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Epigenetic genes and epilepsy — emerging mechanisms and clinical applications

Karen M. J. Van Loo[1,2], Gemma L. Carvill[3], Albert J. Becker[4], Karen Conboy[5,6], Alica M. Goldman[7], Katja Kobow[8], Iscia Lopes-Cendes[9], Christopher A. Reid[10], Erwin A. van Vliet[11,12] and David C. Henshall[5,6]✉

Abstract | An increasing number of epilepsies are being attributed to variants in genes with epigenetic functions. The products of these genes include factors that regulate the structure and function of chromatin and the placing, reading and removal of epigenetic marks, as well as other epigenetic processes. In this Review, we provide an overview of the various epigenetic processes, structuring our discussion around five function-based categories: DNA methylation, histone modifications, histone–DNA crosstalk, non-coding RNAs and chromatin remodelling. We provide background information on each category, describing the general mechanism by which each process leads to altered gene expression. We also highlight key clinical and mechanistic aspects, providing examples of genes that strongly associate with epilepsy within each class. We consider the practical applications of these findings, including tissue-based and biofluid-based diagnostics and precision medicine-based treatments. We conclude that variants in epigenetic genes are increasingly found to be causally involved in the epilepsies, with implications for disease mechanisms, treatments and diagnostics.

Epilepsy is a chronic disorder of the CNS, characterized by recurrent unprovoked seizures and aberrant neuronal network activity. Epilepsy has a worldwide lifetime prevalence of 7.6 per 1,000 individuals, making it one of the most common neurological diseases, and can be broadly grouped into rare epilepsies (caused by single rare, highly penetrant genetic variants) and common epilepsies (caused by multiple genetic variants and/or environmental factors)[1,2]. Establishing the genetic basis of epilepsy in a specific patient is crucial for prognostication and treatment decisions. To date, approximately 1,000 genes have been classified as epilepsy-related, and more than 80 genes have been classified as pure or relatively pure epilepsy genes; that is, their disruption causes conditions in which seizures are the primary clinical feature[3]. Many ion channels are implicated in the rare and common epilepsies, and the majority of our knowledge of the mechanisms underlying epilepsy has come from studying these channels[4]. However, variants in genes encoding other classes of proteins, including enzymes, enzyme modulators, cytoskeletal components, transporters and receptors, as well as factors involved in nucleic acid binding, cell adhesion, signal transduction and membrane trafficking, have also been implicated in a range of epilepsies[3]. In particular, genes that encode proteins with an epigenetic function are increasingly being linked to epilepsies[5]. These so-called epigenetic genes form the focus of this Review.

In this article, we summarize the latest insights into how proteins that regulate epigenetic mechanisms contribute to the epilepsies. Our discussion is structured around five categories of epigenetic mechanisms: DNA methylation, histone modifications, histone–DNA crosstalk, non-coding RNAs and chromatin remodelling. We provide examples of key epigenetic genes that harbour epilepsy-associated variants and discuss the possible mechanisms by which these variants lead to hyperexcitable states and network dysfunction. Furthermore, we outline how cell and animal model systems based on these epigenetic genes might provide new insights into the process of epileptogenesis and how they can be used to test novel therapeutic compounds. Finally, we discuss how these insights could help us to develop new treatments and support diagnosis of epilepsy.

Genetics of epilepsy

The first studies to identify rare single-gene variants in people with epilepsy date back to the 1990s, starting with the finding that pathogenic variants in the neuronal acetylcholine receptor subunit α4 (*CHRNA4*) gene cause

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Key points

- The term epigenetics refers to potentially heritable changes in gene expression that do not involve alterations in the DNA sequence; the key epigenetic processes include DNA methylation, histone modifications and the actions of certain non-coding RNAs.
- Various monogenic forms of epilepsy have been attributed to pathogenic variants in genes encoding factors that regulate chromatin access and the deposition, reading and removal of epigenetic marks.
- Epigenetics-related epilepsies are often accompanied by a range of comorbidities, including intellectual disability.
- Insights from experimental studies in cell and animal models are helping us to understand how epigenetic alterations give rise to neuronal hyperexcitability.
- The findings of this research might yield diagnostic or prognostic biomarkers or treatment strategies, including precision medicine-based treatments.

Polygenic risk scores
The cumulative risk assessment for an individual to develop a particular medical condition, based on the collective influence of multiple genetic variants.

autosomal dominant nocturnal frontal lobe epilepsy[6]. Early gene discoveries were mostly made in familial epilepsies with large pedigrees, which allowed linkage analysis followed by identification of pathogenic variants[7]. Subsequent research has indicated that severe epilepsies beginning in infancy and childhood are mainly attributable to de novo variants[8]. The concept of somatic mosaicism, in which an individual has at least two cell populations with different genotypes, has also been identified as an important cause of rare focal epilepsies[9]. However, our understanding of the genetic architecture of more common epilepsies has lagged behind, as these epilepsies have a more complex mode of inheritance, probably with contributions from multiple 'susceptibility alleles'. Ultra-rare variants in known epilepsy-associated genes, as well as rare structural genomic variants, including microdeletions, are known to contribute to common epilepsies[10–12]. In addition, polygenic risk scores have been developed that can distinguish, at the cohort level, between patients with epilepsy and controls[13].

As our understanding of the genetic architecture of epilepsy grows, so does the number of genes that are known to contribute to disease. To date, rare variants in hundreds of different genes, including many genes related to synapses and ion channels, have been implicated in epilepsy[3]. As we discuss in the sections that follow, an increasing number of causative genetic variants are also being identified in genes with an epigenetic function[5].

Epigenetics — granting access to genes

In 1942, the British scientist Conrad Hal Waddington introduced the term 'epigenetic landscape' into modern biology. He defined epigenetics as "all developmental processes that lie between genotype and phenotype"[14]. Over the past few decades, the definition and usage of the term epigenetics has changed. In modern terms, Waddington's concept of epigenetics has been reinterpreted as all (potentially) heritable changes in gene expression that do not involve alterations in the DNA sequence[15].

To understand how epigenetics can affect gene transcription, we need to understand how a gene is normally 'read'. DNA is wrapped around a protein core consisting of eight positively charged histone proteins (two copies of each of the core histones H2A, H2B, H3 and H4). A nucleosome consists of an approximately 146-bp stretch of DNA wrapped around a histone octamer[16]. Nucleosome organization has an important role in regulating the accessibility of DNA to transcription factors and thereby controls gene expression. An open chromatin structure (euchromatin) is associated with a transcriptionally active state, whereas a less accessible chromatin structure (heterochromatin) is associated with a transcriptionally inactive state[17]. Epigenetic marks, such as DNA methylation, histone modifications and histone variants, collectively regulate the accessibility of the transcriptional machinery by affecting the structure of the chromatin, and help to determine whether a gene is turned on or off[18].

Epigenetic proteins can be divided into several categories. Writers are proteins that introduce chemical modifications on DNA and histones, readers are proteins that identify and interpret such modifications, and erasers are proteins that remove such chemical tags[19]. These epigenetic proteins are increasingly recognized to have pivotal roles in normal physiological functions and development, including in the brain. Since the late 1990s, a range of epigenetic genes have been linked to epilepsies[20]. These genes encode proteins that include regulators of the deposition, reading and erasing of epigenetic marks, and components of the regulatory complexes that shape and organize chromatin.

In the next few sections, we review key epigenetic genes that harbour rare, highly penetrant genetic variants in individuals with severe paediatric-onset epilepsies. The gene products are grouped into five categories that reflect their primary functions, namely, DNA methylation, histone modifications, histone–DNA crosstalk, non-coding RNAs and chromatin remodelling. For each category, we describe the general mechanism that leads to altered gene expression and present two examples of key epigenetic genes and their association with the epilepsies in more detail.

DNA methylation

DNA methylation is the most studied epigenetic process and refers to the addition of a methyl group to a cytosine base in DNA to form 5-methylcytosine. This DNA

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modification, which was first identified by Hotchkiss in 1948 (REF.[21]), occurs in mammalian cells primarily at cytosines in the context of a palindromic CpG dinucleotide sequence. Approximately 70–80% of the CpG cytosines within the mammalian genome are methylated at any given time[22]. DNA methylation marks are located in regulatory sequences such as promoter and enhancer regions, and can also be found in genic and intergenic regions and in transposable and repeat elements[23]. Notably, certain regions of the genome have a higher frequency of CpG dinucleotides than the rest of the genome. These regions are known as CpG islands and are typically located in the promoter regions of housekeeping and many tissue-specific genes[24]. Methylation of these CpG islands can influence gene transcription by affecting the local chromatin configuration: hypermethylation of the islands makes a gene less accessible to the transcriptional machinery, thereby repressing expression, whereas hypomethylation is permissive of gene expression[25].

Several non-mutually-exclusive mechanisms have been postulated to explain how DNA methylation might repress transcription (FIG. 1). First, it could impair binding of transcription factors, as suggested by the observation that CpG methylation blocks binding of the transcriptional regulator protein CTCF to DNA[26]. Second, it could recruit methyl-binding proteins that mediate transcriptional repression, such as methyl-CpG-binding protein 2 (MECP2)[27]. Last, it could affect histone modification patterns[28,29].

DNA methylation patterns are relatively stable over time, starting at the time of blastocyst implantation and being inherited through successive generations of cells[30]. CpG sites are methylated by DNA methyltransferases (DNMT1 for maintenance of methylation, and DNMT3A and DNMT3B for de novo methylation) by transferring a methyl group from *S*-adenyl methionine to a cytosine residue[23]. DNA methylation has major roles in normal development and brain function, including maintenance of genomic stability, X chromosome inactivation (XCI) in females, genomic imprinting, silencing of transposable elements and the repression of gene expression. Certain DNA methylation events have also been linked to synaptic plasticity and might contribute to molecular memory storage[31].

Several genes encoding proteins that can bind to methyl-CpG sites or are directly involved in DNA methylation or demethylation have been implicated in the epilepsies (TABLE 1). In the sections that follow, we discuss neurogenic differentiation factor 2 (NEUROD2) — a transcription factor that is also involved in DNA demethylation at specific binding sites — and the aforementioned MECP2 in more detail.

X chromosome inactivation (XCI). The X chromosome is gene-rich and, as females have two X chromosomes and males have only one, potential discrepancies in gene dosage between the sexes are addressed through inactivation of one of the X chromosomes. This process occurs randomly, and all daughter cells will have the same X chromosome inactivated.

NEUROD2. De novo variants in *NEUROD2* were first observed in two unrelated children with drug-resistant early infantile epileptic encephalopathy and developmental delay[32]. Subsequently, a variant in this gene was described in a third patient who had a neurodevelopmental disorder without seizures[33].

NEUROD2 belongs to the neurogenic basic helix–loop–helix (bHLH) family of transcription factors and has crucial roles in the development and function of the CNS and PNS[34,35]. Specifically, this factor has been implicated in neuronal survival, as well as the balance between synaptic neurotransmission and intrinsic excitability[32]. In the cortex, expression of *NEUROD1*, the closest paralogue of *NEUROD2*, is turned off around birth, whereas *NEUROD2* expression persists postnatally, suggesting that NEUROD2 is also involved in maturational processes and normal neuronal function[36].

Besides acting as a transcription factor, NEUROD2 executes an epigenetic modulatory role. This dual role is regulated by the sequence specificity of enhancer box (E-box) binding motifs for NEUROD2, which lie in the promoter regions of genes that are regulated by this protein. Binding of NEUROD2 to CAGATG E-box motifs, which are specific for NEUROD2, is associated with gene transcription, whereas binding to CAGCTG motifs, which are shared with other bHLH family members, is associated with epigenetic remodelling[37]. NEUROD2 can interact with methylcytosine dioxygenase TET2 and has the capacity to induce DNA demethylation through the oxidation of 5-methylcytosine at NEUROD2 binding sites[38].

Neurod2$^{-/-}$ mice show a wide-based ataxic phenotype, diminished motor performance, impaired growth and severe neuronal cell death, and they die between postnatal days 14 and 35 (REF.[34]). Compared with heterozygous and wild-type littermates, *Neurod2*$^{-/-}$ mice show significantly higher CpG methylation levels concomitant with altered RNA expression levels at Neurod2-targeted genomic regions, supporting the involvement of Neurod2 in DNA methylation remodelling[38]. *Neurod2* knockdown in cultured rodent cortical neurons decreases the frequency of miniature inhibitory postsynaptic currents, indicating a role for Neurod2 in synaptic inhibition[39]. Decreases in miniature inhibitory postsynaptic currents are also observed in *Neurod2*-mutant mice[39]. Neurod2 expression is activity-regulated, as indicated by an increase in Neurod2 transcript and protein levels in the hippocampus in mice treated with pentylenetetrazol — a GABA receptor antagonist that is used as a chemoconvulsant to generate seizures in animal models[40]. These actions seem to be evolutionarily conserved, as reduced Neurod2 expression in genome-edited *Xenopus tropicalis* tadpoles results in abnormal swimming behaviour and spontaneous seizure-like activity[32].

All three patient-specific missense variants (p.E130Q, p.M134T and p.L163P) are located within the bHLH DNA-binding domain of NEUROD2 and are highly conserved[32]. Experiments in an overexpression *Xenopus laevis* tadpole model suggested that these variants act in a loss-of-function manner[32]. Overexpression of wild-type NEUROD2 could induce non-neuronal cells to differentiate into ectopic neuronal populations, whereas overexpression of the mutant forms resulted in a strong reduction in ectopic neurons (p.M134T and p.L163T variants) or complete loss of these neurons (p.E130Q variant)[32,33]. In this functional assay, no differences were observed between the effects of the seizure-associated variants p.M134T and p.E103Q and the neurodevelopmental disorder-only variant p.L163P[33]. Further studies are required to refine the phenotypic spectrum associated with *NEUROD2* genetic variants.

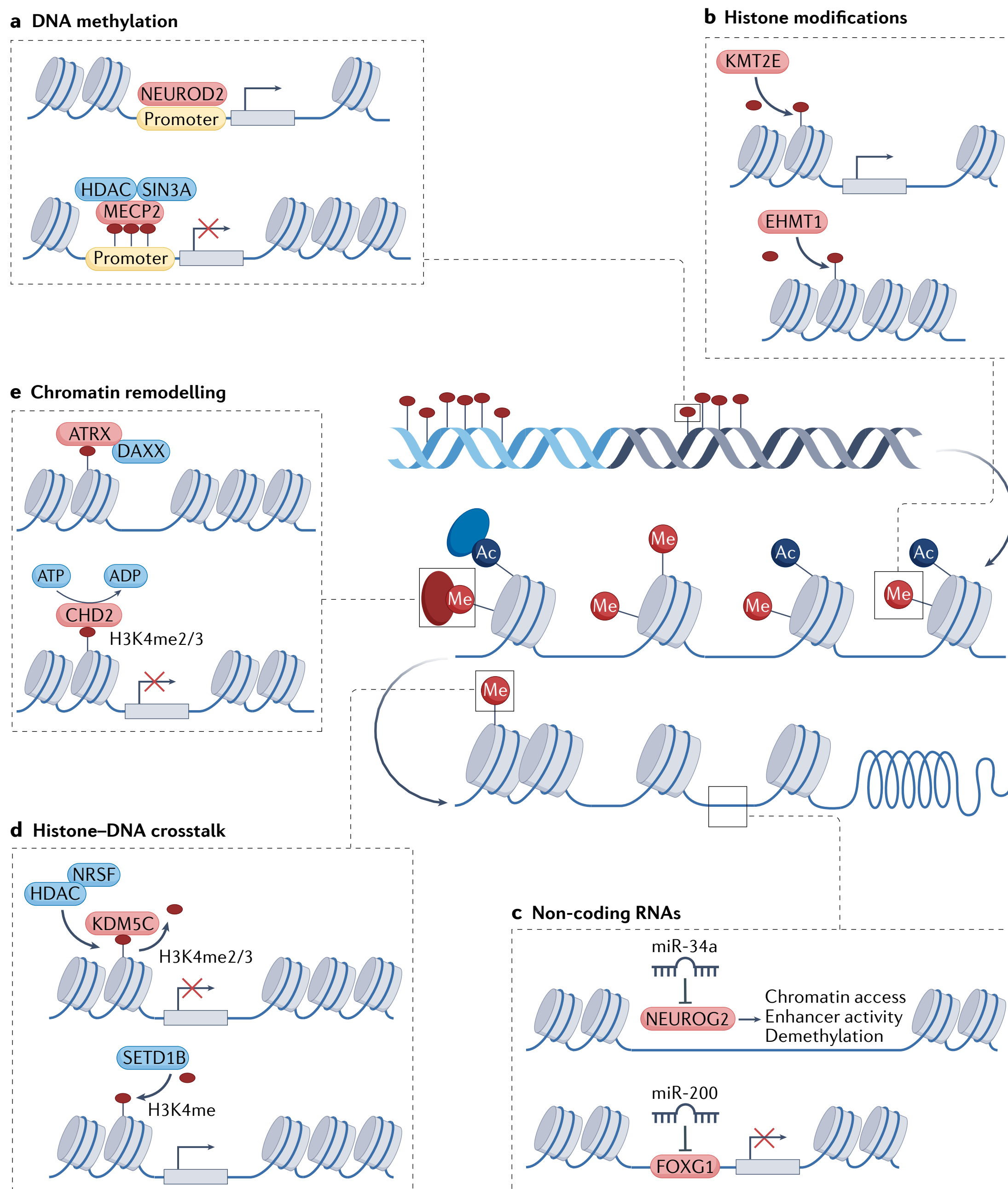

Fig. 1 | **Functions of epigenetic factors implicated in epilepsy.** The figure shows nucleosomes (blue) wrapped in DNA, with epigenetic marks including post-translational modifications indicated on histones. Within the chromatin landscape, various factors with epigenetic functions and the potential mechanisms underlying their involvement in the epilepsies are depicted. **a** | DNA methylation. The transcription factor neurogenic differentiation factor 2 (NEUROD2) is involved in DNA demethylation through the oxidation of 5-methylcytosine at specific sites located in the promoter regions of genes that are regulated by this factor. Methyl-CpG-binding protein 2 (MECP2) binds to methylated cytosine residues and recruits paired amphipathic helix protein SIN3A, resulting in transcriptional repression. **b** | Histone modifications. Inactive histone-lysine *N*-methyltransferase 2E (KMT2E) activates gene expression by affecting H3K4 methylation, whereas histone-lysine *N*-methyltransferase EHMT1 silences gene expression by catalysing H3K9 monomethylation and dimethylation. **c** | Non-coding RNAs. Expression of neurogenin 2 (NEUROG2) and forkhead box protein G1 (FOXG1) can be negatively regulated by miR-34a and miR-200, respectively. **d** | Histone–DNA crosstalk. Lysine-specific demethylase 5C (KDM5C) removes dimethylation and trimethylation of H3K4 and recruits histone deacetylase (HDAC) and neuron-restrictive silencing factor (NRSF), resulting in transcriptional repression. Histone-lysine *N*-methyltransferase SETD1B mediates the methylation of H3K4 and activates gene transcription. **e** | Chromatin remodelling. Together with the transcription cofactor death domain-associated protein 6 (DAXX), transcriptional regulator ATRX can alter nucleosome spacing by regulating histone H3.3 modifications. Chromodomain helicase DNA-binding protein 2 (CHD2) remodels chromatin through modifications of H3K4me2/3.

MECP2. Pathogenic variants in *MECP2* are associated with two different syndromes, both of which have epilepsy and neurodevelopmental delay as core features. Loss-of-function variants cause Rett syndrome (RTT; Online Mendelian Inheritance in Man (OMIM) #312750), whereas duplications encompassing *MECP2* cause *MECP2* duplication syndrome (OMIM #300260). Both syndromes are characterized by severe and progressive neurodevelopmental impairment, and they present with refractory epilepsy, intellectual disability, autism, developmental regression and vasomotor deficits[41–43]. RTT usually manifests in females owing to early lethality in hemizygous males. XCI underlies somatic mosaicism and a varied clinical spectrum in girls. Conversely, *MECP2* duplication syndrome typically affects males but can manifest as behavioural and psychiatric problems in females[43,44].

Epilepsy is a common, varied and disabling symptom in both RTT and *MECP2* duplication syndrome. Focal and generalized tonic–clonic seizures tend to dominate the clinical presentation of epilepsy in these conditions[45]. The cumulative lifetime epilepsy prevalence is close

Table 1 | **Epigenetic and related genes implicated in epilepsy**

Gene	Gene product	Functions of gene product	Number of individuals with pathogenic gene variants worldwide	Proportion of affected individuals with seizures	Refs.
DNA methylation					
MECP2	Methyl-CpG-binding protein 2	Calcium-dependent DNA-binding protein with roles in chromatin organization, transcriptional regulation and control of protein translation via miRNA-mediated mechanisms	Hundreds of thousands	Rett syndrome (60–80%); *MECP2* duplication syndrome (50%)	44,179,180
MBD5	Methyl-CpG-binding domain protein 5	Binds methylated cytosines and recruits the transcriptional repressor SILENZIO	>200	80%	181–183
Histone modifications: readers and writers					
EHMT1	Histone-lysine *N*-methyltransferase EHMT1	Histone methyltransferase	Hundreds of thousands	30%	59
KDM5C	Lysine-specific demethylase 5C	Histone demethylase	<50	40%	78
KMT2E	Inactive histone-lysine *N*-methyltransferase 2E	Histone methyltransferase	<50	Rare	184
SETD1B	Histone-lysine *N*-methyltransferase SETD1B	H3K4 methylase	<10	Most	84
KANSL1	KAT8 regulatory NSL complex subunit 1	Member of histone acetyltransferase (HAT) complex	Hundreds of thousands	25–50%	185
Chromatin remodellers					
ACTL6B	Actin-like protein 6B	Part of the nBAF chromatin remodelling complex	<50	All	186,187
ATRX	Transcriptional regulator ATRX	Chromatin remodeller (SWI/SNF family)	Thousands	30–40%	188
CHD2	Chromodomain helicase DNA-binding protein 2	Chromatin remodeller (CHD family)	>200	Majority (96%)	158
Transcription factors					
ARX	Homeobox protein ARX	Homeodomain transcription factor that regulates KDM5C and affects histone methylation	>50	Majority	189
CUX2	Homeobox protein cut-like 2	Transcription factor	<10	All	190
FOXG1	Forkhead box protein G1	Transcription factor	>50	All	108
NEUROD2	Neurogenic differentiation factor 2	DNA demethylation and transcription activation	<10	Two or three individuals	32,33
SATB2	DNA-binding protein SATB2	Transcription factor	>100	15%	191
TCF4	Transcription factor 4	Transcription factor	Thousands	50%	192
Other					
PURA	Transcriptional activator protein PURα	Transcriptional activator	<100	At least 50%	193
SMC1A	Structural maintenance of chromosomes protein 1A	Cohesin	<50	All	194
MEF2C	Myocyte-specific enhancer factor 2C	Transcriptional activator	<50	Majority	195
NACC1	Nucleus accumbens-associated protein 1	Transcriptional regulator	<10	All	196

to 90% in RTT[46]. The high burden of often medically refractory epilepsy seems to correlate with RTT severity but not with the type of pathogenic *MECP2* variant[47]. Epilepsy also affects around 50% of individuals with *MECP2* duplication syndrome, manifesting as profound epileptic encephalopathy in over 80% of affected individuals. Similar to RTT, the qualitative properties of the *MECP2* duplication do not correlate with the presence, age of onset or severity of epilepsy[42].

The so-called methylated DNA reader MECP2 is an X-linked epigenetic modulator that is important for neurodevelopment, and was first identified in 1992 owing to its ability to bind to methylated cytosines[41,48]. It is a calcium-dependent DNA-binding protein that is involved in chromatin organization, transcriptional regulation and control of protein translation via microRNA (miRNA)-mediated mechanisms[44]. *MECP2* is organized into several structurally distinct domains, including the methyl-CpG-binding domain, where most disease-causing variants are located[48–50].

The broad clinical phenotypes associated with *MECP2* pathogenic variants reflect the multifaceted functions of the protein. As an epigenetic regulator, MECP2 acts as transcriptional repressor by binding to methylated cytosine residues and recruiting the transcriptional repressor SIN3A, histone deacetylases and NCOR–SMRT co-repressor complexes[27]. MECP2 also functions as a transcriptional activator through recruitment of the transcription factor CREB1, which explains why many genes are downregulated rather than upregulated in its absence[42].

The mechanisms leading to epilepsy in individuals with *MECP2* pathogenic variants are complex and only partially understood, although the functioning of the protein is known to be sensitive to gene dosage. Studies in *Mecp2*-deficient animal models and in induced pluripotent stem cells (iPSCs) have demonstrated defects in synaptic development and plasticity, which were related to abnormal neuronal structure, decreased dendritic complexity and immature synaptic spine morphology[48]. Transcriptomic studies have shown that MECP2 is important in over 60 molecular pathways, including the mTOR–PI3K molecular cascade[50]. Impaired synaptic inhibition and dysfunction in the GABAergic pathways has been observed in several model systems. Mecp2 perturbation in animal models can cause reductions in intracellular GABA concentrations and abundance of proteins involved in GABA synthesis (GAD65 and GAD67) and in transmembrane or vesicular GABA uptake (GAT1 and VGAT, respectively). Consequent GABA spillover leads to activation of extrasynaptic GABA receptors, which manifests as increased tonic inhibition and, thus, as a pattern of spike–wave discharges on EEG in mice[45]. Reflex convulsive seizures triggered by routine handling were observed in conditional knockout mice with *Mecp2* deletion in forebrain GABAergic interneurons[45]. However, the process of epileptogenesis is likely to involve additional components, as indicated by an increase in neuronal excitability and seizure susceptibility when deletion of *Mecp2* is restricted to cholinergic neurons[45,51]. Other mechanisms that are potentially relevant to the epilepsy include oxidative damage of mitochondrial proteins[52].

Histone modifications

Post-translational histone modifications, which were first described by Allfrey et al. in 1964 (REF.[53]), represent the second major tier of epigenetic regulation. Modifications of histone proteins include acetylation, phosphorylation, methylation, ubiquitylation and sumoylation[54]. Together, these modifications control gene expression by altering the compactness and accessibility of chromatin[18] (FIG. 1). Each histone can undergo various post-translational modifications, the combination of which constitutes the 'histone code'. The combined effects of these modifications influence the transcriptional state of a gene. The most common histone marks are methylation or acetylation of the fourth, ninth or 27th lysine residue of histone H3 (termed H3K4, H3K9 and H3K27, respectively)[54].

Currently, the transcriptional effects of most individual histone post-translational modifications are poorly understood, although some modifications have a relatively high predictive value. For example, histone acetylation and histone phosphorylation are generally associated with transcriptional activity, whereas histone methylation can be associated either with transcriptional activation (for example, H3K4me) or with transcriptional repression (for example, H3K27me3 or H3K9me2/3, where '2' and '3' refer to dimethylation and trimethylation, respectively)[55,56]. Different histone modifications can affect each other and can also interact with DNA methylation[57].

Several genes that encode proteins involved in histone post-translational modifications have been associated with the epilepsies (TABLE 1). In the next two sections, we describe in detail the contributions of two methyltransferases, histone-lysine *N*-methyltransferase EHMT1 and inactive histone-lysine *N*-methyltransferase 2E (KMT2E), in the context of epilepsy.

EHMT1. Kleefstra syndrome (OMIM #610253)[58] is a rare monogenic neurodevelopmental disorder caused by de novo loss-of-function variants in *EHMT1* or microdeletions of the chromosomal region 9q34.3. This syndrome is characterized by moderate-to-severe intellectual disability, with severe expressive speech delay and variable cardiac and respiratory features. Seizures are reported in about 30% of patients and seizure types include tonic–clonic seizures, absence seizures and complex partial epilepsy[59].

EHMT1 catalyses H3K9 monomethylation (H3K9me1) and dimethylation (H3K9me2) and silences gene expression[60]. Functionally, EHMT1 can heterodimerize with its family member EHMT2 to form a complex[61], which can interact with two zinc-finger proteins, ZNF644 and WIZ, to regulate H3K9 methylation as well as gene repression[62].

The role of EHMT1 in histone methylation has been investigated in detail in mouse zygotes[63]. This work suggests a broader role for EHMT1 in regulating epigenetic reprogramming during embryonic development, with possible effects on H3K27me2, H3K27me3 and H4K16me1 (REFS.[63,64]).

In a mouse model with haploinsufficiency of *Ehmt1*, administration of the NMDA receptor antagonist ketamine produces a distinct pattern of electrophysiological abnormalities, suggesting that reduced expression of *Ehmt1* during development disrupts the formation of neuronal circuits[65]. Furthermore, *Ehmt1*$^{+/-}$ mice show delayed maturation of parvalbumin-positive inhibitory interneurons in sensory cortical areas, leading to delayed maturation of GABAergic transmission, which could promote hyperexcitability in these animals[66].

Consistent with the results from the mouse models, another study demonstrated abnormal neuronal network formation in iPSCs derived from patients with Kleefstra syndrome[67]. In these *EHMT1*-haploinsufficient iPSCs, NMDA receptor subunit 1 (GRIN1) is upregulated, coupled with reduced deposition of the repressive H3K9me2 mark at the *GRIN1* promoter. Furthermore, the neuronal phenotype could be rescued using NMDA receptor blockers, opening up a potential avenue for therapeutic use of these compounds in patients with Kleefstra syndrome.

Using in vitro models, Frega and colleagues have provided evidence that the mechanisms by which *EHMT1* loss-of-function variants cause phenotypes related to developmental delay are shared by pathogenic variants in other epigenetic genes, including *MBD5*, *KMT2C* and *SMARCB1* (REF.[68]). Neurons carrying pathogenic variants in these genes that have been implicated in Kleefstra syndrome spectrum disorders show differential expression of genes linked to synapse function, including upregulation and downregulation of glutamate and GABA receptors, adhesion molecules and postsynaptic density proteins. Therefore, a common mechanism leading to the formation of the hyperactive neuronal network and abnormal excitatory–inhibitory balance might exist in these patients. However, precisely how these different epigenetic modulators contribute — either directly or indirectly — to the transcriptional changes observed in Kleefstra syndrome is still unclear.

KMT2E. Heterozygous variants in the *KMT2E* gene or 7q22.2-22.23 microdeletions encompassing *KMT2E* have been reported in at least 45 patients[69–72] and are associated with a neurodevelopmental disorder characterized by global developmental delay, speech delay, variably delayed intellectual development and subtle dysmorphic features (OMIM #618512)[70]. Some individuals with this condition also have autism, seizures, hypotonia and/or feeding difficulties, and males seem to be affected twice as often as females. Almost all of the reported variants occurred de novo and were mainly truncating. Missense variants have also been reported, but to a much lesser extent, and were linked to more severe phenotypes[69].

KMT2E encodes a member of the lysine *N*-methyltransferase 2 family, which have roles in histone H3K4 methylation and gene activation[73]. This gene family is homologous to the evolutionarily conserved trithorax group and is important for development[74]. Several monogenic neurodevelopmental disorders have been attributed to impaired regulation of H3K4 methylation owing to pathogenic variants in *KMT2A*, *KMT2B*, *KMT2C* or *KMT2D*. However, unlike the other members of the KMT2 family, KMT2E lacks intrinsic methyltransferase activity towards histone substrates[73]. Nevertheless, KMT2E has been suggested to affect H3K4 methylation indirectly by regulating expression of the histone-modifying enzymes LSD1 and SET7/9 (REF.[75]). KMT2E is highly expressed in the brain, particularly during fetal development, and is involved in a broad spectrum of biological processes, including cell cycle progression, genome stability, adult haematopoiesis and spermatogenesis[73].

Protein-truncating variants of *KMT2E* have been proposed to lead to haploinsufficiency, whereas missense variants result in altered KMT2E binding properties[69]. No functional testing of pathogenic variants has been performed so far, however, and no neurological phenotype has been reported in *Kmt2e*-deficient mouse models (the observed features include growth restriction and impaired haematopoiesis). Nevertheless, the evidence to date indicates that disruptive variants in *KMT2E* are a notable cause of neurodevelopmental abnormalities, including seizures[69].

Histone–DNA crosstalk

DNA methylation and histone post-translational modifications should not be seen as two independent epigenetic processes, as they often work hand in hand to regulate gene transcription. DNA methylation seems to have a role in regulating histone post-translational modifications and, in turn, histone deacetylation and methylation can influence DNA methylation[57]. We have already briefly addressed the ability of MECP2 to bind to methylated cytosines and simultaneously recruit repressor complexes containing histone deacetylases[27]. In the two sections that follow, we describe two additional proteins, lysine-specific demethylase 5C (KDM5C) and histone-lysine *N*-methyltransferase SETD1B (FIG. 1), that can influence histone post-translational modifications as well as DNA methylation in the epilepsies.

KDM5C. Males with X-linked *KDM5C* pathogenic variants present with mild-to-severe intellectual disability, often accompanied by characteristic facial features, short stature, spasticity and behavioural disturbances (OMIM #300534). Epilepsy is present in about 40% of individuals with these variants, though the seizure phenotypes are poorly characterized[76,77]. A smaller subset of more mildly affected females has also been described[78].

KDM5C is involved in regulation of epigenetic marks; specifically, it removes dimethylation and trimethylation of H3K4 but does not act on H3K9, H3K27, H3K36, H3K79 or H4K20 (REF.[79]). KDM5C participates in transcriptional repression of neuronal genes by recruiting the histone deacetylases HDAC1 and HDAC2, the histone methyltransferase EHMT2, and neuron-restrictive silencing factor (NRSF)[80]. Interestingly, depletion of KDM5C using RNA interference-mediated approaches revealed increased H3K4me3 at the sodium channel type 2 (*SCN2A*) and synapsin promoters, concomitant with increased expression of both genes[80]. In addition, *KDM5C* pathogenic variants result in altered genomic DNA methylation in comparison with control

individuals, supporting the idea of functional interdependence between H3K4 modification and DNA methylation[81].

Nonsense and missense *KDM5C* mutations have been found in patients with the aforementioned X-linked intellectual development disorder, suggesting that loss of gene function is the pathogenic mechanism[76]. The effects of these variants on *KDM5C* expression, stability and catalytic activity have been studied in patient-derived cells. Missense variants were shown to reduce protein demethylase activity, further supporting a loss-of-function disease mechanism[82]. Moreover, disruption of the mouse *Kdm5c* gene recapitulates the adaptive and cognitive abnormalities that are observed in X-linked intellectual disability, including impaired social behaviour and memory, and aggression. Furthermore, *Kdm5c*-knockout mouse brains exhibit impaired dendritic arborization, spine abnormalities and altered transcriptomes[83].

SETD1B. De novo microdeletions in the 12q24.3 region encompassing *SETD1B*, as well as de novo nonsense, frameshift and missense variants affecting the highly conserved catalytic SET domain of the SETD1B protein, have been linked to syndromic intellectual disability and epilepsy (OMIM #619000)[84–87] This neurodevelopmental disorder is characterized by global developmental delay, intellectual disability, language delay with regression and autism spectrum disorder. Of the individuals with a pathogenic variant, around 80% present with generalized seizures at onset[88].

SETD1B is a component of a histone methyltransferase complex that specifically mediates the methylation of H3K4 (REF.[89]). In vitro data suggest a specific role in H3K4 trimethylation — an evolutionarily conserved chromatin mark involved in gene activation.

In mice, deletion of *Setd1b* leads to retardation of development after embryonic day 7.5, with death occurring before embryonic day 11.5 (REF.[90]). There are five known paralogues of SETD1B, none of which is redundant in activity. No models recapitulating haploinsufficiency of *Setd1b* have yet been electrophysiologically characterized to provide details on downstream epileptogenic changes. However, DNA methylation changes have been reported in patients with pathogenic variants in *SETD1B*, with a shift towards hypermethylation in comparison with healthy controls[91]. Therefore, we can hypothesize that a lack of SETD1B catalytic activity and subsequent loss of H3K4 trimethylation in gene promoters supports DNMT activity and de novo DNA methylation at the respective loci.

Non-coding RNAs

A relatively recently discovered epigenetic mechanism is the regulation of gene expression by non-coding RNAs[92,93]. The term non-coding RNAs refers to RNAs that are transcribed but do not code for or cannot be translated into functional proteins. Several classes of non-coding RNAs have epigenetic functions, including miRNAs, enhancer RNAs, PIWI-interacting RNAs and natural antisense transcripts[94].

The most studied of these non-coding RNA is the miRNA family. Typically, miRNAs are 19–24 nucleotides long and control gene expression through post-transcriptional gene silencing via sequence-specific binding to the 3′-untranslated region of mRNA transcripts in the cytoplasm. As a consequence, the bound mRNA is degraded or the translation of the target gene is repressed. In addition to regulation at the post-transcriptional level, miRNAs have also been detected in the nucleus, where they are involved in regulating gene expression at the transcriptional level[95]. For example, miR-320 was shown to epigenetically silence expression of the cell cycle gene *POLR3D* by mediating recruitment of argonaute 1, polycomb group component EZH2 and H3K27me3 to the *POLR3D* promoter[95].

To date, approximately 2,300 bona fide human mature miRNAs have been identified, and they are predicted to regulate one-third or more of all proteins[96], probably including MECPs, histone methyltransferases and deacetylases, as well as chromatin remodelling proteins. Although most miRNAs do not have direct epigenetic effects, several studies have found that aberrant miRNA expression can regulate histone modifications and induce chromatin remodelling[95,97–99]. Other studies have shown that miRNAs can also affect CpG methylation by regulating the expression of DNA methylases[100–102].

Extensive evidence exists for a role for miRNAs in experimental and human epilepsy[103], and in resected brain tissue from patients with temporal lobe epilepsy, several miRNAs displayed nuclear localization[104,105]. To date, no genetic variants in miRNAs themselves have been identified in patients with epilepsy. However, as more studies are focusing on identifying pathogenic variants in non-coding RNAs, new forms of genetic variation linked to the epilepsies are likely to emerge. To highlight the relevance of miRNAs to the epilepsies, we describe two miRNA-related cascades that are associated with transcription factors that harbour pathogenic variants in individuals with paediatric epilepsies (FIG. 1).

The miR-200–FOXG1 cascade. Forkhead box protein G1 (*FOXG1*)-related disorders are characterized by severe intellectual disability, absent speech with autistic features, and epilepsy[106,107]. Similar to MECP2, FOXG1 is sensitive to gene dosage, and both haploinsufficiency and increased gene expression are associated with neurodevelopmental disorders including epilepsy[108]. In children with pathogenic loss-of-function *FOXG1* variants, the recognized clinical features include microcephaly, developmental delay, severe cognitive disabilities, early-onset dyskinesia and hyperkinetic movements, stereotypies, epilepsy and cerebral malformation[108]. By contrast, children with *FOXG1* duplications are typically normocephalic and have normal brain MRI scans[108]. In both conditions, seizure onset typically occurs in early childhood, but is typically earlier in the case of *FOXG1* duplications[108]. However, individuals with loss-of-function variants are more likely to present with refractory seizures[108].

FOXG1 is a well-conserved DNA-binding transcription factor that belongs to the forkhead family and is encoded by a gene on chromosome 14q12. *FOXG1* is highly expressed in neural tissues during brain development and regulates the formation of cortical GABAergic

circuits during a critical early postnatal period[109]. The protein functions as a transcriptional repressor, either directly or through the regulation of miR-200 biogenesis[110]. In addition, FOXG1 was found to interact with the DROSHA complex — core proteins of the miRNA-processing pathway[110].

As homozygous mutations in *Foxg1* lead to perinatal lethality in mice, heterozygous *Foxg1*$^{+/-}$ mice are used in experimental studies. In these mice, the size of the cerebral hemispheres is reduced, and the animals also display alterations in cortical layering and autism spectrum disorder-like features[111]. Electrophysiological recordings in the primary motor cortex or hippocampus of *Foxg1*$^{+/-}$ mice showed an overall increase in the frequency of high-amplitude spikes and the occurrence of electrographic seizures in comparison with controls[111,112]. By contrast, recordings in primary motor cortex neurons showed downregulation of spontaneous glutamatergic transmission, as indicated by reduced frequency and amplitude of miniature excitatory postsynaptic currents[112]. This finding could be attributable to reduced expression of the ionotropic AMPA receptor GluA1 and of PSD95, a multifunctional organizer of excitatory synapse structure[113].

These seemingly contradictory findings might reflect homeostatic plasticity, in which spontaneous synaptic transmission is reduced to compensate for pathologically enhanced excitability of a whole brain structure[113]. Furthermore, overactivation of AKT–S6 signalling (indicating of an overactive mTOR pathway) and increased expression of vesicular glutamate transporter 2 (a glutamate transporter isoform that is specific to subcortical afferents) was found in *Foxg1*$^{+/-}$ mice, whereas expression of the potassium–chloride cotransporter KCC2 (a key regulator of intracellular chloride homeostasis) was reduced, indicating an increased excitation–inhibition ratio[112].

Interestingly, a diet supplemented with the C7 triglyceride triheptanoin rescued hippocampal hyperexcitability and seizure susceptibility in *Foxg1*$^{+/-}$ mice, probably via anaplerosis, that is, replenishment of the tricarboxylic acid cycle with intermediate products. This diet has already been used successfully in various mitochondrial pathologies, including MECP2-dependent RTT, and has also shown anticonvulsant effects in several experimental models[111].

The miR-34a–NEUROG2 cascade. Although genetic variants in the neurogenin 2 gene (*NEUROG2*) have not been identified in individuals to date, a study by Avansini and colleagues indicated that *NEUROG2* is negatively regulated by the non-coding RNA miR-34a[114], which was found to be downregulated in surgical tissue from patients with focal cortical dysplasia type IIb (FCD IIb). Moreover, *NEUROG2* is upregulated in FCD IIb[114]. FCD is a developmental malformation frequently associated with severe seizure disorders[115]. In FCD IIb, a characteristic pattern of histopathological abnormalities is observed, including dysmorphic neurons with balloons cells[116].

NEUROG2 is a member of the bHLH transcription factor family[117] and can facilitate chromatin looping, increase chromatin accessibility, mediate enhancer activity and influence DNA demethylation[118]. During development, NEUROG2 is expressed predominantly during neurogenesis and is downregulated during the transition from neurogenesis to gliogenesis[119–121]. Abnormal regulation of NEUROG2 during brain development could disrupt this transition, culminating in the presence of the types of abnormal cells that are seen in FCD IIb[122,123]. NEUROG2 is also involved in the development of glutamatergic neurons[124,125]. Therefore, increased expression of NEUROG2 might enhance synaptic excitatory glutamatergic neurotransmission, thereby altering the balance between excitation and inhibition in the brain and ultimately leading to epilepsy[126].

NEUROG2 has also been linked to the modulation of transcriptional regulators[117,122–124], thereby affecting downstream genes such as *RND2*, which is overexpressed in FCD II[114]. *RND2* encodes RHO-family GTPase 2, which has been shown to regulate actin cytoskeleton organization during the migration of excitatory neurons[127]. Furthermore, RND2-deficient neurons in mice fail to transition from the multipolar to the bipolar stage[128], suggesting that downregulation of RND2 is detrimental to cortical development and can result in the formation of abnormal neural circuits, leading to the type of abnormal cortical synaptic connectivity seen in FCD IIb[126].

Chromatin remodelling

In mammalian cells, chromatin structure and gene expression are interconnected: condensation of chromatin to form heterochromatin restricts the access of transcription factors to DNA, and loosening of the chromatin structure to form euchromatin permits transcriptional activation. Besides the CpG methylation status and post-translational modifications of histone proteins, nucleosome remodellers can influence nucleosome organization and dynamics. Chromatin remodellers are crucial for spatial and temporal control of gene expression, and can alter nucleosome spacing and density or facilitate histone variant exchange[129]. Consequently, variants in remodelling genes can influence many cellular functions, including DNA replication and repair and cell proliferation and differentiation. In the sections that follow, we describe two key epilepsy-related chromatin remodelling proteins, transcriptional regulator ATRX and chromodomain helicase DNA-binding protein 2 (CHD2) (FIG. 1), and their relationships to the epilepsies.

ATRX. The X-linked α-thalassaemia mental retardation syndrome (OMIM #301040; also known as ATR-X syndrome) is a rare congenital disorder caused by X-linked recessive variants in *ATRX*, which were first identified in 1995 (REFS.[130,131]). ATR-X has a characteristic phenotype that includes severe psychomotor retardation, facial dysmorphism, genital abnormalities and seizures, and is commonly associated with α-thalassaemia[131–134].

ATRX encodes a chromatin-remodelling protein that is part of a broad family of ATP-dependent chromatin remodellers[130]. ATRX has been linked to DNA methylation[135,136] and, together with the transcription cofactor DAXX, it can also epigenetically regulate

Histone variant exchange
Histone variants confer different structural properties to the nucleosome. Exchange of these histone variants can promote or weaken nucleosome stability and/or permit more or less DNA to be wrapped around the nucleosome.

Chromodomain
A functional protein domain commonly found in chromatin remodellers and other proteins that associate with chromatin.

Telomeric
Towards the telomeres — the very ends of the linear chromosome.

Pericentromeric
The centromere is the region of the chromosome to which the microtubules of the mitotic spindle are attached during cell division. Pericentromeric regions lie either side of the centromere.

Chromocentres
Large heterochromatic regions of densely packed DNA, mostly satellite DNA and other repetitive regions, as well as histone proteins.

histone H3.3 modification at telomeric and pericentromeric heterochromatic regions[137–139]. In addition, ATRX can interact with MECP2 and with cohesin, another important regulator of chromatin structure. Together, these proteins can bind to chromatin and silence a subset of imprinted genes[140,141].

Studies in mice lacking *Atrx* demonstrated that this gene is required for proper development of the brain[130]. *Atrx* deletion in the early developing brain leads to DNA replication stress, telomere instability, extensive cell death and microcephaly[142–144]. No clear seizure phenotype has emerged from animal studies; however, *Atrx* deletion causes changes in the morphology of dendritic spines, which are important contact points for excitatory neurotransmission, and also leads to short-term and long-term plasticity deficits, which are cellular correlates of learning and memory[145–147]. Consistent with these findings, behavioural analysis of various conditional *Atrx*-knockout mice revealed impaired contextual and spatial memory, recapitulating the intellectual impairment seen in patients with ATR-X syndrome[145,146,148].

Most variants that cause ATR-X syndrome occur in the two main domains of ATRX — the ARX–DNMT3–DNMT3L domain (a zinc-finger domain involved in the recognition of histone marks) and the catalytic ATPase–helicase domain (the enzymatic core of the protein) — and no clear genotype–phenotype correlations have been observed[149]. Expression-based assays have demonstrated that most variants in *ATRX* cause a reduction in protein levels[150,151]. Experiments utilizing recombinant proteins have also demonstrated reduced ATPase activity and altered localization to chromocentres[150,152,153]. The role of *ATRX* is clearly complex and we require a more comprehensive understanding of both direct and indirect effects of disease variants to facilitate targeted therapy.

CHD2. A link between *CHD2*, intellectual disability and epilepsy was first reported in 2012 (REF.[154]), and subsequent studies identified *CHD2* pathogenic variants as a cause of developmental and epileptic encephalopathies[8,155,156]. This disorder is characterized by early-onset (6 months to 4 years of age) seizures that are typically drop attacks, myoclonus and rapid-onset seizures of various types. Photosensitivity is common and the seizures are highly refractory to antiseizure medications[157]. *CHD2* variants have also been implicated in other neurodevelopmental phenotypes, including intellectual disability and autism spectrum disorder[158].

CHD2 is a chromodomain helicase DNA-binding protein that is a member of one of four subfamilies of chromatin-remodelling proteins. This subfamily contains eight other members (CHD1 and CHD3–9)[159–161]. CHD2 contains several important functional domains, including paired chromodomains and an ATPase–helicase domain, which mediate recognition of and binding to epigenetically marked regions of DNA (H3K4me2/3 sites)[162]. CHD2 is recruited to transcriptional start sites and also assembles nucleosomes[161]. The effects of CHD2 recruitment are context-dependent and can result in promotion of gene transcription. Functional studies have shown that disrupting the function of CHD2 affects the expression of hundreds of genes, including master regulators such as NRSF. In addition, CHD2 has been implicated in DNA repair pathways[163], which might also be relevant to mechanisms of epileptogenesis.

The mechanisms through which loss of CHD2 function promotes epilepsy are not fully understood but are likely to be secondary to disruption of neurodevelopment and dysregulation of gene expression. CHD2 is expressed in various organs, including the placenta, lung, skeletal muscle and brain[159]. In the developing mouse brain, CHD2 was shown to be highly expressed in progenitor regions for inhibitory neurons[164]. In the mature brain, the protein was found in most excitatory and inhibitory neurons and oligodendrocytes, but not in astrocytes.

In a mouse line with a heterozygous loss-of-function mutation in *Chd2* (*Chd2*$^{+/-}$), the brain appeared normal in terms of size and patterning, but the animals displayed molecular and neurophysiological deficits, as well as reduced numbers of GABAergic and glutamatergic neurons[164]. Loss of *Chd2* was associated with differential expression of more than 600 genes, including many linked to epilepsy and intellectual disability. The mice exhibited changes in the properties of both inhibitory and excitatory neurons and also showed EEG abnormalities, including increased alpha and gamma power. However, spontaneous seizures were not observed, suggesting that this mouse model does not precisely recapitulate the clinical presentation in humans.

Studies on specific treatments for CHD2-related epilepsies are limited, although Kim and colleagues found that transplantation of GABAergic progenitors rescued some cognitive phenotypes in the *Chd2*$^{+/-}$ mouse model[164].

Therapeutic implications

Many of the genes described in this Review exhibit specific DNA methylation patterns, known as epigenetic signatures or episignatures, in individuals with neurodevelopmental disorders[165]. These episignatures are detected by performing DNA methylation-based arrays in peripheral blood samples and may be used to reclassify variants of uncertain significance in specific genes as either pathogenic, if the episignature is present, or benign if not. In the future, these signatures might also be useful for therapeutic targeting and precision medicine-based treatments[166,167]. Once the disease-associated gene is identified, treatment strategies can be developed to address the epigenetic impairment directly or to compensate for the affected molecular pathway. The potential reversibility of epigenetic profiles makes epigenetic therapy an attractive prospect for epilepsies in which epigenetic mechanisms have been implicated[56]. Modulating the mechanisms involving DNA methylation, histone acetylation and miRNA expression might retard epileptogenesis and reverse chronic epilepsy in such cases[168]. Several drugs targeting epigenetic mechanisms have already been approved to treat cancer[169], but their applications in the epilepsy field have yet to be fully explored.

In experimental models of epilepsy, several epigenetic agents have shown promising effects, including the

HDAC inhibitors valproic acid and trichostatin A, and the DNMT inhibitor zebularine[168]. In addition, epigenetic agents can increase the efficacy of antiseizure medications. For example, the HDAC inhibitor sodium butyrate was shown to improve the anticonvulsant activity of two antiseizure medications, the non-competitive NMDA receptor antagonist dizocilpine (MK-801) and the benzodiazepine receptor agonist flurazepam[170,171]. However, the epigenetic mechanisms that are influenced by these enzymes operate in all cell types throughout the body, so a cell-specific manipulation approach that targets only the cells and mechanisms underlying epileptogenesis will be required.

Another promising epigenetic-based therapy — though still some way from the clinic — is based on a CRISPR–Cas9 methylation editing technique, in which sequence-specific guide RNAs are used to target methylation-modifying enzymes to affected genes, thereby enabling normal expression levels to be restored[172]. Also the use of antisense oligonucleotides to target miRNAs has great potential. Initial experiments in animal models demonstrated that targeting of miRNAs could result in effective suppression of spontaneous recurrent seizures[173,174]. Clinical trials based on miRNA antagonism in other diseases[175,176] help to support the feasibility of such an approach for the human epilepsies.

Conclusions and future directions

Over the past decade, tremendous progress has been made in the field of epilepsy genetics, driven in part by advances in next-generation sequencing technologies. As we highlight in this Review, a subset of newly identified epilepsy-associated genes encode proteins with epigenetic functions. We summarize current knowledge of a selection of these genes — a more comprehensive list can be found in TABLE 1. Besides factors with clear epigenetic functions, several other proteins, including transcription factors, work in concert with epigenetic factors, and pathogenic variants in these proteins have also been associated with epilepsy. The most recent genome-wide association studies in genetic generalized epilepsies implicate transcription factors and a histone modification gene in these common epilepsies[177].

The developments discussed here demonstrate that disturbed epigenetic profiles have been identified in both human and experimental epilepsy. Such epigenetic signatures might have diagnostic value and could be used as clinical biomarkers in individuals with epilepsy. In particular, epigenetic factors that are easily detectable in biofluids, including plasma and cerebrospinal fluid, could provide potent diagnostic biomarkers. Though still challenging to detect in a clinical setting, blood miRNAs show strong promise as epigenetic biomarkers of epilepsy[105,178].

In summary, the epigenetic machinery could represent a valuable new platform for the discovery of optimal clinical biomarkers and the development of precision medicine-based therapeutic treatments in the epilepsies. More research is required to discover new variants in epigenetic genes and to understand how these variants contribute to pathogenesis. Many of the epigenetic genes described in this Review show comparable properties in humans and experimental animal models, and further studies in cell and animal models should provide new insights into the molecular and cellular mechanisms underlying epigenetic variation and its role in epileptogenesis.

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The authors declare no competing interests.

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Review criteria
Genes were selected on the basis of PubMed searches using the terms "epilepsy" AND "epigenetic", and the searches were combined with the terms "mutation" or "variant". The genes and their pathogenic variants were prioritized according to relevance. Epigenetic genes that have been implicated in the severe, early-onset paediatric epilepsies, in which gene discovery has been the most robust and effective in establishing bona fide causative genetic variants for these conditions, were included. An example of how epigenetic processes might be perturbed more broadly in epilepsy, including in the more common focal epilepsies, was also included, using the example of the miR-34a–NEUROG2 cascade that has been implicated in focal cortical dysplasia.

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