

EPILEPSY IN  
HUMANS,  
LABORATORY  
RODENTS AND  
DOGS:  
COMPARATIVE  
GENETIC ASPECTS

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## **ABSTRACT -**

**Epilepsy is a severe chronic neurological disorder defined by recurrent seizures. The disease is categorized according to underlying cause and in primary (idiopathic) epilepsy no obvious anatomic neurological deficit is found and is suspected to have a hereditary basis in many cases. In humans, many rare epilepsy syndromes have been found to be the result of channelopathies of both voltage- and ligand-gated ionchannels. The discovery of epigenetics and its involvement in many (neurological) diseases, including epilepsy, has provided a new angle for future research of pathogenesis and possibly even novel therapies.**

**This paper focuses on idiopathic epilepsy, known or proposed epileptogenic mechanisms in humans , laboratory rodent models for epilepsy and dogs, an animal of which little is known about its epileptogenic background but may prove promising as an animal model for epilepsy in the future.**

## **INTRODUCTION**

Epilepsy is a severely debilitating neurological disease in both humans and animals. Since the dawn of the human species people have been afflicted by epilepsy and it has been recognized since the earliest medical writings, going as far back as 400 b.c. when Greek philosopher Hippocrates writes the first book on epilepsy, *On the Sacred Disease*. In this book he is the first to recognize epilepsy as a disease. However, history has proven not everyone agreed on his view and since ancient times epilepsy patients have undergone many cruelties and unorthodox therapies with the aim to take away the evil that was considered to be inside them. It was not until the 19<sup>th</sup> century epilepsy regained attention as a medical disorder and only with the introduction of the first anti-epileptic drug, Phenobarbital, under the name of Luminal, in 1912 serious attempts were made to combat epilepsy from a medical perspective (Chilemi, 2005).

Epilepsy is a disorder defined by recurrent seizures (LeCouteur and Child, 1989). Seizures are characterized as transitory disturbance of brain function as a result of hypersynchronous neuronal discharge (DeLahunta, 1977). Epileptic disorders can be subdivided into different categories according to causality and symptoms. In idiopathic or primary epilepsy no obvious anatomical neurologic deficit can be located and is suspected to be hereditary, which is in contrast with acquired or secondary epilepsy in which a specific initiator of the seizures can be identified (Thomas 2000). Seizures can be generalized or partial, consequently with or without loss of conscience during the seizure (Thomas, 2000).

Epilepsy is a common chronic neurological disorder in many species. Based on clinical criteria, more than 50 distinct forms of epilepsy have been defined in humans (Reid et al., 2009). Epilepsy is the most common acquired chronic neurological disorder in humans (Chandler K, 2006) and the lifetime risk of developing epilepsy is 2-6% (Sander et al 1996). Idiopathic epilepsy is considered hereditary and approximately 40% of epilepsy syndromes in humans are believed to be idiopathic (Gardiner M, 2008). In dogs the incidence of epilepsy varies from 0.5% to 5.7% in some highly affected breeds (Chandler K, 2006). Over 100 inbred rodent strains exist modeling a wide variety of epileptic disorders (Baraban, 2007), which has been of great help identifying genes involved in many rare Mendelian forms of idiopathic epilepsy in humans. Most of the genes involved in these rare epileptic disorders have been discovered to encode mutant dysfunctional ion channels, which is consistent

with the theory that seizures are a result of dysfunctional neuronal discharge (Reid et al, 2009). However, monogenetic channelopathies are considered to be only one piece of the puzzle. More recent research has focused on epigenetics and the realization that the brain may be more plastic than previously thought has shed a new light on causal factors in epileptogenesis. Moreover, most idiopathic epilepsies segregate in a complex, polygenetic fashion and animal models have suggested that quantitative susceptibility loci influencing the seizure threshold may be numerous.

The goal of this paper is to review the current state of knowledge with regard to multispecies pathophysiology, etiology and genetics of idiopathic epilepsy. Furthermore, using synteny principles an attempt will be made to predict gene loci for epilepsy in species, using the dog as an example, in which familiarity is assumed and in some breeds proven but the mode of inheritance and gene loci have yet to be discovered.

## **EPILEPTOGENESIS IN HUMANS**

Seizures are the result of increased excitability in neurons, resulting in neuronal hypersynchronicity in large groups of neurons in the brain. Epilepsy is often considered to be an imbalance between excitation and inhibition. Both an increase in excitation and a decrease in inhibition can result in hyperexcitability, leading to epileptiform activity in the brain (Chandler, 2006).

All of the genes thus far identified as causing idiopathic epilepsy are molecular components of neuronal signaling, most often encoding for components of ion channels. Over a decade ago the first ion channel mutation associated with human epilepsy was discovered (Steinlein et al., 1995). So far, mutations in at least 25 genes have been described, although evidence regarding their pathogenic role in naturally occurring epilepsy has yet to be established. Mutations have been found for proteins involved in the functional structure of sodium, potassium, calcium and rarely in chloride voltage-gated channels. Also, other human monogenic epilepsies were found to be caused by aberrant ligand-gated ion channels stimulated by GABA, glutamate, acetylcholine and serotonin. Because most of the genes identified to date for all the uncommon forms of human epilepsy involve voltage- or ligand-gated ion channel subunits "channelopathies" may also be responsible for more common forms of human (and animal) idiopathic epilepsy (Steinlein, 1998). However, pedigree analysis has implicated that polygenic modes of inheritance are most likely responsible for the most common forms of idiopathic epilepsy (Steinlein, 1998).

Interneurons are thought to play an important role in orchestrating an adequate neuronal response by preventing uncoordinated discharge of the highly interconnected neurons of the brain (Chandler, 2006). Interneuronal activity usually results in GABAergic inhibitory postsynaptic impulses and a defect in any of the genes coding for functional neuroconductive proteins could therefore potentially lead to decreased inhibition. A commonly used group of anti-epileptic drugs are benzodiazepines, further strengthening the belief that GABAergic inhibition could play an essential role in the pathogenesis of epilepsy (Chandler, 2006).

Moreover, if dysfunctional ion channels lead to seizures then defects in the release machinery of synaptic vesicles may also cause disruption of normal conductivity and can therefore potentially lead to seizures. The large protein families that mediate vesicle trafficking and exocytosis have been shown to include several genes of epilepsy (Noebels, 2003).

Revolutionary has been the dogmatic switch in the belief of the brain as a fixed and immutable structure, to a more nuanced view of the brain as a fairly plastic, malleable organ. Structural brain

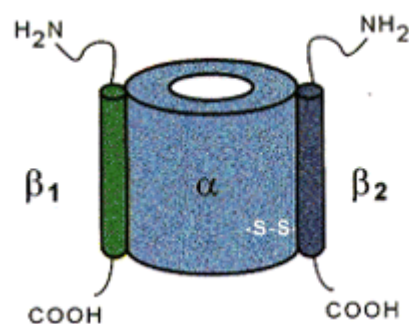
plasticity is based on neural activity, including adult neurogenesis and other morphological changes at the level of individual dendritic spines and synapses (Naegele, 2009). More recent research has focused on epigenetic mechanisms involved in epileptogenesis, both in idiopathic and acquired forms of epilepsy, which may possibly dilute the distinction between the two forms in the future.

## Voltage-gated channelopathies

After neuronal stimulation voltage-gated sodium ion channels in the plasma membrane of a neuron are activated and an action potential is produced, causing a shift in membrane potential which leads to synaptic release of neurotransmitters and –modulators. The release of these substances in turn leads to impulse propagation to neighbouring neurons and is the basis of neuronal communication.

### *Sodium channels*

Voltage gated sodium channels are responsible for initiation of the action potential in neurons. Additional inactivation gates within the pores of sodium channels close to stop ion flow. This produces a refractory period which prevents further voltage-induced sodium channel opening. The sodium channel consists of 4  $\alpha$ -subunits and 2 ancilliary  $\beta$ -subunits. The  $\alpha$ -subunit proteins form the pore of the sodium channel, the  $\beta$ -subunits interact and are multifunctional. A range of human monogenic epilepsies are the result of mutations in both  $\alpha$ - and  $\beta$ -subunits. Of the 13 genes coding for sodium channel components, mutations in 3 subunits (Nav1.1, 1.2,  $\beta$ 1) have been associated with epilepsy (Reid et al, 2009).



General 3-D structure of the voltage-gated sodium channel.

Source: Marban et al, 1998. For complete reference see Literature.

SCN1A encodes the  $\alpha$ 1 (Nav1.1)-subunit that forms a fast inactivating voltage-dependent sodium channel. Mutations in SCN1A cause the devastating Dravet syndrome and generalized epilepsy with febrile seizures plus (GEFS+) in humans (Reid et al, 2009). Gain-of-function mutations of sodium channel subunits result in several maladjusted actions: accelerated recovery from inactivation (Spampanato et al, 2001), a negative shift in the voltage-dependence of activation and inactivation (Spampanato et al, 2004a), less use-dependent decline in sodium channel amplitude (Cossette et al, 2003) and an increase in persistent sodium current leading to defects in membrane repolarization

(Spampanato et al, 2004b) which ultimately results in a reduction of voltage required to fire action potentials. As mentioned earlier, mutations resulting in hyperexcitability of neurons are predicted to lead to seizures. Paradoxically, loss-of-function mutations resulting in a decrease in excitability have also been reported in epileptic syndromes. Heterozygous SCNA1 knock-out mice developed spontaneous seizures (Yu et al, 2006). In these mice, it turned out sodium currents were essentially unchanged in hippocampal excitatory pyramidal neurons, but the current was reduced in inhibitory interneurons. Dysfunctional inhibitory interneurons have been postulated to cause neuronal hyperexcitability. Apparently, the ability of specific functional and topographic neuron clusters to compensate for loss of the Nav1.1-subunit varies between cell types (Noebels, 2003).

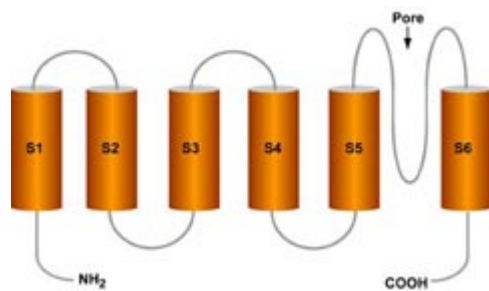
SCN2A, coding for Nav1.2 is also associated with epilepsy in human (GEFS+) and mouse models (temporal lobe epilepsy) and is reported to slow the inactivation of the channel and augment sodium

current like mutations in SCN1A do (Sugawara et al, 2001). Recently, a modifying gene was found in transgenic mouse model *Scn2a(Q54)* on mouse chromosome 19, the candidate gene being *Kcnv2*, a voltage-gated potassium channel subunit. Modifier genes affecting the epilepsy phenotype of this mouse may contribute to the variable expressivity and penetrance of epilepsy patients with sodium channel mutations (Bergren et al. 2009).

Furthermore, mutations in SCN1B have been described. In human and mice SCN1B encodes the ancillary  $\beta 1$  subunit which interacts with both Nav1.1 and Nav1.2 subunits.  $\beta 1$  subunits are multifunctional and modulate channel gating by regulating the level of channel expression and potentially act as a cell adhesion molecule. A C121W mutation in SCN1B causes GEFS+ in human (Wallace et al, 1998). The mutation causes a reduced rate of inactivation of  $\beta$ -associated  $\alpha$  subunits, leading to increased persistent sodium current and thus a lower threshold for action potential firing.

### Potassium channels

Potassium channel subunit proteins are encoded in a fivefold larger number of genes than the sodium channel, with over 70 subunits discovered to date (Reid et al, 2009). Genes are distributed



Voltage-gated potassium channel alpha-subunit. Four alpha subunits form the pore of the channel.

Source: Hoy, 2006. For complete reference see literature

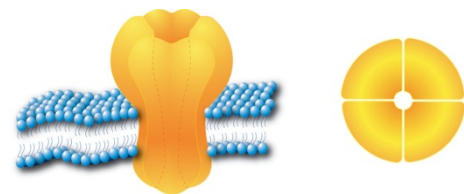
into subfamilies depending on the number of transmembrane domains in each subunit and epilepsy-causing mutations have been described in several of these subfamilies. The diverse distribution and combinations of subunit proteins makes potassium channelopathy categorization far more complex. (Noebels, 2003).

Mutations of subunits can lead to altered assembly, targeting, and kinetics of the channel but non-epileptogenic phenotypes have also been described (Noebels, 2003). Reduction of potassium current leads to a relative depolarized state of the neurons involved.

However, overexpression of the Kv1.1 channel, encoded by KCNA1 in mice and man, has also led to an epileptic

phenotype, possible due to coordinate changes in the expression of other subunit family members (Sutherland et al, 1999).

KCNO2 and KCNO3 encode voltage-activated potassium channels in human and transgenic mice. These channels produce a M-current, which is a non-inactivating voltage-gated potassium current that is open during prolonged depolarization and mediates the medium after-hyperpolarisation conductance (Reid et al, 2009).



3-D structure of the Shaker potassium channel  
Source: Hoy, 2006. For complete reference see literature

Mutations in KCNO2 and rarely in KCNO3 have led to a form of autosomal dominant epilepsy in human, benign familial neonatal seizures (BFNS) and over 50 mutations have been described (Reid et al, 2009 for references).

Activation of this so-called M-channel stabilizes membrane potential and thereby leads to a limited action potential firing. Loss-of-function mutations would therefore be expected to increase neuronal excitability. A study with transgenic mice showed a reduced medium after-polarisation in a group of hippocampal pyramidal neurons of these mice, resulting in reduced spike-frequency adaptation seen

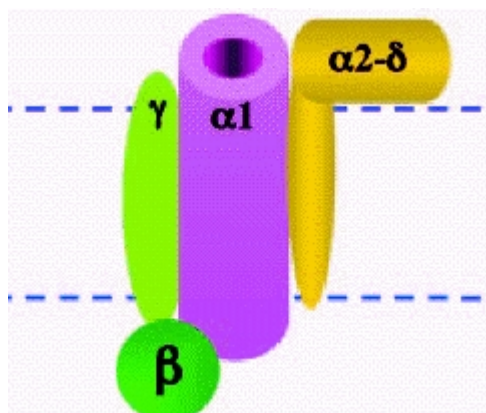
on electro-encephalography (EEG) which may explain the spontaneous seizures in this animal model (Peters et al, 2005). The specific regional localization of these mutated potassium channels in excitatory pyramidal neurons in the middle layers of neocortex and hippocampal formation may be related to a lack of expression of KCNQ1 and KCNQ4 in these regions, which can compensate for loss of function of KCNQ2 and 3 in other regions of the brain (Saganich et al, 2001).

Another potassium channel gene linked to epilepsy is KCNAB2, which encodes for a major regulatory subunit of the Shaker-type channel, a voltage-gated potassium channel type. A mutation in this gene contributes to mental retardation and seizures (Heilstedt et al, 2002), as loss of the  $\beta$ 2 subunit prolongs membrane repolarisation (McCormack et al, 2002). The gene encoding a GABA  $\delta$  subunit lies close to the KCNAB2 gene and it is suggested cooperation of the two genes may potentiate excitability (Noebels, 2003).

A gain-of-function mutation in KCNMA1, encoding the poreforming subunit of the BK channel (Kca1.1) was found in a family suffering of generalized epilepsy and paroxysmal dyskinesia (Du et al, 2005). The BK channel is a voltage-gated potassium channel that is gated by depolarization but also by intracellular calcium. The mutated BK channel was found to be five times more sensitive to calcium and hyperexcitability of neurons may be caused by a shorter repolarisation period leading to faster recovery thereby allowing a higher frequency of action potentials (Reid et al, 2009).

### Calcium channels

Voltage-gated calcium channels are separated into 2 categories: low voltage and high voltage-gated calcium channels. Electrophysiological studies indicate there are at least six calcium channel types: L-,



General 3-D structure of the voltage-gated calcium channel.

Source: Snutch TP, 2005. For complete reference see Literature

N-, P-, Q-, R- and T-type (Catterall, 2000). Distinction is made upon their different  $\alpha$ 1 pore-forming subunit. Dysfunctional calcium channels are involved in epileptogenesis. Mutations, however, do not directly lower membrane potential threshold but control central synaptic neurotransmitter release and promote hypersynchronization by secondary changes in electrocircuit behavior (Noebels, 2003). Mutations in subunits of calcium channels are rare in human epilepsy and when present cause childhood absence epilepsy (CAE). Two potential mutations in the human ortholog of the lethargic gene in the mouse, CACNB4, have been found in association with human epilepsy (Gardener, 2008). Over 30 mutations of a specific T-type channel

(Cav3.2 subunit) are known in humans and the mutated gene involved is CACNA1H, resulting in idiopathic generalized epilepsy (IGE) (Reid et al, 2009 for references). Other neurological diseases associated with mutations in calcium channels, more specifically CACNA1A, are familial hemiplegic migraine (FHM1), episodic ataxia type 2 (EA2), spinocerebellar ataxia type 6 (SCA6) (Gardener, 2008). Dysfunction of calcium channels and their effect on the central nervous system have been widely studied in spontaneous mouse models and will be discussed later in the section about animal models of epilepsy.

## Chloride channels

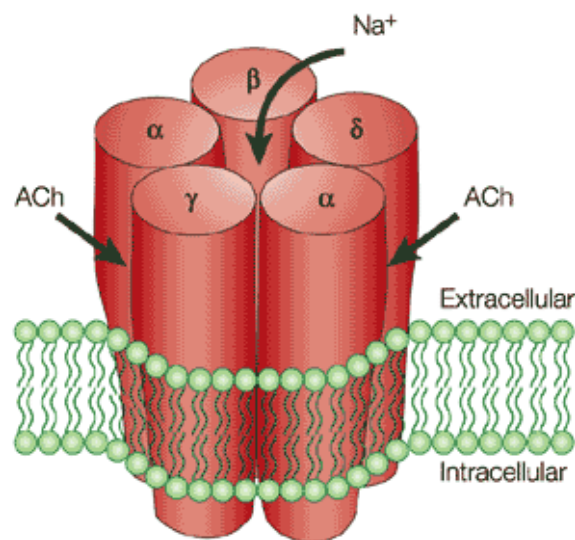
Chloride channels are involved in a broad range of functions, such as stabilization of the membrane potential in neurons, synaptic inhibition, cell volume regulation, transepithelial transport, extracellular and vesicular acidification, and endocytic trafficking (Jentsch et al, 2002). CLCN encodes a chloride channel (CLC-2), which is gated by hyperpolarization. Mutations in CLCN have been described in patients with common forms of idiopathic generalized epilepsy. Mutations lead to a premature stop codon, an atypical splicing event, and a non-synonymous amino acid change (Gardener, 2008). The role CLC-2 within the central nervous system is still unclear. Haploinsufficiency results in a reduced number of active channels. Loss-of-function, either through haploinsufficiency of dominant negative effects, may increase hyperexcitability by reducing the chloride gradient that is essential for GABA inhibition. However, CLC-2 knock-out mice have not shown a reduced threshold of seizures or spontaneous seizures (Blanz et al, 2007).

## Ligand-gated channelopathies

Mutations of ligand-gated channelopathies have also been described in the pathogenesis of epilepsy. Ligand-gated channels are permeable to specific ions, depending on their role in neurocommunication.

### Nicotinic acetylcholine receptors

Nicotinic acetylcholine receptors (nAChR) are permeable to sodium, potassium and calcium and are widely distributed in the central nerve system. One of the major roles of these receptors is to modulate neurotransmitter release, including glutamate and GABA. A total of 17 subunits have been



General 3-D structure of the nACh receptor.

Source: Karin A, 2002. For complete reference see Literature

found, with 10  $\alpha$ -, 4  $\beta$ -,  $\gamma$ ,  $\delta$ - and  $\epsilon$ -subunits (Kalamida et al, 2007). Mutations in  $\alpha$ - and  $\beta$ -subunits have been described for epilepsy (Reid et al, 2009).

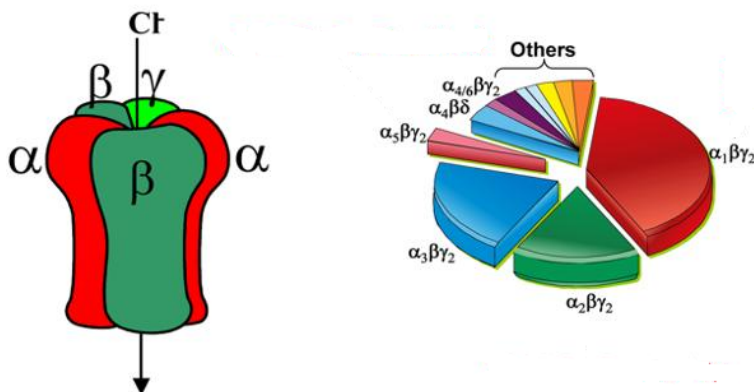
In humans a clinical partial myoclonic epilepsy syndrome, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), is caused by mutations in *CHRNA4* and *CHRNA2*, encoding the  $\alpha$ 4- and  $\beta$ 2-subunit of the nACh-receptor (Reid et al, 2009, for references). These two subunits combine to form the most abundant isoform of nAChR in the brain, which are found pre- and postsynaptically on pyramidal neurons and interneurons. A gain-of-function has been suggested for mutations in *CHRNA4* and *CHRNA2*, resulting in a higher sensitivity of the nAChR to acetylcholine. This leads to an increase of calcium influx presynaptically and consequently

an increase in neurotransmitter release (Reid et al, 1998). Another mechanism has been proposed that suggests that an impaired calcium-mediated modulation occurs, most prominently in excitatory synapses (Rodrigues-Pinguet et al, 2005). A knock-in mouse model has shown an over 20 fold

increase in nicotine-evoked synaptic release exclusively at inhibitory synapses on to groups of pyramidal neurons in the cortex. A model proposes enhanced GABA<sub>A</sub>-inhibition (hyper)synchronizes layer II /III pyramidal cells as a result of cholinergic activation of mutant nAChRs (Klaassen et al, 2006), which shows that enhanced inhibition can also synchronize neurons to become epileptogenic. This paradoxical concept is further discussed by Mann et al (2008).

### GABA receptors

The GABA receptor is the predominant inhibitory neurotransmitter of the central nervous system. Three types of GABA receptors exist, the ionotropic GABA<sub>A</sub> and GABA<sub>C</sub> receptor and the metabotropic GABA<sub>B</sub> receptor. GABA receptors are ligand-gated chloride channels and activation has an extra- and postsynaptic hyperpolarizing action, depending on the GABA receptor type being activated. The chloride permeant pore is formed from various combinations of proteins formed of combinations of  $\alpha$ 1-6,  $\beta$ 1-3,  $\gamma$ 1-3,  $\delta$ -,  $\epsilon$ -,  $\pi$ -,  $\theta$  and  $\rho$ 1-3 subunits. There is remarkable receptor heterogeneity, with subtype combinations varying in different brain regions and cell types, and during different times in ontogeny (Brooks-Kayal et al, 2009). The most common GABA<sub>A</sub> receptor is a  $\alpha$ 1  $\beta$ 2  $\gamma$ 2 complex (Reid et al, 2009).



General 3-D structure of the GABA receptor and the relative distribution of the different GABA receptor types within the mammalian body.

Source: Belelli D. See Literature for complete reference

The alpha and gamma subunits are most frequently implicated to the epileptic phenotype. Mutations in GABG2, encoding the  $\gamma$ 2 subunit, cause GEFS+, Dravet syndrome, CAE and febrile seizures in humans (Reid et al, 2009). A mutation in GABRA1, encoding the  $\alpha$ 1-subunit, causes a rare dominant form of a fairly common epilepsy syndrome in adolescents: juvenile myoclonic epilepsy (JME) (Cossette et al, 2002). The R43Q mutation of the  $\gamma$ 2 subunit results in altered

benzodiazepine sensitivity, receptor kinetics, and the assembly, trafficking and cell surface expression of GABA receptors (Reid et al, 2009, for references). These changes result in a reduction of GABA<sub>A</sub> receptor-mediated chloride current and thus lead to hyperexcitability. A knock-in mouse model carrying the R43Q mutation showed absence seizures as in human patients but also showed evidence of developmental changes that can alter neuronal network excitability (Chiu et al, 2008), proposing another epileptogenic mechanism for GABA receptor mutations. The K289 mutation of the same  $\gamma$ 2 subunit is associated with childhood generalized tonic clonic epilepsy with febrile seizures (Baulac et al, 2001). This mutation alters deactivation kinetics of the GABA<sub>A</sub> receptor (Bianchi et al, 2002) and leads to a reduction in receptor surface expression by retenting the nonfunctional subunit within the endoplasmic reticulum (Harkin et al, 2002)). A A322D mutation of the gene encoding the  $\alpha$ 1 subunit, GABRA1, results in loss-of-function of the receptor through relative desensitvity to GABA, accelerated deactivation, reduction in cell surface expression (Krampfl et al, 2005) most likely the result of rapid endoplasmic reticulum, associated degradation (Gallagher et al, 2007).

Human patients that harbor a  $\gamma 2$  subunit mutation usually have epilepsy triggered by fever (Reid et al, 2009). This subunit is responsible for receptor trafficking which led to the theory that temperature may affect surface expression of GABA<sub>A</sub> receptors carrying the mutated  $\gamma 2$  subunit (Kang et al, 2006). Experiments have shown that increasing temperature selectively decreases GABA<sub>A</sub> receptor expression in vivo, a mechanism that may be responsible for the onset of seizures. It is, however, uncertain whether this can be regarded as a general mechanism for febrile seizures (Berkovic and Petrou, 2006)). Prolonged seizures, as in status epilepticus, result in alterations in the expression and membrane localization of several GABA<sub>A</sub> receptor subunits ( $\alpha 1$ ,  $\alpha 4$ ,  $\gamma 2$ , and  $\delta$ ) in hippocampal dentate granule neurons of animal models (Brooks-Kayal et al, 2009) and this mechanism may possibly be responsible for seizure-induced aggravation of temporal lobe epilepsy in humans. Variation in developmental onset of seizures in GABA mutations may be caused by the fact that GABA has a depolarizing action in early brain development (Noebels, 2003). Deletion of chloride transporter KCC2, which mediates the conversion of GABAergic transmission from excitatory to inhibitory, also produces an epileptic phenotype (Woo et al, 2002).

### *Glutamate receptors*

Although activity-dependent plasticity of glutamate receptors is well recorded and an essentially inescapable feature of epileptic brain once seizures begin, evidence for primary genetic mutations in this pathway remains scant. Receptor deletion mutants are less viable and only a few mutations have been linked to epileptogenesis by gene targeting studies (Noebels, 2003).

Mutation of the GluRB subunit of the AMPA-sensitive, calcium-impermeable glutamate receptor permits formation of AMPA receptors that miss a critical pore-lining site that confers calcium impermeability and a three-fold attenuation of single-channel conductance. Mice that bear this mutation have AMPA receptors with high calcium permeability and as a result have severe seizures and cell death (Brusa et al, 1995).

Impaired glial uptake of glutamate in targeted EAAT2 glutamate transporter deficient mice leads to excitotoxicity and seizures (Tanaka et al, 1997). Mice deleted for neuronally expressed EAAT1 show seizures, possible as a result of insufficient glutamate available for GABA synthesis (Sepkuty et al, 2002).

### *Serotonin receptors*

Activation of the 5HT<sub>2c</sub> receptor in cortical neurons reduces both the rapidly inactivating and persistent sodium channel currents through a protein kinase C (PKC)-dependent mechanism, lowering neuronal excitability (Carr et al, 2002). Loss of inhibition of these currents may enhance excitability and targeted deletion of the serotonin 5HT<sub>2c</sub> receptor gene is associated with seizures and a reduced threshold for audiogenic seizures (Noebels, 2003).

**Table 2** Relevant genes in epilepsies associated with minimal or no structural brain abnormalities

Type	Gene	References
Juvenile myoclonic epilepsy	Linkage to <i>GABRD</i>	Dibbens et al. (2004), Feng et al. (2006)
	Linkage to <i>BRD2</i> (RING3)	Cavalleri et al. (2007), Durner et al. (1991), Greenberg et al. (1988), Pal et al. (2003), Sander et al. (1997)
	Mutations in <i>GABRA1</i>	Cossette et al. (2002)
	Mutations in <i>CLCN2</i>	Haug et al. (2003)
	Mutations in <i>EFHC1</i>	Suzuki et al. (2004)
Childhood absence epilepsy	Mutations in <i>CACNB4</i>	Escayg et al. (2000)
	Linkage to: <i>GABRA5</i> , <i>GABRB3</i> , <i>GABRG3</i>	Robinson et al. (2002)
	Linkage to <i>CACNG3</i>	Everett et al. (2007b); Robinson et al. (2002)
	Linkage to 8q24	Fong et al. (1998)
	Mutations in <i>GABRG2</i>	Wallace et al. (2001)
	Mutations in <i>GABRA1</i>	Maljevic et al. (2006)
	Mutations in <i>CACNA1H</i>	Chen et al. (2003)
Genetic epilepsy with febrile seizures plus	Mutations in <i>SCN1B</i>	Audenaert et al. (2003)
	Mutations in <i>CLCN2</i>	Combi et al. (2009), Everett et al. (2007a)
	Mutations in <i>SCN1B</i>	Wallace et al. (1998)
	Mutations in <i>SCN1A</i>	Baulac et al. (1999), Moulard et al. (1999)
	Mutations in <i>GABRG2</i>	Baulac et al. (2001)
	Mutations in <i>GABRD</i>	Dibbens et al. (2004)
Severe myoclonic epilepsy of infancy and severe myoclonic epilepsy borderland	Mutations in <i>SCN1A</i>	Claes et al. (2001)
	Mutations in <i>SCN2A</i> (later onset and no febrile seizures)	Kamiya et al. (2004)
	Mutations in <i>GABRG2</i>	Harkin et al. (2002)
Temporal lobe epilepsy (mesial)	Linkage to <i>IL-1<math>\beta</math></i>	Kanemoto et al. (2000)
	Failure to replicate linkage to <i>IL-1<math>\beta</math></i>	Buono et al. (2001), Cavalleri et al. (2005), Heils et al. (2000), Jin et al. (2003)
	Linkage to <i>APOE</i>	Briellmann et al. (2000)
	Linkage to <i>PDYN</i>	Stogmann et al. (2002)
	Linkage to <i>GABBR1</i>	Gambardella et al. (2003)
	Linkage to <i>PRNP</i>	Walz et al. (2003)
	Failure to replicate linkage to <i>APOE</i> , <i>PDYN</i> , <i>GABBR1</i> and <i>PRNP</i>	Cavalleri et al. (2005)
	Mutation in <i>SCN1B</i>	Scheffer et al. (2007)
	Mutations in <i>LGII</i>	Otman et al. (1995), Pizzuti et al. (2003)
	Autosomal dominant temporal lobe epilepsy with auditory features	Mutations in <i>CHRNA4</i>
Mutations in <i>CHRN2</i>		Phillips et al. (2001)
Mutations in <i>CRH</i>		Combi et al. (2005)
Mutations in <i>CHRNA2</i>		Aridon et al. (2006)
Familial partial epilepsy with variable foci	Linkage to 2q	Scheffer et al. (1998)
	Linkage to 22q	Callenbach et al. (2003)

Source: Andrade, DM 2009. See Literature for complete reference.

## Epigenetic mechanisms of epilepsy

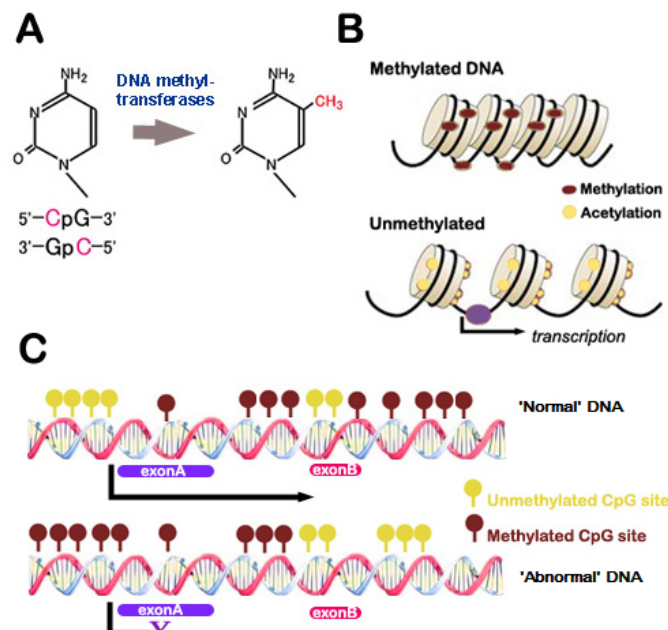
Many of the mutations described so far relate to the direct onset of seizures, but can only partially account for the variation in (syndromal) epileptic phenotype seen between and within epilepsy syndromes. Furthermore, they fail to explain the development of epilepsy in acquired diseases such as trauma, CNS infections, hypoxic-ischemic and metabolic disorders, tumors, and vascular abnormalities.

Complementary studies have begun to elucidate the molecular and cellular bases for epileptogenesis, which is associated with complex temporal and spatial abnormalities of neural network structure and activity mediated by activation of immediate early genes (IEGs), posttranslational modification of proteins, and other alterations in profiles of gene expression and function (Qureshi et al. 2010). These abnormalities can eventually lead to deregulated neural circuits and predispose to neuronal hypersynchronicity. Epigenetics, the study of inherited changes in phenotype or gene expression by molecular mechanisms other than changes in underlying DNA sequence (Bird, 2007), has received increasing attention from the scientific community and a wide array of clinical syndromes, including neurodevelopmental, neuropsychiatric, and neurodegenerative disorders as well as cancer, which are also comorbidities in many epileptic syndromes (Mehler, 2008), are believed to be caused by epigenetic regulatory networks. These networks play an essential role in modulating aspects of cellular and organismal development, homeostasis, ageing

and even transgenerational inheritance (Mehler, 2008).

### DNA methylation

DNA methylation is an epigenetic mechanisms involved in regulating global and local gene transcription, maintaining genomic integrity, facilitating X-chromosome inactivation (Barr Body) and mediating genomic imprinting (Robertson, 2005).



**A.** Methylation by DNA methyltransferases at CpG islands. **B.** DNA demethylation relaxes chromatin structure allowing histone acetylation and the binding of transcriptional complexes. **C.** abnormal cells are characterised by hypermethylation of CpG islands and general DNA hypomethylation.

Source: Taylor SM, 2006. For complete reference see Literature

DNA methylation is catalyzed by DNA methyltransferases (DNMTs) that transfer methyl groups from S-adenosylmethionine (SAM) to cytosine residues which results in the formation of 5-methylcytosine, primarily located at CpG dinucleotide-containing regulatory sequences (Robertson, 2005). Members of DNMT promote *de novo* methylation and maintain methylation marks.

Methylation effects include inhibition of gene transcription and indirectly transcriptional silencing mediated by methyl-CpG-binding domain proteins (MBDs). MBDs are DNA binding proteins that bind to methylated loci, recruit additional epigenetic regulatory factors and in this way modulate local and long-range chromatin structural and functional dynamics (Qureshi et al. 2010). Expression of DNMTs and MBDs occur throughout life, regulating neural cell fate specification, maturation and survival and

regulate activity-dependent synaptic plasticity. All of these processes are implicated in epileptogenesis (Sharma et al., 2008).

Rett syndrome, an X-linked autism spectrum disorder that is associated with severe epilepsy is caused by a mutation in MeCP2, a member of the MBDs, resulting in aberrant expression and function of this protein. MeCP2 associated with other protein complexes which leads to global transcriptional activation through interaction with transcriptional activator CREB, and selective regulation of activity-dependent BDNF (Brain Derived Neurotrophic Factor) transcription. BDNF is a growth factor implicated in epileptogenesis. Aside from its importance in neuronal cell survival and plasticity (Huang et al., 2001), BDNF modulates excitatory and inhibitory synaptic transmission by inhibiting GABA<sub>A</sub>-receptor-mediated post-synaptic currents (Tanaka et al. 1997). MeCP2 also has functions that link DNA methylation with other epigenetic processes such as chromatin architecture and dynamics and posttranscriptional RNA processing. Hence, aberrant MeCP2 can contribute to epilepsy through malfunctional mediation of global genome organization as well as local epigenetic processes. Apart from this MeCP2 also regulates genes that are independently implicated in epilepsy and related comorbidities. For example, Rett syndrome animal models show deregulation of

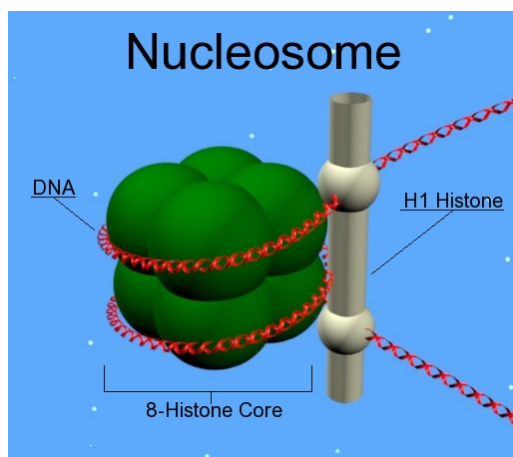
neurotransmitter biosynthesis and promotion of the differentiation and maturation of various neural cell types (Urdinguio et al. 2008).

Other epilepsy syndromes associated with deregulated DNA methylation are temporal lobe epilepsy, Prader-Willi and Angelman syndrome. Prader-Willi syndrome and Angelman syndrome are autism spectrum disorders caused by mutations in chromosome 15q11-13 of respectively the paternal and maternal chromosome. Genomic imprinting is responsible for the selective inactivation of the functional chromosome, leading to these syndromes. UBE3 (Ubiquitin Conjugating Enzyme 3) and GABA receptor subunits are encoded in this chromosome region and both protein structures are implicated in epileptogenesis (Hogart et al. 2007, Hogart et al. 2009).

In temporal lobe epilepsy (TLE), high levels of DNA methylation in the promoter region of neural factor Reelin (RELN) and decreased levels of RELN protein in hippocampal regions are associated with the pathogenesis of this disorder (Kobow et al. 2009). RELN is an extracellular matrix molecule that is essential in cell positioning, neuronal migration during brain development and maturation, and in synaptic plasticity during adulthood. In the hippocampus RELN is crucial for the proper migration and laminar patterning of granule cells and for the maintenance of integrity of the dentate gyrus in the adult phase. Decreased RELN protein concentration in the hippocampus are responsible for causing granule cell dispersion (GCD), a feature often found in temporal lobe epilepsy (Haas, et al. 2010). Dynamic alterations of DNA methylation are also found in animal models of epileptogenesis induction. Methylation of the BDNF promoter results in upregulation of BDNF levels (Aid et al. 2007) and inhibition of DNA methyltransferases in hippocampal neurons results in suppression of neuronal excitability and network activity (Nelson et al. 2008). This is in part because NMDA-receptor activation demethylates the BDNF promoter. Interesting is the involvement of MeCP2 in this process (Nelson et al. 2008), which once again suggests the importance of this MBD in epileptogenesis.

### *Chromatin architecture and dynamics*

Chromatin actively controls DNA accessibility, provides for local and long-range interaction between genes, gene clusters, and regulatory elements. It also compartmentalizes genomic and epigenetic



3-D structure of the nucleosome, the basic unit of chromatin.

Source: Hill M, 2008. For complete reference see Literature

factors and processes taking place in the nucleus (Qureshi et al. 2010). Chromatin is an important epigenetic mechanism as it promotes genomic programs like DNA replication, DNA repair and recombination, maintenance of genomic integrity, inactivation of particular regions in chromosomes or even entire chromosomes, and regulates gene expression. Like DNA methylation, chromatin is essential in nervous system development, homeostasis and plasticity (Mehler, 2008; Urdinguio et al., 2009). The basic unit of chromatin is the nucleosome, which is constructed of an octamer of histone proteins. This structure is subject to modulation by integrated epigenetic mechanisms that include histone modifications, nucleosome repositioning and remodeling. Histone-modifying enzymes acetylate,

methylate, phosphorylate, biotinylate, ubiquitylate, SUMOylate, and ADP-ribosylate and each of these modifications may have specific functional consequences.

Nucleosome repositioning enzymes promote the movement and reorganization of gene regulatory regions and more widespread chromatin remodeling in eu- or heterochromatin, the former being actively transcribed and the latter being transcriptionally inactive but playing a part in the regulation of developmental processes and maintenance of genomic integrity (Kouzarides, 2007).

A number of mutations in genes responsible for chromatin modification and remodeling have been implicated in epileptogenesis. The ATRX enzyme is a chromatin-remodeling enzyme that interacts with a number of other epigenetic factors, including MeCP2, leading to heterochromatin formation, DNA repair, and chromosome segregation. Impaired ATRX results in alpha thalassemia mental retardation associated with epilepsy in approximately 30% of patients. The underlying mechanism may include disturbances in inhibitory interneuron survival and differentiation and can therefore lead to an imbalance between excitation and inhibition of neural networks (Medina et al., 2009). KDM5C is a histone demethylase enzyme that also plays a role in chromatin remodeling and gene regulation. Mutations in KDM5C are also associated with epilepsy and mental retardation (Abidi et al., 2008). KDM5C interacts with the master epigenetic regulator repressor element-1 silencing transcription factor/neuronal restrictive silencer factor (REST/NRSF) (Tahiliani et al., 2007). REST is a molecular platform to which many factors may be recruited to participate in epigenetic remodeling and modification. REST is responsible for regulation of many factors involved in epileptogenesis, such as growth factors, ion channels, neurotransmitter receptors, gap junctions, neurosecretory vesicles and neural development and adult neurogenesis (Qureshi and Mehler, 2009). Interestingly, REST target genes include *Cacnb4*, *Cacng3* encoding calcium channel subunits; *Chrn2*, which encodes an acetylcholine receptor subunit; *Clcn2*, encoding a chloride channel protein; and *Gabra1*, *Gabra2*, encoding GABA-receptor subunits (Abrajano et al., 2009).

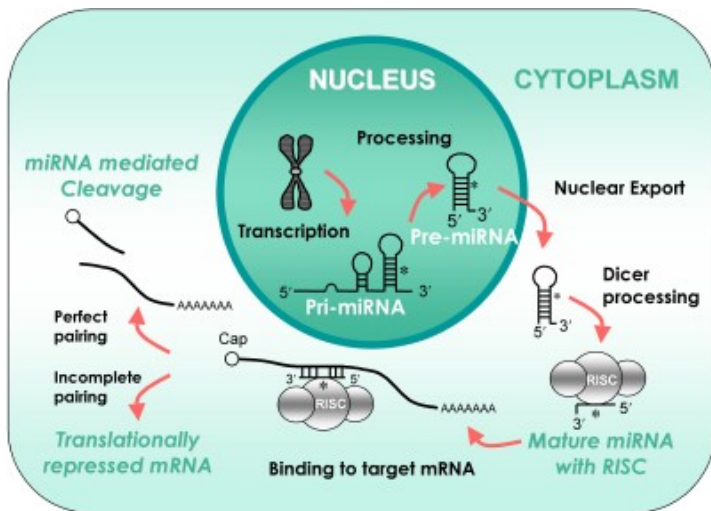
Also associated with histone modification dysfunction leading to epilepsy is biotin deficiency (Hassan and Zemleni, 2006). Besides being an essential metabolic co-enzyme, chromatin regulation relies in part on histone biotinylation and deficiency of biotin is associated with epilepsy, and also hypotonia, ataxia, mental retardation and fetal malformations (Zemleni et al., 2008).

Indirect evidence also shows that chromatin regulatory factors are relevant to epileptogenesis. Chromatin alterations are found in animal models of epileptogenesis, such as drug- and electroconvulsive seizure-induced epilepsies. SKF82958, a dopaminergic receptor agonist, pilocarpine, a muscarinic acetylcholine receptor agonist, and kainic acid, a glutamate receptor agonist, induce histone modifying chromatin remodeling in hippocampal neurons (Crosio et al., 2003). These modifications are linked to mitogen-activated protein kinase (MAPK) pathways and immediate early gene transcription, which are mechanisms that mediate epileptogenesis. Kainic acid exposure leads to epigenetic regulation of GluR2 (a glutamate receptor) and results in mRNA downregulation mediated by REST (Jia et al., 2006).

Other studies of electroconvulsive-seizure induced animal models of epilepsy show that the CREB gene promoter is target to selective histone modifications (Tsankova et al., 2004). CREB is a transcriptional activator that is postulated to modulate many cellular processes that include the differential expression of GABA<sub>A</sub> receptor subunits in the hippocampus, which suggests that epigenetic modulation is important in modulation of transcription of key neurotransmitters and consequently for the balance between excitation and inhibition of neurons in the brain (Qureshi et al. 2010).

## Regulatory noncoding RNAs

In the eukaryote genome a separate group of non-protein-coding RNAs (ncRNAs) exists that act to couple nuclear and cytoplasmic processes and form functional networks with a broad range of



Schematic overview of cellular functions of miRNAs. RISC: RNA-induced silencing complex (RISC) where the miRNA and its mRNA target interact. Source: Vertis biotechnology AS. For complete reference see Literature.

effects on DNA methylation, chromatin architecture, transcriptional regulation, posttranscriptional RNA processing and translation (Mattick et al., 2009). Two types of ncRNAs are implicated in epileptogenesis. MicroRNAs (miRNAs) act as environmental biosensors and play key roles in neural differentiation, maintenance and plasticity (Schratt, 2009). A single miRNA can target large numbers of mRNAs and regulate them through different sequence-specific interactions. miRNAs bind to mRNAs repressing translation of these transcripts or sequestering them for storage or degradation (Qureshi et al., 2010).

Fragile X mental retardation protein (FMRP) is a factor associated with the miRNA pathway and is encoded by the FMR1 gene, which is mutated in Fragile X syndrome (FXS), a mental retardation syndrome associated with epilepsy in approximately 20% of patients. FMRP plays a key role in modulating synaptic function and plasticity. In Fragile X syndrome, GABAergic and glutaminergic synaptic dysfunctions at mRNA and protein level underlie neuronal hyperexcitability causing epilepsy (Hagerman and Stafstrom, 2009). An epileptogenic factor, AP3M2, revealed sequence variation of which one of these SNPs associated with generalized epilepsy and GEFS+ in humans was located in a binding site for miR-422a, suggesting that this SNP may disrupt the functional relationship between certain miRNA pathways and the AP3M2 mRNA which can lead to epilepsy (Huang et al., 2007). miRNA expression is also dynamically modulated in animal models of epileptogenesis (Liu et al., 2010). Kainic acid seizure induction elevated certain miRNA levels in blood en brain while downregulating other types of miRNA. Many of these miRNAs are implicated in pathways of cell death, development, cell cycle, cell morphology and gene expression (Liu et al., 2010). These miRNAs may serve as novel clinical biomarkers and candidates for epilepsy therapies (Qureshi et al., 2010). Long ncRNAs (lncRNAs) are implicated in the regulation of chromatin remodeling, transcription, and posttranscriptional RNA processing (Mercer et al., 2009). Evf2 is a lncRNA that may play a role in epileptogenesis. It is important for transcriptional regulation of DLX5 and DLX6, transcription factors that are responsible for the development of subtypes of GABAergic neurons in the forebrain (Bond et al., 2009). Evf2 mouse mutants show reduced numbers of GABAergic interneurons in the hippocampus and dentate gyrus leading to reduced synaptic inhibition (Bond et al., 2009).

## RNA editing

RNA editing is an epigenetic mechanism that is responsible for RNA post-transcriptional processing (Mehler and Mattick, 2007). It modifies nucleotides in RNA molecules, promoting molecular diversity

and environmental plasticity in transcripts. By editing mRNA transcripts amino acid coding potential can change and hence protein function. Different editing events occur. A to I editing, converting adenosine to inosine, is catalyzed by adenosine deaminases (ADARs). These enzymes are highly environmentally responsible and they edit transcripts involved in critical neuronal processes. A to I editing is described in mRNA that encode ion channels and neurotransmitter receptors (Qureshi et al., 2010). The editing process is a mechanism for fine-tuning gating, permeability, kinetics, intracellular trafficking, and assembly of these factors. The majority of RNA editing occurs in untranslated areas of protein-coding transcripts and in ncRNA transcripts. Abnormalities in RNA editing are associated with a range of CNS disorders that include amyotrophic lateral sclerosis, schizophrenia, stroke, and depression and abnormal profiles of RNA editing have been implicated in human and animal epilepsy as well (Mehler, 2008). For example, A to I editing for AMPA receptor subunit GluR2 is significantly increased in hippocampal tissue of temporal lobe epilepsy patients This results in increased permeability of these glutamate receptors for calcium, leading to excitotoxicity and severe neuronal dysfunction (Vollmar et al., 2004). Transgenic mice with aberrant GluR2 mRNA editing profiles have glutamate receptors with abnormal calcium permeability and these mice suffer from fatal epilepsy (Brusa et al., 1995). Knock-out mice that lack ADAR2 suffer from severe epilepsy because of underediting of an GluR site (Higuschi et al., 2000).

## **ANIMAL MODELS OF EPILEPTIC DISORDERS**

Animal models for seizures and epilepsy have played a fundamental role in our understanding of physiological and behavioral changes associated with epilepsy in human and have stood at the basis of the development of antiepileptic treatment strategies and antiepileptic drugs (AED) that are still prescribed today.

In vivo animal models have been categorized into models of seizures and those of epilepsy (Sarkisian, 2001). Epilepsy is defined as multiple spontaneously recurring seizures and induction of seizures alone is not sufficient to be considered a model for epilepsy (Engel, 1992). Following these criteria, species that are used as animal models in epilepsy research are the rat, mouse, dog, cat, chicken, rabbit and baboon (Sarkisian, 2001). More recently, fruit flies, worms and zebrafish have been added to the category (Baraban, 2007). However, independent of the type of model being used the questions regarding epilepsy remain the same. The questions relate to causes of cellular hypexcitability, synchronous neuronal discharge and rhythmic activity, alterations from transition from interictal to ictal state, mechanisms and side effects of AEDs, and behavioral disruptions caused by or associated with seizures (Mody and Schwartzkroin, 1997).

Most, if not all causative epilepsy genes in man have their counterparts in transgenic or knock-out mice. However, even before such technology became prominent spontaneously mutated and then inbred rodent strains with epileptic phenotype or seizure susceptibility were available for research. The next section elaborates on spontaneous rodent and dog models of epilepsy. Knock-out mouse models will not be separately discussed. For a complete overview of genes associated with seizures in mutant mice see table 2.

## Mice

A number of spontaneous mouse mutants have mutations in the different subunits:  $\alpha 1a$  (*Cav2.1*, *tottering*, *leaner*, *rocker*, *rolling-nagoya*),  $\beta 4$  (*lethargic*),  $\alpha 2\delta$  (*ducky*), and  $\gamma 2$  (*stargazer*, *waggler*) (Reid et al, 2009). These mouse models all produce generalized absence seizures and are seizures are effectively blocked with ethosuximide, a pharmaceutical that reduces sodium current in non-inactivating sodium channels (Gomora JC et al, 2001).

Spatial and temporal effects typify the plastic character of calcium channelopathy (Noebels, 2003).

Most central synapses in the brain rely on both N- and P/Q-type calcium channels.

The spontaneous mutant mouse model, the *tottering* (*tg*) mouse, has a mutation of the *Cav2.1* channel, a P/Q type calcium channel, that reduces calcium current owing to defective membrane targeting and/or kinetics (Noebels, 2003). Blockade of N-type channels in this animal model leads to impaired neurotransmitter release at the CA3-CA1 synapse in the hippocampus, which primarily consists of pyramidal cells (Qian et al, 2000). Other parts of the brain are not affected suggesting a role for N-type channels in rescuing neurotransmitter release in these synapses (Noebels, 2003).

Similarly, rescue of the mutated  $\beta 4$  subunit in the *lethargic* mouse mutant is region-dependent where unaffected regions of the brain are able to reshuffle and express  $\beta 1$  and  $\beta 3$ . These subunits are responsible for the rescue of adequate physiological interaction with two sites on both N- and P-type  $\alpha 1$  subunits, who normally interact with the  $\beta 4$  subunit (Burgess et al, 1999).

Mouse model *stargazer* (*stg*) has a mutation in *CACNG2*, encoding a transmembrane  $\gamma 2$  subunit.  $\gamma 2$  subunits interact with calcium channel  $\alpha 1$  units (*Cav3.1*) and loss of  $\gamma 2$  leads to a seizure phenotype in this mutant mouse due to changes of both high- and low-voltage calcium current (Letts et al, 1998). One function of the  $\gamma 2$  subunit is related to its intracellular PDZ binding domain, a structural domain of 80-90 amino-acids involved in intracellular signaling. The PDZ binding domain allows interaction with PSD proteins that have a scaffolding function at excitatory synapses and regulate the glutaminergic AMPA receptor surface expression (Steinlein, 2008).

Temporal effects of seizure onset during development are believed to be caused by a conversion of N- and P-type dependence in the thalamus to pure P-type expression in this area vulnerable to epileptic activity (Noebels, 2003)

Furthermore, mutated high voltage-activated calcium channel in *tottering* (*CACN1A*), *lethargic* (*CACNB4*) and *stargazer* (*CACNG2*) cause a downstream effect on low voltage-activated currents in postsynaptic T-type calcium channels, which are preferentially located in neuronal dendrites where they define neuronal excitability by supporting burst firing and boosting of synaptic inputs. Increase in calcium current of these T-type channels causes hyperexcitability, especially within the thalamus where this type of channel plays an important regulating neuronal excitability (Zhang et al, 2002).

None of the mutated subunits directly interact with the  $\alpha 1$  subunits that form T-type channels, suggesting a complex substrate for epileptogenesis in these models (Noebels, 2003).

Other spontaneous mouse models are the *mocha2j* (*mh2j*) mouse and the slow-wave epilepsy (SWE) mouse (Gardener, 2008). The *mocha2j* mouse is a mouse model for the Hermansky-Pudlak syndrome, a spike-wave epilepsy syndrome with tonic-clonic seizures. This mouse model exhibits a neurological phenotype that includes hyperactivity, an epileptiform EEG and changes in the basic function of the hippocampus. The *mh2j* gene encodes an adaptin, the AP3 $\delta$  subunit of a complex involved in sortin molecules destined for synaptic vesicles. In *mocha* brain, as a result of dysfunctional AP3 $\delta$  the ZnT-3 transporter is reduced resulting in absence of vesicular zinc

sequestration (Kantheti et al, 2003). Zinc may play a role as a messenger involved in excitatory synaptic transmission and plasticity, but the exact mechanism is unclear.

The slow-wave epilepsy mouse has a mutation in the *slc9a1* gene encoding NHE1, a gene involved in sodium-hydrogen exchange in neurons (Cox et al, 1997). The maintenance of pH homeostasis in the CNS is of key importance for proper execution and regulation of neurotransmission.

### Rats

A methodology to introduce mutations into the rat genome did not exist until recently (Voigt et al, 2009) and therefore up until now this animal has not lent itself so well for research into epileptogenesis as has the mouse. Two rat models have been investigated for many years: the genetic absence epilepsy rat from Strassbourg (GAERS) and the Wistar Albino Glaxo rat (WAG/Rij). Both strains exhibit spike wave discharges (SWDs) in corticothalamic circuits and respond to the commonly used anti-epileptic drugs used in human medicine (Gardener, 2008). In contrast to the many mouse models for epilepsy, these rats show a complex inheritance pattern and quantitative loci have been localized associated with the epileptic phenotype. In the GAERS strain, 3 quantitative trait loci (QTLs) have been localized on rat chromosome 4, 7 and 8. These QTLs influence specific SWD characteristics. Comparative genetics have linked these QTLs to regions in mouse chromosome 9 and several ion channels map to these GAERS QTLs, including *Cacng2* (the Stargazin gene), *Kcnj4* (a potassium channel), and *Scn2b* (a sodium channel subunit, see 'channelopathies') (Rudolf et al, 2004). Sequencing the rat homologue of KCNK9, a potassium channel, revealed an additional alanine residue within the C-terminal intracellular domain (Holter et al, 2005). The human homologue of KCNK9 lies on chromosome 8q24 and is mapped to the ECA1 locus, a gene associated with childhood absence epilepsy (Gardener, 2008).

A genome-wide scan using microsatellite loci in the WAG/Rij rat strain demonstrated QTLs on chromosomes 5 and 9, associated independently with the two specific types of SWDs this rat strain exhibits. Comparative genome mapping to the mouse associates these QTLs with *kcnab2* (a potassium channel subunit) and *slc9a1* (the gene mutated in the SWE mouse)(Gauguier et al, 2004).

Table 1. Genes associated with spontaneous or handling-associated seizures in mutant mice<sup>a</sup>

Gene	Encoded protein	Gene	Encoded protein
<i>Acp2</i>	Acid phosphatase 2, lysosomal	<i>Htr2c</i>	5-hydroxytryptamine (serotonin) receptor 2C
<i>Adam22</i>	A disintegrin and metalloproteinase domain 22	<i>Htra2</i>	HtrA serine peptidase 2
<i>Aldh5a1</i>	Aldheyde dehydrogenase family 5, subfamily A1	<i>Itp1</i>	Inositol 1,4,5-triphosphate receptor 1
<i>Amph</i>	Amphiphysin	<i>Jrk</i>	Jerky
<i>Ank3</i>	Ankyrin 3, epithelial	<i>Kcna1</i>	Potassium voltage-gated channel, shaker-related subfamily, member 1
<i>Ap3b2</i>	Adaptor-related protein complex 3, $\beta$ 2 subunit	<i>Kcna2</i>	Potassium voltage-gated channel, shaker-related subfamily, member 2
<i>Ap3m2</i>	Adaptor-related protein complex 3, $\mu$ 2 subunit	<i>Kcnj6</i>	Potassium inwardly-rectifying channel, subfamily J, member 6
<i>Asc1</i>	Anterior suture cataract 1	<i>Kcnj9</i>	Potassium inwardly-rectifying channel, subfamily J, member 9
<i>Atcay</i>	Ataxia, cerebellar, Cayman type homolog (human)	<i>Kcnj10</i>	Potassium inwardly-rectifying channel, subfamily J, member 10
<i>Atxn7</i>	Ataxin 7	<i>Kcnq2</i>	Potassium voltage-gated channel, subfamily Q, member 2
<i>Bax</i>	Bcl2-associated X protein	<i>Myo5a</i>	Myosin Va
<i>Bckdha</i>	Branched chain ketoacid dehydrogenase E1, $\alpha$ polypeptide	<i>Neurod2</i>	Neurogenic differentiation 2
<i>Bmi1</i>	Bmi1 polycomb ring finger oncogene	<i>Npy</i>	Neuropeptide Y
<i>Bruno4</i>	Bruno-like 4, RNA binding protein	<i>Nr4a3</i>	Nuclear receptor subfamily 4, group A, member 3
<i>Bsn</i>	bassoon	<i>Numb</i>	Numb gene homolog ( <i>Drosophila</i> )
<i>Cacna1a</i>	Calcium channel, voltage-dependent, P/Q type, $\alpha$ 1A subunit	<i>Numbl</i>	Numb-like
<i>Cacna2d2</i>	Calcium channel, voltage-dependent, $\alpha$ 2/delta subunit 2	<i>Otx1</i>	Orthodenticle homolog 1 ( <i>Drosophila</i> )
<i>Cacnb4</i>	Calcium channel, voltage-dependent, $\beta$ 4 subunit	<i>Pcm1</i>	Protein-L-isospartate (D-aspartate) O-methyltransferase 1
<i>Cacng2</i>	Calcium channel, voltage-dependent, gamma subunit 2	<i>Pitpna</i>	Phosphatidylinositol transfer protein, $\alpha$
<i>Cdk5r1</i>	Cyclin-dependent kinase 5, regulatory subunit (p35) 1	<i>Plaur</i>	Plasminogen activator, urokinase receptor
<i>Chrm4</i>	Cholinergic receptor, nicotinic, $\alpha$ polypeptide 4	<i>Plcb1</i>	Phospholipase C, $\beta$ 1
<i>Clcn3</i>	Chloride channel 3	<i>Plp1</i>	Proteolipid protein (myelin) 1
<i>Cnp</i>	2',3'-cyclic nucleotide 3' phosphodiesterase	<i>Pmp22</i>	Peripheral myelin protein
<i>Cplx1</i>	Complexin 1	<i>Ppt1</i>	Palmitoyl-protein thioesterase 1
<i>Cstb</i>	Cystatin B	<i>Psap</i>	Prosaposin
<i>Ctnnb1</i>	Catenin (cadherin associated protein), $\beta$ 1	<i>Pten</i>	Phosphatase and tensin homolog
<i>Ctsd</i>	Cathepsin D	<i>Pura</i>	Purine rich element binding protein A
<i>Dbnl</i>	Drebrin-like	<i>Qk</i>	Quaking
<i>Dbp</i>	D site albumin promoter binding protein	<i>Scg5</i>	secretogranin V
<i>Drd1a</i>	Dopamine receptor D1A	<i>Serpine2</i>	Serine (or cysteine) peptidase inhibitor, clade E, member 2
<i>Emx1</i>	Empty spiracles homolog 1 ( <i>Drosophila</i> )	<i>Sgce</i>	Sarcoglycan, epsilon
<i>Epm2a</i>	Epilepsy, progressive myoclonic epilepsy, type 2 gene $\alpha$	<i>Scn1a</i>	Sodium channel, voltage-gated, type I, $\alpha$
<i>Fgf14</i>	Fibroblast growth factor 14	<i>Scn1b</i>	Sodium channel, voltage-gated, type I, $\beta$
<i>Fyn</i>	Fyn proto-oncogene	<i>Scn2a</i>	Sodium channel, voltage-gated, type-2 $\alpha$
<i>Gabbr1</i>	Gamma-aminobutyric acid (GABA-B) receptor, 1	<i>Scn8a</i>	Sodium channel, voltage-gated, type VIII, $\alpha$
<i>Gabbr2</i>	Gamma-aminobutyric acid (GABA-B) receptor 2	<i>Shh</i>	Sonic hedgehog
<i>Gabrb3</i>	Gamma-aminobutyric acid (GABA) B receptor 3	<i>Slc12a5</i>	Solute carrier family 12, member 5
<i>Gabrg2</i>	Gamma-aminobutyric acid (GABA-A) receptor gamma	<i>Slc13a1</i>	Solute carrier family 13 (sodium/sulphate symporters), member 1
<i>Gad2</i>	Glutamic acid decarboxylase 2	<i>Slc1a2</i>	Solute carrier family 1 (glial high affinity glutamate transporter), member 2
<i>Gjb1</i>	Gap junction protein, $\beta$ 1	<i>Slc25a12</i>	Solute carrier family 25 (mitochondrial carrier, Aralar), member 12
<i>Gjc2</i>	Gap junction protein, gamma 2	<i>Slc2a1</i>	Solute carrier family 2 (facilitated glucose transporter), member 1
<i>Gnao1</i>	Guanine nucleotide binding protein, $\alpha$ O	<i>Slc9a1</i>	Solute carrier family 9 (sodium/hydrogen exchanger), member 1
<i>Gng3</i>	Guanine nucleotide binding protein (G protein), gamma 3	<i>Snap25</i>	Synaptosomal-associated protein 25
<i>Gpr98</i>	G-protein coupled receptor, 98 (a.k.a. <i>Mass1</i> )	<i>Sod2</i>	Superoxide dismutase 2, mitochondrial
<i>Gria2</i>	glutamate receptor, ionotropic, AMPA2 ( $\alpha$ 2)	<i>Sox1</i>	SRY-box containing gene 1
<i>Gria3</i>	glutamate receptor, ionotropic, AMPA2 ( $\alpha$ 3)	<i>Syn1</i>	Synapsin I
<i>Grm7</i>	glutamate receptor, metabotropic 7	<i>Syn2</i>	Synapsin II
<i>Helt</i>	Hey-like transcription factor (zebrafish)	<i>Synj1</i>	Synaptojanin 1
<i>Hexa</i>	Hexosaminidase A	<i>Ta2</i>	T-cell acute lymphocytic leukemia 2
<i>Hexb</i>	Hexosaminidase B	<i>Tef</i>	Thyrotroph embryonic factor
<i>Hif</i>	Hepatic leukemia factor	<i>Ube3a</i>	Ubiquitin protein ligase E3A
		<i>Usp18</i>	Ubiquitin-specific peptidase 18

<sup>a</sup>Results were tabulated from a standard phenotype query of the Mouse Genome Informatics database (<http://www.informatics.jax.org>).

Source: Frankel, WN, 2009. For further reference see Literature.

## Dogs

Epilepsy is the most common chronic neurological disorder in dogs, and the prevalence is estimated to be from 0.5% to 5.7% (Chandler, 2005, for references). It affects nearly 35 of the 150 different purebred dog breeds (Gardener, 2008). The prevalence among breeds varies, with some breeds highly affected. Any breed, including mix-breed dogs can be affected. Based on pedigree analysis, a genetic basis for idiopathic epilepsy is suspected in a number of breeds, including the Beagle (Biefelt et al, 1971), Belgian Tervuren (Oberbauer et al, 2003), Keeshond (Hall et al, 1996), British Alsatian

(Falco et al, 1974), Labrador Retriever (Jaggy et al, 1998), Golden Retriever (Srenk et al, 1996), Irish wolfhound (Casal et al, 2006), Vizsla (Patterson et al, 2003) Bernese Mountain dog (Kathmann et al, 1999), Lagotto Romagnolo dogs (Jokinen et al, 2007), English Springer spaniel (Patterson et al, 2005), Standard Poodles (Licht et al, 2007) and a recently published article includes Border Collies (Hülsmeier et al, 2010) to the long list of dog breeds affected by idiopathic epilepsy.

Epilepsy in the dog usually presents between the age of 1 year and 5 years old, but seizure onset has been described in dogs younger and older. The highest incidence is between 1 and 3 years old (Patterson et al, 2003). With increasing age, the possibility of underlying pathology as a cause of seizures (i.e. secondary epilepsy) rises. In the past, generalized tonic-clonic seizures were considered the most common type of seizure in dogs with idiopathic epilepsy, but more recent observations reveal that a variety of focal-onset seizures, including secondarily generalized seizures, can occur in dogs and some individuals have more than one type of seizure (Chandler, 2006). Even though most seizures appear to occur spontaneously, they may be precipitated by a variety of factors. In human patients, sleep deprivation, emotional stress, menstruation, missed medication, and concurrent illness are recognized, and similar factors may also play a role in triggering seizures in animals (Thomas, 2010).

Pedigree studies of the different epilepsy-prone canine breeds have not been able to elucidate the exact mode of inheritance and it is believed different genetic backgrounds exist between breeds or even between different families within breeds (Licht et al, 2007). Segregation analysis, however, have been mostly consistent with a form of recessive inheritance (Hülsmeier et al, 2010). Postulated models for the mode of inheritance of canine idiopathic epilepsy include an autosomal recessive trait for Keeshonds (Hall et al, 1996), a single gene of major effect for Belgian Tervurens and sheepdogs (Oberbauer et al, 2003), a polygenic recessive trait for Labrador Retrievers (Jaggy et al, 1998) and Golden Retrievers (Srenk et al, 1996), and one autosomal recessive gene combined with a sex-linked repressor in Beagles (Biefelt et al, 1971). Gender differences have been reported in some breeds, with males being more affected than females (Chandler, 2006). However, a sex-linked mutation has been ruled out for most breeds and at most a sex-modifying gene may be responsible for this gender imbalance.

Only in the case of the Border Collie, Irish Setter and miniature wirehaired Dachshund have causative genes for epileptic syndromes been discovered (Gardener, 2008).

Over 5% of wirehaired Dachshund suffer from an autosomal recessive progressive myoclonic epilepsy, which in 2005 was shown to be the canine equivalent to a the more severe teenage-onset form of epilepsy, Lafora disease (Lohi et al, 2005). Lafora disease is caused by mutations of EPM2A on chromosome 6q24, and EPM2B on chromosome 6p22. Homozygosity and linkage analysis have mapped the canine disease locus on canine chromosome 35. This chromosome is syntenic with human chromosome 6q21-25. A PCR with clone of canine EPM2B failed in affected dachshund dogs on the 5' of the genes single exon. It was shown a tandem dodecamer (D) repeat expansion of 19 to 26 copies of this sequence is the responsible mutation within the EPM2B gene, causing a 900 times reduced Epm2b mRNA level. As a result, Lafora bodies, starch-like polyglucosans, precipitate in cytoplasm of cells in various organs in patients and in neurons in the brain. The exact pathogenesis of Lafora disease and its canine counterpart, however, remains uncertain.

The Irish Setter is affected by a NCL (neuronal ceroid lipofuscinosis)-like disease, a neurodegenerative disease leading to seizures, cognitive decline and visual impairment from 1 to 2 years of age

(Koppang, 1973). The causative gene locus was mapped to canine chromosome 37 (CFA37) and megablast search located the gene as a homologue of the human gene CLN8, associated with NCL in humans (Katz et al, 2005).

NCL was also described in the Australian Border Collie in 1991 (Studdert et al, 1991). Positive linkage was found to the region on CFA22 in a region of conserved syteny with human chromosome HSA13q, containing CLN5. The canine CLN5 revealed a nonsense mutation (Q206X) within exon 4, predicting truncated protein and correlating with the disease (Melville et al, 2005).

Determining heritability and mode of inheritance in dogs is useful for a number of reasons (Oberbauer et al, 2003). First, selection programs can be established to minimize the incidence of

Gene	human chromosome	canine chromosome
<b>Potassium channels</b>		
KCNO2	20q	24
KCNO3	8q	13
KCNA1	12p	27
KCNAB2	1p	5
KCNMA1	10q	4
KCNJ4	22q	10
<b>Sodium channels</b>		
SCN1A	2q	36
SCN2A	2q	36
SCN1B	19q	1
SCN2B	11q	5
<b>Chloride channels</b>		
CLCN2	3q	34
GABRA1	5q	4
GABRG2	5q	4
<b>Calcium channels</b>		
CACNA1H	16p	6
CACNG2	22q	10
CACNB4	2q	19
<b>Neuronal nicotinic Ach receptors</b>		
CHRNA4	20q	24
CHRNA2	1q	7
<b>Other epileptogenic genes</b>		
slc9a1	1p	2
mh2j	unknown	unknown

epilepsy within a breed if the heritability is of sufficient magnitude. Second, the identification of a major locus can open the possibility to create a marker linked to the epileptic phenotype which can then be used for breeding programmes. Last but certainly not least, identification of epileptogenic mechanisms in the canine may shed a light on genetic mechanisms of human epilepsy. *Canis familiaris*, to which all domestic dog breeds belong to has a genome size of 2.8 Gb, similar in size to the 3 Gb human genome (Shearin et al, 2010). The average nucleotide heterozygosity, when considered across dog breeds, is  $8 \times 10^{-4}$ , which is essentially the same high level of nucleotide diversity reported in the human population. However, the genetic diversity within any breed is significantly lower (Parker et al, 2007). This loss of diversity is

evident in the extensive linkage disequilibrium (LD), the nonrandom association of alleles at two or more loci, among dog breeds (Sutter et al, 2004). Genome-wide association studies (GWAS) can be done in the dog with considerably fewer SNPs compared to a similar study of the human genome as in humans linkage equilibrium usually extends over only short distances (Andersson, 2008). When analyzing a locus for mutations in multiple breeds with the same ancestral mutation the region investigated can also be reduced, sometimes from Mb to Kb (Shearin et al, 2010, for references), which also significantly reduces the amount of effort that has to be put into the search for causative mutations of a disease, such as epilepsy.

## CONCLUSIONS

Neurological diseases, such as epilepsy, know a long history of mostly empirical therapies and arguably the greatest breakthroughs on research into epileptogenesis have occurred in the last 25 years. However, an ultimate cure for epilepsy has yet to be discovered. A major problem in this regard is the heterogeneity of the disease and its causes. The concept of epilepsy as a channelopathy is relatively new. However, although many genes are linked to ion channels, channelopathies cannot fully explain the epileptic phenotype, especially when complex inheritance is suspected.

To summarize, mutations in voltage- and ligand gated channels alter neuron sensitivity to impulses, making them more vulnerable to develop spontaneous and/or induced seizures. Both increased excitability and decreases inhibition feature in epileptogenesis. Topographic variation in receptor expression exists, making some brain areas more vulnerable to seizures than others and hence giving rise to a great number of specific epileptic syndromes. Also, temporal variation in receptor expression exists, which may explain the characteristics of certain epilepsy syndromes, such as those associated with febrile seizures in neonates and children that stop to occur in later life.

Historically, a distinction between acquired and idiopathic, (genetic) epilepsy is made. With the recent attention given to epigenetics and the knowledge gained in this relatively new field of genetic research, the old causal epilepsy categorization may soon reach its expiration date. Our plastic brain, both in early nervous system development but also in later life, give us the ability to learn and to functionally remodel our brain in response to biological and environmental factors, many of which are still unknown or if recognized the epileptogenic background is usually enigmatic. This feature of the human and animal brain, however, can also deform the brain and give rise to epilepsy, as is shown in animal models of epilepsy and seen in acquired epilepsy in humans. Epigenetics have even been associated with a potential cure of epilepsy, but the future will have to prove the validity of this hypothesis.

It should be clear by now animal models have been absolutely essential in epilepsy research. Especially the mouse, due to its great potential for mutagenesis, has given great insight into the biophysiological, -chemical and molecular background of epileptogenic mechanisms. However, the dog is now considered a promising new animal model for many diseases, including epilepsy. The canine genome has recently been uncovered and the variety of breeds in combination with a lack of diversity within breeds makes them ideal models, not only for monogenic traits but especially genetic research of complex and/or polygenetic traits. Although most genes involved in epileptogenesis in the dog have yet to be discovered, when found the combination of GWAS and canine-human homology studies may significantly reduce the amount of time needed to discover the genes responsible for epileptic syndromes in human, greatly accelerating scientific progress in the search for a cure of epilepsy. The dog would then again prove he is indeed man's best friend.

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