

Epigenetics explained: a topic “primer” for the epilepsy community by the ILAE Genetics/ Epigenetics Task Force

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ABSTRACT – Epigenetics refers broadly to processes that influence medium to long-term gene expression by changing the readability and accessibility of the genetic code. The Neurobiology Commission of the International League Against Epilepsy (ILAE) recently convened a Task Force to explore and disseminate advances in epigenetics to better understand their role and intersection with genetics and the neurobiology of epilepsies and their co-morbidities, and to accelerate translation of these findings into the development of better therapies. Here, we provide a topic primer on epigenetics, explaining the key processes and findings to date in

experimental and human epilepsy. We review the growing list of genes with epigenetic functions that have been linked with epilepsy in humans. We consider potential practical applications, including using epigenetic signals as biomarkers for tissue- and biofluid-based diagnostics and the prospects for developing epigenetic-based treatments for epilepsy. We include a glossary of terms, FAQs and other supports to facilitate a broad understanding of the topic for the non-expert. Last, we review the limitations, research gaps and the next challenges. In summary, epigenetic processes represent important mechanisms controlling the activity of genes, providing opportunities for insight into disease mechanisms, biomarkers and novel therapies for epilepsy.

Key words: epileptogenesis, chromatin remodelling, DNA methylation, histone modifications, non-coding RNA

Epigenetics is a word that has, within just a few years, become common to hear at epilepsy conferences. But what does it *really* refer to? In 2017, the Neurobiology Commission of the International League Against Epilepsy (ILAE) established a new Task Force that was given the specific remit of exploring the topic, how it intersects with ongoing genetic discoveries about causes of epilepsy and whether it will lead to new therapeutic approaches. Why a “primer” on epigenetics? We are in the midst of an exponential phase of discovery about how gene expression is controlled. This has been driven, in part, by advances in the field of cell biology and gene sequencing technology. Together, these allow researchers to ask questions about the molecular mechanisms which shape the gene expression landscape. This not only helps our understanding of the neurobiology of disease but offers opportunities to discover biomarkers and new treatments for patients. Researchers in the areas of neurodevelopment and psychiatry have been first to embrace and apply these technologies to the brain. Practical applications may soon reach the clinic, including therapies that target epigenetic processes, molecular diagnostics and gene panels. We, in the epilepsy field, risk falling behind and must catch up, fast.

The Genetics-Epigenetics Task Force recognised a first step was to provide an overview of the subject and its potential practical applications. Our target audience are clinical colleagues and basic scientists interested in the mechanisms and treatment of epilepsy. We select examples of work from within the field and outside, including studies by expert members of the Task Force. The result, we hope, is a clear and understandable set of definitions and explanations of the main mechanisms at work. We include a glossary of terms (*Box 1*) and Frequently Asked Questions (FAQs, *Box 2*).

We review some of the key findings, provide evidence that epigenetic processes underlie epileptogenesis and the epileptic state, and look at prospects for epigenetic-based therapies and biomarkers. Finally, we

discuss the limitations of the work to date, particularly around translation to clinical practice, and some of the next questions that should be asked and, hopefully, answered (*Box 3*).

The manuscript is addressing between others, issues corresponding to the knowledge domain 7.0 (Biology of Epilepsy) and competency 1.2 (related to understanding genetic testing procedures) of the ILAE curriculum (Blümcke *et al.*, 2019).

What is epigenetics?

Before tackling epigenetics, let us briefly step back and ask what makes a neuron a neuron? The functional properties of a cell are generated by the genes that are expressed. Just because you *have* a gene that codes for something (say an ion channel) does not mean a particular cell will *make* the protein for which it encodes. The brain transcribes about 80% of all the genes in our genome (Kang *et al.*, 2011; Hawrylycz *et al.*, 2012). Some genes are transcribed by all cells regardless of specialized function while others are not. For example, neurons express various channel proteins that allow the generation and propagation of action potentials. An efficient way to tackle this is for genes that are never needed to be more or less permanently switched off while those needed often are kept in a ready-to-go state. To produce a protein, transcription factors must bind to a promoter region of a gene and the RNA (ribonucleic acid) polymerase machinery must assemble, generating a messenger RNA that, following a series of further processing and trafficking events, will be translated into a protein. That first step – getting access and reading the code for transcription – is where epigenetics occurs. *Epigenetics* (*Box 1*) is the umbrella term for the collection of biochemical processes which influence the readability of the genetic code. Put another way, epigenetic processes determine whether or not a gene can be “read”. This is achieved by controlling the structure of chromatin – the packaged DNA inside the nucleus – which in turn

Box 1. Glossary

CpG island: A region of DNA in which CG dinucleotides are particularly abundant. The C and G here are not opposite each other (base-paired) but lie on the same strand of DNA. These are common sites for DNA methylation (“p” indicates that the C and G are connected by a phosphodiester bond). There are about 30,000 such CpG islands in the human genome.

DNA methylation: A major epigenetic mark: methylation of DNA (of which there are four types) leads to the recruitment of silencing or activator protein complexes that influence transcription at that particular site. Methyl groups are deposited by DNA methyltransferases and removed by demethylases.

Epigenetics: Umbrella term for the collection of biochemical processes which influence the medium-to-long-term gene expression landscape by controlling chromatin structure and function and thus the readability (accessibility to transcriptional machinery) of the genetic code.

Epigenome: The entirety of all the epigenetic marks on DNA.

Euchromatin: A chromatin state that is transcriptionally permissive; loosely packed nucleosomes in an open state where transcription is most active.

Heterochromatin: Densely packed chromatin that is closed to active transcription.

Histones: A group of five families of proteins (H1, H2A, H2B, H3 and H4) that package DNA into nucleosomes and have important functions in gene expression.

Histone variant: An isoform of the canonical histones. Their introduction into the nucleosome also serves to alter gene transcription.

Histone post-translational modifications: The collection of covalent modifications of histones which influences their structure and function. These include acetylation, hydroxylation, methylation and phosphorylation.

Non-coding RNA: A diverse family of RNAs that are actively transcribed but do not code for proteins. Many function within the nucleus to promote or repress gene transcription. MicroRNAs are a form of short non-coding RNA that function mainly in the cytoplasm to reduce protein expression by binding to complementary sequences in messenger RNAs.

Nucleosome: The basic structural unit by which genomic DNA is packed in chromosomes; a 146-base-pair stretch of DNA wrapped around a histone octamer core.

3D genome: The concept of a “spatial genome”, with highly organised distribution of chromatin within the nucleus and looping that brings scaffolding and other regulatory elements together to mediate transcription.

dictates accessibility of the genetic code to transcription. Epigenetics works, primarily, through adding or removing chemical tags on either DNA or on the proteins around which DNA is wrapped (histones). The nucleus of our cells possess an extensive assortment of proteins and RNAs which work together to apply, read and wipe off these marks. These epigenetic marks cause physical changes to chromatin, making it more open (termed *euchromatin*) or less open (*heterochromatin*) which makes transcription easier or harder, respectively. Together, this creates an appropriately stable but still responsive gene expression environment in the cell. While epigenetic marks are heritable and can be passed on during cell division, many of the underlying mechanisms are functional and responsive to stimuli in post-mitotic cells, including neurons.

The complexity of epigenetics is staggering. For example, it is estimated that there are ~600 potential modifications to histones that can affect transcription. While we have a very good understanding of some epigenetic processes such as the inactivation of the spare X chromosome in female cells, we do not have answers to how most other individual or collective epigenetic marks shape gene expression in health and disease. This lack of knowledge is slowly being resolved and

it is possible that a fairly complete “epigenome” will be possible to map for most diseases, including the epilepsies. There is also a strong age-dependent aspect to epigenetics, with chromatin becoming increasingly “closed” as we age, including within the brain (Berson *et al.*, 2018). The implications of this are currently unknown. For the purposes of our Task Force, we will use the following definition of the epigenome, based on that of the NIH: “*The chemical modifications added to the entirety of one’s DNA (genome) as a way to regulate the activity (expression) of all the genes.*”

The basic epigenetic processes

There are three main categories of biochemical mediators of the epigenetic control of gene expression. All are important in brain development, function and disease. These are:

- methylation of DNA;
- histone modifications and variants;
- and non-coding RNAs.

Each comprises a varying degree of complexity in terms of the mediators involved and their influence on

Box 2. FAQs (Frequently Asked Questions)***Where does the word epigenetics originate from?**

ANSWER: The term *epi* is derived from the Greek prefix *ἐπί* (*epí*, “over or on top of”), referring to an additional layer of instructions that lies over or on top of DNA and controls gene expression. The word *epigenetics* was introduced in 1942 by the British developmental biologist, paleontologist, geneticist, embryologist and philosopher Conrad Hal Waddington, relating it to the 17th century concept of “epigenesis”: the complex of developmental processes between the genotype and phenotype.

How does epigenetics work?

ANSWER: Epigenetics works, primarily, through adding or removing chemical tags on either DNA or on the proteins around which DNA is wrapped (histones). Certain types of non-coding RNA are also involved.

What is the significance of epigenetics?

ANSWER: Epigenetics plays an important role during development and maintaining genome homeostasis. Any disturbance of the epigenetic processes can have major adverse health and behavioural consequences.

What are the main epigenetic processes? (and what are not?)

ANSWER: DNA methylation, histone modifications and certain non-coding RNAs as well as silencing factors. Most transcription factors are therefore not part of the epigenetic machinery. MicroRNAs are also not really epigenetic factors but some other small non-coding RNAs (e.g. enhancer RNAs) are.

Can epigenetics be transmitted from generation to generation?

ANSWER: Yes, this is called (transgenerational) epigenetic inheritance. This is the transmission of information from one generation to the next that affects the traits of offspring without alteration of the primary structure of DNA.

Can we selectively manipulate any epigenetic processes?

ANSWER: Yes. We already have “epigenetic drugs”. There are small molecule inhibitors of histone deacetylases (HDAC inhibitors) in clinical use and trials (mainly for non-CNS diseases). Many more are in development. We can also selectively target sites of epigenetic modification (*i.e.* epigenetic engineering using CRISPR/Cas; although this has not yet reached humans).

Do any epilepsy treatments work via an epigenetic mechanism?

ANSWER: Possibly. Sodium valproate is an HDAC inhibitor but its anti-seizure effects seem unrelated to this activity. The ketogenic diet may reduce DNA methylation in the brain and thus re-activate previously silenced genes.

(*) Note: The manuscript is addressing, between others, issues corresponding to the knowledge domain 7.0 (Biology of Epilepsy) and competency 1.2 (related to understanding genetic testing procedures) of the ILAE curriculum (Blümcke *et al.*, 2019).

gene transcription and are facilitated by a further set of proteins that form chromatin remodelling complexes (Dulac, 2010; Jakovcevski and Akbarian, 2012).

DNA methylation

DNA methylation refers to the addition of a methyl (-CH₃) group that occurs on cytosine bases (at the carbon 5 position [5mC]) in the genetic code (*figure 1*). This may occur at various sites within and outside the coding region of a gene. It can occur in regions that contain promoter elements for a gene, but can also occur at exon/intron boundaries and intergenic (between genes) regions. Some regions of the genome are particularly densely methylated, such as those encoding transposons (DNA sequences that can change their position within a genome). The primary biological functions of DNA methylation includes maintenance of genomic stability by silencing transposable elements (including retrotransposons), the monoallelic expression of imprinted genes, X chromosome inactivation in female cells, and the

selective exposure of promoters and enhancers of genes to transcription factors (Edwards *et al.*, 2017; Greenberg and Bourc’his, 2019). A number of genes have been identified that undergo changes in methylation in response to synaptic activity. Thus, DNA methylation marks support the gene expression programmes that underlie synaptic plasticity, producing lasting and responsive adjustment to the expression of certain genes (Sweatt, 2013). DNA methylation is mediated by DNA methyltransferases (DNMT1, 3A and 3B). The process is reversible through base excision repair proteins (Edwards *et al.*, 2017).

Histone post-translational modification and variants

Packaging the approximately six billion bases of DNA within our cell’s nucleus is a major challenge. It is achieved by winding DNA around a set of proteins called histones and then packing individual units,

Box 3. Outstanding research questions

- In which cells do epigenetic changes occur in epilepsy? Is the epigenetic machinery more or less active/important in particular cell types?
- What are the expression patterns and various histone modifications in experimental and human epilepsy and what controls their patterning?
- What are the roles (and therapeutic potential) of the various forms of non-coding RNAs in transcriptional control?
- What is the overall influence of a given set of epigenetic marks for a specific gene? Can this be predicted?
- Do epileptogenic injuries re-shape the 3D genome?
- Which epigenetic marks are potential biomarkers (and what type of biomarkers -diagnostic, risk/prognostic, theranostic; are they suitable?). Can some epigenetic marks be monitored non-invasively? (e.g. in blood, CSF).
- Can we correct aberrant gene expression in a genetic or acquired epilepsy by specific or global targeting of the epigenetic machinery?
- Are there mutations in other genes with epigenetic functions that may contribute to unsolved epilepsies?
- Which experimental technologies and approaches are applied in epigenetic research?
- What is the relationship between genetics and epigenetics?

termed nucleosomes, together to eventually form a chromosome (*figure 1*). Importantly, densely packed nucleosomes are inaccessible to the machinery of transcription whereas loosely-packed chromatin can be read (*i.e.* genes transcribed). The nucleosome comprises a 146 base-pair stretch of DNA around an octamer of histone proteins; two copies each of H2A, H2B, H3 and H4. The amino terminals (tails) of these histones protrude from the nucleosome and, along with the core region, are subject to modifications, including acetylation, phosphorylation, methylation and ubiquitylation. These biochemical additions lead to changes in chromatin compaction, in part, by altering charges on the proteins so that they move closer or further apart. The influence of these modifications is complex and context-specific and predicting their effects is challenging. The transcriptional landscape cannot [yet] be determined by reading the overall histone code. However, we know that increased acetylation of histones is associated with increased transcription. This is mediated by acetyl transferases and the mark is removed by histone de-acetylases (HDACs; thus, a histone deacetylase inhibitor would tend to promote gene expression; see below). Other histone marks

are associated with reduced gene expression, such as methylation of lysine residues on H3 (H3K9me2, H3K27me3). In addition to post-translational modifications, variants of the histones can replace the canonical histones in the nucleosome. This promotes unique interactions with other factors that shape chromatin structure (Berson *et al.*, 2018). From a research perspective, it is possible to study either individual histone changes or globally assess histone changes, for example, by breaking up modified histones and the segments of DNA to which they are localised.

Non-coding RNAs

The third major category of the epigenetic process is the role of non-coding RNAs. This is a highly heterogeneous class of RNAs that are transcribed but do not code for proteins (unlike messenger RNA). They are split into short (or small) non-coding RNAs (<200 nucleotides in length) and long non-coding RNAs (200 nucleotides or longer). Not all non-coding RNAs are involved in epigenetic processes. For example, ribosomal and transfer RNAs are involved in translation of proteins and are located in the cytoplasm.

The non-coding RNAs that mediate epigenetic effects are considered to belong to the main classes of short and long non-coding RNAs (based on a length of 200 bases). Long non-coding RNAs have been identified which, upon transcription, serve as structural bridges to bring together segments of chromatin called “loops” (St Laurent *et al.*, 2015). The bringing together of such loops can be an important influence on gene transcription. Long non-coding RNAs can also cause changes to histone modifications, directly influence DNA methylation, and alter other aspects of chromatin structure (Hanly *et al.*, 2018). A number of long non-coding RNAs have been identified with roles in brain function, influencing synaptic structure and neurophysiological properties of neurons (Bernard *et al.*, 2010; Barry *et al.*, 2017). Over the last two decades, several types of small non-coding RNAs with epigenetic functions have been discovered. These include microRNA (miRNA), piwi-interacting RNA (piRNA), enhancer RNA (eRNA) and natural antisense transcripts (NAT). The most widely studied class of short RNA are miRNAs. These mainly function outside the nucleus, binding to mRNA to reduce protein production (*i.e.* acting post-transcriptionally). They generally do not directly perform epigenetic functions. Aberrant expression of miRNAs can, however, lead to alterations in the proteins that serve epigenetic functions, for example by restricting the activity of chromatin remodelling enzymes (Wei *et al.*, 2017). Indeed, miRNAs are capable of targeting genes that control epigenetic pathways, including histone methyltransferases,

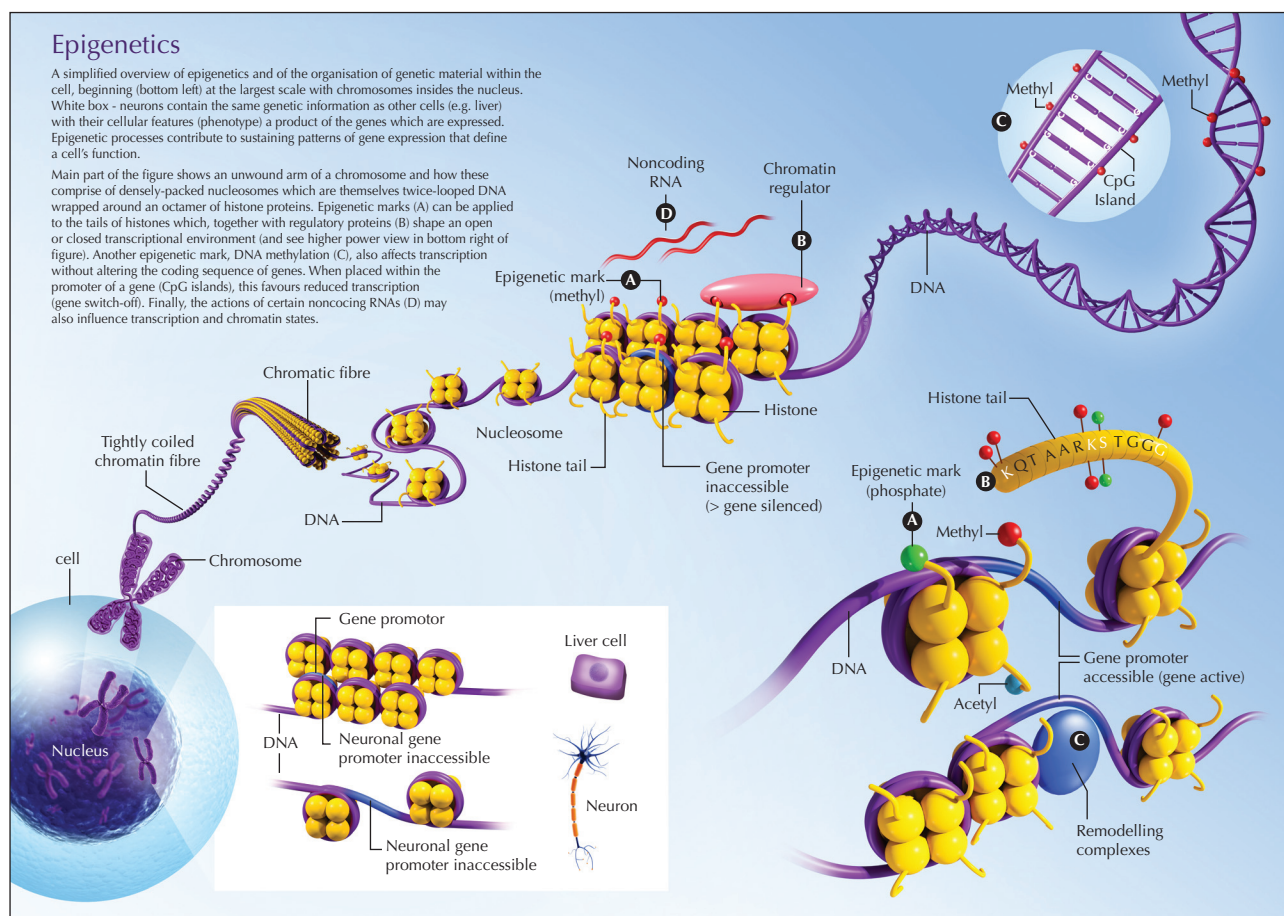


Figure 1. Overview of epigenetic mechanisms. A simplified overview of epigenetics and organisation of genetic material within the cell, beginning (bottom left) at the largest scale with chromosomes inside the nucleus. The white box shows neurons containing the same genetic information as other cells (e.g. in the liver) with their respective cellular features (phenotype); a product of the genes which are expressed. Epigenetic processes contribute to sustaining patterns of gene expression that define a cell's function. The main part of the figure shows an unwound arm of a chromosome and how this is comprised of densely packed nucleosomes which are themselves twice-looped DNA wrapped around an octamer of histone proteins. Epigenetic marks (A) can be applied to the tails of histones which, together with regulatory proteins (B) shape an open or closed transcriptional environment (see higher power view in bottom right of figure). Another epigenetic mark, DNA methylation (C), also affects transcription without altering the coding sequence of genes. When placed within the promoter of a gene (CpG islands), this favours reduced transcription (gene switch-off). Finally, the actions of certain non-coding RNAs (D) may also influence transcription and chromatin states.

methyl CpG binding proteins and histone deacetylases (Lewis *et al.*, 2005; Sato *et al.*, 2011).

Among other small non-coding RNAs, piRNAs have been reported to be involved in epigenetic regulation by modulating histone modifications and DNA methylation (Esteller, 2011; Luteijn and Ketting, 2013). Enhancer RNAs (eRNA) are a class of short non-coding RNA that act within the nucleus to promote or oppose transcription of specific genes. They possess some classic epigenetic characteristics since they can modify histones, may contribute to a more open chromatin structure and help bring enhancers and promoters together through chromatin looping (Ding *et al.*, 2018). NATs are transcribed from the opposite strand of DNA from the side that encodes a gene

and compete locally to reduce abundance of the coding mRNA (Wahlestedt, 2013). Both long and short non-coding RNAs have been explored for therapeutic potential in epilepsy (see *below*).

Other mediators of epigenetic processes

In addition to the three main processes described in the previous paragraphs, a number of additional mediators are involved in epigenetic functions. These include the chromatin remodelling factors that are recruited to a genomic location after a gene promoter is methylated, which perform the subsequent mechanical aspects of condensing chromatin. Transcription factors – both activators and repressors – often work in

tandem with epigenetic factors. For example, neuron restrictive silencing factor (NRSF) is attracted to sites with specific sequences whereupon it attracts histone deacetylases to mediate gene silencing (Huang *et al.*, 1999).

Epigenetic mechanisms in epilepsy - neurobiological implications

All three major epigenetic processes have been observed, and found altered, in experimental and human epilepsy (Henshall and Kobow, 2015). This includes changes to DNA methylation, histone post-translational modifications, and changes to the expression or activity of non-coding RNAs. Researchers have performed both focused work, looking at how epigenetic marks at specific loci affect gene expression as well as several studies that have attempted to characterise the totality of an epigenetic mark across the entire genome. Collectively, studies about epigenetic changes in epilepsy indicate that epigenetic processes serve some role in shaping the gene expression landscape during epileptogenesis and in chronic epilepsy. However, our understanding of the mechanisms involved in applying, reading and removing epigenetic marks is poor and we do not yet have the ability to control these or predict their effects which will be essential for any epigenetic-based treatment for epilepsy.

DNA methylation in epilepsy

The DNA methylation landscape has been mapped in both experimental and human epilepsy. The first genome-wide study of DNA methylation in human epilepsy determined that most DNA methylation was unchanged in hippocampal samples from patients with temporal lobe epilepsy (TLE) compared to control brain (Miller-Delaney *et al.*, 2015). Select increases (hyper-) and decreases (hypo-) in methylation were found but few of these occurred around genes that had conceivable links to pathomechanisms of epilepsy. Moreover, altered DNA methylation was often not predictive of associated changes in levels of gene transcription, meaning other factors were important (Miller-Delaney *et al.*, 2015). Important caveats in this study were small sample size and technical bias introduced when comparing surgically-obtained material to autopsy control. Kobow *et al.* performed the first genome-wide analysis of DNA methylation in chronic epileptic rats and identified epilepsy-associated genome-wide DNA methylation changes which correlated well with gene expression. That is, genes that were highly methylated showed a reduction in expression and vice versa (Kobow *et al.*,

2013). In a follow up study, Debski *et al.* found genome-wide changes in DNA methylation to be a common feature of several different pro-epileptogenic insults. These epigenetic changes were unlikely to be an epiphenomenon as they appeared highly specific with distinct aetiology-dependent DNA methylation patterns observed across different acquired epilepsy models (*i.e.* pilocarpine, amygdala stimulation, and traumatic brain injury [Debski *et al.*, 2016]). DNA methylation profiling may, therefore, be clinically useful to diagnose and stratify epilepsy patients. This was recently tested and validated in epilepsy patients with different focal cortical dysplasia (FCD) subtypes, TLE patients and non-epilepsy controls (Kobow *et al.*, 2019). Where there are discrepancies between changes in methylation and gene expression, a possible explanation may derive from competing epigenetic mechanisms besides DNA methylation. Further research is required to better understand what drives these changes in DNA methylation marks and how they influence, or not, gene expression in epilepsy.

Technological advances are transforming capabilities to map the gene expression landscape. Among key recent advances is the ability to study the 3D genomic landscape using techniques such as ATAC-Seq (Assay for Transposase-Accessible Chromatin Sequencing) (Buenrostro *et al.*, 2013). This allows mapping of all open and closed chromatin sites in the genome, significantly improving our understanding of mammalian gene regulation in normal development and in pathological conditions (Bonev *et al.*, 2017). These techniques provide an exciting opportunity to extend our understanding of gene to genome interaction and gene regulation in epilepsy.

Histones in epilepsy

A number of studies have reported post-translational histone modifications in epilepsy. The earliest work reported acetylation changes to histones located at the loci of glutamate receptors and brain derived neurotrophic factor (Huang *et al.*, 2002). These studies revealed that histone modulation influences transcription of genes that regulate brain excitability. Other studies have also identified phosphorylation of histones within the hippocampus after experimental status epilepticus (Crosio *et al.*, 2003; Sng *et al.*, 2006). There are now methods that can globally assess histone modifications. These typically involve fragmenting chromatin and using an antibody specific to a certain histone modification to pull out regions followed by sequencing. There has not yet been a global characterisation of histone modifications in experimental or human epilepsy.

Non-coding RNAs in epilepsy

Both long and short non-coding RNAs have been reported to be dysregulated in experimental and human epilepsy. There is evidence linking several long non-coding RNAs to the control of neuronal excitability, including BC1 (Zhong *et al.*, 2009), MALAT1 (Bernard *et al.*, 2010) and NEAT1 (Barry *et al.*, 2017). Profiling studies using gene arrays and RNA sequencing have revealed the dysregulation of numerous long non-coding RNAs (Lee *et al.*, 2015; Miller-Delaney *et al.*, 2015; Jang *et al.*, 2018; Xiao *et al.*, 2018) and short non-coding RNAs (Henshall *et al.*, 2016; Korotkov *et al.*, 2017; Srivastava *et al.*, 2017; Cava *et al.*, 2018; Mills *et al.*, 2019) in experimental and human epilepsy. However, few functions have been assigned to these transcripts. The only class of short non-coding RNA for which we have extensive evidence of roles in epilepsy is miRNA (Henshall *et al.*, 2016). Some miRNAs that function in an “epigenetic way” have been identified, including miR-320 (Kim *et al.*, 2008; Catalanotto *et al.*, 2016). However, these have not been manipulated in experimental models to date, making it difficult to assign a biological role. Moreover, studies that have tested miRNA manipulations in seizure models have not investigated whether this produces changes to the epigenetic landscape, an area that could be explored in the future. Intriguingly, a number of miRNAs are mis-localised to the nucleus in the hippocampus of patients with drug-resistant temporal lobe epilepsy (Kan *et al.*, 2012; Raoof *et al.*, 2018). This could indicate that miRNAs acquire new (epigenetic) functions in epilepsy. Further research is needed to explore the expression and function of other non-coding RNAs including eRNA which serves more traditional epigenetic roles.

Epilepsy genes with epigenetic functions

Mutations in a growing number of genes which are neither ion channels, receptors nor other classic epilepsy genes, but instead encode proteins that serve epigenetic functions, have been identified as responsible for genetic forms of epilepsy (table 1, figure 2). The list of clinical phenotypes includes epileptic encephalopathies, intellectual disability syndromes and autism spectrum disorders with associated severe or occasional seizure phenotypes. A good example is *CHD2*, mutations in which cause a Dravet-like fever associated epileptic encephalopathy (Suls *et al.*, 2013). This gene codes for an ATP-dependent helicase with chromatin remodelling function (Carvill *et al.*, 2013; Sulis *et al.*, 2013). Re-modellers such as the CHD protein family alter gene expression by modification of chromatin structure, meaning

that they can help to improve accessibility of the transcriptional apparatus to the DNA template either by moving, ejecting or restructuring nucleosomes. In an energy-dependent process, the remodelling complexes reposition (*i.e.* slide, twist or loop) nucleosomes along the DNA, expel histones away from DNA or facilitate exchange of histone variants, and thus create regions of DNA for gene activation (Marfella and Imbalzano, 2007). *ATRX*, that encodes another chromatin re-modeller, was found to be mutated in Alpha-thalassemia/mental retardation (*ATRX*), which is associated with seizures in about one third of patients (Gibbons, 2006). Mutations in *SMARCA4*, *ATRX* and other re-modeller genes have also been found to cause Coffin-Siris syndrome, with structural brain abnormalities and seizures as typical features (Tsurusaki *et al.*, 2012).

Histone modifying enzymes and readers of histone tags are frequently identified in intellectual disability syndromes with associated epilepsy. The protein encoded by *EHMT1* is a histone methyltransferase that is implicated in learning and memory, and was recently shown to be mutated in Kleefstra syndrome (Iwase *et al.*, 2017). *KDM5* encodes a histone demethylase, and as such functions to erase methylation marks from histones. Its function in the brain is not fully determined, but a mutation in this gene has been associated with X-linked intellectual disability (Poeta *et al.*, 2013). Insertion/missense mutations of the transcription factor *ARX* have been linked to epileptic encephalopathy, lissencephaly and X-linked intellectual disability as well as functions including regulation of histone methylation patterns.

Mutations in genes that regulate DNA methylation have also been linked to epilepsy. *MECP2* encodes a typical reader of DNA methylation that when mutated is the cause of classic Rett syndrome (Amir *et al.*, 1999). Seizures are reported in up to 90% of affected females, with generalized tonic-clonic seizures and partial complex seizures being most common. Notably, *MECP2* duplication syndrome is commonly associated with an epileptic encephalopathy and intellectual disability (Marafi *et al.*, 2019). This indicates an intolerance of both lower and higher amounts of certain epigenetic factors. *MBD5* encodes another DNA methylation binding protein and was identified as causing intellectual disability and epilepsy in patients with 2q23.1 microdeletion syndrome. Variation in *CDKL5* that codes for a cyclin-dependent kinase has been associated with an X-linked dominant early infantile epileptic encephalopathy (Weaving *et al.*, 2004). The *CDKL5* protein has been proposed to interact with *MECP2* and *DNMT1*, thereby influencing gene expression and DNA methylation (Carouge *et al.*, 2010).

A number of studies have reported variants in non-coding RNAs in epilepsy cases, including miRNAs

Table 1. Genes with epigenetic functions that, when mutated, cause epilepsy or neurodevelopmental diseases with an associated seizure phenotype.

Gene	Full name	Function(s)	Clinical phenotype	Ref
ARX	Aristaless related homeobox	Homeodomain transcription factor regulating KDM5C, affects histone methylation	Early infantile epileptic encephalopathy (EIEE1)/ West syndrome; X-linked lissencephaly (LISX2)	(Kato <i>et al.</i> , 2007)
ATRX	ATRX, Chromatin remodeller	Chromatin remodeller (SWI/SNF family)	Alpha-thalassemia/mental retardation syndrome X-linked	(Iwase <i>et al.</i> , 2017)
BRD2	Bromodomain containing 2	Reader of histone acetylation marks	Juvenile myoclonic epilepsy (JME)	(Pal <i>et al.</i> , 2003)
CHD2	Chromodomain helicase DNA-binding protein 2	Chromatin remodeller (CHD family)	Childhood-onset epileptic encephalopathy (COEE)	(Carvill <i>et al.</i> , 2013; Suls <i>et al.</i> , 2013)
EHMT1	Euchromatin histone methyltransferase 1	Histone methyltransferase	Kleefstra syndrome (OMIM #610253)	(Iwase <i>et al.</i> , 2017)
FMR1	Fragile X mental retardation 1	RNA binding protein in miRNA pathway	Fragile X-syndrome (OMIM #300624)	(Verkerk <i>et al.</i> , 1991)
FTO	FTO, Alpha-ketoglutarate-dependent dioxygenase	N(6)-methyladenosine (m6A) RNA demethylase	Growth retardation, developmental delay and facial dysmorphism (OMIM #612938)	(Boissel <i>et al.</i> , 2009; Rowles <i>et al.</i> , 2012)
KDM5C	Lysine demethylase 5C	Histone demethylase	X-linked mental retardation (XLMR)	(Poeta <i>et al.</i> , 2013)
KMT2D	Lysine methyltransferase 2D	Histone methyltransferase	Kabuki syndrome	(Kurahashi <i>et al.</i> , 2017)
MBD5	Methyl-binding domain 5	Methyl-DNA binding protein	Intellectual disability, autosomal dominant 1 (MRD1); 2q23.1 microdeletion syndrome with seizures	(Talkowski <i>et al.</i> , 2011)
MECP2	Methyl CpG binding protein 2	Methyl-DNA binding protein	Rett syndrome (OMIM #312750)	(Iwase <i>et al.</i> , 2017)
PRDM8	PR/SET domain 8	Histone methyltransferase	Progressive myoclonic epilepsy (PME)	(Turnbull <i>et al.</i> , 2012)

(Manna *et al.*, 2013; Cui *et al.*, 2015; Manna *et al.*, 2016), but these have yet to be replicated. *Figure 2* provides an example of how multiple genes associated with epilepsy may be linked through epigenetic processes. It may be difficult to anticipate how a mutation affecting an epigenetic enzyme or modulator may influence seizure development. Studying the 3D genome in e.g. mice or cell lines with an epigenetic mutation may help to unravel long-ranging effects of these mutations (Barutcu *et al.*, 2016) and better explain the connection to altered brain functions.

Epigenetic cross-talk in epilepsy

While the three major categories of epigenetic process are presented separately, they should not be thought of as functioning in silos. The main epigenetic processes do not act in isolation but instead interact and converge. As mentioned earlier, histone modifications can attract or expel components that regulate DNA methylation. As a result, assessing single epigenetic marks is often not predictive of overall gene expression when, in practice, each mark is likely to influence

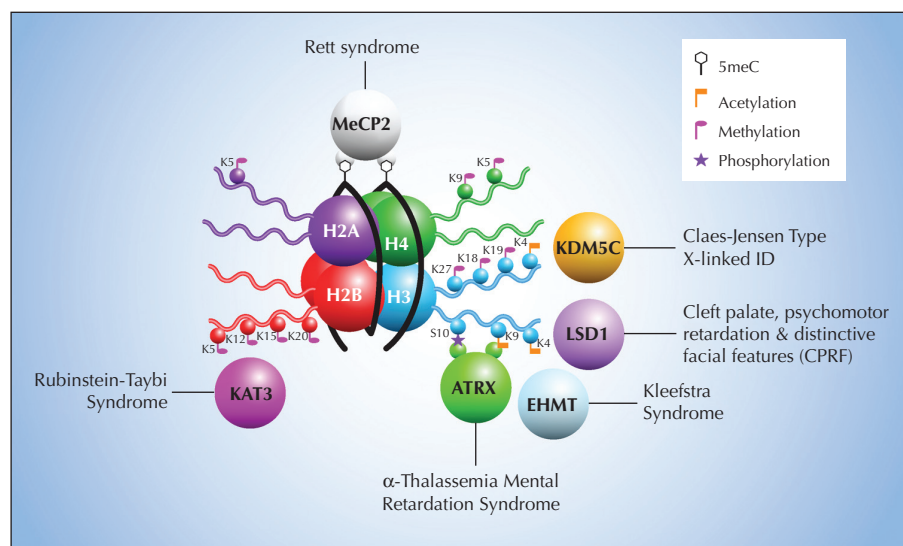


Figure 2. Examples of novel genes linked to epilepsy and their epigenetic functions. An example of the close relationship between chromatin function and proteins, that are mutated in patients with known syndromes often associated with intellectual disability and epilepsy, is presented. The figure shows the nucleosome, comprising a histone core and DNA coiled around it (black lines). Indicated on the histones and DNA are epigenetic marks including post-translational modifications which affect compaction and transcriptional access. Surrounding this are six proteins and their potential physical interaction sites and the known diseases caused by mutations in these. Adapted, with permission, from Iwase et al (Iwase et al., 2017).

others. Such “collective” approaches to assessing epigenetic influences will be needed in the epilepsy field but have not been undertaken. One or two examples of over-lapping epigenetic mechanisms have been reported, including the observation that a subset of differentially expressed miRNAs and long non-coding RNAs were influenced by DNA methylation in epilepsy patients with hippocampal sclerosis (Miller-Delaney et al., 2015).

In which cells do epigenetic changes occur?

Studying epigenetic processes *in vitro* enables researchers to be confident of the cell types involved because relatively pure cell populations can be studied. This has confirmed roles for epigenetics in neuronal function and plasticity. However, all of the *in vivo* data we have for epilepsy, including genome-wide analyses of DNA methylation, is based on bulk tissue. We therefore do not know the cell source of the “signal” – it could be neurons (but which types?) or glia or other cells. The machinery necessary for epigenetic processes is not equally divided among cell types. For example, expression of DNMT1 and H3A is highly enriched in neurons and hardly detectable in glia (see www.brain-map.org). In theory, neurons and glia do not have parity in terms of how much the transcription of their genes is influenced by epigenetic processes – both adding and removing epigenetic marks. This will be important to resolve in the future, and the

technology to separate individual neurons from bulk tissue and assay their epigenome will provide a means to determine where the epigenetic changes are really happening.

How do epigenetic changes lead to neuronal excitability and seizures?

Fundamentally, changes in neuronal network behaviour on a millisecond timeframe are responsible for the generation of seizures. However, the process by which a neuronal network becomes more susceptible to generating seizures can occur across a broad temporal scale. Furthermore, epigenetic changes by their nature have the potential to have far-reaching consequences on cellular function. Acute changes caused by epigenetic factors could include altered voltage and/or ligand-gated ion channel expression that would alter a neuron’s moment-to-moment excitability. Epigenetic changes could also initiate cell death signalling (e.g. apoptotic) cascades over longer periods that result in neurodegeneration and imbalances in network excitability. An additional raft of consequences could be caused by epigenetic insults occurring early in development, including changes in axon guidance, synaptogenesis and neuronal migration, differentiation and proliferation leading to long-term changes in network stability. Attributing a causative action of a particular epigenetic-mediated change on neuronal function is therefore challenging. Syndromes in which causes have been isolated

to a single “epigenetic gene” (*table 1*) are likely to have a narrower spectrum of cellular consequences. Genetically-modified mice for the study of “epigenetic genes” are available and are beginning to provide some insight into cellular mechanisms of excitability. As always, we do not know the extent to which animal model findings will translate to human epilepsy. Interestingly, physiological studies have indicated that cognitive deficits in murine models based on disease-causing epigenetic genes can be reversed in adulthood (Guy *et al.*, 2007). This could have important treatment implications suggesting that a more dynamic role of epigenetic changes is responsible for at least some of the pathological outcomes in neurodevelopmental disease. In particular, studies have broadly implicated altered synaptic plasticity in neurodevelopmental disease caused by mutations of epigenetic genes (e.g. *MECP2*) (Johnston *et al.*, 2015; Iwase *et al.*, 2017). This understanding has motivated clinical studies aimed at targeting the pathomechanisms. For example, there is a marked increase in expression of the N-methyl-D-aspartate (NMDA) receptor, a glutamate receptor, in *MeCP2* knock-out mice (Blue *et al.*, 2011) that recapitulates clinical findings in Rett patients (Blue *et al.*, 1999). Dextromethorphan, an NMDA antagonist, has been shown to improve speech in Rett patients, but did not reduce seizure liability (Smith-Hicks *et al.*, 2017). Clearly, we are some way off fully elucidating the mechanisms that cause increased excitability in “epigenetic disease”. There may well be different mechanisms causing distinct clinical phenotypes (cognition vs seizures). The therapeutic time window in which treatment needs to be given will differ for different epigenetic insults. Converging pathomechanisms (e.g. synaptic plasticity) in some epigenetic diseases suggest that common treatment strategies may be possible.

Diagnostic implications

There are a number of potential diagnostic applications of the discoveries on epigenetic marks in epilepsy. Rodent studies clearly show that DNA methylation patterns differ between aetiologies (Debski *et al.*, 2016). This could have practical implications in tissue diagnostics. Resected tissue has been reported to contain unique epigenetic signatures that could form the basis of classification alongside traditional histological findings (Miller-Delaney *et al.*, 2015; Kobow *et al.*, 2019). Epigenetic marks and factors are also detectable in circulating biofluids. This has been best explored for miRNAs which, due to their tissue-specific expression, stability and amenity to assay, have been studied the most (Enright *et al.*, 2018). A number of miRNAs are found at altered levels in human

epilepsy, including in plasma and cerebrospinal fluid, and research suggests the levels of these molecules change in response to therapies (Trelinska *et al.*, 2016; Raoof *et al.*, 2018). However, the analysis of circulating miRNAs is challenging, which relates both to the lack of standardized sampling protocols and to analysis of miRNAs. Recently, a protocol for standardization of procedures for discovery of circulating miRNA biomarkers in preclinical models has been proposed (van Vliet *et al.*, 2017). This may facilitate clinical biomarker discovery. The technology to detect miRNAs in a point-of-care setting and commercial opportunities for miRNA-based diagnostics is being explored. Other epigenetic marks may also have diagnostic value. Distinct DNA methylation patterns in blood samples have been reported in patients with epilepsy compared to controls (Long *et al.*, 2017). Again, the relative stability of DNA methylation would seem an ideal attribute for a biomarker.

Therapeutic implications and limitations

The discovery, in 2001, that sodium valproate had HDAC inhibitor activity (Gottlicher *et al.*, 2001) drove excitement that this commonly used treatment for epilepsy may work through an epigenetic mechanism. However, other HDAC inhibitors lacked this property (Hoffmann *et al.*, 2008). Nevertheless, there continues to be significant interest in using epigenetic drugs to treat brain diseases, including epilepsy. A number of anti-cancer therapies have been approved which have epigenetic mechanisms of action, including other HDAC inhibitors and inhibitors of DNA methylation. Whether any of these drugs could be re-purposed for use in epilepsy is unknown but could be the focus of future research.

A number of epigenetic manipulations have shown promising effects in experimental epilepsy models. Several teams have explored global approaches to modulate the amount of DNA methylation in animals (Ryley Parrish *et al.*, 2013; Williams-Karnesky *et al.*, 2013). Results have been conflicting to date, however, with both increased and decreased excitability being reported. What may be needed is a context-specific or targeted approach to increase or decrease DNA methylation. Elegant experimental studies in cells and animal models using the genome editing tool, CRISPR (clustered regularly interspaced short palindromic repeats), recently demonstrated this was possible. Researchers tagged an enzyme that either increased or decreased DNA methylation onto a CRISPR variant and directed it to specific sites in the genome whereupon the epigenetic mark was modified (Liu *et al.*, 2016). If critical genes are epigenetically silenced in epilepsy, this type of approach could be

considered. For example, the gene encoding Reelin is hypermethylated in epilepsy patients with granule cell dispersion (Kobow *et al.*, 2009). Re-activating the gene could be an approach to restore secretion of this protein and perhaps arrest granule cell layer dispersion. Genome-wide methylation mapping of the hippocampus in patients with temporal lobe epilepsy identified multiple hyper-methylated genes (Miller-Delaney *et al.*, 2015). Although few had biologically plausible links to epilepsy, they (or others) might be targets for this type of selective approach in the future. This is also seen in brain tissue from various animal models of acquired epilepsy (Kobow *et al.*, 2013; Debski *et al.*, 2016).

Targeting of specific non-coding RNAs is another therapeutic approach that might be tried. Non-coding RNAs can be manipulated using antisense oligonucleotides (OGNs). These can be designed to be extremely potent and selective. CUR-1916 is the first OGN targeting a non-coding RNA to reach clinical trials for epilepsy. The OGN targets the natural antisense transcript that is produced by *SCN1A* which encodes the sodium channel that is most often mutated in patients with Dravet syndrome (Hsiao *et al.*, 2016). By blocking the *SCN1A*-NAT, CUR-1916 is expected to upregulate the levels of the *SCN1A* transcript. Given that the majority of mutations cause loss-of-function in epilepsy (and other neurological diseases), this therapeutic approach has particular promise. The main problem with all OGNs, however, is delivery. They cannot pass from the circulation into the brain as they are too large to cross the [intact] blood-brain barrier. They must therefore be injected intrathecally. The results of trials of this and other OGNs are eagerly anticipated. There are a number of important challenges with manipulating epigenetic processes that may limit clinical translation. As mentioned in the preceding text, the machinery for epigenetic change is present in all cell types, therefore cell-specific manipulations may need to be developed so that epigenetic marks are changed in the right cell. Expression of epigenetic components is much higher in dividing cells, therefore cells within the neurogenic niche in the brain (e.g. the subgranular zone of the dentate gyrus) may be affected most. Then there are timing aspects. Aging is associated with a trend toward a closed transcriptional state (Berson *et al.*, 2018). Conversely, the developing brain may be highly susceptible to epigenetic manipulations, which creates opportunities but also risks. Finally, epigenetic systems are excellent at storing memories and brain tissue may retain a form of memory for seizure activity. We may need to “wipe clean” epigenetic marks in order to get better outcomes. For example, to prevent previously epileptogenic tissue from re-starting hyper-excitable behaviour following resective surgery for drug-resistant epilepsy.

Summary, prospects, challenges and next steps

Epigenetic processes shape the gene expression landscape by controlling access to the genetic code. It is increasingly important that we think about epigenetics when considering mechanisms controlling gene expression. There is growing evidence that this process is disturbed following epileptogenic injuries, and some of the recently discovered epilepsy genes encode proteins that perform epigenetic functions. Epigenetic marks persist in the chronic epileptic state, perhaps stabilizing aberrant gene expression. Epigenetic marks may serve as diagnostic biomarkers, and epigenetic processes lend themselves to therapeutic targeting. However, the bulk of data in our field has come from animal models which may not extrapolate to human epilepsies.

How do we see the field progressing and where are the opportunities? (*Box 3: Outstanding Research Questions*). Technology will continue to drive discoveries in this area. Elucidating the epigenome of epilepsy is a key challenge. We need more solid data on each of the major epigenetic processes in experimental and human epilepsy. Combining deep sequencing with proteomics and other emerging technologies, such as single-cell sequencing, could provide us with the epigenome at individual cell resolution. Beyond being a scientific achievement, this will help us to see where therapies could be deployed, for example to “re-activate” a silenced gene or silence pathogenic genes. It may indicate a need to direct therapies toward specific cells types. Alongside precision epigenetic therapies, there may still be a place for broad-acting epigenetic drugs which have yet to be tested in clinical trials. It is likely that many more non-coding RNAs exist that regulate transcription and chromatin structure, and these may harbour new targets in the future. Achieving these aims will require multi-disciplinary approaches. Last, what is the role of epigenetic processes in presumed genetic epilepsies and could correction of these be a novel approach to therapy? The results should provide a better understanding of how gene expression is altered in epilepsy and may be corrected, with the development of innovative molecular diagnostics and therapeutics. □

Author contributions.

DCH wrote the manuscript with KK, CAR and EAvV. AJB, AMG, GLC, SH, IL-C, HMK, AP and MRJ contributed to concepts or literature analyses, and read and approved the final manuscript.

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References

- Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 1999; 23: 185-8.
- Barry G, Briggs JA, Hwang DW, *et al.* The long non-coding RNA NEAT1 is responsive to neuronal activity and is associated with hyperexcitability states. *Sci Rep* 2017; 7: 40127.
- Barutcu AR, Lajoie BR, Fritz AJ, *et al.* SMARCA4 regulates gene expression and higher-order chromatin structure in proliferating mammary epithelial cells. *Genome Res* 2016; 26: 1188-201.
- Bernard D, Prasanth KV, Tripathi V, *et al.* A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression. *EMBO J* 2010; 29: 3082-93.
- Berson A, Nativio R, Berger SL, Bonini NM. Epigenetic regulation in neurodegenerative diseases. *Trends Neurosci* 2018; 41: 587-98.
- Blue ME, Naidu S, Johnston MV. Development of amino acid receptors in frontal cortex from girls with Rett syndrome. *Ann Neurol* 1999; 45: 541-5.
- Blue ME, Kaufmann WE, Bressler J, *et al.* Johnston MV Temporal and regional alterations in NMDA receptor expression in Mecp2-null mice. *Anat Rec (Hoboken)* 2011; 294: 1624-34.
- Blümcke I, Arzimanoglou A, Beniczky S, Wiebe S. Roadmap for a competency-based educational curriculum in epilepsy: report of the Epilepsy Education Task Force of the International League Against Epilepsy. *Epileptic Disord* 2019; 21(2): 129-40.
- Boissel S, Reish O, Proulx K, *et al.* Loss-of-function mutation in the dioxygenase-encoding FTO gene causes severe growth retardation and multiple malformations. *Am J Hum Genet* 2009; 85: 106-11.
- Bonev B, Mendelson Cohen N, Szabo Q, *et al.* Multiscale 3D genome rewiring during mouse neural development. *Cell* 2017; 171: 557-572.e524.
- Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat Methods* 2013; 10: 1213-8.
- Carouge D, Host L, Aunis D, Zwiller J, Anglard P. CDKL5 is a brain MeCP2 target gene regulated by DNA methylation. *Neurobiol Dis* 2010; 38: 414-24.
- Carvill GL, Heavin SB, Yendle SC, *et al.* Targeted resequencing in epileptic encephalopathies identifies *de novo* mutations in CHD2 and SYNGAP1. *Nat Genet* 2013; 45: 825-30.
- Catalanotto C, Cogoni C, Zardo G. MicroRNA in control of gene expression: an overview of nuclear functions. *Int J Mol Sci* 2016; 17: E1712.
- Cava C, Manna I, Gambardella A, Bertoli G, Castiglioni I. Potential role of miRNAs as theranostic biomarkers of epilepsy. *Mol Ther Nucleic Acids* 2018; 13: 275-90.
- Crosio C, Heitz E, Allis CD, Borrelli E, Sassone-Corsi P. Chromatin remodeling and neuronal response: multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. *J Cell Sci* 2003; 116: 4905-14.
- Cui L, Tao H, Wang Y, *et al.* A functional polymorphism of the microRNA-146a gene is associated with susceptibility to drug-resistant epilepsy and seizures frequency. *Seizure* 2015; 27: 60-5.
- Debski KJ, Pitkanen A, Puhakka N, *et al.* Etiology matters – Genomic DNA methylation patterns in three rat models of acquired epilepsy. *Sci Rep* 2016; 6: 25668.
- Ding M, Liu Y, Liao X, Zhan H, Liu Y, Huang W. Enhancer RNAs (eRNAs): new insights into gene transcription and disease treatment. *J Cancer* 2018; 9: 2334-40.
- Dulac C. Brain function and chromatin plasticity. *Nature* 2010; 465: 728-35.
- Edwards JR, Yarychivska O, Boulard M, Bestor TH. DNA methylation and DNA methyltransferases. *Epigenet Chromatin* 2017; 10: 23.
- Enright N, Simonato M, Henshall DC. Discovery and validation of blood microRNAs as molecular biomarkers of epilepsy – ways to close current knowledge gaps. *Open Epilepsia* 2018; 3(4): 427-36.
- Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet* 2011; 12: 861-74.
- Gibbons R. Alpha thalassaemia-mental retardation, X linked. *Orphanet J Rare Dis* 2006; 1: 15.
- Gottlicher M, Minucci S, Zhu P, *et al.* Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J* 2001; 20: 6969-78.
- Greenberg MVC, Bourc'his D. The diverse roles of DNA methylation in mammalian development and disease. *Nat Rev Mol Cell Biol* 2019; 20: 590-607.

- Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological defects in a mouse model of Rett syndrome. *Science* 2007;315:1143-7.
- Hanly DJ, Esteller M, Berdasco M. Interplay between long non-coding RNAs and epigenetic machinery: emerging targets in cancer? *Philos Trans R Soc Lond B Biol Sci* 2018;373:20170074.
- Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, et al. An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature* 2012;489:391-9.
- Henshall DC, Kobow K. Epigenetics and epilepsy. *Cold Spring Harb Perspect Med* 2015;5:a022731.
- Henshall DC, Hamer HM, Pasterkamp RJ, et al. MicroRNAs in epilepsy: pathophysiology and clinical utility. *Lancet Neurol* 2016;15:1368-76.
- Hoffmann K, Czapp M, Loscher W. Increase in antiepileptic efficacy during prolonged treatment with valproic acid: role of inhibition of histone deacetylases? *Epilepsy Res* 2008;81:107-13.
- Hsiao J, Yuan TY, Tsai MS, et al. Upregulation of haploinsufficient gene expression in the brain by targeting a long non-coding RNA improves seizure phenotype in a model of Dravet syndrome. *EBioMedicine* 2016;9:257-77.
- Huang Y, Myers SJ, Dingleline R. Transcriptional repression by REST: recruitment of Sin3A and histone deacetylase to neuronal genes. *Nat Neurosci* 1999;2:867-72.
- Huang Y, Doherty JJ, Dingleline R. Altered histone acetylation at glutamate receptor 2 and brain-derived neurotrophic factor genes is an early event triggered by status epilepticus. *J Neurosci* 2002;22:8422-8.
- Iwase S, Berube NG, Zhou Z, et al. Epigenetic etiology of intellectual disability. *J Neurosci* 2017;37:10773-82.
- Jakovcevski M, Akbarian S. Epigenetic mechanisms in neurological disease. *Nat Med* 2012;18:1194-204.
- Jang Y, Moon J, Lee ST, et al. Dysregulated long non-coding RNAs in the temporal lobe epilepsy mouse model. *Seizure* 2018;58:110-9.
- Johnston M, Blue ME, Naidu S. Recent advances in understanding synaptic abnormalities in Rett syndrome. *F1000Res* 2015;4:F1000.
- Kan AA, van Erp S, Derijck AA, et al. Genome-wide microRNA profiling of human temporal lobe epilepsy identifies modulators of the immune response. *Cell Mol Life Sci* 2012;69:3127-45.
- Kang HJ, Kawasawa YI, Cheng F, et al. Spatio-temporal transcriptome of the human brain. *Nature* 2011;478:483-9.
- Kato M, Saitoh S, Kamei A, et al. A longer polyalanine expansion mutation in the ARX gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara syndrome). *Am J Hum Genet* 2007;81:361-6.
- Kim DH, Saetrom P, Snove Jr. O, Rossi JJ. MicroRNA-directed transcriptional gene silencing in mammalian cells. *Proc Natl Acad Sci U S A* 2008;105:16230-5.
- Kobow K, Jeske I, Hildebrandt M, et al. Increased reelin promoter methylation is associated with granule cell dispersion in human temporal lobe epilepsy. *J Neuropathol Exp Neurol* 2009;68:356-64.
- Kobow K, Kaspi A, Harikrishnan KN, et al. Deep sequencing reveals increased DNA methylation in chronic rat epilepsy. *Acta Neuropathol* 2013;126:741-56.
- Kobow K, Ziemann M, Kaipananickal H, et al. Genomic DNA methylation distinguishes subtypes of human focal cortical dysplasia. *Epilepsia* 2019;60(6):1091-103.
- Korotkov A, Mills JD, Gorter JA, van Vliet EA, Aronica E. Systematic review and meta-analysis of differentially expressed miRNAs in experimental and human temporal lobe epilepsy. *Sci Rep* 2017;7:11592.
- Kurahashi N, Miyake N, Mizuno S, et al. Characteristics of epilepsy in patients with Kabuki syndrome with KMT2D mutations. *Brain Dev* 2017;39:672-7.
- Lee DY, Moon J, Lee ST, et al. Dysregulation of long non-coding RNAs in mouse models of localization-related epilepsy. *Biochem Biophys Res Commun* 2015;462:433-40.
- Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005;120:15-20.
- Liu XS, Wu H, Ji X, et al. Editing DNA methylation in the mammalian genome. *Cell* 2016;167:233-247 e217.
- Long HY, Feng L, Kang J, et al. Blood DNA methylation pattern is altered in mesial temporal lobe epilepsy. *Sci Rep* 2017;7:43810.
- Luteijn MJ, Ketting RF. PIWI-interacting RNAs: from generation to transgenerational epigenetics. *Nat Rev Genet* 2013;14:523-34.
- Manna I, Labate A, Mumoli L, et al. Relationship between genetic variant in pre-microRNA-146a and genetic predisposition to temporal lobe epilepsy: a case-control study. *Gene* 2013;516:181-3.
- Manna I, Labate A, Borzi G, et al. An SNP site in pri-miR-124, a brain expressed miRNA gene, no contribution to mesial temporal lobe epilepsy in an Italian sample. *Neurol Sci* 2016;37:1335-9.
- Marafi D, Suter B, Schultz R, Glaze D, Pavlik VN, Goldman AM. Spectrum and time course of epilepsy and the associated cognitive decline in MECP2 duplication syndrome. *Neurology* 2019;92:e108-14.
- Marfella CG, Imbalzano AN. The Chd family of chromatin remodelers. *Mutat Res* 2007;618:30-40.
- Miller-Delaney SF, Bryan K, Das S, et al. Differential DNA methylation profiles of coding and non-coding genes define hippocampal sclerosis in human temporal lobe epilepsy. *Brain* 2015;138:616-31.
- Mills JD, van Vliet EA, Chen BJ, et al. Coding and non-coding transcriptome of mesial temporal lobe epilepsy: Critical role of small non-coding RNAs. *Neurobiol Dis* 2019;134:104612.

- Pal DK, Evgrafov OV, Tabares P, Zhang F, Durner M, Greenberg DA. BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. *Am J Hum Genet* 2003; 73: 261-70.
- Poeta L, Fusco F, Drongitis D, *et al.* A regulatory path associated with X-linked intellectual disability and epilepsy links KDM5C to the polyalanine expansions in ARX. *Am J Hum Genet* 2013; 92: 114-25.
- Raoof R, Bauer S, El Naggar H, *et al.* Dual-center, dual-platform microRNA profiling identifies potential plasma biomarkers of adult temporal lobe epilepsy. *EBioMedicine* 2018; 38: 127-41.
- Rowles J, Wong M, Powers R, Olsen M. FTO, RNA epigenetics and epilepsy. *Epigenetics* 2012; 7: 1094-7.
- Ryley Parrish R, Albertson AJ, Buckingham SC, *et al.* Status epilepticus triggers early and late alterations in brain-derived neurotrophic factor and NMDA glutamate receptor Grin2b DNA methylation levels in the hippocampus. *Neuroscience* 2013; 248C: 602-19.
- Sato F, Tsuchiya S, Meltzer SJ, Shimizu K. MicroRNAs and epigenetics. *FEBS J* 2011; 278: 1598-609.
- Smith-Hicks CL, Gupta S, Ewen JB, *et al.* Randomized open-label trial of dextromethorphan in Rett syndrome. *Neurology* 2017; 89: 1684-90.
- Sng JC, Taniura H, Yoneda Y. Histone modifications in kainate-induced status epilepticus. *Eur J Neurosci* 2006; 23: 1269-82.
- Srivastava PK, Roncon P, Lukasiuk K, *et al.* Meta-analysis of microRNAs dysregulated in the hippocampal dentate gyrus of animal models of epilepsy. *eNeuro* 2017; 4, pii: ENEURO.0152-17.2017.
- St Laurent G, Wahlestedt C, Kapranov P. The Landscape of long noncoding RNA classification. *Trends Genet* 2015; 31: 239-51.
- Suls A, Jaehn JA, Kecskes A, *et al.* De novo loss-of-function mutations in CHD2 cause a fever-sensitive myoclonic epileptic encephalopathy sharing features with Dravet syndrome. *Am J Hum Genet* 2013; 93: 967-75.
- Sweatt JD. The emerging field of neuroepigenetics. *Neuron* 2013; 80: 624-32.
- Talkowski ME, Mullegama SV, Rosenfeld JA, *et al.* Assessment of 2q23.1 microdeletion syndrome implicates MBD5 as a single causal locus of intellectual disability, epilepsy, and autism spectrum disorder. *Am J Hum Genet* 2011; 89: 551-63.
- Trelinska J, Fendler W, Dachowska I, *et al.* Abnormal serum microRNA profiles in tuberous sclerosis are normalized during treatment with everolimus: possible clinical implications. *Orphanet J Rare Dis* 2016; 11: 129.
- Tsurusaki Y, Okamoto N, Ohashi H, *et al.* Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome. *Nat Genet* 2012; 44: 376-8.
- Turnbull J, Girard JM, Lohi H, *et al.* Early-onset Lafora body disease. *Brain* 2012; 135: 2684-98.
- van Vliet EA, Puhakka N, Mills JD, *et al.* Standardization procedure for plasma biomarker analysis in rat models of epileptogenesis: focus on circulating microRNAs. *Epilepsia* 2017; 58: 2013-24.
- Verkerk AJ, Pieretti M, Sutcliffe JS, *et al.* Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991; 65: 905-14.
- Wahlestedt C. Targeting long non-coding RNA to therapeutically upregulate gene expression. *Nat Rev Drug Discov* 2013; 12: 433-46.
- Weaving LS, Christodoulou J, Williamson SL, *et al.* Mutations of CDKL5 cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. *Am J Hum Genet* 2004; 75: 1079-93.
- Wei JW, Huang K, Yang C, Kang CS. Non-coding RNAs as regulators in epigenetics (Review). *Oncol Rep* 2017; 37: 3-9.
- Williams-Karnesky RL, Sandau US, Lusardi TA, *et al.* Epigenetic changes induced by adenosine augmentation therapy prevent epileptogenesis. *J Clin Invest* 2013; 123: 3552-63.
- Xiao W, Cao Y, Long H, *et al.* Genome-wide DNA methylation patterns analysis of noncoding RNAs in temporal lobe epilepsy patients. *Mol Neurobiol* 2018; 55: 793-803.
- Zhong J, Chuang SC, Bianchi R, *et al.* BC1 regulation of metabotropic glutamate receptor-mediated neuronal excitability. *J Neurosci* 2009; 29: 9977-86.