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Genetic models of focal epilepsies

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Highlights

- Several focal epilepsy syndromes are proven to be monogenic disorders.
- Mutations in *CHRNA4*, *CHRNB2*, *CHRNA2* and *KCNT1* cause autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE).
- Mutations in *LGI1* cause autosomal dominant epilepsy with auditory features (ADEAF).
- This review provides an update of the mutational spectrum in these genes.
- We review cellular and genetic animal models generated for autosomal dominant focal epilepsies.

ABSTRACT

Focal epilepsies were for a long time thought to be acquired disorders secondary to cerebral lesions. However, the important role of genetic factors in focal epilepsies is now well established. Several focal epilepsy syndromes are now proven to be monogenic disorders. While earlier genetic studies suggested a strong contribution of ion channel and neurotransmitter receptor genes, later work has revealed alternative pathways, among which the mammalian target of rapamycin (mTOR) signal transduction pathway with *DEPDC5*. In this article, we provide an update on the mutational spectrum of neuronal nicotinic acetylcholine receptor genes (*CHRNA4*, *CHRNB2*, *CHRNA2*) and *KCNT1* causing autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), and of *LGI1* in autosomal dominant epilepsy with auditory features (ADEAF). We also emphasize, through a review of the current literature, the contribution of *in vitro* and *in vivo* models developed to unveil the pathogenic mechanisms underlying these two epileptic syndromes.

KEYWORDS

genetic focal epilepsies, cellular and animal models, CHRNA4, CHRNB2, KCNT1, LGI1, DEPDC5

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ABBREVIATIONS

ACh = acetylcholine

ADEAF = autosomal dominant epilepsy with auditory features

ADNFLE = autosomal dominant nocturnal frontal lobe epilepsy

CHRNA4, CHRNB2, CHRNA2 = α4, β2 and α2 subunits of nicotinic acetylcholine receptors

KCNT1 = potassium channel, subfamily T, member 1

LGI1 = leucine-rich, glioma inactivated 1

nAChR = nicotinic acetylcholine receptor

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1. INTRODUCTION

Twenty years have now passed since the first identification of a mutated gene in inherited focal epilepsies. In 1995, a mutation in CHRNA4, encoding the α4 subunit of the nicotinic acetylcholine receptor (nAChR), was identified in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Steinlein et al., 1995). Subsequently, mutations in CHRNB2 and CHRNA2, encoding respectively the β2 and α2 subunits of nAChRs, were reported in a subset of ADNFLE families (Aridon et al., 2006; De Fusco et al., 2000), reinforcing the commonly held view that monogenic focal epilepsies belonged to the channelopathies. Since then, great advances have been made in gene discovery, revealing a genetic heterogeneity that extended pathophysiological mechanisms to non-ion channel biological pathways in focal epilepsies. The first mutations in a non-ion channel gene, leucine-rich glioma inactivated 1 (LGI1), were identified in families with autosomal dominant epilepsy with auditory features (ADEAF) (Kalachikov et al., 2002; Morante-Redolat et al., 2002). More recently, whole-exome sequencing identified mutations in the potassium channel KCNT1 gene in families with severe forms of ADNFLE with intellectual disability and psychiatric features (Heron et al., 2012). Lately, mutations in the DEPDC5 (dishevelled, Egl-10 and pleckstrin domain-containing protein 5) gene have been linked to diverse focal epileptic phenotypes, ranging from apparently nonlesional focal epilepsies to malformation-associated focal epileptic syndromes (for review (Baulac, 2014)). DEPDC5 is the major cause for familial focal epilepsy with variable foci (FFEVF), an autosomal dominant syndrome characterized by focal epileptic seizures arising from different cortical regions in different family members (Dibbens et al., 2013; Ishida et al., 2013). DEPDC5 mutations were also reported in ~13 % of a cohort of ADNFLE families (Picard et al., 2014) as well as families with focal epilepsies and focal cortical dysplasia (Baulac et al., 2015). Finally, a causative role for the corticotrophin-releasing hormone (CRH) gene has also been suggested in ADNFLE (Sansoni et al., 2013).

We will review the respective contribution of in vitro and in vivo models to the understanding of the pathogenic mechanisms of genetic focal epilepsies. Due to the current lack of functional studies concerning mutations in DEPDC5, we will focus on CHRNA4, CHRNB2, CHRNA2, KCNT1 and LGI1. This choice will allow us to exemplify both gain- and loss-of-function pathogenic mechanisms in focal epilepsies caused by mutations in ion channel and non-ion channel genes.

2. INVESTIGATION METHODS AND MODELS OF GENETIC FOCAL EPILEPSIES

There is an abundant literature concerning the experimental procedures to induce seizures either chemically or by electroshocks. These models of acquired epilepsy will not be covered in this review. Instead, we will focus on investigation methods of genetic focal epilepsies, which follow a logical progression from cellular to animal models, offering specific and complementary advantages.

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Basically, *in vitro* models are needed to assess if a given genetic variant is responsible for the phenotype, while intact living organisms allow us to integrate pathomechanisms in neuronal networks and to recapitulate the clinical features observed in the patients. In addition, *ex vivo* techniques, including acute brain slices and organotypic cultures, are particularly used in epilepsy research paired with electrophysiology, but these will not be covered in this review. We provide a brief overview of the methods and models that are used step-by-step to link a mutation to its pathological consequences, together with their advantages and limitations.

2.1 Types of disease-causing mutations

Mutations are typically classified in different types, including most commonly missense, nonsense, splice-site and frameshift mutations. Missense mutations (a non-synonymous substitution) lead to an amino acid exchange in the protein sequence, which may modify the functional properties of the protein. Mutations found in ion channel genes are typically missense. In this case, the pathomechanism is defined as a gain-of-function. Additional dominant-positive or dominant-negative effects can act on associated proteins. The positional clustering of mutations along the gene can also provide useful clues regarding functional domains of the protein playing a key role in the pathology. In contrast, nonsense, splice-site and frameshift mutations lead to the introduction of a premature stop codon that may result in the truncation of the protein or its absence due to nonsense-mediated decay (NMD) degradation, the pathogenic mechanism being most likely a complete loss-of-function. Thus, the type of mutations constitutes a first piece of information to select the appropriate model and assess their functional consequences on the function of the protein of interest.

2.2 Input of cellular models

2.2.1 Heterologous expression systems

Once a genetic variant has been identified, the initial step is to determine if and how this variant affects protein function. This stage typically implies the generation of cellular models, in which rapid results may be obtained. When studying ion channels, heterologous expression systems are frequently used, in particular *Xenopus* oocytes and non-neuronal mammalian cell lines (for instance, human embryonic kidney [HEK-293T] cells). Expression of cDNA encoding wild-type (WT) or mutant proteins precedes *in vitro* analysis of their functional properties, to be explored with electrophysiological techniques. The advantage of using heterologous cells is the absence of the endogenous protein of interest, which facilitates the interpretation of the functional consequences of mutations. *In vitro* expression systems usually allow the distinguishing of neutral variants (with no effects on the trafficking/expression or on the intrinsic properties of the channel) and disease-causing variants. However, species cDNA (human or rodent), type of expression vectors, transfection rates and cell types may influence the results and thus differ between studies. One major

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disadvantage of these heterologous systems resides in their differences from the physiological (i.e. neuronal) environment, which strongly influences the functions of proteins.

2.2.2 Neuronal expression systems

When studying epilepsy, neuronal cells are often indispensable to study the expression, intracellular trafficking and binding characteristics of a protein of interest, either in its endogenous form or overexpressed as a mutant or WT form. Neuronal systems mainly include a restricted number of neuronal cell lines (for instance PC12, N2a and SH-SY5Y cells) and primary neuronal cultures from rodents. Although conceptually interesting, the use of the latter is limited since their viability is restricted in time and transfection is inefficient. Besides these technical issues, this approach also presents scientific drawbacks. First, expression of endogenous proteins complicates the study of overexpressed mutated proteins: for example, other ion channels with closely related functions can mask the detection of subtle defects in the one studied. Second, maturation stages of primary cultured neurons, mostly embryonic, may differ from pathogenic mature neurons in patients. Finally, though closer to neural physiology than heterologous systems, an essential component for epileptogenesis, seizure generation and propagation is missing in cultured neurons: the architectural organization of neural networks and cerebral structures. Taken together, heterologous and neuronal expression systems are precious basic tools to assess the effect of a given mutation at single-molecule/cell levels. Yet, more complex models, namely intact living organisms, are required to explore pathophysiological mechanisms underlying genetic focal epilepsies at fully integrated levels.

2.3 Animal models

Genetically engineered animal models are particularly suitable to understand the neurobiology of a specific inherited epilepsy syndrome. Mouse models have become standard *in vivo* models but, zebrafish and rat models have also more sporadically emerged. A diversification of available genetic animal models will certainly occur in the coming years with the advancement in gene-editing technologies, such as TALENs (transcription activator-like effector nucleases) and CRISPRs (clustered regularly interspaced short palindromic repeats) which can add, disrupt or change targeted sequences of DNA. Animal models are required to fulfill a number of validity criteria, including construct validity, face validity and predictive validity criteria (Grone and Baraban, 2015). Animals should recapitulate the causal mechanisms underlying the epilepsy syndrome (i.e. the genetic defect), and with an appropriate design mimic the human mutation (construct validity). Knockout (KO) models, in which the target gene is disrupted by homologous recombination or by newer gene-editing techniques such as CRISPRs, are suitable to mimic a complete loss-of-function mechanism (when modeling nonsense and frameshift mutations). Constitutive (or global) KO delete permanently the gene of interest in all cells of a given organism. Conditional knockouts inactivate gene expression

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in a specific target tissue and/or in a time-controlled manner using a Cre/loxP strategy. Knock-in (KI) models, defined by the replacement of endogenous alleles by mutated alleles, are used to reproduce missense mutations, and are therefore particularly useful in the case of gain-of-function pathomechanisms and to link a given mutation to a specific phenotype. Heterozygous KI models are also relevant models to replicate dominant-positive or -negative effects. Overexpressing (OE) transgenic models, consisting of random insertion of several copies of the gene of interest (mutated or WT), have been widely used in the past because of the short development time to produce them, but tend to be replaced by knock-in models that are more physiological. Face validity is the ability to reproduce the phenotypic features of the human disorder. Animal models of epilepsy should ideally display spontaneous and recurrent seizures that reflect their human equivalents in terms of age at onset and semiology (both behavioral manifestations and electroencephalogram (EEG) patterns). Lastly, predictive validity refers to the ability of an animal model to correctly identify the efficacy of novel antiepileptic drugs. To date, none of the existing animal models of monogenic focal epilepsies fully meet all validity criteria and replicate the human disorder. However, despite this and technical difficulties, including time constraints and costs, animal models continue to play an essential part in elucidating molecular, functional and anatomical aspects of genetic focal epilepsies. Numerous genetically engineered animal models already exist, among which scn1a-/- mice (Yu et al., 2006), scn1b-/- mice (Chen et al., 2004) and gabrg2-KI (Tan et al., 2007), which mutations in Human lead to generalized seizures and occasionally focal seizures. In this review, we will only focus on models reported to reproduce autosomal dominant focal epilepsies.

3. ADNFLE MUTATIONS IN NICOTINIC ACETYLCHOLINE RECEPTOR GENES

3.1 Genetic basis of autosomal dominant frontal lobe epilepsy (ADNFLE)

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) has been reported in hundreds of families. ADNFLE is a syndrome characterized by clusters of sleep-related motor seizures and an average age at onset between 8 and 12 years. Ictal semiology includes a wide spectrum of motor manifestations, ranging from brief tonic events to major hyperkinetic episodes (Marini and Guerrini, 2007; Picard and Scheffer, 2012). Although ADNFLE was initially described as a rather benign and clinically homogeneous epilepsy, psychiatric symptoms and cognitive impairment appear to also take part in the broad ADNFLE phenotype, with intra- and inter-familial variability. Carbamazepine is the first choice drug for controlling seizures in ADNFLE patients, but drug resistance is reported in about 30% of patients (Picard et al., 2000; Picard et al., 2009). Cases of sporadic nocturnal frontal lobe epilepsy (NFLE) also exist, with clinical manifestations indistinguishable from familial forms (Combi et al., 2004).

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A limited number of disease-causing mutations in three genes (*CHRNA2*, *CHRNA4* and *CHRNB2*) encoding the respective α2, α4 and β2 subunits of the nAChR have been associated with ADNFLE families and sporadic NFLE patients. To date, a total of six distinct mutations in *CHRNA4*, six in *CHRNB2* and two in *CHRNA2* have been reported (Table 1). To note, S280F and S284L mutations in *CHRNA4* appear to be mutational hot spots since several families and sporadic cases carry these specific mutations. The second mutation in *CHRNA2* (I297F) was recently identified in a large family, confirming the involvement of this gene in ADNFLE (Conti et al., 2015). Five *CHRNA4* mutations cluster in the second transmembrane (TM2) domain and five *CHRNB2* mutations are located either at the TM2-TM3 loop or within TM3; the two *CHRNA2* mutations are located in TM1 and TM2. The TM2-TM3 region plays an essential part in controlling the opening and closing kinetics of nAChRs. Mutations in *CHRNA4*, *CHRNB2* and *CHRNA2* collectively account for only a minority of ADNFLE families, indicating that ADNFLE is genetically heterogeneous (Steinlein, 2014). All ADNFLE mutations in *CHRNA2*, *CHRNA4* and *CHRNB2* are missense to the exception of a three base pair insertion in *CHRNA4* leading to an additional leucine residue in the protein sequence (L291dup). There are no loss-of-function mutation reported so far.

3.2 *In vitro* models: towards a gain-of-function effect

Neuronal nAChRs are pentameric ligand-gated ion channels that response to endogenous neuromodulator acetylcholine (ACh) and exogenous nicotine. These receptors are encoded by nine α and three β subunit genes and are widely distributed throughout the mammalian brain with various combinations of five subunits, $\alpha4_2/\beta2_3$ heteromers being one of the most frequent combinations in the brain. Neuronal nAChRs are preferentially expressed in presynaptic and preterminal localizations, modulating excitatory and inhibitory neurotransmitter release. Postsynaptic and nonsynaptic nAChRs also play important roles and may influence neuronal excitability. The broad action of nAChRs may explain their involvement in a variety of physiological processes (including learning and memory and regulation of mood) or in drug dependence, Alzheimer's or Parkinson's diseases, and may also explain cognitive and psychiatric symptoms associated with ADNFLE (Dani and Bertrand, 2007). Numerous electrophysiological studies in heterologous expression systems including *Xenopus* oocytes and mammalian cells, have attempted to elucidate how disease-causing mutations may alter the properties of nAChRs. Due to the heterogeneity of expression systems, cDNA species and homozygous or heterozygous simulated situation, comparisons between studies are difficult. Nevertheless, most studies agreed that mutations in *CHRNA4*, *CHRNB2* or *CHRNA2* have no or little effect on the assembly and membrane expression of nAChRs in heterologous cells (Figl et al., 1998; Kuryatov et al., 1997; Phillips et al., 2001). In contrast, mutations cause alterations of nAChR functional properties, although divergent conclusions have been reported. Several studies have

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investigated the consequences of the recurrent S280F-α4 mutation in heterologous systems, when expressed at the homozygous state. Different observations have been made, ranging from S280F-α4 inducing a faster desensitization (Bertrand et al., 1998; Figl et al., 1998; Kuryatov et al., 1997; Weiland et al., 1996), a decreased ACh sensitivity (Bertrand et al., 1998) or a use-dependent potentiation of nAChRs (Figl et al., 1998; Kuryatov et al., 1997), suggesting both gain- or loss-of-function mechanisms. Similarly, L291dup-α4, when expressed at the homozygous state, resulted both in negative effect by lowering Ca^{2+} permeability (Figl et al., 1998; Steinlein et al., 1997), and positive effects by increasing ACh sensitivity (Bertrand et al., 1998; Steinlein et al., 1997) and inducing a use-dependent potentiation of the ACh response (Figl et al., 1998). When expressed at the heterozygous state, to mimic the situation in humans, a convergent gain-of-function effect emerged for CHRNA4 and CHRNB2 mutations (Table 1). S284L-α4 and V287M-β2 variants caused a reduced dependence of the ACh response to Ca^{2+} and an increased sensitivity to ACh (Bertrand et al., 2002; Phillips et al., 2001; Rodrigues-Pinguet et al., 2003). Increased sensitivity to ACh was also caused by S280F-α4, L291dup-α4 and T293I-α4 mutations, and M301V-β2, V308A-β2 and I312M-β2 mutations (Bertrand et al., 2005; Bertrand et al., 2002; Hoda et al., 2008; Leniger et al., 2003), leading to activation of nAChRs at low concentrations of ACh, thus increasing their probability of opening (Bertrand et al., 2002; Hoda et al., 2008; Leniger et al., 2003; Phillips et al., 2001; Teper et al., 2007). In contrast, the effects of α2-mutations on nAChR properties are still uncertain since I279N-α2 resulted in a gain-of-function consisting of an increased sensitivity to the exogenous agonist nicotine (Aridon et al., 2006), whereas the I297F-α2 mutation caused a decreased nicotine-induced current (Conti et al., 2015), when expressed heterozygously in HEK-293T cells.

In summary, *in vitro* studies confirmed the pathogenicity of ADNFLE mutations and revealed a gain-of-function of mutated nAChRs. Although diverse effects have been reported, they all tended towards an overall increase in nAChR function. The prolonged activation of presynaptic nAChRs may lead to an increased neurotransmitter release. The link between this putative enhanced synaptic transmission and hyperexcitability still needs to be further characterized, and would probably benefit from investigations in neuronal expression systems. Due to the diversity of receptor location in neurons and subunit composition in the brain, *in vivo* studies are essential to explore mutational effects at synaptic and network levels.

3.3 Murine models of ADNFLE

3.3.1 nAChR mutations induce seizures and sleep-related phenotypes

Numerous murine models of ADNFLE including knock-in (KI) and overexpressing (OE) transgenic mouse and rat models of CHRNA4 and CHRNB2 have been generated (Table 2) (also reviewed in (Steinlein, 2010)). Most murine models of ADNFLE presented an epileptic phenotype, either

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spontaneously or induced by nicotine. None of them entirely recapitulated the ADNFLE phenotype but they mimicked some of the clinical features of patients. In contrast, homozygous knockout mice of Chrna4 (Ross et al., 2000), Chrnb2 (Picciotto et al., 1995) or Chrna2 (Lotfipour et al., 2013) gene did not display spontaneous or nicotine-induced epileptic phenotype supporting the hypothesis that complete loss-of-function of nAChR subunits did not underlie ADNFLE.

3.3.1.1 Nicotine-induced seizures

The first genetically-engineered animal model was a KI mouse named L9'A-α4 corresponding to L287A-α4 (Fonck et al., 2005). Although no disease-causing mutations have been reported in the corresponding human amino acid L283, its proximity to reported mutations in TM2 made this model relevant to ADNFLE. Despite the absence of a spontaneous epileptic phenotype, heterozygous L9'A-α4 KI mice exhibited a hypersensitivity to nicotine-induced seizures (Fonck et al., 2005). Subsequently, two KI mouse models of ADNFLE mutations were generated: both heterozygous L291dup-α4 and S280F-α4 KI mice also exhibited increased sensitivity to nicotine (Klaassen et al., 2006). Homozygous V287L-β2 KI mice (O'Neill et al., 2013) and heterozygous S280F-α4 KI mice (Teper et al., 2007) displayed dystonic arousal complexes (DAC), characterized by stereotypical head movements, body jerking and forelimb dystonia and resembled dystonic head and limb elements of ADNFLE patients. Heterozygous L9'A-α4 KI mice (Fonck et al., 2005) and S284L-α4 OE rats (Zhu et al., 2008) exhibited focal seizures in response to high-dose injections of nicotine whereas control animals displayed generalized seizures. Overall, quantitative and qualitative differences of response to nicotine-induced seizures are common features of rodent models engineered with nAChR mutations.

3.3.1.2 Spontaneous epileptic seizures

Spontaneous seizures with a typical EEG activity pattern were reported in two distinct α4 murine models: L291dup-α4 KI mice and S280F-α4 KI mice (Klaassen et al., 2006), and in one β2 mouse model (V287L-β2 OE mice) (Manfredi et al., 2009). Heterozygous L291dup-α4 KI and S280F-α4 KI mice showed frequent spontaneous seizures (several/hour), ranging from brief periods of behavioral arrest to long periods of rhythmic, jerking motion of all limbs and loss of equilibrium with falls (Klaassen et al., 2006). Interestingly, another strain of S280F-α4 KI mice did not present spontaneous seizures (Teper et al., 2007), reflecting the importance of the genetic background on top of engineered mutations in seizure susceptibility. The spontaneous epileptic phenotype observed in V287L-β2 OE mice consisted of very frequent interictal spikes of high amplitude and seizures. The severity depended on the transgene expression level since a mouse strain with low expression only displayed rare and brief spontaneous seizures while another strain with higher expression had more frequent and longer episodes (Manfredi et al., 2009).

Additionally, a rat model, S284L-α4 OE, was produced. S284L-α4 OE rats exhibited a spontaneous epileptic phenotype consisting of frequent interictal discharges and seizures occurring

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on average once a week (Zhu et al., 2008). Interictal discharges, integrated in the thalamocortical loop, originated from the sensorimotor cortex and propagated to the ventrolateral nucleus of the thalamus and basolateral amygdala. Seizures were described as paroxysmal arousals (brief episodes with frightened expression), paroxysmal dystonia (brief episodes of dystonic posturing), and epileptic wandering (ambulation with head shaking and bizarre movements). In contrast to most ADNFLE cases, patients carrying the S284L-α4 mutation are not good responders to carbamazepine (Combi et al., 2004). Interestingly, the pharmacological response of S284L-α4 OE rats was similar to that of patients since carbamazepine was ineffective, while diazepam and zonisamide reduced interictal discharge frequency (Zhu et al., 2008). Altogether, seizure phenotype and antiepileptic drug response indicated that S284L-α4 OE rats are a pertinent model for ADNFLE (Steinlein, 2010).

3.3.1.3 Sleep-related phenotype

Circadian rhythm was explored in L9'A-α4 KI and V287L-β2 KI mice. In both models, the activity-rest cycle was found to be altered. Sleep-wake transitions were more frequent in homozygous L9'A-α4 KI mice whereas brief awakenings, lasting a few seconds, were more frequent in both heterozygous and homozygous animals compared to WT mice (Fonck et al., 2005). Homozygous V287L-β2 KI mice displayed an increased activity during the light cycle that suggested disturbances in sleep patterns (Xu et al., 2011). Interestingly, the frequent interictal discharges and the seizures exhibited by S284L-α4 OE rats occurred exclusively during slow-wave sleep, as in patients with NFLE (Zhu et al., 2008).

3.3.2 Dysfunction of GABAergic transmission

Heterozygous L291dup-α4 and S280F-α4 knock-in mice showed a robust increase in nicotinic-evoked GABAergic cortical inhibition by increasing synaptic release of GABA in layer II/III pyramidal cells from frontal cortex slices. In contrast, nicotine had no effect on excitatory transmission. The authors hypothesized that seizure generation resulted from a hypersynchronization of cortical pyramidal cells that follows recovery from the massive inhibition (Klaassen et al., 2006). Conversely, in S284L-α4 OE rats, an attenuation of GABAergic transmission was demonstrated before seizure onset (Zhu et al., 2008). Recordings from layer V pyramidal cells of the sensorimotor cortex (the focus region) before seizure onset (4 weeks after birth) showed a lower frequency of spontaneous inhibitory postsynaptic currents in S284L-α4 OE rats compared to WT without changes in excitatory transmission. It was suggested that this initial reduction of GABAergic transmission could in turn explain the increased glutamate release showed by S284L-α4 OE rats at seizure onset (8 weeks of age), in particular during sleep (Yamada et al., 2013; Zhu et al., 2008). Taken together, animal models suggested that ADNFLE initial pathogenic mechanisms rely on dysfunctional GABAergic transmission.

3.3.3 Epileptogenesis is restricted to a critical period

V287L-β2 OE mice overexpressed the CHRNB2 transgene under the drive of an inducible neuronal-specific tetracycline promoter, allowing the transgene to be reversibly silenced by doxycycline

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administration (Manfredi et al., 2009). While untreated mice showed interictal spikes and spontaneous seizures, silencing the transgene during the two first postnatal weeks prevented the emergence of seizures. However, administration of doxycycline to adult mice had no effect on seizures. Accordingly, diuretic furosemide administration to S284L-α4 OE rats before seizure onset reduced both the proportion of rats exhibiting seizures and the seizure frequency, while it had no significant effect when administered after seizure onset (Yamada et al., 2013). It thus seems that epileptogenesis in ADNFLE models occurs during a critical period in young animals and that early expression of mutated nAChRs causes long-lasting structural and/or functional alterations that lead to seizures. Interpretation of recent data obtained from neocortical cultures of V287L-β2 OE mice suggested that *CHRNB2* mutations affect the dynamics of synaptic transmission, especially during its maturation, thus agreeing with the hypothesis of functional defects (Gullo et al., 2014). In conclusion, all cellular and animal models of nAChR genes-related to ADNFLE excluded a complete loss-of-function of mutated alleles. Mutated nAChR subunit genes cause abnormal functional properties that may cause a dominant-negative effect on associated subunits in pentameric nAChRs. GABAergic transmission was found to be altered in several animal models exhibiting spontaneous seizures. However, numerous questions remain unresolved in order to understand seizure emergence in ADNFLE. Among them, the link between a given mutation and its phenotypic expression, including comorbidities, still needs to be addressed, as well as the focal nature of seizures considering the ubiquitous expression of nAChRs in the central nervous sytem.

4. ADNFLE MUTATIONS IN POTASSIUM CHANNEL KCNT1 GENE

The KCNT1 channel underlies a Na^+- gated K^+ channel which is specifically blocked by quinidine (Kim et al., 2014). *KCNT1* mutations were recently linked to severe ADNFLE presenting an earlier mean age of onset and association with intellectual disabilities and psychiatric features (Heron et al., 2012). So far, four missense mutations have been reported in *KCNT1* in NFLE: 3 inherited and one *de novo*. Interestingly, mutations in *KCNT1* also cause malignant migrating partial seizures of infancy (MMPSI) and Ohtahara syndrome, two severe forms of early-onset epilepsies (Barcia et al., 2012; Martin et al., 2014). The electrophysiological properties of ADNFLE-associated *KCNT1* mutations (M896I, R398Q, Y796H and R928C) and of MMPSI mutations (R428Q, A934T and P924L) were examined in *Xenopus* oocytes. All disease-causing *KCNT1* mutations produced higher currents than the WT, causing a marked increase in function (Milligan et al., 2014). The effect was even greater in MMPSI mutations than ADNFLE mutations, suggesting a correlation between the *in vitro* effects and the severity of the phenotype. However, the existence of an identical mutation identified in both a patient with MMPSI and a patient with ADNFLE, suggests that other factors influence the phenotype (Kim et al., 2014). To

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date, more than 30 cases of epilepsy patients have been reported with a KCNT1 mutation in a wide phenotypic spectrum indicating KCNT1 may not be a specific gene for ADNFLE. Preliminary clinical evidence shows that quinidine may be an effective medication for epilepsy syndromes due to gain-of-function mutations in KCNT1 (Bearden et al., 2014)

5. ADEAF MUTATIONS IN LGI1 GENE

5.1 Genetic basis of autosomal dominant epilepsy with auditory features (ADEAF)

ADEAF refers to an inherited focal epilepsy with a relatively benign outcome and a good response to standard antiepileptic drugs (Michelucci et al., 2009; Winawer et al., 2000). ADEAF is a well-defined and homogenous condition consisting of adolescence / early adulthood-onset lateral temporal seizures. Seizures are predominantly characterized by auditory auras and by a high propensity to secondary generalized tonic-clonic seizures. Aphasic seizures, alone or in association with auditory symptoms, and visual hallucinations are also frequently reported in ADEAF patients. Conventional magnetic resonance imaging (MRI) is normal.

Leucine-rich, glioma inactivated 1 (LGI1) gene was first shown to be interrupted by a chromosome 10 translocation in a glioblastoma cell line (Chernova et al., 1998). A few years later, two groups discovered LGI1 disease-causing mutations in families with ADEAF presenting linkage to chromosome 10q (Kalachikov et al., 2002; Morante-Redolat et al., 2002). Currently, 45 ADEAF families and 3 sporadic cases have been reported listing 42 distinct LGI1 mutations (Table 3). These 42 mutations, including 27 missense (64%), 7 frameshift (17%), 4 splice-site (10%), 2 nonsense mutations (4.5%) and 2 deletions (4.5%), are evenly distributed along the gene. A loss-of-function is the most likely general pathogenic mechanism underlying LGI1-related epilepsy given that more than a third of mutations lead to the introduction of a premature termination codon. The rather homogeneous phenotype displayed by patients is in accordance with this common mechanism. No correlation has been found between the mutation type or its position within the gene and particular clinical features (Nobile et al., 2009; Rosanoff and Ottman, 2008). LGI1 mutations account for 30 to 50% of ADEAF families, suggesting a still unresolved genetic heterogeneity. In addition to its role in inherited epilepsies, LGI1 is involved in a subset of patients with acquired autoimmune limbic encephalitis (Irani et al., 2010; Lai et al., 2010). Autoimmune encephalitis refers to an adult-onset neurological disorder associated with antibodies against variable extracellular epitopes, including LGI1, previously attributed to voltage-gated potassium channels (VGKC). Limbic encephalitis is characterized by neurologic and psychiatric symptoms: memory loss and confusion, seizures and hippocampal abnormalities.

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LGI1 was the first non-ion channel gene to be directly linked to epilepsy in humans. The protein is composed of a signal peptide at the N-terminus, followed by four leucine-rich repeat (LRR) domains flanked by two cysteine clusters and seven copies of a repeat of about 45 residues named EAR (epilepsy-associated repeat) or EPTP (epitempin). LGI1 belongs to a family of genes including three other paralogs: LGI2, LGI3 and LGI4 (Scheel et al., 2002; Staub et al., 2002). Although these paralogs are pertinent candidates for epilepsies, no disease-causing mutations have been reported so far (Kegel et al., 2013).

5.2 Exploring LGI1 function using cellular models

5.2.1 ADEAF mutations impair secretion *in vitro*

A secretion-competent heterologous expression system, LGI1 transfected HEK-293T cells, provided the first experimental evidence that LGI1 was secreted (Senechal et al., 2005). Subsequently, secretion was confirmed to be a distinctive feature of LGI1 in different mammalian cell types such as COS7 (Sirerol-Piquer et al., 2006), CHO and PC12 cells (Chabrol et al., 2007; de Bellescize et al., 2009) and cultured hippocampal neurons (Fukata et al., 2006). LGI1 protein is N-glycosylated in the endoplasmic reticulum (ER) and matured through the secretory pathway. Interestingly, all ADEAF-causing mutations tested, with the exception of three missense mutations (R407C, S473L, R474Q) (Striano et al., 2011; Yokoi et al., 2015), impaired the secretion of the protein in transfected mammalian cells (Table 3). Secretion-defective mutant proteins were unstable and accumulated in the ER and Golgi complex before premature degradation (Sirerol-Piquer et al., 2006). In a recent study of 19 secretion-defective LGI1 missense mutations, variability in the strength of the secretion was reported (Yokoi et al., 2015). As an alternative mechanism, LGI1 secretion-competent mutation (S473L) showed a much weaker binding to one of its protein partners, ADAM22, compared to wild-type LGI1 (Yokoi et al., 2015). These studies confirmed that LGI1-related epilepsy resulted from a loss-of-function mechanism and demonstrated the decisive role played by extracellular LGI1 in the pathogenesis.

5.2.2 Identification of LGI1 binding partners

LGI1 protein was copurified with the voltage-gated potassium channel subunit Kv1.1 from rat brain lysate (Schulte et al., 2006). Presynaptic A-type channels, of which Kv1.1 is a major constituent, are responsible for adjusting synaptic transmission in the central nervous system by regulating neurotransmitter release. The functional relevance of LGI1-Kv1.1 interaction was investigated in *Xenopus* oocytes: LGI1 slowed the inactivation of Kv1.1-Kvβ1 channels by interacting with the cytosolic Kvβ1 subunit. Conversely, truncated LGI1 failed to prevent Kvβ1-mediated inactivation (Schulte et al., 2006). In the mean time, in the search for PSD-95 binding partners, LGI1 was copurified from rat brain lysate with ADAM22 (A Disintegrin And Metalloprotease domain)

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transmembrane protein (Fukata et al., 2006). Colocalization of LGI1 and ADAM22 at the cell surface of transfected mammalian cells and hippocampal neurons was further confirmed (Fukata et al., 2006; Fukata et al., 2010). ADAM22 is a brain specific metalloproteinase lacking catalytic activity (Sagane et al., 2008) and is currently considered as the main receptor of LGI1. The direct interaction is mediated by EPTP domains of LGI1 and the extracellular domain of ADAM22. Two additional LGI1 ligands were identified from transfected mammalian cells and mouse brain purification: ADAM23 and ADAM11, two other neuronal catalytically-deficient ADAM family members (Fukata et al., 2010; Owuor et al., 2009; Sagane et al., 2008). A hypothesis that still needs to be proven postulates that LGI1 forms a transsynaptic bridge between presynaptic ADAM23 and postsynaptic ADAM22 proteins (Fukata et al., 2010). It was recently reported that auto-antibodies against LGI1 from patients with limbic encephalitis inhibited the binding of LGI1 to ADAM22 and ADAM23 in transfected mammalian cells (Ohkawa et al., 2013). Therefore, the pathophysiology of LGI1-related limbic encephalitis, also probably relies on a loss-of-function mechanism. Interestingly, membrane-surface clustering of Kv1 channels, including Kv1.1, is mediated by PSD-95 and ADAM22 (Kim et al., 1995; Ogawa et al., 2010). It is likely that Kv1 channels, ADAM22, PSD-95 and LGI1 form part of a multi-protein complex.

5.2.3 Role of LGI1 in the regulation of neurite growth

The LRR domains of LGI1 are close homologues of the secreted SLIT proteins involved in axonal growth and guidance in the nervous system (Blockus and Chedotal, 2014; Krex et al., 2002). Based on the assumption that structural similarities shared by LGI1 and SLIT might underlie functional similarities, a study showed that LGI1 antagonized myelin-induced inhibition of neurite outgrowth and growth cone collapse in both cultures from rat cerebellar granule neurons and chick dorsal root ganglion (DRG) sensory neurons. LGI1 mediated this action by binding in vitro to Nogo receptor 1 (NgR1), a transmembrane protein, in association with ADAM22 (Thomas et al., 2010). Another study has shown that LGI1 stimulated neurite outgrowth of DRG and hippocampal neurons through its interaction with ADAM23, even in the absence of myelin substrate (Owuor et al., 2009). Taken together, these data suggest that LGI1 contributes in vitro to shape neurite morphology.

In summary, in vitro models have confirmed that LGI1 is a secreted protein, and demonstrated that the vast majority of ADEAF-mutations prevent its secretion. In addition, the identification of its binding partners, including ADAM22, ADAM23, ADAM11, Kv1.1 and NgR1, provided the first elements to understand LGI1 function(s).

5.3 Animal models of ADEAF

5.3.1 Animal models of LGI1-deficiency are epileptic

5.3.1.1 Lgi1 knockdown in zebrafish

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Morpholinos (MO) were used to reduce the expression of lgi1a, one of the two LGI1 homologues in zebrafish. High-dose MO targeting lgi1a triggered seizure-like behaviors consisting of hyperactivity and erratic swimming, reduced brain and eye size, and high mortality rates (Teng et al., 2010). Lack of electrophysiological recordings precluded to firmly conclude that morphant lgi1a fishes exhibited epileptic seizures. With low-dose MO, morphant fishes were morphologically normal, and were more vulnerable to convulsant pentylenetetrazole (PTZ)-induced seizures than control animals (Teng et al., 2010). The correlation between the severity of the phenotype and the reduction of lgi1a expression was consistent with the loss-of-function hypothesis. Additionally, the structural neural abnormalities induced by an embryonic lack of lgi1a in zebrafish suggested that these abnormalities may contribute to the pathomechanisms of seizures. Knockdown of the lgi1b gene produced developmental abnormalities but no spontaneous seizure-like behavior. However, lgi1b morphants were more susceptible to PTZ-induced hyperactivity (Teng et al., 2011).

5.3.1.2 Lgi1 mouse models (Table 4)

Three independent strains of knockout (KO) mice that completely lack Lgi1 were generated, on two genetic backgrounds (C57Bl/6 and albino C57Bl/6). All strains of homozygous KO (Lgi1-/-) mice presented early-onset frequent spontaneous seizures (Chabrol et al., 2010; Fukata et al., 2006; Yu et al., 2010). Lgi1-/- had normal behavior and appearance at birth. During the second postnatal week, spontaneous epileptic seizures emerged, accompanied by EEG abnormalities with a generalized pattern (Yu et al., 2010) or that may originated in the hippocampus (Chabrol et al., 2010). A strong reduction of body weight was observed in Lgi1-/- mice that died at 2–3 weeks of age (Chabrol et al., 2010; Fukata et al., 2006; Yu et al., 2010). No major neuroanatomical abnormalities were detected before seizure onset (P10). However, after recurrent seizures, Lgi1-/- mice displayed neuronal loss, mossy fiber sprouting, astrocyte reactivity and granule cell dispersion in the hippocampus (Chabrol et al., 2010). Heterozygous KO mice (Lgi1+/-) were fertile, had no spontaneous seizure and survived normally. However, they were more susceptible to sound-induced (Chabrol et al., 2010) or PTZ-induced seizures (Fukata et al., 2006). In summary, Lgi1+/- mice recapitulate the genetic cause and mimic the human condition with an auditory epileptogenic trigger, while Lgi1-/- mice present early onset spontaneous seizures with a probable origin in the temporal lobe structures. It is interesting to note that homozygous KO mouse models of several LGI1 binding partners, namely ADAM22, ADAM23 and Kv1.1, exhibited a phenotype with epilepsy and premature death similar to that of Lgi1-/- mice (Mitchell et al., 2001; Owuor et al., 2009; Smart et al., 1998). Transgenic mice overexpressing either WT (Lgi1-OE-WT) or a truncated form of Lgi1 protein (Lgi1-OE-835delC) were engineered. No spontaneous seizures were detected in either of the lines but Lgi1-OE-835delC mice were more susceptible to PTZ-induced seizures than WT littermates and Lgi1-OE-WT mice (Zhou et al., 2009). Recently, mice overexpressing a mutant Lgi1 transgene with an ADEAF missense mutation (either the

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secretion-defective mutant E383A or the secretion-competent mutant S473L) were produced on an Lgi1-/- background (Yokoi et al., 2015). Similarly to Lgi1-/- mice, both Lgi1-/-;Lgi1-OE-E383A and Lgi1-/-;Lgi1-OE-S473L mice presented frequent spontaneous seizures and premature death, confirming that the secretion-competent S473L mutation is deleterious. In contrast, Lgi1+/-;Lgi1-OE-E383A and Lgi1+/-;Lgi1-OE-S473L mice had no spontaneous seizures, but increased susceptibility to PTZ-induced seizures, a similar phenotype to that of Lgi1+/- mice, suggesting that ADEAF-causing mutations do not act as dominant-negative (Yokoi et al., 2015).

5.3.1.3 Lgi1 knock-in rats (Table 4)

Rats carrying the missense mutation L385R at a highly conserved residue in the fourth EPTP domain close to the ADEAF E383A mutation were generated by ENU (N-ethyl-N-nitrosourea) mutagenesis (Baulac et al., 2012). Spontaneous and frequent epileptic seizures, typically consisting of hypertonic trunk, limb and tail postures and clonies of all limbs or jerking, were observed from postnatal day P10 in all homozygous KI rats ($Lgi1^{L385R/L385R}$). They concomitantly failed to gain weight and died prematurely before P17. Reminiscent of the Lgi1+/- mouse phenotype, $Lgi1^{+/L385R}$ rats were fertile and survived normally. No spontaneous epileptic seizure was ever observed, but they presented high vulnerability to audiogenic seizures, like ADEAF patients. Mutant protein L385R-Lgi1 was not detectable in rat brain lysates from $Lgi1^{L385R/L385R}$ rats and was reduced by half in $Lgi1^{+/L385R}$ compared to control littermates, suggesting instability and premature degradation of the mutant protein (Baulac et al., 2012). A similar observation was done in Lgi1-OE-E383A mice indicating that Lgi1-E383A mutant protein may be misfolded (Yokoi et al., 2015).

5.3.2 LGI1-deficiency induces glutamatergic transmission defects

Murine models have also allowed great advances in the understanding of pathogenic mechanisms underlying LGI1-related epilepsy, pointing toward a defect of glutamatergic transmission. The first study, in transgenic Lgi1-OE mice, revealed a role in the postnatal maturation of glutamatergic synapses both pre- and postsynaptically as well as in the structural pruning of glutamatergic neurons (Zhou et al., 2009). Moreover, in Lgi1-OE-WT mice, retinogeniculate axon pruning was accelerated in the developing visual system (Zhou et al., 2012). The role of LGI1 in glutamatergic transmission was further confirmed by studies in KO mice. Fukata and colleagues reported that the binding of LGI1 to ADAM22 enhances the number of AMPA receptors at the synapse, therefore increasing the glutamatergic transmission (Fukata et al., 2006). Electrophysiological recordings of CA1 hippocampal pyramidal neurons on brain slices revealed that the amplitude of miniature excitatory postsynaptic currents (mEPSCs) and the AMPA/NMDA ratio were decreased in Lgi1-/- mice, whereas mEPSCs frequency was unaltered (Fukata et al., 2010). In contrast, Yu and colleagues reported opposite results, with increased frequency of mEPSCs but no change in amplitude, and increased amplitude of both evoked AMPA and NMDA currents, suggesting a presynaptic defect due to increased release of

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glutamate (Yu et al., 2010). One reason for these contradicting conclusions may result from different seizure histories and seizure-induced brain damages present in Lgi1-/- mice (Anderson, 2010). In summary, acute brain slices of *Lgi1* mouse models all agreed that LGI1 plays a role in the glutamatergic transmission. Recent work from our laboratory confirmed the link between LGI1 and excitatory transmission with *in vivo* studies in *Lgi1* conditional KO mice models. *Lgi1* deletion, restricted to cortical glutamatergic neurons from embryogenesis (Emx1-cKO mice), was sufficient to induce a spontaneous severe epileptic phenotype and premature death within the first month after birth, close to that of Lgi1-/- mice (Boillot et al., 2014). In contrast, neither spontaneous seizures nor increased seizure susceptibility to PTZ were observed when *Lgi1* was specifically deleted in GABAergic parvalbumin interneurons (PV-cKO mice) (Boillot et al., 2014). This study demonstrated *in vivo* that glutamatergic neurons are the main contributors to the pathogenesis of LGI1-related epilepsy.

5.3.3 LGI1 is essential during the whole life

Another fundamental question is whether the role of LGI1 in epileptogenesis is purely neurodevelopmental or whether depletion of the protein in adult can also trigger seizures. Another strain of conditional deletion of *Lgi1* restricted to adult forebrain glutamatergic neurons was produced (CaMKII-cKO mice) (Boillot et al., 2014). Occasional spontaneous seizures with EEG abnormalities were recorded in CaMKII-cKO mice showing that loss of Lgi1 beyond the early postnatal developmental period can also trigger seizures later in life. This study demonstrated that LGI1 is critical during the whole life, as it was suggested by the adult-onset of limbic encephalitis with LGI1 autoantibodies.

In summary, both *in vitro* functional evidence and the spontaneous epileptic phenotype consistently presented by all *Lgi1*-deficient animal models supported that complete loss-of-function is the most convincing mechanism for *LGI1* mutations. Multiple functions of LGI1 have been proposed thanks to cellular and animal models, although the direct link with epilepsy still remains to be specified. Currently, the process of confirming *in vivo* the interesting avenues provided by *in vitro* data is the key step to fully resolve LGI1 function. Among them, it seems essential to establish whether LGI1 has distinct roles throughout life and how the disruption of LGI1 molecular complex integrity leads to a dysfunction of glutamatergic transmission.

5. CONCLUSIONS

Seizures are the visible end point of a long chain of deficient mechanisms going from molecular to network levels. Epilepsy genes constitute an entry point into the pathogenic processes underlying disturbed excitability leading to seizures, in particular in focal epileptic syndromes. Targeted

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mutagenesis is therefore relevant to provide *in vitro* and *in vivo* experimental models of epilepsy that can then be studied following a logical progression. Once the pathogenicity of a given mutation is established, the aim is to link its functional consequences to network hyperexcitability. In this review, we detailed the contribution of cellular and animal models to the understanding of two monogenic focal epilepsies, ADNFLE and ADEAF. Mutations in ion channel genes encoding nAChRs and *KCNT1* lead to a gain-of-function whereas *LGI1* mutations cause a complete loss-of-function. Studying impacts of mutations in specific causative genes is crucial to decipher the neurobiology of epilepsy. However, the biological diversity of epilepsy genes is very rapidly growing, raising the question of whether this time-consuming approach should be applied for each new identified gene, even though new genetic engineering technologies hasten the generation of experimental models. One part of the answer resides in the identification of common molecular pathways, which would make the exploration of a given mutation relevant to the global comprehension of an epileptic syndrome. Genetic modifiers add a level of complexity and may explain inter-individual variability of phenotypes. Heterologous expression systems lack this genetic diversity while the genetic background of animal models is still poorly taken into account. Neurons derived from patients' stem cells present a very interesting option to study epilepsy gene mutations in the context of patients' genetic backgrounds.

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Gene	Mutation	Location	References	Models	Coexpressed subunit	Main effect on AChR properties	Pathomechanism	References
CHRNA4	I275F	TM2	(Wang et al., 2014)	Not studied				
	S280F	TM2	(Saenz et al., 1999; Steinlein et al., 1995) (McLellan et al., 2003; Steinlein et al., 2000)	Xenopus oocytes	β2	increased ACh sensitivity and desensitization to ACh, reduced Ca2+ permeability	gain-of-function	(Bertrand et al., 2002)
	S284L	TM2	(Cho et al., 2003; Phillips et al., 2000) (Rozycka et al., 2003; Sansoni et al., 2012)	Xenopus oocytes	β2	increased ACh sensitivity	gain-of-function	(Bertrand et al., 2002)
				Xenopus oocytes	β2	reduced Ca2+ dependence of the ACh response	gain-of-function	(Rodrigues-Pinguet et al., 2003)
	L291dup	TM2	(Steinlein et al., 1997)	Xenopus oocytes	β2	increased ACh sensitivity, reduced Ca2+ permeability	gain-of-function	(Bertrand et al., 2002)
	T293I	TM2	(Leniger et al., 2003)	Xenopus oocytes	β2	increased ACh sensitivity	gain-of-function	(Leniger et al., 2003)
	R336H	second intracellular domain	(Chen et al., 2009)	Not studied				
CHRNB2	V287L	second extracellular domain	(De Fusco et al., 2000)	HEK-293T cells	α4	retardation of channel desensitization	gain-of-function	(De Fusco et al., 2000)
	V287M	second extracellular domain	(Diaz-Otero et al., 2008; Phillips et al., 2001)	Xenopus oocytes	α4	increased ACh sensitivity	gain-of-function	(Phillips et al., 2001) (Bertrand et al., 2002)
				Xenopus oocytes	β2	reduced Ca2+ dependence of the ACh response	gain-of-function	(Rodrigues-Pinguet et al., 2003)
	L301V	TM3	(Hoda et al., 2008)	Xenopus oocytes	α4	increased ACh sensitivity	gain-of-function	(Hoda et al., 2008)
	V308A	TM3	(Hoda et al., 2008)	Xenopus oocytes	α4	increased ACh sensitivity	gain-of-function	(Hoda et al., 2008)
	I312M	TM3	(Bertrand et al., 2005; Cho et al., 2008)	Xenopus oocytes	α4	increased ACh sensitivity	gain-of-function	(Bertrand et al., 2005)
	V337G	second intracellular domain	(Liu et al., 2011)	Not studied				
CHRNA2	I279N	TM1	(Aridon et al., 2006)	HEK-293T cells	β4	increased nicotine sensitivity	gain-of-function	(Aridon et al., 2006)
	I297F	TM2	(Conti et al., 2015)	HEK-293T cells	β2 or β4	reduced nicotine-elicited current density	loss-of-function	(Conti et al., 2015)

Table 1: ADNFLE mutations in nicotinic receptor genes and their in vitro impact on nAChR functional properties.

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Gene	Species	Model	Mutation	Genotype	Genetic background	Epileptic seizures		References
						Spontaneous	Nicotine-induced	
CHRNA4	Mouse	KO	Null allele	-/-	BALB/C, CF1, C57Bl/6	No	No	(Ross et al., 2000)
		KI	"L9'A"	+/-	C57Bl/6	No	Yes	(Fonck et al., 2005)
		KI	S280F	+/-	Not available	Yes	Yes	(Klaassen et al., 2006)
		KI	L291dup	+/-	Not available	Yes	Yes	(Klaassen et al., 2006)
		KI	S280F	+/-	CD1/129Sv or C57Bl/6	No	Yes	(Teper et al., 2007)
	Rat	OE	S284L		Sprague Dawley	Yes	Yes	(Zhu et al., 2008)
CHRNB2	Mouse	KO	Null allele	-/-	C57Bl/6, DBA/2	No	No	(Picciotto et al., 1995)
		KI	V287L	-/-	C57Bl/6	No	Yes	(O'Neill et al., 2013; Xu et al., 2011)
		OE	V287L		FVB	Yes	NA	(Manfredi et al., 2009)
CHRNA2	Mouse	KO	Null allele	-/-	C57Bl/6	No	No	(Lotfipour et al., 2013)

Table 2: Murine models of ADNFLE linked to nAChR subunit genes.

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References	Mutation	Exon	Mutation type	Domain	Effect on secretion
(Berkovic et al., 2004)	c.124T>G	1	missense	LRR-NT	defective
(Ottman et al., 2004)	c.124T>C	1	missense	LRR-NT	defective
(Gu et al., 2002; Pizzuti et al., 2003)	c.136T>C	1	missense	LRR-NT	defective
(Lee et al., 2013)	c.137G>T	1	missense	LRR-NT	defective
(Sadleir et al., 2013)	c.245T>C	2	missense	LRR1	not studied
(Ottman et al., 2004)	c.329C>A	3	missense	LRR1	defective
(Striano et al., 2008)	c.365T>A	4	missense	LRR2	defective
(Di Bonaventura et al., 2011)	c.365T>C	4	missense	LRR2	defective
(Di Bonaventura et al., 2009)	c.367G>A	4	missense	LRR2	defective
(de Bellescize et al., 2009)	c.377_379del	4	missense	LRR2	defective
(Di Bonaventura et al., 2011; Michelucci et al., 2007)	c.406C>T	4	missense	LRR2	defective
(Hedera et al., 2004)	c.435C>G	5	missense	LRR3	defective
(Pisano et al., 2005)	c.461T>C	5	missense	LRR3	defective
(Di Bonaventura et al., 2011)	c.535T>C	6	missense	LRR-CT	defective
(Michelucci et al., 2003)	c.598T>C	6	missense	LRR-CT	defective
(Chabrol et al., 2007)	c.695T>C	7	missense	EPTP1	defective
(Dazzo et al., 2015)	c.856T>C	8	missense	EPTP2	not studied
(Ottman et al., 2004)	c.893T>C	8	missense	EPTP2	defective
(Fertig et al., 2003)	c.953T>G	8	missense	EPTP3	defective
(Dazzo et al., 2015)	c.1118T>C	8	missense	EPTP4	not studied
(Leonardi et al., 2011)	c.1138A>G	8	missense	EPTP4	defective
(Kalachikov et al., 2002)	c.1148A>C	8	missense	EPTP4	defective
(Striano et al., 2011)	c.1219C>T	8	missense	EPTP4	competent
(Michelucci et al., 2003)	c.1295T>A	8	missense	EPTP5	defective
(Berkovic et al., 2004; Kawamata et al., 2009)	c.1418C>T	8	missense	EPTP6	competent
(Kawamata et al., 2009)	c.1421G>A	8	missense	EPTP6	competent
(Heiman et al., 2010)	c.1477G>A	8	missense	EPTP6	defective
(Dazzo et al., 2015)	microdeletion	2	deletion		
(Hedera et al., 2004)	c.329del	3	frameshift		
(Kalachikov et al., 2002)	c.360-3C>A	intron 3	splice site		
(Berghuis et al., 2013; Chabrol et al., 2007)	c.431+1G>A	intron 4	splice site		
(Sadleir et al., 2013)	c.432-2_436del	intron 4	splice site		
(Fanciulli et al., 2012)	deletion	1-4	deletion		
(Heiman et al., 2010)	c.598del	6	frameshift		
(Kalachikov et al., 2002)	c.611del	6	frameshift		
(Sadleir et al., 2013)	c.673G>T	6	nonsense		
(Michelucci et al., 2003; Morante-Redolat et al., 2002)	c.758del	7	frameshift		
(Kobayashi et al., 2003)	c.839-2A>G	intron 7	splice site		
(Kalachikov et al., 2002)	c.1050_1051del	8	frameshift		
(Bisulli et al., 2004; Morante-Redolat et al., 2002)	c.1420C>T	8	nonsense		
(Heiman et al., 2010)	c.1636_1637del	8	frameshift		
(Kalachikov et al., 2002)	c.1639dup	8	frameshift		

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Table 3: ADEAF mutations in LGI1 and impact on secretion of missense mutations.

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Species	Model	Mutation	Genotype	Genetic background	Epileptic seizures		References
					Spontaneous	Induced	
Mouse	OE	truncated		C57Bl/6	No	Yes (PTZ)	(Zhou et al., 2009)
	OE	WT		C57Bl/6	No	No (PTZ)	(Zhou et al., 2009)
	KO	Null allele	-/-	C57Bl/6	Yes		(Chabrol et al., 2010)
	KO	Null allele	+/-	C57Bl/6	No	Yes (sound)	(Chabrol et al., 2010)
	KO	Null allele	-/-	C57Bl/6	Yes		(Fukata et al., 2010)
	KO	Null allele	+/-	C57Bl/6	No	Yes (PTZ)	(Fukata et al., 2010)
	KO	Null allele	-/-	albino C57Bl/6	Yes		(Yu et al., 2010)
	cKO	Null allele	Emx1	C57Bl/6	Yes		(Boillot et al., 2014)
	cKO	Null allele	CaMKII	C57Bl/6	Yes	Not studied	(Boillot et al., 2014)
	cKO	Null allele	PV	C57Bl/6	No	No (PTZ)	(Boillot et al., 2014)
Rat	KI	L385R	-/-	F344/NSlc	Yes		(Baulac et al., 2012)
	KI	L385R	+/-	F344/NSlc	No	Yes (sound)	(Baulac et al., 2012)

Table 4: Murine models of ADEAF linked to LGI1.

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