated the novel utility of the longitudinal, discordant MZ twin model, to facilitate a deeper appreciation of the complex neurobiology of SECTS. The genetic architecture of SECTS is complex and is likely to involve an interplay between genes and environment, in part mediated by epigenetics.

1. Introduction

Self-limited epilepsy with centrotemporal spikes (SECTS), previously termed benign rolandic epilepsy, is an age-dependent focal epilepsy of childhood. Onset is usually between 5 and 10 years of age and characterized by unilateral sensorimotor seizures, normal neurological development and the electroencephalogram (EEG) trait of centrotemporal spikes.

Historically, SECTS was thought to be caused by genetic factors (Bray and Wiser, 1965; Heijbel et al., 1975). A purely genetic basis for SECTS has been challenged over the years by the paucity of affected relatives and lack of concordance in monozygotic (MZ) twins (Vadlamudi et al., 2004, 2006).

To date, genes such as *GRIN2A*, *DEPDC5*, *ELP4*, *BDNF* and *KCNQ2* have been implicated in SECTS. For a number of these genes the data is not strong or the observations are in cases on the severe end of the phenotypic spectrum of focal epilepsies, often with associated neurological impairment (Xiong and Zhou, 2017).

The role of environmental factors needs consideration in SECTS, potentially mediated by epigenetic mechanisms such as DNA methylation. Recently, epigenetic analyses of MZ twins discordant for neurodevelopmental disorders such as cerebral palsy (Mohandas et al., 2018) have identified differences in DNA methylation in relevant genes. Whilst epigenetic states have a significant tissue-specific component, recent evidence has shown that for some brain disorders including epilepsies, epigenetic analyses of peripheral tissues are of value

Abbreviations: MZ, Monozygotic; DMP, Differentially methylated probe; DMR, Differentially methylated region; EWAS, Epigenome-wide association study

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Table 1
Characteristics of the affected twins with SECTS.

Twin individual	1*	2	3*
Sex	Female	Male	Female
Age of seizure onset (years)	8	10	7
Age at last seizure (years)	11	14	10
Age when second samples taken (years)	23	14	29
Seizure semiology	Gagging noises, drooling, focal seizures	drooling, focal seziures	focal seizures
EEG	Left centro-temporal spikes	Right centro-temporal spikes	Right centro-temporal spikes
Brain MRI & Neurological examination	Normal	Normal	Normal
Family history	Maternal aunt seizures of uncertain nature, mother nocturnal events	No	No

*Vadlamudi et al. 2004.

(Karsten et al., 2011; Yuen et al., 2018).

DNA methylation analysis of Guthrie cards (heel-prick test cards routinely taken shortly after birth) enables identification of epigenetic marks that occur prior to the disease onset supporting the mark being a potential cause rather than effect of the disease, an inference further supported if the mark persists after disease onset.

In this pilot study, we analysed genome-wide DNA methylation in three pairs of MZ twins discordant for SECTS. Our primary aim was to identify gene regions that show SECTS-specific differences in DNA methylation across all pairs at birth and further observe if any of these or other differences was present post diagnosis.

2. Material and methods

2.1. Study participants

Ethics approval was obtained from the Mater Health Services (ethics approval number HREC/13/MHS/114). SECTS-discordant MZ twin pairs were recruited through Twins Research Australia (https://www.twins.org.au) and the Epilepsy Research Centre Database (http://www.epilepsyresearch.org.au). Zygosity testing confirmed monozygosity. Informed consent was obtained from each twin individual. Diagnoses were confirmed with clinical and EEG findings. Blood spots from stored neonatal Guthrie cards were sourced and blood samples post diagnosis (aged 14–29 years) were collected (Table 1).

2.2. DNA methylation analysis

Following genomic DNA extraction from all of the samples, an epigenome-wide analysis was performed, using the Illumina Infinium EPIC array. Data was analysed through a Bioconductor workflow for analyzing DNA methylation array data (Mohandas et al., 2018). Differentially methylated probes (DMPs) were considered significant if the false discovery rate (FDR) threshold was < 0.1 and if the unadjusted p-value $< 1 \times 10^{-4}$. Differentially methylated regions (DMRs), which are multiple proximal CpGs that are concordantly differentially methylated, were identified using the *bumphunter* package in R and ranked by bootstrap-based family-wide error rates (fwer), which is the proportion of permutations that have at least one region as extreme as the observed region.

3. Results

3.1. DNA methylation differences at birth

Data exploration using principal component analysis and multi-dimensional scaling was performed to estimate the sources of variation in the data. Known covariates including age, sex, birth weight, birth order, medication and gestational age were tested to capture similarities and variations between data samples. DMPs were identified but none were considered statistically significant, as they were not within the false discovery rate threshold of 0.1.

To minimise type 1 errors, we focussed on identifying DMRs, each identified as more than 3 probes within a distance of < 1 kb and effect sizes (difference in DNA methylation) of greater than 5%. With our limited sample size, we found no significant DMRs (family-wise error rate; fwer < 0.1) at birth.

Within the top ten DMR-associated genes ranked by fwer, those previously associated with neurodevelopmental disorders, included *TRIM39*, *RNF144A* and *S100A8*. The other top-ranked DMRs are shown in Table 2. The top-ranked DMR, *LYPD8* (fwer = 0.78; p-value = 4.63×10^{-05}) is located on chromosome 1, and included seven probes with average within-pair DNA methylation differences between 8.2% and 13.6%.

3.2. Post-diagnostic DNA methylation analysis

Within the top ten DMR-associated genes ranked by fwer, those previously associated with neurodevelopmental disorders included *OR8U8* and *ITGBL1*, *TBCD* and *ABCC5*. The other top-ranked DMRs are shown in Table 2.

3.3. DNA methylation analysis independent of time

To identify epigenetic differences that differ between SECTS-affected twins and their co-twins from birth through to diagnosis, we compared all SECTS-discordant twin pairs, independent of sample collection time. This enabled greater power for analysis through a technical replication of samples in each group (samples from the same twin taken at the different time points).

Within the top ten DMR-associated genes ranked by fwer, those previously associated with neurodevelopmental disorders included, *BAIAP2, RXRA, SP110* and *RNH1*. The top-ranked DMR, *LYPD8* (family-wise error rate; fwer = 0.83; p-value = 5.22×10^{-04}), is located on chromosome 1 and included six probes with average DNA methylation differences between 5.5% and 8.8%. This gene was also identified as the top DMR in analysis at birth but was not identified as a top ranked DMR post diagnosis. For this DMR, there were greater within-pair DNA methylation differences seen at birth compared with post-diagnosis (average DNA methylation differences of 11% and 1.8% respectively) and across both time points. Comparison of the average differences in DNA methylation is shown in Fig. 1. Other top-ranked DMRs are shown in Table 2.

Table 2
Top-ranking DMRs from each analysis with average DNA methylation difference, p-value, associated gene and number of probes within differentially methylated gene region. "+" means affected hypermethylated compared with the unaffected and "-" means unaffected hypermethylated compared with the affected.

	At birth				Post-diagnosis				Combined			
	Gene (chromosome: location)	p.value	fwer	Average DNA methylation difference (%)	Gene (chromosome: location)	p.value	fwer	Average DNA methylation difference (%)	Gene	p.value	fwer	Average DNA methylation difference (%)
DMRs	LYPD8 (7 probes)	4.63E-05	0.784	10.98	KRTAP6-2 (4 probes)	6.83E-04	0.98	−11.97	LYPD8 (6 probes)	5.22E-04	0.825	6.73
	TRIM39 (7 probes)	4.97E-05	0.784	10.13	AF127936.7 (4 probes)	7.64E-04	0.996	−10.44	MARCH8 (4 probes)	2.05E-03	0.94	9.07
	RNF144A (7 probes)	5.11E-05	0.788	9.71	OR8U8 (3 probes)	3.12E-03	1	−10.79	DYRK4 (3 probes)	3.32E-03	0.96	11.45
	S100A8 (6 probes)	1.03E-04	0.812	12.94	ITGBL1 (3 probes)	3.44E-03	1	−10.44	SP110 (3 probes)	3.47E-03	0.96	11.37
	TREM1 (7 probes)	5.34E-05	0.824	7.68	RP11-359E19.1 (3 probes)	3.70E-03	1	−11.90	RXRA (4 probes)	2.39E-03	0.955	8.36
	ZIM2/PEG3 (7 probes)	5.35E-05	0.824	−7.49	TBCD (3 probes)	3.74E-03	1	−12.48	BAIAP2 (4 probes)	2.39E-03	0.955	8.29
					CCDC172 (3 probes)	4.15E-03	1	−10.36	RNH1 (5 probes)	1.17E-03	0.945	6.43

4. Discussion

The genetic architecture of SECTS is complex and is likely to involve an interplay between genes and environment, in part mediated by epigenetics. Whilst no gene regions reached our stringent threshold for significance in our limited discordant twin sample, the potential role of epigenetics is supported by the observation that many of the identified genes have been previously reported in epilepsies and also have large effect sizes.

The *LYPD8* gene was associated with a top-ranked DMR at birth and also across both time points. LYPD8 has a wide range of cellular and immune functions and is expressed in the colon and prefrontal cortex (Loughner et al., 2016). To date, there is no clinical/phenotypic data for gene variants for this gene. The overlap of DMR findings associated with *LYPD8* gene at different time points, with a reduction in this difference with time, suggests this change may have been present from early life and could be a transient effect, which is interesting in light of the fact that SECTS is an age-dependant disorder.

The general lack of overlap of DMRs at birth and post diagnosis may indicate a short-lived effect of SECTS-specific epigenetic programming at birth or the alteration of epigenetic marks by other environmental influences such as age or medications after birth.

We acknowledge that the sample size is small and limits understanding of epigenetic mechanisms associated with SECTS. Larger sample sizes of 25 twin pairs or more are preferable to detect a mean DNA methylation effect size of at least 8% methylation (FDR = 0.05) (Pei-Chien Tsai, 2015). The use of blood rather than brain tissue, can limit understanding of biological mechanisms underlying SECTS, however, epigenetic patterns in blood have been shown to be of value (Karsten et al., 2011; Yuen et al., 2018) and blood is readily accessible.

5. Conclusions

Although limited by small numbers, we have demonstrated the novel utility of the longitudinal, discordant MZ twin model, to facilitate a deeper appreciation of the complex neurobiology of SECTS.

Ethics approval and consent to participate

Informed consent was obtained from each twin individual. A multicentre ethics approval was obtained with the lead site being Mater Health Services (ethics approval number HREC/13/MHS/114).

Availability of data

The datasets used and/or analysed during the current study are available from the corresponding author on request.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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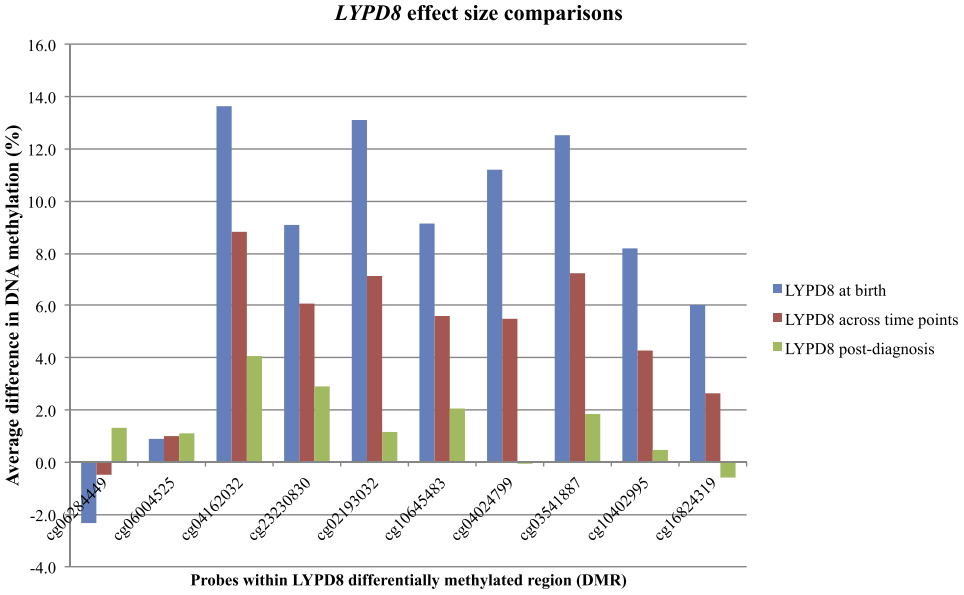

Fig. 1. Comparison of average DNA methylation differences (SECTS affected minus unaffected) for *LYPD8* DMR at birth, post-diagnosis and across both time points. X-axis represents the probes within the differentially methylated region of 556 base pairs (cg04162032 to cg03541887) and the neighbouring probes surrounding the DMR and Y-axis represents the average difference in DNA methylation.

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