Ion Channel Defects in Idiopathic Epilepsies

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Abstract: Idiopathic epilepsies are genetically determined diseases of the central nervous system characterized by typical epileptic seizures and EEG abnormalities but not associated with structural brain lesions. In recent years, an increasing number of mutations associated with idiopathic epilepsy syndromes were identified in genes encoding subunits of voltage- or ligand-gated ion channels. These encouraging results provide a plausible pathophysiological concept, since ion channels form the basis for neuronal excitability and are the major targets for anticonvulsive pharmacotherapy. The first epilepsy genes were identified for rare autosomal dominant syndromes within large pedigrees. Recently, a few mutations were also found for the frequent classical forms of idiopathic generalized epilepsies (IGE), for example absence or juvenile myoclonic epilepsy. The mutations can affect ion channels which on one hand have been known since several decades to be crucial for neuronal function, such as the voltage-gated sodium channel or the $GABA_A$ receptor, or on the other hand were newly identified within the last decade as KCNQ potassium channels or the ClC-2 chloride channel. Functional studies characterizing the molecular defects of the mutant channels point to a central role of GABAergic synaptic inhibition in the pathophysiology of IGE. Furthermore, newly discovered genes may be suitable as novel targets for pharmacotherapy such as KCNQ channels for the anticonvulsant drug retigabine. Altogether, these genetic and pathophysiological investigations will enhance our knowledge about the understanding of epileptogenesis and can help to improve anticonvulsive therapy.

Key Words: Ion channel, epilepsy, genetics, electrophysiology, patch clamp.

INTRODUCTION

Epilepsy is one of the most common neurological disorders affecting up to 3% of the world's population during lifetime [1]. The disease is characterized by recurring unprovoked epileptic seizures resulting from synchronized electrical discharges of neurons within the central nervous system (CNS). With regard to the complicated nature and the many different functions of the brain, the number of clinically differentiable seizures types is large. The symptoms of a seizure, the so-called seizure semiology, depends for example on age, the underlying cause and the brain region involved. Accordingly, epileptic semiology can include only mild feelings of the patient himself that are not visible for other individuals (such as seen with an epigastric aura), but also short-lasting black outs (such as known for absence or complex-partial seizures), or severe generalized tonic-clonic convulsions. The most important features used to classify epileptic seizures and epileptic syndromes are (i) the origin of the seizure/epilepsy which can be focal or generalized and (ii) the underlying cause which can be symptomatic (for example due to cortical malformations, brain tumors or stroke) or idiopathic, i.e. genetic [2, 3].

Idiopathic epilepsy syndromes are characterized by typical seizures, such as absences or myoclonic seizures, and distinct abnormalities of the electroencephalogram (EEG), such as 3-4 Hz generalized spike and wave discharges or generalized polyspikes. These patients usually have a normal magnetic resonance imaging of the brain without an epileptogenic lesion. Within the recent 10 years, the first genes have been identified causing mostly rare monogenic idiopathic epilepsy syndromes, such as autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) or benign familial neonatal convulsions (BFNC). Very recently, mutations were also identified in a few families with the most common idiopathic generalized epilepsies (IGE), which are childhood and juvenile absence epilepsy (CAE, JAE), juvenile myoclonic epilepsy (JME) and epilepsy with Grand-Mal seizures on awakening (EGMA). Almost all of the genes identified so far encode ion channels. This is very well understandable from a pathophysiological point of view, as ion channels provide the basis for both the electrophysiological excitability of neuronal cell membranes and the communication between neurons: Axonal conduction is mediated by voltage-gated channels generating the action potential and signal transduction from cell to cell by ligand-gated channels mediating synaptic transmission. Therefore, any mutation-induced channel malfunction may alter brain excitability and induce epileptic seizures. Underlining this important role of ion channels in epileptology, most anticonvulsant drugs that are in clinical use today modulate different types of ion channels. Best known examples are the use-dependent block of voltage-gated sodium channels by phenytoine, carbamazepine or lamotrigine and the enhancement of GABAergic synaptic inhibition by benzodiazepines, barbiturates, tiagabine or vigabatrine.

This review summarizes epileptic syndromes which are associated with mutations in ion channels with regard to their clinical presentation, genetic findings, and functional studies, which have been performed to prove the pathogenicity of mutations and elucidate the molecular pathophysiology. All

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other idiopathic epilepsy syndromes will not be discussed in this review. Table **1** gives an overview about all syndromes for which linkage to a specific chromosomal region alone or also the responsible genes have been identified. Other recent reviews on this topic are given by [4-6]. As probably not all of the potential readers are sufficiently familiar with ion channels, the review will start with an overview about the most important structural and functional features of these proteins.

Table 1. Susceptibility Loci and Affected Genes Described for Various Idiopathic Epilepsy Syndromes

Disease	Abbreviation	Locus	Gene
Benign familial neonatal convulsions	BFNC1/EBN1 BFNC2/EBN2	20q13.3 [37, 106] 8q24 [38]	*KCNQ2* [39, 40, 48, 49, 108-112] *KCNQ3* [41, 49, 113]
Benign familial neonatal/infantile convulsions	BFNIC	2q23-24.2 [59]	*SCN2A* [59]
Benign familial infantile convulsions (BFIC)	BFIC1 BFIC2 BFIC3	19q [114] 16p12-q12 [115, 116] 2q23-24.2 [117]	- - -
BFIC with familial hemiplegic migraine	BFIC/FHM	1q21-23 [118]	*ATP1A2* [118]
Infantile Convulsions with paroxysmal choreoathetosis	ICCA	16p12-q12 [119-121]	-
Rolando epilepsy/paroxysmal Dystonia/writer`s cramp	RE-PED-WC	16p12-q12 [122]	-
Benign epilepsy with centro-temporal spikes (Rolandic epilepsy)	BECTS	15q24 [123]	-
Generalized epilepsy with febrile seizures plus ($GEFS^+$)	GEFS1	19q13.1 [67]	*SCN1B* [67, 124]
$GEFS^+$	GEFS2	2q23-24.2 [125-128]	*SCN1A* [69, 78, 129-134]
Severe myoclonic epilepsy of infancy (Dravet syndrome)	SMEI	2q23-24.2	*SCN1A* [79, 135-140]
Intractable childhood epilepsy with generalized tonic-clonic seizures	ICEGTC	2q23-24.2	*SCN1A* [66]
$GEFS^+$ / childhood absence epilepsy with febrile seizures	GEFS3/CAE+FS	5q31.1-33.1 [84, 85]	*GABRG2* [84-87]
Febrile seizures	FEB1 FEB2 FEB3 FEB4	8q13-21 [141] 19p13.3 [142] 2q23-24[127] 5q14 [143]	- - - *MASS1* [144]
Juvenile myoclonic epilepsy	JME	2q22-23	*CACNB4* [99]
Idiopathic generalized epilepsy	IGE	2q36 [91]	-
Idiopathic generalized epilepsy	IGE	3q26 [91]	*CLCN2* [96]
Juvenile myoclonic epilepsy	JME JME/EJM1	5q34-35 [90] 6p21 [145-147]	*GABRA1* [90] -
Idiopathic generalized epilepsy / childhood absence epilepsy	IGE/CAE/ECA1	8q24 [148-150]	-
Idiopathic generalized epilepsy	IGE	14q23 [91]	-
Juvenile myoclonic epilepsy	JME/EJM2	15q14 [151]	-
Childhood absence epilepsy	CAE	16p13.3	*CACNA1H* [102]
Idiopathic generalized epilepsy and episodic ataxia type 2	IGE+EA-2	19q13	*CACNA1A* [100]
Benign adult famililial myoclonic epilepsy	BAFME1 BAFME2	8q24 [152] 2p11.1-q12.2 [153]	- -
Autosomal dominant nocturnal frontal lobe epilepsy	ADNFLE/EFNL1 ADNFLE/EFNL3 ADNFLE/EFNL2	20q13 [20] 1q21 [22, 23] 15q24 [157]	*CHRNA4* [21, 27, 30, 154-156] *CHRNB2* [22, 23] -
Autosomal dominant lateral temporal lobe epilepsy	ADLTE	10q23-26 [158]	*LGI1* [159]
Familial partial epilepsy with variable foci	FPEVF	22q11-12 [160]	-

STRUCTURE AND FUNCTION OF ION CHANNELS

Ion channels are transmembrane proteins allowing the passive flow of Na^+, K^+, Cl^- or Ca^{2+} ions along their concentration gradient. They are controlled either by voltage and contribute to action potentials or by ligands – as specific neurotransmitters – and contribute to synaptic transmission. Distinct and structurally conserved classes of ion channels emerged during evolution to mediate these and other processes of excitability. Most important and best studied are the groups of voltage-gated cation channels and of ligand-gated channels [7], which will be explained in more detail below.

Voltage-Gated Cation Channels

Voltage-gated cation channels have pores that are selective for K^+, Na^+ or Ca^{2+} ions. Their main α-subunit constitutes structures for gating and permeation while the accessory subunits called β, γ, or δ have only modifying effects. The α-subunits have a tetrameric structure consisting of four homologous domains (I-IV) each with six transmembrane segments (S1-S6). In Na^+- and Ca^{2+}-channel α-subunits, the entire subunit is encoded by one gene so that the domain arrangements are always the same. In contrast, K^+ channel genes encode only one domain and by co-assembly with domains encoded by other K^+ channel genes, the tetramers can be of high diversity. Of special importance for the voltage-gated cation channels are the voltage sensitive structures, the S4 segments of all domains. These are located around the ion-selective pore-forming structures constituted by S5-S6 loops (Figs. **3**, **4**).

There are three main conformational states of voltage-gated channels, a closed resting state, an open conducting state, and a closed inactivated state. At the resting potential, the channels are in the resting state. Upon membrane depolarization, the voltage sensors move outward opening the 'activation gate' of the channel on a time scale of milliseconds. Sustained depolarization leads to channel closing by spontaneous inactivation. The channels cannot be directly activated again from this state, i.e. they are refractory. The inactivation gate is therefore different from the activation gate. Upon membrane repolarization, inactivated channels recover from inactivation (Fig. **1**). Variations of kinetics, voltage dependence of channel gating, or the amount of functional protein in the membrane are typical modifying properties of the smaller β-, γ- or δ-subunits [8-10].

In an action potential, the steep depolarizing phase is mediated by activation of the Na^+ inward current, whereas membrane repolarization is due to both fast inactivation of Na^+ channels and activation of the outward K^+ current. Consequently, disruption of fast Na^+ channel inactivation or a decrease in K^+ conductance can lead to slowed or incomplete repolarization of the cell membrane resulting in

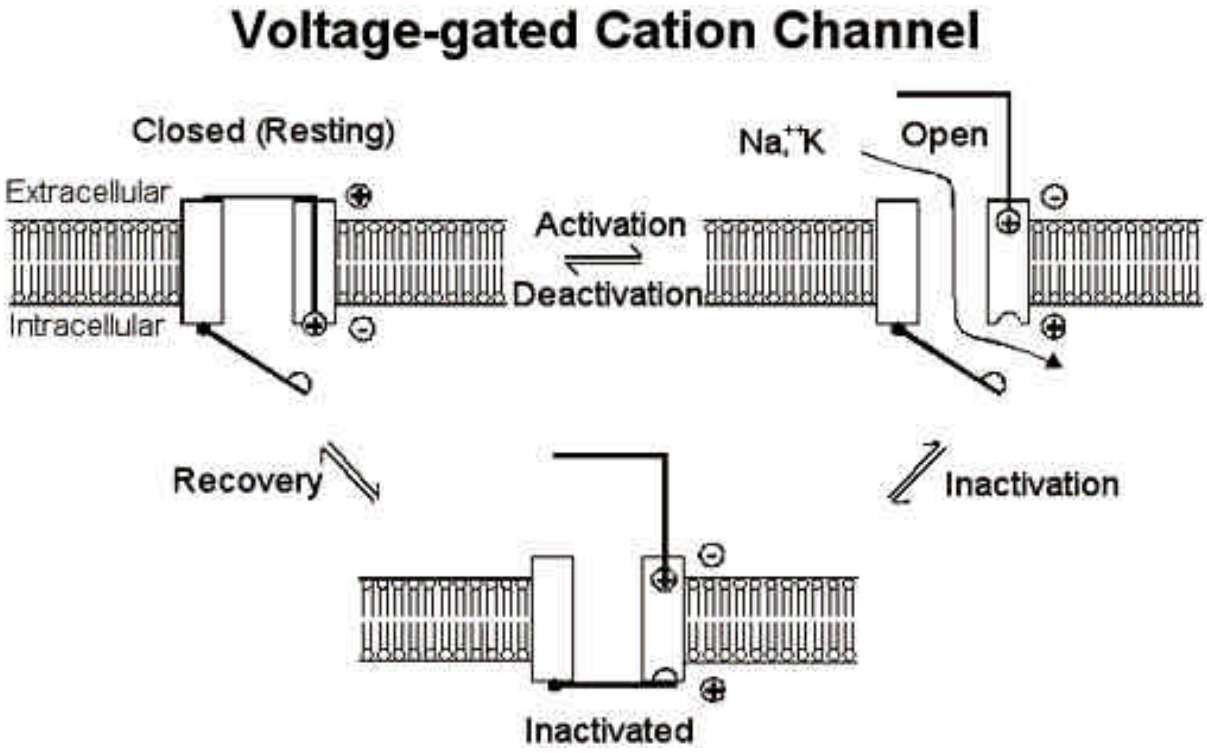


Fig. (1). The three main conformational states of voltage-gated cation channels. From a closed resting state at a hyperpolarized membrane potential, channels open upon depolarization conveyed by outward movement of the voltage sensors opening the activation gate. With ongoing depolarization, some channels, such as the voltage-gated Na^+ channel, inactivate by binding of the inactivation gate to its receptor site which is exposed when the voltage sensors move outward. From the inactivated state, they can only recover upon repolarization of the cell membrane before they are available for another opening.

hyperexcitability and spontaneous series of action potentials. Main functions of neuronal voltage-gated Ca^{2+} channels are the regulation of transmitter release in presynaptic nerve terminals.

There are several genes encoding different Na^+ channel α-subunits (*SCN1A-SCN12A*) that show tissue-specific expression patterns: *SCN4A* is expressed in skeletal muscle and *SCN5A in* heart muscle. Four of these subunits (*SCN1A, SCN2A, SCN3A* and *SCN8A*) are highly expressed in brain [11]. This tissue specificity explains the restricition of symptoms of Na^+ channel mutations to certain tissue types, for example *SCN1A* mutations generate febrile and afebrile seizures but not cardiac conduction defects. Voltage-gated K^+ channels, show less homology to one another than Na^+ channels and are therefore grouped into several families according to their gating properties. For example there are inactivating (e.g. *KCNA1*) and non-inactivating (e.g. *KCNQ1-5*) K^+ channels both of which are relevant to neuronal excitability (reviewed in [10, 12]).

Ligand-Gated Ion Channels

Ligand-gated channels are activated by neurotransmitters such as acetylcholine (ACh), γ-amino butyric acid (GABA), glycine, glutamate or nucleotides. Classically, these channels are tetrameric or pentameric associations of subunits of equivalent structure. The subunits all have either two or four transmembrane segments named M1-M4 whereby the ion-conducting pore is formed by the M2 segments (Figs. **2**, **5**). The pore is not selective for a specific ion type but may be permeable either to cations, as in excitatory ACh or glutamate receptors, or to anions, such as in inhibitory GABA or glycine receptors. Neurotransmitter affinitiy is dependent on amino acid sequences in many different regions of the channel. There are three main conformational states of these ligand-gated channels: resting, open, and desensitized. Activation occurs by binding of the transmitter, and desensitization (refractoriness) is brought about by prolonged exposure to the neurotransmitter. Removal of the transmitter leads to recovery from desensitization [13].

Of special relevance to the context of epilepsy are the nicotinic ACh receptors (nAChR) which have a pentameric structure of two α- and three β-subunits (Fig. **2**). In brain, altogether eight α- ($\alpha_{2\text{-}9}$) and three β- ($\beta_{2\text{-}4}$) subunit isoforms are known to be expressed. The α_4- and β_2- subunits encoded by *CHRNA4* and *CHRNB2*, are especially abundant, so it is not surprising that mutations therein cause disease, i.e. autosomal dominant nocturnal frontal lobe epilepsy [14]. Pentameric GABA receptors are also involved in causing epilepsy. There are several different subunits of $GABA_A$ receptors ($\alpha_{1\text{-}6}$, $\beta_{1\text{-}3}$, $\gamma_{1\text{-}3}$, δ, ε, π, $\rho_{1\text{-}3}$). The subunit composition most abundantly found in brain is probably $2\alpha_1 2\beta_2 1\gamma_2$ [15, 16].

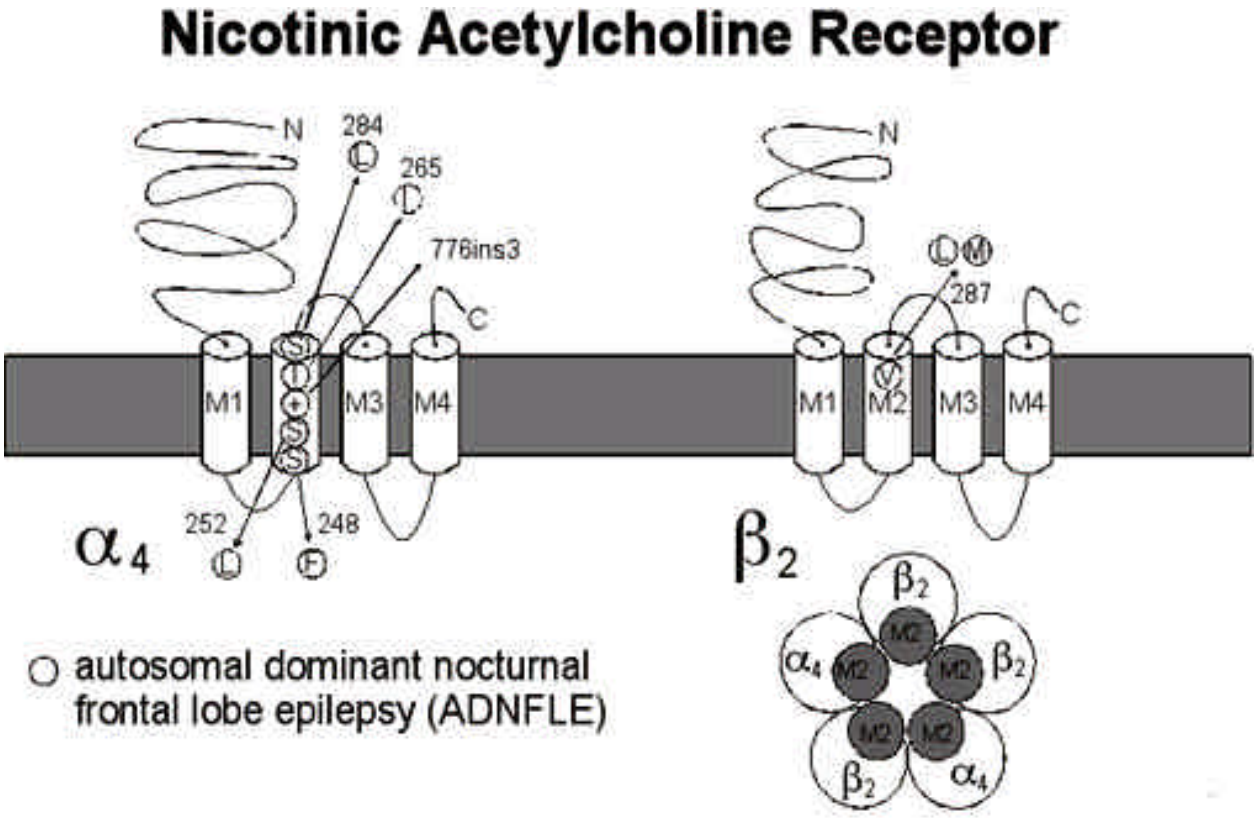


Fig. (2). Proposed structure of the nicotinic acetylcholine receptor with mutations found in patients with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (references in Table 1). Each of the 5 subunits contains 4 transmembrane regions (M1-M4), the M2 segments constitute the ion pore and the long extracellular N-terminal part of the α-subunit contains the binding site for acetylcholine.

AUTOSOMAL DOMINANT NOCTURNAL FRONTAL LOBE EPILEPSY (ADNFLE)

Clinical Picture

ADNFLE has an autosomal dominant mode of transmission and a penetrance of 70-80% [17, 18]. The onset is during childhood. The disorder is characterized by brief partial seizures typically occurring during the night and showing hyperkinetic or tonic manifestations. Ictal video-electroencephalographic studies have revealed that the seizures originate from the frontal lobe. ADNFLE has been often misdiagnosed as paroxysmal nocturnal dyskinesia, or other sleep disorders such as night terrors, nightmares, or hysteria [19].

Genetics

The first linkage study performed in a large Australian family was suggestive of a locus on chromosome 20q13.2 [20]. Later, a mutation was identified in the *CHRNA4* gene encoding the α_4-subunit of a neuronal nicotinic acetylcholine receptor (nAChR), representing the first ion channel mutation in an inherited form of epilepsy [21]. In the mean time, altogether five *CHRNA4* mutations and two mutations in *CHRNB2* encoding the homologous β_2-subunit of neuronal nAChR [22, 23] have been identified. All known mutations are located in the pore-forming M2 transmembrane segments (Fig. **2**, Table **1**).

Pathophysiology

Functional expression of most of the known mutations in Xenopus oocytes or human embryonic kidney (HEK) cells revealed different effects on gating of heteromeric $\alpha_4\beta_2$ channels leading either to a gain-of-function or a loss-of-function. The pathomechanism is still not clear up to now. A decrease of the overall channel activity by enhanced desensitization, slowed recovery from desensitization, reduced single channel conductance and reduced permeability for Ca^{2+} ions was described for the S248F and the 776ins3 mutations in the α_4-subunit [24-26]. However, further studies of the same mutations also revealed a use-dependent potentiation to repetitive ACh-expositions or a 10-fold increase in ACh-sensitivity that increase channel activity [26-28]. First studies of the mutations in the β_2-subunit revealed gain-of-function mechanisms. The V287L mutation showed a profound slowing of desensitization kinetics [22] and V287M showed a 10-fold increase in ACh-sensitivity [23]. Recently, an increased sensitivity to ACh was found to be a molecular mechanism common for six mutations in α_4- or β_2-subunits and therefore postulated as the disease-causing one [29, 30]. In contrast, another investigation of five mutations proposed a reduction of the Ca^{2+} sensitivity of the ACh response, thus a loss-of-function, as a common pathomechanism [31].

Transgenic mice generated with either a knock-out or knock-in of the α_4–subunit were not reported to develop seizures [32, 33]. However, the knock-in mice showed a decreased threshold for ACh-induced seizures [34], fitting well with the hypothesis of an increased ACh-sensitivity of the mutations. Thus, a gain-of-function of the nAChR might be the relevant pathomechanism for ADNFLE. How these changes can induce nocturnal frontal lobe seizures remains to be elucidated. Thalamic neurons under cholinergic control, which are part of thalamocortical loops playing a crucial role for rhythmic activities during sleep and also including predominantly the frontal lobe, could be responsible for the pathophysiology of this disease [14].

BENIGN FAMILIAL NEONATAL CONVULSIONS (BFNC)

Clinical Picture

BFNC is a rare dominantly inherited epileptic syndrome with a penetrance of 85% [35, 36]. The seizures manifest within the first weeks of life and typically disappear spontaneously after weeks to months. The neurological examination, interictal EEG and development of these children are often normal. The prognosis is benign with a low risk of about 15% of recurring seizures later in life.

Genetics

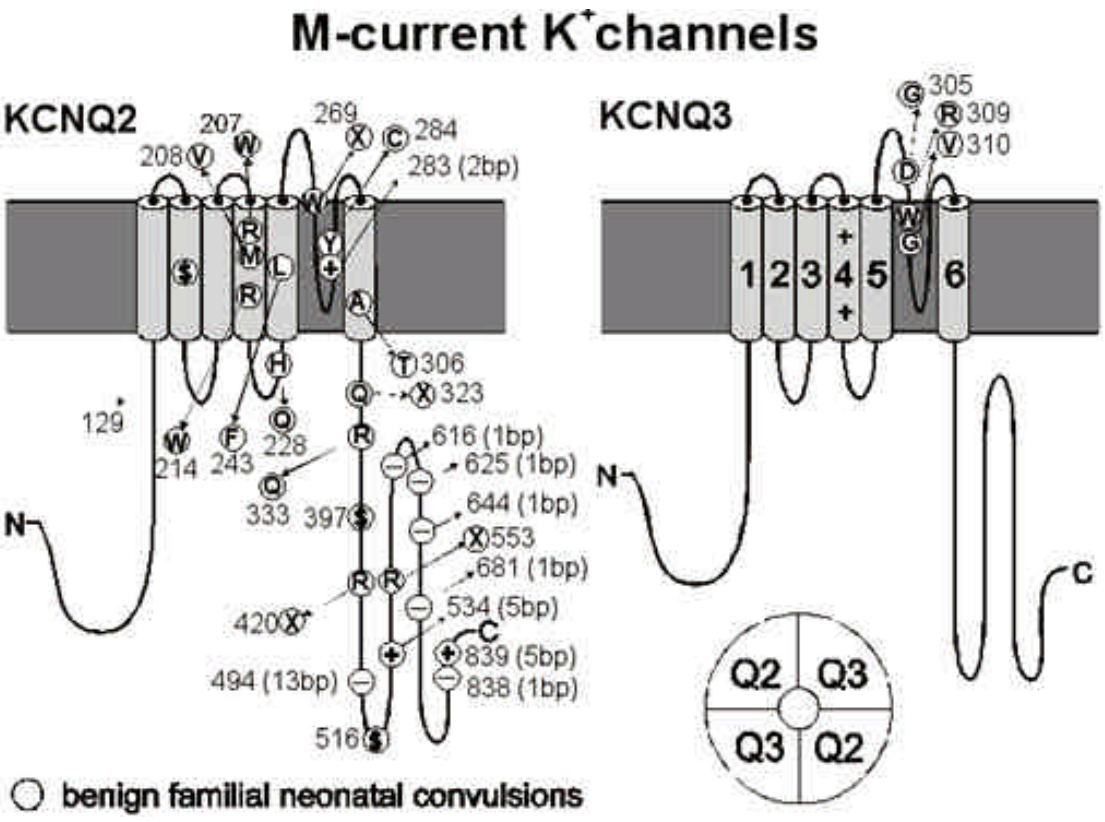
Two loci on chromosome 20 [37] and chromosome 8 have been described [38]. In both regions, genes encoding voltage-gated potassium channels of the KCNQ family are located. Two groups independently discovered the first mutations in *KCNQ2* (20q13.3 [39, 40]) and *KCNQ3* (8q24 [41]). All mutations known up to date are summarized in Fig. **3**.

Pathophysiology

The KCNQ gene family encodes delayed rectifier K^+ channels that are mainly expressed in heart muscle (*KCNQ1*), in the central nervous system (*KCNQ2-5*), the inner ear (*KCNQ4*) and skeletal muscle (*KCNQ5*) (reviewed by [42]). They are slowly activated upon long-lasting membrane depolarizations, for example with trains of action potentials, and control the membrane potential near the threshold for the action potential. In this way, KCNQ channels can regulate the frequency of neuronal firing and play a role in repolarization during the late phase of the cardiac action potential. Mutations in four of the five genes identified cause inherited diseases. *KCNQ1* mutations cause cardiac arrhythmia in the long QT syndrome [43], *KCNQ2* and *KCNQ3* mutations epileptic seizures in BFNC (see above) and *KCNQ4* mutations congenital deafness [44]. Functional expression of the known mutations revealed a consistent reduction of the resulting potassium current in *KCNQ1-4* [39, 44-49] (Fig. **4**). This can lead to a membrane depolarization in the subthreshold range in neurons or to an impaired membrane repolarization in cardiac myocytes explaining the occurrence of hyperexcitability in the affected tissues.

However, the effects of the mutations on current reduction were greatly dependent on channel type. Whereas *KCNQ1* and *KCNQ4* mutations exhibited strong dominant negative effects on WT channels [44-46], *KCNQ2* and *KCNQ3* mutations usually did not but instead produced the disease by a haploinsufficiency mechanism [39, 47-49]. There is only one report with suggestive evidence of a dominant negative effect of a *KCNQ2* mutation on the co-expressed wildtype [49].

(A)



(B)

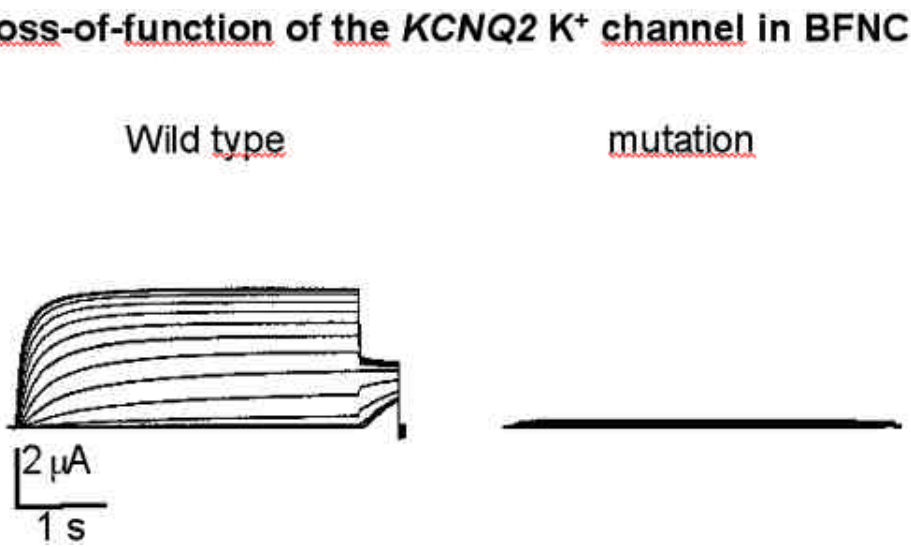


Fig. (3). (A) Proposed structure of the voltage-gated *KCNQ2* and *KCNQ3* K^+ channels containing mutations causing benign familial neonatal convulsions (BFNC) (references in Table 1). The channels are built of six transmembrane segments (S1-S6). The voltage sensors are constituted by the S4 segments containing positively charged residues, and the ion conducting pore by S5 and S6 segments and the connecting P-loop. The long carboxy (C)-terminus contains an interaction domain enabling *KCNQ2* and *KCNQ3* channels to form heterotetramers as indicated in the inset (lower right). \$: splice site mutation; +: insertion; -: deletion; bp: base pairs. (B) Functional consequences of a BFNC-causing mutation when expressed in Xenopus oocytes. The mutation 2513delG is a single base pair deletion located at amino acid (aa) position 838 (A), just 7 aa before the regular stop codon. It induces a frame shift, change of the last 7 aa and prolongation by another 56 aa before the new stop codon. This mutation largely reduces the resulting potassium current by 20-fold as seen in the raw current traces in (A) and for the average of many such experiments in (B). Coexpression of both wild type (WT) and mutant channels did not result in significantly less than 50% of WT current, suggesting that there was no dominant negative effect of the mutation (modified after [48]).

It has been shown that *KCNQ2* and *KCNQ3* channels interact with each other [50] and constitute the so-called 'M-current', a neuronal K^+ current known since several decades to play an important role in the regulation of the firing rate of neurons [51]. In Xenopus oocytes, coexpression of WT and mutant *KCNQ2* with WT *KCNQ3* channels in a 1:1:2 ratio resulted in a current reduction of only 20-25% compared to co-expression of both wildtypes in equimolar ratios [47]. Therefore, relatively small changes of the M-current are sufficient to cause epileptic seizures in the neonatal period.

Disease-causing mutations in KCNQ channels are clustered in the pore region and in the C-terminus (Fig. **3A**). Mutations in the pore reduce the K^+ current by affecting ion conductance, whereas mutations in the C-terminus affect assembly of α-subunits to heteromeric channels. First evidence suggestive for the co-assembly region was given by a C-terminal *KCNQ1* mutation causing the Jervell and Lange-Nielson syndrome which disrupted assembly of *KCNQ1* channels [52]. Later experiments using chimeras between *KCNQ1*, *KCNQ2* and *KCNQ3* channels confirm that the co-assembly region is located in the C-terminus [53, 54]. Presumably, correct tetramerization is necessary for insertion of the channel complex into the outer cell membrane. This would explain the reduced surface expression of a *KCNQ2* mutant truncating the C-terminus. In contrast, pore mutations in *KCNQ2* and *KCNQ3* do not seem to additionally affect surface expression [55].

The question remains as to why the reduced *KCNQ2/KCNQ3* K^+ current results in seizures preferentially during the neonatal period. An explanation might be the differential expression of potassium channels during maturation. On one hand, upregulation of these channels during development could gradually lead to a critical amount of *KCNQ* channels needed for adequate control of the subthreshold membrane potential. A small reduction of the potassium current by mutations may then be sufficient to cause seizures in the neonatal period, when the potassium current is still on a low level, but not in the adult period, when *KCNQ* channels are abundantly available. Alternatively, upregulation of other potassium channels might help to compensate for the deficit later on. Expression of a shorter, non-functional splice variant of *KCNQ2* exclusively in fetal brain, which attenuates *KCNQ2* and *KCNQ3* channels upon coexpression [56], could be another reason for the age-dependent phenotype.

BENIGN FAMILIAL INFANTILE CONVULSIONS (BFIC) AND BENIGN FAMILIAL NEONATAL/ INFANTILE CONVULSIONS (BFNIC)

A well established epilepsy syndrome very similar to BFNC is benign familial infantile convulsions (BFIC) [57, 58], in which partial epileptic seizures with or without secondary generalization develop in clusters between 3 and 12 months of age. The syndrome is also inherited as an autosomal dominant trait, affected individuals develop normally, and adults usually do not develop seizures. The syndrome can be associated with other inherited disorders of the central nervous system, as paroxysmal dyskinesia or migraine. Several different loci were described but a responsible gene has not been identified so far (Table **1**).

Scheffer and colleagues then described two families in which seizures occurred in the first weeks of life, just in between the typical age of manifestation of BFNC and BFIC. They named this syndrome BFNIC (or BFNIS for BFNI seizures). In both families they identified mutations in the gene *SCN2A* encoding one of the α-subunits of voltage-gated sodium channels expressed in mammalian brain [59] (Fig. **4A**). The mutations have not been functionally examined so far.

Generalized epilepsy with febrile seizures plus ($GEFS^+$), severe myoclonic epilepsy of infancy (SMEI, Dravet syndrome) and intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC)

Clinical Picture

$GEFS^+$ was first described in 1997 and 1999 [60, 61]. It is an autosomal dominant childhood-onset syndrome featuring febrile convulsions and a variety of afebrile epileptic seizure types within the same pedigree. Two thirds of affected individuals were diagnosed as febrile convulsion syndrome (FS), often combined with febrile seizures persisting after the 6^{th} year of life or in combination with afebrile generalized tonic-clonic seizures (called 'FS^+'). Other phenotypes characterized by additional seizure semiologies as 'FS^+ with absences', 'FS^+ with myoclonic seizures', 'FS^+ with atonic seizures' or myoclonic astatic epilepsy (MAE) were described in one third of the patients. Partial epilepsies occurred in rare cases ('FS^+ with temporal lobe epilepsy'). The penetrance was about 60%.

Severe myoclonic epilepsy of infancy, as first described by Dravet [62], is characterized by clonic and tonic-clonic seizures in the first year of life that are often prolonged and associated with fever. In the cause of the disease, patients develop afebrile generalized myoclonic, absence or tonic-clonic seizures, but also simple and complex partial seizures do occur. Developmental stagnation with dementia occurs in early childhood. In contrast to $GEFS^+$, the syndrome is usually resistant to pharmacotherapy. SMEI patients sometimes have a family history of febrile or afebrile seizures and there are families in which $GEFS^+$ and SMEI overlap, so that SMEI can be regarded as the most severe phenotype of the $GEFS^+$ spectrum [63, 64].

A similar severe epilepsy syndrome of childhood is intractable childhood epilepsy with generalized tonic clonic seizures (ICEGTC) [65]. Onset and clinical course including mental retardation are as in SMEI, but myoclonic seizures do not occur. Cases of ICEGTC with other family members affected by $GEFS^+$ were described [66]. Thus, as also outlined below with regard to the genetic results, the $GEFS^+$ spectrum extends from simple febrile seizures to the variety of severe epilepsy syndromes of childhood as ICEGTC, MAE and SMEI.

Sodium Channel Defects in $GEFS^+$, SMEI and ICEGTC

The first genetic defect in this group of diseases was discovered in a large $GEFS^+$ pedigree by [67]. The authors described linkage to chr 19q13 and identified a point mutation within the gene *SCN1B* encoding the β_1-subunit of the voltage-gated Na^+ channel. The mutation (C121W, Fig. **4A**)

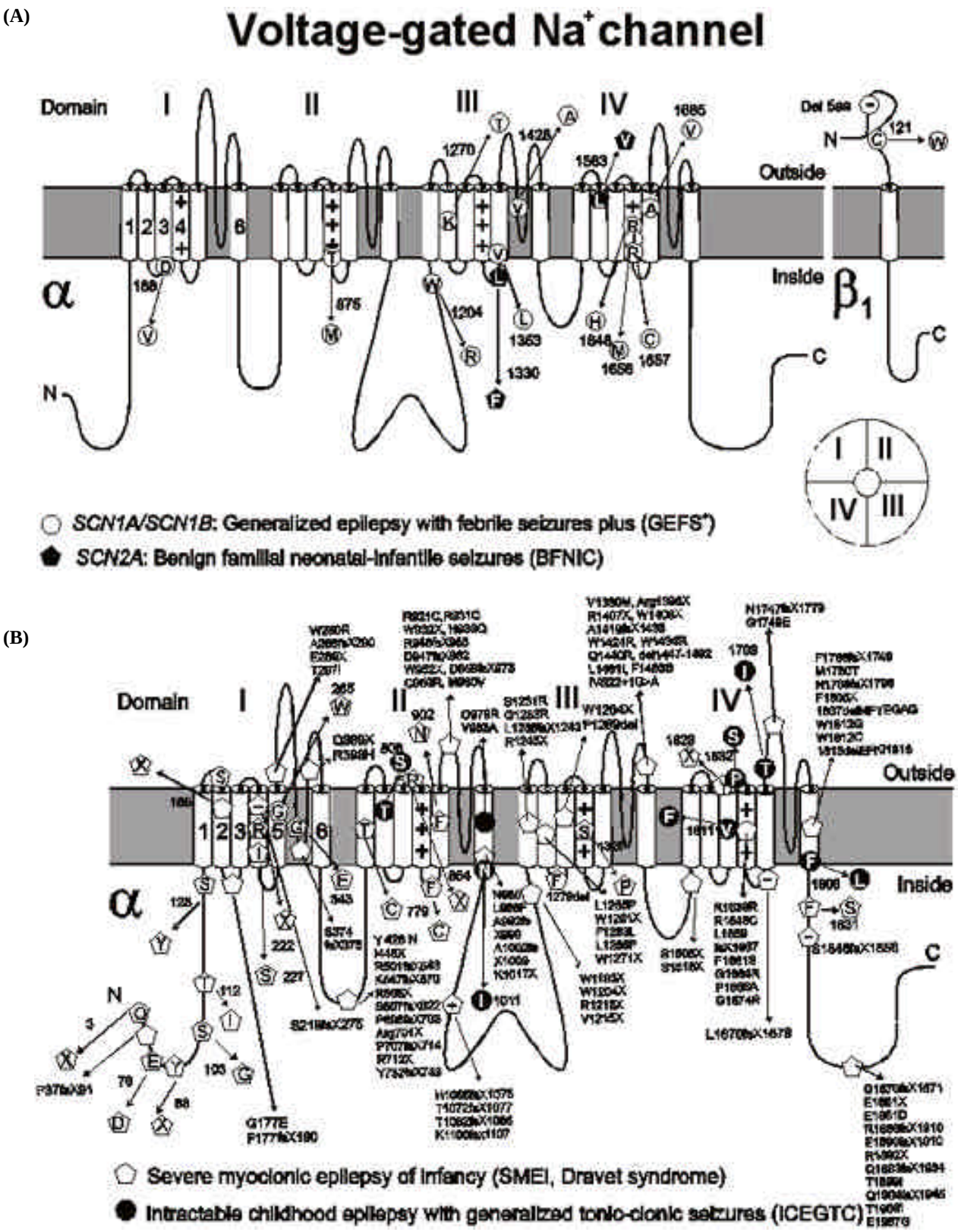


Fig. (4). Proposed structure of the voltage-gated Na^+ channel α- and β_1-subunits encoded by the *SCN1A*, *SCN2A* (α) and *SCN1B* genes. The mutations cause $GEFS^+$ or BFNIC (A), or SMEI or ICEGTC (B) (references in Table 1). Na^+ channels are built similar to K^+ channels like shown in Fig. 3A. They have 4 highly homologous repeats (I-IV) with 6 transmembrane segments each (S1-S6) forming a central pore (lower right in A).

disrupts a disulfide bridge changing the secondary structure of the β_1-subunit extracellular loop and leading to a loss of β-subunit function [67, 68].

Subsequently, several groups found linkage to a cluster of genes encoding neuronal Na^+ channel α-subunits on chromosome 2q21-33 (Table **1**) and the first two point mutations were detected in *SCN1A* predicting amino acid changes within the voltage sensors (S4 segments) of domains II and IV [69] (Fig. **4A**). Many more *SCN1A* mutations have been described since then in $GEFS^+$ families (Fig. **4A**) and there is evidence for further genetic as well as clinical heterogeneity [70, 71], beside the mutations in the $GABA_A$ receptor mentioned below.

A few of these mutations have been expressed in human embryonic kidney cells or Xenopus oocytes revealing both gain- and loss-of-function mechanisms. Gain-of-function alterations so far described were an acceleration of recovery from inactivation shortening the refractory period after an action potential (R1648H) [72, 73], increased persistent sodium currents predicting membrane depolarization (T875M, W1204R, R1648H) [74], hyperpolarizing shift in window current (W1204R) [75], and resistance to the decrease in channel activity upon high frequency depolarizations (D188V) [76]. However, loss-of-function mechanisms were described in part for the same mutations such as enhanced fast and slow inactivation (T875M, R1648H) [72, 73, 77] or a depolarizing shift of the steady-state activation curve (I1656M, R1657C) [78] which all reduce the amount of available sodium channels. Even a complete loss-of-function was described for two other $GEFS^+$ point mutations (V1353L, A1685V) [78]. Hence, loss-of-function mechanisms seem to predominate for $GEFS^+$ which is in agreement with the genetic findings in SMEI, as will be outlined below.

In contrast to the point mutations found in $GEFS^+$ families, most of the SMEI patients carry de novo nonsense mutations predicting truncated proteins without function [79] (Fig. **4B**). One SMEI point mutation was also shown to yield non-functional channels when expressed in human embryonic kidney cells [78]. The point mutations found for ICEGTC patients [66] have not been functionally examined so far. Interestingly, the sodium channel blocker lamotrigine, the only drug of this class which is in use in patients with idiopathic generalized epilepsies, deteriorates the clinical situation in SMEI patients; in particular, it can lead to an increased number of myoclonic seizures [80], similar as in juvenile myoclonic epilepsy [81]. This observation confirms that SMEI is a loss-of-function sodium channel disorder caused by haploinsufficiency of *SCN1A* and from a genetic and clinical point of view – like ICEGTC – a severe allelic variant of $GEFS^+$. The percentage of SMEI patients carrying sodium channel mutations is high but varies between 35 and 100% in different studies (references in Table **1**).

As a loss-of-function of a voltage-gated sodium channel decreases membrane excitability, it seems paradoxical that such mutations can cause epilepsy. However, when acting predominantly on inhibitory neurons, this effect could well be responsible for the occurrence of hyperexcitability in neuronal circuits inducing epileptic seizures. Since *SCN1A* was not described to be expressed selectively in inhibitory neurons [82, 83], a predominant effect of *SCN1A* mutations on inhibitory neurons is not evident and has to be demonstrated by further investigations.

$GABA_A$ Receptor Defects in $GEFS^+$ and Absence Epilepsy with Febrile Seizures

Confirming the hypothesis of an 'inhibited inhibition' (disinhibition) as a pathophysiological mechanism for $GEFS^+$, subsequently mutations were found in the γ_2-subunit of the $GABA_A$ receptor (gene *GABRG2*) in two $GEFS^+$ families. One of these families presented with a typical $GEFS^+$ phenotype (FS and FS^+; mutation K289M [84]), the other one with a frequent combination of FS and absence seizures beside other epilepsy syndromes described for $GEFS^+$ (mutation R43Q [85]). Only two more mutations were found in other samples up to now, one of those in a $GEFS^+$ family with one affected individual suffering from SMEI [86]. However, a bilineal inheritance with another yet unknown mutation in this patient was postulated so that it is questionable that *GABRG2* can be considered as a SMEI gene. The other mutation occurred in a family in which all affected individuals were diagnosed as having childhood absence epilepsy (CAE) and FS [87] (Fig. **5**).

Functional studies of all *GABRG2* mutations expressed in Xenopus oocytes or mammalian cell lines (γ_2-subunits together with α_1- and β_2- or β_3-subunits) revealed a more or less pronounced loss-of-function as a common pathogenic mechanism. The main gating defects described for the K289M mutation were a decrease in the macroscopic GABA-response and an increased rate of deactivation [84, 88]. For the R43Q mutation, results from two different groups were controversial, which may be explained by the use of different β-subunits for functional studies. A reduction of the benzodiazepine potentiation and increased rate of desensitization [85, 89] could not be confirmed [88]. Instead, a reduced macroscopic current was found by the other group [88]. The other two mutations presumably lead to a complete loss-of-function. Q351X did not yield measurable currents and confocal imaging with green-fluorescent protein (GFP)-tagged receptors revealed no surface expression of the truncated protein [86]. The second mutation alters a splice donor site predicting a non-functional protein as well [87]. Hence, all $GABA_A$ receptor mutations directly reduce the main mechanism for neuronal inhibition in the brain which can well explain the occurrence of epileptic seizures.

IDIOPATHIC GENERALIZED EPILEPSIES (IGE)

Clinical Picture

The four classical and most common subtypes of IGE are childhood and juvenile absence epilepsy (CAE, JAE), juvenile myoclonic epilepsy (JME), and epilepsy with Grand-Mal seizures on awakening (EGMA) [3]. Absence seizures in CAE manifest typically around the 6^{th} year of life. They are of short duration, usually about 10 s, and characteristically occur in clusters of up to 100 seizures a day. The EEG shows typical 3-4 Hz generalized spike and wave discharges. During adolescence, generalized tonic-clonic seizures can develop. Absence seizures in JAE are principally similar. The age of manifestation is around puberty and the number of seizures is considerably lower, the typical clusters are usually not observed. Generalized

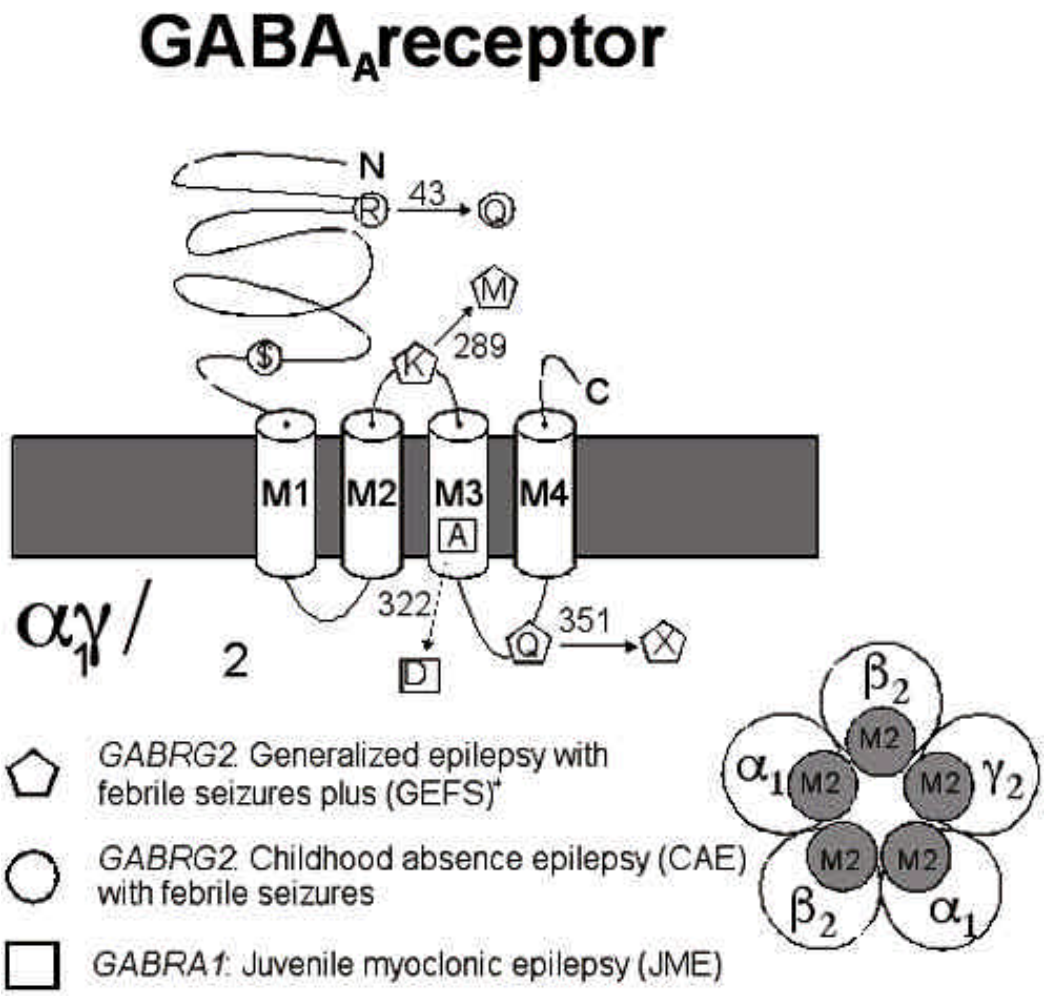


Fig. (5). Proposed transmembrane structure of the $GABA_A$ receptor with mutations causing $GEFS^+$, CAE with febrile seizures or JME (references in Table 1) (\$: splice site mutation). The main $GABA_A$ receptor in the brain is a pentamer (as the nAChR, compare Fig. 2), composed of 2 α_1-, 2 β_2- and 1 γ_2-subunits (inset lower right). Each subunit has 4 transmembrane segments (M1-M4), the M2 segments forming the ion pore.

tonic-clonic seizures occur more frequently than in CAE and some patients develop additional myoclonic seizures. Generalized myoclonic seizures with bilateral myoclonic jerks in particular of the upper extremities and without loss of consciousness are the hallmark of JME. The disease also manifests during puberty. Typically, seizures develop after awakening and can be provoked by sleep deprivation. Generalized tonic-clonic seizures occur in about 75% of patients. The EEG mostly shows generalized polyspikes. Epilepsy with Grand-Mal (generalized tonic-clonic) seizures on awakening develops in adolescence. Seizures usually occur in the first hours after the patients awake, independent of the day-time.

Genetics – General Aspects

In contrast to the syndromes discussed in the other parts of this article, IGE follows a complex genetic inheritance with the exception of rare large families presenting as an apparent dominant trait. While some of these families might really have a monogenic background, in most of them probably a major gene effect dominates the familial disease pattern which in addition depends on other modifying genes or on the genetic background. Different approaches have been used to identify IGE-causing genes. Linkage has been performed in single large families with many affected individuals, as well as in large samples of small, nuclear families. Furthermore, direct candidate gene approaches have been used. The results of these studies are summarized in Table **1**. Using all of these three approaches, the first mutations in ion channel encoding genes have been identified recently.

$GABA_A$ Receptor Defects

In addition to mutations in *GABRG2* causing $GEFS^+$ or CAE with febrile seizures, a mutation was identified in *GABRA1*, the gene encoding the α_1-subunit of the $GABA_A$ receptor, in a large family with pure JME [90]. As described above for all mutations in the γ_2-subunit, the α_1-subunit mutation, A322D (Fig. **5**), leads to a pronounced loss-of-function of the $GABA_A$ receptor. The main molecular

mechanisms are a shift in the GABA sensitivity by two orders of magnitude and probably a reduced surface expression [90]. Thus, the spectrum of epilepsy syndromes which can be caused by direct GABAergic disinhibition extends from classical IGE phenotypes to febrile seizures.

Chloride Channel Defects

A second IGE gene identified, and the first one in which mutations are associated with all four classical IGE subtypes, is *CLCN2* encoding the voltage-gated Cl^- channel ClC-2. A search for mutations in this gene was initiated after a genome scan in nuclear IGE families had shown significant linkage to chromosome 3q26 at which *CLCN2* is located [91]. ClC-2 has been proposed to play an important role in neuronal Cl^- homeostasis [92, 93], which is crucial for GABAergic inhibition, and therefore *CLCN2* was chosen as an excellent candidate gene. $GABA_A$ receptors are synaptic ion channels which open in response to GABA to induce a passive flow of Cl^- ions. Their potential to inhibit the postsynaptic neuron therefore critically depends on the transmembrane Cl^- gradient. Since an inflow of Cl^- ions hyperpolarizes the cell membrane whereas an outflow leads to a depolarization, $[Cl^-]_i$ has to be kept on a low level to enable and maintain an inhibitory GABA response. ClC-2 is a voltage-gated Cl^- channel with strong expression in brain [94, 95]. The channel is activated upon membrane hyperpolarization at potentials more negative than the Cl^- equilibrium potential (E_{Cl}) and thus constitutes an exclusive efflux pathway for Cl^- ions at an increased $[Cl^-]_i$, well suited to maintain an inhibitory GABA response [92, 96].

Three mutations within *CLCN2* were described in three unrelated families affected with JME or EGMA, CAE or EGMA, or JAE, respectively. The mutations predict (i) a premature stop codon (M200fsX231), (ii) an atypical splicing (del74-117), and (iii) a single amino acid substitution (G715E). Functional characterization of all three mutations in a mammalian cell line (tsA201) revealed distinct explanations for their pathogenic phenotypes. M200fsX231 and

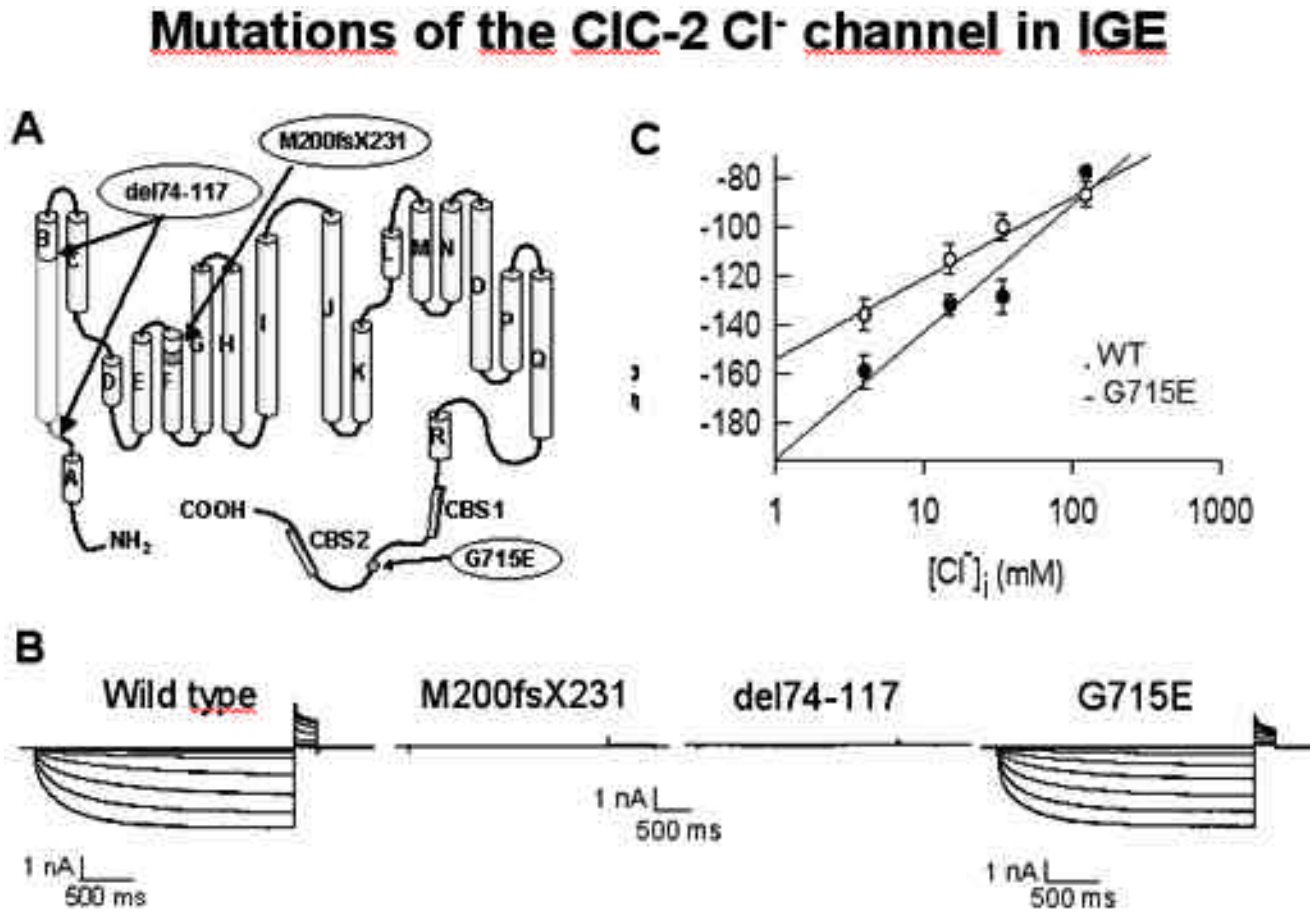


Fig. (6). (A) Proposed transmembrane structure of the ClC-2 Cl^- channel derived from the crystal structure of a bacterial Cl^- channel [161] with two mutations (M200fsX231, G715E) found in two independent IGE families and a splice variant lacking exon 3 (del74-117) which is expressed at higher levels due to an intronic deletion identified in a third IGE family [96]. (B) Cl^- currents recorded with the whole-cell patch clamp technique from transfected human embryonic kidney cells (tsA201). M200fsX231 and del74-117 cause a complete loss-of-function, whereas G715E shows Cl^- currents similar to the wild type channel but with an altered voltage- and Cl^--dependent gating as shown in (C). (C) Midpoint of voltage-dependent activation ($V_{0.5}$) plotted against the internal Cl^- concentration. In the range of physiological Cl^- concentrations the activation curve shifted in the depolarizing direction for G715E compared to the wild type (modified after [96]).

del74-117 caused a dominant negative loss-of-function of ClC-2 channels which should decrease the transmembrane Cl^- gradient and lead to a reduced inhibitory GABA response. G715E lead to a depolarizing shift of voltage- and Cl^--dependent activation of the channel, i.e. a gain-of-function mechanism, inducing membrane depolarization and hyperexcitability due to an increased Cl^- outflow. Hence, both mechanisms finally should induce a hyperexcitability of the postsynaptic membrane in GABAergic synapses which can explain the occurrence of epileptic seizures [96]. These results underline an important role of ClC-2 in GABAergic inhibition and confirm the crucial role of GABAergic inhibition in the pathophysiology of idiopathic epilepsies which has already been discussed in previous paragraphs of this article.

Furthermore, two recent reports point to similar molecular pathophysiological aspects in mesial temporal lobe epilepsy, the most frequent form of acquired partial epilepsies. For both (i) human epileptic tissue derived from patients undergoing epilepsy surgery due to hippocampal sclerosis and (ii) a bihippocampal animal model with induction of an epileptic mirror focus, it could be shown that epileptic activity goes along with a paradoxical GABAergic excitation and shift in the chloride equilibrium potential [97, 98]. This might be explained by a secondary change of chloride transport proteins or channels as ClC-2.

Calcium Channel Mutations

Finally, direct candidate gene approaches also lead to the discovery of mutations in ion channel encoding genes in IGE patients. One mutation was discovered in a patient with JME in the gene *CACNB4*, encoding the β_4-subunit of the high voltage-gated L-type Ca^{2+} channel, predicting a premature stop codon (R482X), another mutation in a father and son affected with JAE (C104F). The latter mutation was also detected in another family with episodic ataxia type II (EA-2). Functional studies revealed small differences in channel gating for R482X and both mutations lead to little but significant reduction in current size [99]. Similarly, a mutation was found in *CACNA1A*, predicting a R1820X truncation of the α_1-subunit of the same channel complex, in a patient suffering from IGE, episodic and progressive ataxia and cognitive impairment. Functional studies as well showed impaired channel function [100]. Naturally occurring mutations in different subunits of the same channel complex are well known to cause epilepsy with generalized spike and wave discharges in several mouse models [101].

In a chinese study with CAE patients, 20 different mutations were recently discovered in *CACNA1H*, the gene encoding the α_1-subunit of a low-voltage-activated T-type Ca^{2+} channel [102]. The first functional studies of five of these mutations indicate a gain-of-function for some of them [103]. The results are very interesting with regard to the pathophysiology of absence seizures. Thalamic neurons in thalamo-cortical loops are thought to generate the rhythmic activity underlying generalized spike and wave discharges and T-type Ca^{2+} channels play a crucial role for the depolarizing phase of the rhythmic activity in these thalamic neurons [104]. Ethusuximide, an efficient drug in the treatment of absence seizures, blocks T-type Ca^{2+} channels. A knock-out mouse of another T-type Ca^{2+} channel α_1-subunit (gene *CACNA1G*) is resistant to the induction of spike and wave discharges [105]. Thus, a gain-of-function is the expected pathomechanism such as already observed for some of the mutations in *CACNA1H* associated with CAE [103].

IMPLICATIONS FOR THERAPY

The discovery of genetic defects and in particular the electrophysiological characterization of mutant ion channels elucidates pathophysiological concepts of hyperexcitability in the brain. This knowledge enables new therapeutic strategies by antagonizing the epilepsy-causing mechanisms. In particular, the defective proteins can be used as new pharmacological targets. In the cause of identifying the genetic defect of BFNC, a completely novel approach of anticonvulsive treatment emerged from identifying retigabine as an activator of M-currents conducted by *KCNQ2* and *KCNQ3* K^+ channels. Retigabine shifts the voltage dependence of activation of these channels by about 20 mV in the hyperpolarizing direction so that they are active at the resting membrane potential [106]. This stabilizes the cell membrane via hyperpolarization towards the K^+ equilibrium potential, a very potent anticonvulsive mechanism, which is not in clinical use up to now.

CONCLUDING REMARKS

In summary, a tremendous progress has been made in recent years to understand the genetic and pathophysiologic basis of idiopathic epilepsies. After the discovery of the first gene defects in rare monogenic syndromes, we now get the first positive and encouraging results also for the common epilepsy syndromes. Both focal and generalized idiopathic epilepsies are almost exclusively caused by ion channel mutations (Table **1**). This emerges as a plausible pathophysiological concept, since defects of ion transport by mutations in channel genes usually only affect the excitability of neurons and neuronal networks but do not induce structural lesions. GABAergic inhibition plays a central role in the pathogenesis of idiopathic generalized epilepsies and there are parallels to pathophysiolgical concepts of acquired focal epilepsy syndromes. These results strongly enhance our knowledge about the etiology and pathogenesis of the epilepsies and hopefully will contribute to develop new therapies. One such novel anticonvulsive mechanism, the activation of a neuronal potassium conductance, could already be elucidated on the molecular level by the identification of a new epilepsy gene.

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REFERENCES

[1] Hauser WA, Annegers JF, Rocca WA. Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester, Minnesota. Mayo Clin Proc 1996; 71: 576-86.

[2] Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised clinical and electroencephalographic classification of epileptic seizures. Epilepsia 1981; 22: 489-501.

[3] Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 1989; 30: 389-99.

[4] Mulley JC, Scheffer IE, Petrou S, Berkovic SF. Channelopathies as a genetic cause of epilepsy. Curr Opin Neurol 2003; 16: 171-6.

[5] Noebels JF. The biology of epilepsy genes. Annu Rev Neurosci 2003; 26: 599-625.

[6] Steinlein OK. Channelopathies can cause epilepsy in man. Eur J Pain 2002; 6: 27-34.

[7] Hille B. Ionic channels of excitable membranes. Sunderland: Sinauer. 3rd edition, 2001

[8] Siegelbaum SA, Koester J. Ion channels. In: Kandel ER, Schwartz JH, Jessel MT Eds, Principles of neural science. New York, Mc Graw Hill. 2000; 105-24

[9] Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 2000; 26: 13-25.

[10] Lehmann-Horn F, Jurkat-Rott K. Voltage-gated ion channels and hereditary disease. Physiol Rev 1999; 79: 1317-72.

[11] Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, *et al*. Nomenclature of voltage-gated sodium channels. Neuron 2000; 28: 365.

[12] Chandy KG, Gutman GA. Voltage-gated K^+ channel genes. In: North RA Ed, Handbook of Receptors and Channels. Ligand- and voltage-gated ion channels. Boca Raton, CRC Press 1995; 1-71

[13] Kandel ER, Siegelbaum SA. Synaptic integration. In: Kandel ER, Schwartz JH, Jessel MT Eds, Principles of neural science. New York, Mc Graw Hill. 2000; 207-28

[14] Hogg RC, Raggenbass M, Bertrand D. Nicotinic acetylcholine receptors: from structure to brain function. Rev Physiol Biochem Pharmacol 2003; 147: 1-46.

[15] Mehta AK, Ticku MK. An update on GABA-A receptors. Brain Res Brain Res Rev 1999; 29: 196-217.

[16] Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Hoger H, *et al*. Structure and subunit composition of GABA(A) receptors. Neurochem Int 1999; 34: 379-85.

[17] Scheffer IE, Bhatia KP, Lopes-Cendes I, Fish DR, Marsden CD, Andermann E, *et al*. Autosomal dominant nocturnal frontal lobe epilepsy: a distinct clinical disorder. Brain 1995; 118: 61-73.

[18] Picard F, Baulac S, Kahane P, Hirsch E, Sebastianelli R, Thomas P, *et al*. Dominant partial epilepsies. A clinical, electrophysiological and genetic study of 19 European families. Brain 2000; 123: 1247-62.

[19] Scheffer IE, Bhatia KP, Lopes-Cendes I, Fish DR, Marsden CD, Andermann F, *et al*. Autosomal dominant frontal epilepsy misdiagnosed as sleep disorder. Lancet 1994; 343: 515-17.

[20] Phillips HA, Scheffer IE, Berkovic SF, Hollway GE, Sutherland GR, Mulley JC. Localization of a gene for autosomal dominant nocturnal frontal lobe epilepsy to chromosome 20q 13.2. Nat Genet 1995; 10:117-8.

[21] Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, Sutherland GR, *et al*. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. Nat Genet 1995; 11: 201-3.

[22] De Fusco M, Becchetti A, Patrignani A, Annesi G, Gambardella A, Quattrone A, *et al*. The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. Nat Genet 2000; 26: 275-6.

[23] Phillips HA, Favre I, Kirkpatrick M, Zuberi SM, Goudie D, Heron SE, *et al.* CHRNB2 is the second acetylcholine receptor subunit associated with autosomal dominant nocturnal frontal lobe epilepsy. Am J Hum Genet 2001; 68: 225-31.

[24] Weiland S, Witzemann V, Villarroel A, Propping P, Steinlein O. An amino acid exchange in the second transmembrane segment of a neuronal nicotinic receptor causes partial epilepsy by altering ist desensitization kinetics. FEBS Lett 1996; 398: 91-6.

[25] Kuryatov A, Gerzanich V, Nelson M, Olale F, Lindstrom J. Mutation causing autosomal dominant nocturnal frontal lobe epilepsy alters Ca^{2+} permeability, conductance, and gating of human alpha4beta2 nicotinic acetylcholine receptors. J Neurosci 1997; 17: 9035-47.

[26] Bertrand S, Weiland S, Berkovic SF, Steinlein OK, Bertrand D. Properties of neuronal nicotinic acetylcholine receptor mutants from human suffering from autosomal dominant nocturnal frontal lobe epilepsy. Brit J Pharmacol 1998; 125: 751-60.

[27] Steinlein OK, Magnusson A, Stoodt J, Bertrand S, Weiland S, Berkovic SF, *et al*. An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy. Hum Mol Genet 1997; 6: 943-7.

[28] Figl A, Viseshakul N, Shafaee N, Forsayeth J, Cohen BN. Two mutations linked to nocturnal frontal lobe epilepsy cause use-dependent potentiation of the nicotinic Ach response. J Physiol 1998; 513: 655-70.

[29] Bertrand D, Picard F, Le Hellard S, Weiland S, Favre I, Phillips H, *et al*. How mutations in the nAChRs can cause ADNFLE epilepsy. Epilepsia 2002; 43: 112-22.

[30] Leniger T, Kananura C, Hufnagel A, Bertrand S, Bertrand D, Steinlein OK. A new Chrna4 mutation with low penetrance in nocturnal frontal lobe epilepsy. Epilepsia 2003; 44: 981-5.

[31] Rodrigues-Pinguet N, Jia L, Li M, Figl A, Klaassen A, Truong A, *et al*. Five ADNFLE mutations reduce the Ca2+ dependence of the mammalian alpha4beta2 acetylcholine response. J Physiol 2003; 550: 11-26.

[32] Ross SA, Wong JY, Clifford JJ, Kinsella A, Massalas JS, Horne MK, *et al.* Phenotypic characterization of an alpha 4 neuronal nicotinic acetylcholine receptor subunit knock-out mouse. J Neurosci 2000; 20: 6431-41.

[33] Labarca C, Schwarz J, Deshpande P, Schwarz S, Nowak MW, Fonck C, *et al*. Point mutant mice with hypersensitive alpha 4 nicotinic receptors show dopaminergic deficits and increased anxiety. Proc Natl Acad Sci USA 2001; 98: 2786-91.

[34] Fonck C, Nashmi R, Deshpande P, Damaj MI, Marks MJ, Riedel A, *et al*. Increased sensitivity to agonist-induced seizures, straub tail, and hippocampal theta rhythm in knock-in mice carrying hypersensitive alpha 4 nicotinic receptors. J Neurosci 2003; 23: 2582-90.

[35] Ronen GM, Rosales TO, Connolly M, Anderson VE, Leppert M. Seizure characteristics in chromosome 20 benign familial neonatal convulsions. Neurology 1993; 43: 1355-60.

[36] Plouin P. Benign idiopathic neonatal convulsions (familial and non-familial): open questions about these syndromes. In: Wolf P Ed, Epileptic seizures and syndromes. London, John Libbey & Co. 1994; 193-201

[37] Leppert M, Anderson VE, Quattlebaum T, Stauffer D, O'Connell P, Nakamura Y, *et al*. Benign familial neonatal convulsions linked to genetic markers on chromosome 20. Nature 1989; 337: 647-8.

[38] Lewis TB, Leach RJ, Ward K, O'Connell P, Ryan SG. Genetic heterogeneity in benign familial neonatal convulsions: identification of a new locus on chromosome 8q. Am J Hum Genet 1993; 53: 670-5.

[39] Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, *et al*. A potassium channel mutation in neonatal human epilepsy. Science 1998; 279: 403-6.

[40] Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, *et al*. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nat Genet 1998; 18: 25-29.

[41] Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ, *et al*. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nat Genet 1998; 18: 53-5.

[42] Jentsch TJ. Neuronal KCNQ potassium channels: physiology and role in disease. Nat Neurosci Rev 2000; 1: 21-30.

[43] Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, *et al*. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet 1996; 12: 17-23.

[44] Kubisch C, Schroeder BC, Friedrich T, Lütjohann B, El-Amraoui A, Martin S, *et al*. KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. Cell 1999; 96: 437-46.

[45] Chouabe C, Neyroud N, Guicheney P, Lazdunski M, Romey G, Barhanin J. Properties of KvLQT1 K+ channel mutations in Romano-Ward and Jervell and Lange-Nielsen inherited cardiac arrhythmias. EMBO J 1997; 16: 5472-9.

[46] Wollnik B, Schroeder BC, Kubisch C, Esperer HD, Wieacker P, Jentsch TJ. Pathophysiological mechanisms of dominant and recessive KVLQT1 K+ channel mutations found in inherited cardiac arrhythmias. Hum Mol Gen 1997; 6: 1943-9.

[47] Schroeder BC, Kubisch C, Stein V, Jentsch TJ. Moderate loss of function of cyclic-AMP-modulated KCNQ2/KCNQ3 K^+ channels causes epilepsy. Nature 1998; 396: 687-90.

[48] Lerche H, Biervert C, Alekov AK, Schleithoff L, Lindner M, Klingler W, *et al*. A reduced K^+ current due to a novel mutation in KCNQ2 causes neonatal convulsions. Ann Neurol 1999; 46: 305-12.

[49] Singh NA, Westenskow P, Charlier C, Pappas C, Leslie J, Dillon J, *et al*. BFNC Physician Consortium. KCNQ2 and KCNQ3 potassium channel genes in benign familial neonatal convulsions: expansion of the functional and mutation spectrum. Brain 2003; 126: 2726-37.

[50] Yang W-P, Levesque PC, Little WA, Conder ML, Ramakrishnan P, Neubauer MG, *et al*. Functional Expression of two KvLQT1-related potassium channels responsible for an inherited idiopathic epilepsy. J Biol Chem 1998; 273: 19419-23.

[51] Wang HS, Pan Z, Shi W, Brown BS, Wymore RS, Cohen IS, *et al*. KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. Science 1998; 282: 1890-3.

[52] Schmitt N, Schwarz M, Peretz A, Abitbol I, Attali B, Pongs O. A recessive C-terminal Jervell and Lange-Nielsen mutation of the KCNQ1 channel impairs subunit assembly. EMBO J 2000; 19: 332-40.

[53] Schwake M, Jentsch TJ, Friedrich T. A carboxy-terminal domain determines the subunit specificity of KCNQ K+ channel assembly. EMBO Rep 2003; 4: 76-81.

[54] Maljevic S, Lerche C, Seebohm G, Alekov AK, Busch AE, Lerche H. C-terminal interaction of KCNQ2 and KCNQ3 K^+ channels. J Physiol 2003; 548: 353-60.

[55] Schwake M, Pusch M, Kharkovets T, Jentsch TJ. Surface expression and single channel properties of KCNQ2/KCNQ3, M-type K^+ channels involved in epilepsy. J Biol Chem 2000; 275: 13343-8.

[56] Smith JS, Ianotti CA, Dargis P, Christian EP, Aiyar J. Differential expression of KCNQ2 splice variants: implications to M-current function during neuronal development. J Neurosci 2001; 21: 1096-103.

[57] Watanabe K, Yamamoto N, Negoro T, Takaesu E, Aso K, Furune S, *et al*. Benign complex partial epilepsies in infancy. Pediatr Neurol 1987; 3: 208-11.

[58] Vigevano F, Fusco L, Di Capua M, Ricci S, Sebastianelli R, Lucchini P. Benign infantile familial convulsions. Eur J Pediatr 1992; 151: 608-12.

[59] Heron SE, Crossland KM, Andermann E, Phillips HA, Hall AJ, Bleasel A, *et al*. Sodium-channel defects in benign familial neonatal-infantile seizures. Lancet 2002; 360: 851-2.

[60] Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. Brain 1997; 120: 479-90.

[61] Singh R, Scheffer IE, Crossland K, Berkovic SF. Generalized epilepsy with febrile seizures plus: a common childhood-onset genetic epilepsy syndrome. Ann Neurol 1999; 45: 75-81.

[62] Dravet C. Les éplepsies graves de l'enfant. Vie Med 1978; 8: 543-8.

[63] Singh R, Andermann E, Whitehouse WP, Harvey AS, Keene DL, Seni MH, *et al*. Severe myoclonic epilepsy of infancy: extended spectrum of GEFS+? Epilepsia 2001; 42: 837-44.

[64] Scheffer IE. Severe infantile epilepsies: molecular genetics challenge clinical classification. Brain 2003; 126: 513-4.

[65] Seino M, Higashi T. A group of patients with infant onset secondary generalized epilepsy characterized by refractory grand mal seizures. [Japanese]. In: Kimura M Ed, Mental and physical disability research group sponsored by Ministry of Health and Welfare. Report of research group of children's health and environment (1978). 1979; 79-80

[66] Fujiwara T, Sugawara T, Mazaki-Miyazaki E, Takahashi Y, Fukushima K, Watanabe M, *et al*. Mutations of sodium channel alpha subunit type 1 (SCN1A) in intractable childhood epilepsy with frequent generalized tonic-clonic seizures. Brain 2003; 126: 531-46.

[67] Wallace RH, Wang DW, Singh R, Scheffer IE, George AL Jr, Phillips HA, *et al*. Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta1 subunit gene SCN1B. Nat Genet 1998; 19: 366-70.

[68] Meadows LS, Malhotra J, Loukas A, Thyagarajan V, Kazen-Gillespie KA, Koopman MC, *et al*. Functional and biochemical analysis of a sodium channel beta1 subunit mutation responsible for generalized epilepsy with febrile seizures plus type 1. J Neurosci 2002; 22: 10699-709.

[69] Escayg AP, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, *et al*. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS$^+$2. Nat Genet 2000; 24: 343-5.

[70] Lerche H, Weber YG, Baier H, Jurkat-Rott K, Kraus de Camargo O, Ludolph AC, *et al*. Generalized epilepsy with febrile seizures plus: Further heterogeneity in a large family. Neurology 2001; 57: 1191-8.

[71] Bonanni P, Malcarne M, Moro F, Veggiotti P, Buti D, Ferrari AR, *et al*. Generalized epilepsy with febrile seizures plus (GEFS+): clinical spectrum in seven Italian families unrelated to SCN1A, SCN1B, and GABRG2 gene mutations. Epilepsia 2004; 45: 149-58.

[72] Alekov AK, Rahman MM, Mitrovic N, Lehmann-Horn F, Lerche H. A sodium channel mutation causing epilepsy in man exhibits subtle defects in fast inactivation and activation *in vitro*. J Physiol 2000; 529: 533-9.

[73] Spampanato J, Escayg A, Meisler MH, Goldin AL. Functional effects of two voltage-gated sodium channel mutations that cause generalized epilepsy with febrile seizures plus type 2. J Neurosci 2001; 21: 7481-90.

[74] Lossin C, Wang DW, Rhodes TH, Vanoye CG, George AL Jr. Molecular basis of an inherited epilepsy. Neuron 2002; 34: 877-84.

[75] Spampanato J, Escayg A, Meisler MH, Goldin AL. Generalized epilepsy with febrile seizures plus type 2 mutation W1204R alters voltage-dependent gating of Na(v)1.1 sodium channels. Neuroscience 2003; 116: 37-48.

[76] Cossette P, Loukas A, Lafreniere RG, Rochefort D, Harvey-Girard E, Ragsdale DS, *et al*. Functional characterization of the D188V mutation in neuronal voltage-gated sodium channel causing generalized epilepsy with febrile seizures plus (GEFS). Epilepsy Res 2003; 53: 107-17.

[77] Alekov AK, Rahman MM, Mitrovic N, Lehmann-Horn F, Lerche H. Enhanced inactivation and acceleration of activation of the sodium channel associated with epilepsy in man. Eur J Neurosci 2001; 13: 2171-6.

[78] Lossin C, Rhodes TH, Desai RR, Vanoye CG, Wang D, Carniciu S, *et al*. Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel SCN1A. J Neurosci 2003; 23: 11289-95.

[79] Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. *De novo* mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. Am J Hum Genet 2001; 68: 1327-32.

[80] Guerrini R, Dravet C, Genton P, Belmonte A, Kaminska A, Dulac O. Lamotrigine and seizure aggravation in severe myoclonic epilepsy. Epilepsia 1998; 39: 508-12.

[81] Biraben A, Allain H, Scarabin JM, Schuck S, Edan G. Exacerbation of juvenile myoclonic epilepsy with lamotrigine. Neurology 2000; 55: 1758.

[82] Westenbroek RE, Merrick DK, Catterall WA. Differential subcellular localization of the RI and RII Na+ channel subtypes in central neurons. Neuron 1989; 3: 695-704.

[83] Goldin AL. Resurgence of sodium channel research. Annu Rev Physiol 2001; 63: 871-94.

[84] Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme JF, *et al*. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. Nat Genet 2001; 28: 46-8.

[85] Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, *et al*. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 2001; 28: 49-52.

[86] Harkin LA, Bowser DN, Dibbens LM, Singh R, Phillips F, Wallace RH, *et al*. Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. Am J Hum Genet 2002; 70: 530-6.

[87] Kananura C, Haug K, Sander T, Runge U, Gu W, Hallmann K, *et al*. A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. Arch Neurol 2002; 59: 1137-41.

[88] Bianchi MT, Song L, Zhang H, Macdonald RL. Two different mechanisms of disinhibition produced by GABAA receptor

mutations linked to epilepsy in humans. J Neurosci 2002; 22: 5321-7.

[89] Bowser DN, Wagner DA, Czajkowski C, Cromer BA, Parker MW, Wallace RH, *et al*. Altered kinetics and benzodiazepine sensitivity of a GABAA receptor subunit mutation [gamma 2(R43Q)] found in human epilepsy. Proc Natl Acad Sci USA 2002; 99: 15170-5.

[90] Cossette P, Liu L, Brisebois K, Dong H, Lortie A, Vanasse M, *et al*. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. Nat Genet 2002; 31: 184-9.

[91] Sander T, Schulz H, Saar K, Gennaro E, Riggio MC, Bianchi A, *et al*. Genome search for susceptibility loci of common idiopathic generalised epilepsies. Hum Mol Genet 2000; 9: 1465-72.

[92] Staley KJ. The role of an inwardly rectifying chloride conductance in postsynaptic inhibition. J Neurophysiol 1994; 72: 273-84.

[93] Staley KJ, Smith R, Schaack J, Wilcox C, Jentsch TJ. Alteration of GABAA receptor function following gene transfer of the ClC-2 chloride channel. Neuron 1996; 17: 543-51.

[94] Thiemann A, Gründer S, Pusch M, Jentsch TJ. A chloride channel widely expressed in epithelial and non-epithelial cells. Nature 1992; 365: 57-60.

[95] Cid L, Montrose-Rafizadeh C, Smith DI, Guggino WB, Cutting GR. Cloning of a putative human voltage-gated chloride channel (CLC-2) cDNA widely expressed in human tissues. Hum Mol Genet 1995; 4: 407-13.

[96] Haug K, Warnstedt M, Alekov AK, Sander T, Ramirez A, Poser B, *et al*. Mutations in CLCN2 encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. Nat Genet 2003; 33: 527-32.

[97] Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy *in vitro*. Science 2002; 298: 1418-21.

[98] Khalilov I, Holmes GL, Ben-Ari Y. *In vitro* formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. Nat Neurosci 2003; 6: 1079-85.

[99] Escayg AP, De Waard M, Lee DD, Bichet D, Wolf P, Mayer T, *et al*. Coding and noncoding variation of the human calcium-channel β4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. Am J Hum Genet 2000; 66: 1531-9.

[100] Jouvenceau A, Eunson LH, Spauschus A, Ramesh V, Zuberi SM, Kullmann DM, *et al*. Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. Lancet 2001; 358: 801-7.

[101] Burgess DL, Noebels JL. Single gene defects in mice: the role of voltage-dependent calcium channels in absence models. Epilepsy Res 1999; 36: 111-22.

[102] Chen Y, Lu J, Pan H, Zhang Y, Wu H, Xu K, *et al*. Association between genetic variation of CACNA1H and childhood absence epilepsy. Ann Neurol 2003; 54: 239-43.

[103] Khosravani H, Altier C, Simms B, Hamming KS, Snutch TP, Mezeyova J, *et al*. Gating effects of mutations in the Cav3.2 T-type calcium channel associated with childhood absence epilepsy. J Biol Chem 2004; 279: 9681-4.

[104] Westbrook GL. In: Kandel ER, Schwartz JH, Jessel TM. Principles of neural science. McGraw Hill 2000; 910-35.

[105] Kim D, Song I, Keum S, Lee T, Jeong MJ, Kim SS, *et al*. Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking alpha(1G) T-type Ca(2+) channels. Neuron 2001; 31: 35-45.

[106] Rundtfeld C, Netzer R. The novel anticonvulsant retigabine activates M-currents in Chinese hamster ovary-cells transfected with human KCNQ2/3 subunits. Neurosci Lett 2000; 282: 73-6.

[107] Malafosse A, Leboyer M, Dulac O, Navelet Y, Plouin P, Beck C, *et al*. Confirmation of linkage of benign familial neonatal convulsions to D20S19 and D20S20. Hum Genet 1992; 89: 54-8.

[108] Biervert C, Steinlein OK. Structural and mutational analysis of KCNQ2, the major gene locus for benign familial neonatal convulsions. Hum Genet 1999; 104: 234-40.

[109] del Giudice EM, Coppola G, Scuccimarra G, Cirillo G, Bellini G, Pascotto A. Benign familial neonatal convulsions (BFNC) resulting from mutation of the KCNQ2 voltage sensor. Eur J Hum Genet 2000; 8: 994-7.

[110] Lee WL, Biervert C, Hallmann K, Tay A, Dean JC, Steinlein OK. A KCNQ2 splice site mutation causing benign neonatal convulsions in a Scottish family. Neuropediatrics 2000; 31: 9-12.

[111] Dedek K, Kunath B, Kananura C, Reuner U, Jentsch TJ, Steinlein OK. Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K(+) channel. Proc Nat Acad Sci USA 2001; 98: 12272-7.

[112] Li HY, Tang BS, Zhang AM, Cao QH, Meng GL, Jiang H, *et al*. A novel mutation of KCNQ2 gene in a Chinese family with benign familial neonatal convulsions. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2003; 20: 482-5.

[113] Hirose S, Zenri F, Akiyoshi H, Fukuma G, Iwata H, Inoue T, *et al*. A novel mutation of KCNQ3 (c.925T-->C) in a Japanese family with benign familial neonatal convulsions. Ann Neurol 2000; 47: 822-6.

[114] Guipponi M, Rivier F, Vigevano F, Beck C, Crespel A, Echenne B, *et al*. Linkage mapping of benign familial infantile convulsions (BFIC) to chromosome 19q. Hum Mol Genet 1997; 6: 473-7.

[115] Caraballo R, Pavek S, Lemainque A, Gastaldi M, Echenne B, Motte J, *et al*. Linkage of benign familial infantile convulsions to chromosome 16p12-q12 suggests allelism to the infantile convulsions and choreoathetosis syndrome. Am J Hum Genet 2001; 68: 788-94.

[116] Weber YG, Berger A, Bebek N, Maier S, Karafyllakes S, Meyer N, *et al*. Benign familial infantile convulsions (BFIC): linkage to chromosome 16p12.2-q12.2 in 14 families. Epilepsia 2004, in press.

[117] Malacarne M, Gennaro E, Madia F, Pozzi S, Vacca D, Barone B, *et al*. Benign familial infantile convulsions: mapping of a novel locus on chromosome 2q24 and evidence for genetic heterogeneity. Am J Hum Genet 2001; 68: 1521-6.

[118] Vanmolkot KR, Kors EE, Hottenga JJ, Terwindt GM, Haan J, Hoefnagels WA, *et al*. Novel mutations in the Na+, K+-ATPase pump gene ATP1A2 associated with familial hemiplegic migraine and benign familial infantile convulsions. Ann Neurol 2003; 54: 360-6.

[119] Szepetowski P, Rochette J, Berquin P, Piussan C, Lathrop GM, Monaco AP. Familial infantile convulsions and paroxysmal choreoathetosis: a new neurological syndrome linked to the pericentromeric region of human chromosome 16. Am J Hum Genet 1997; 61: 889-98.

[120] Lee WL, Tay A, Ong HT, Goh LM, Monaco AP, Szepetowski P. Association of infantile convulsions with paroxysmal dyskinesias (ICCA syndrome): confirmation of linkage to human chromosome 16p12-q12 in a Chinese family. Hum Genet 1998; 103: 608-12.

[121] Swoboda KJ, Soong B, McKenna C, Brunt ER, Litt M, Bale JF Jr, *et al*. Paroxysmal kinesigenic dyskinesia and infantile convulsions: clinical and linkage studies. Neurology 2000; 55: 224-30.

[122] Guerrini R, Bonanni P, Nardocci N, Parmeggiani L, Piccirilli M, De Fusco M, *et al.* Autosomal recessive rolandic epilepsy with paroxysmal exercise-induced dystonia and writer's cramp: delineation of the syndrome and gene mapping to chromosome 16p12-11.2. Ann Neurol 1999; 45: 344-52.

[123] Neubauer BA, Fiedler B, Himmelein B, Kampfer F, Lassker U, Schwabe G, *et al*. Centrotemporal spikes in families with rolandic epilepsy: linkage to chromosome 15q14. Neurology 1998; 51: 1608-12.

[124] Audenaert D, Claes L, Ceulemans B, Lofgren A, Van Broeckhoven C, De Jonghe P. A deletion in SCN1B is associated with febrile seizures and early-onset absence epilepsy. Neurology 2003; 61: 854-6.

[125] Baulac S, Gourfinkel-An I, Picard F, Rosenberg-Bourgin M, Prud'homme JF, Baulac M, *et al*. A second locus for familial generalized epilepsy with febrile seizures plus maps to chromosome 2q21-q33. Am J Hum Genet 1999; 65: 1078-85.

[126] Moulard B, Guipponi M, Chaigne D, Mouthon D, Buresi C, Malafosse A. Identification of a new locus for generalized epilepsy with febrile seizures plus (GEFS+) on chromosome 2q24-q33. Am J Hum Genet 1999; 65: 1396-1400.

[127] Pfeiffer A, Thompson J, Charlier C, Otterud B, Varvil T, Pappas C, *et al.* A locus for febrile seizures (FEB3) maps to chromosome 2q23-24. Ann Neurol 1999; 46: 671-8.

[128] Lopes-Cendes I, Scheffer IE, Berkovic SF, Rousseau M, Andermann E, Rouleau GA. A new locus for generalized epilepsy with febrile seizures plus maps to chromosome 2. Am J Hum Genet 2000; 66: 698-701.

[129] Escayg A, Heils A, MacDonald BT, Haug K, Sander T, Meisler MH. A novel SCN1A mutation associated with generalized epilepsy with febrile seizures plus--and prevalence of variants in patients with epilepsy. Am J Hum Genet 2001; 68: 866-73.

[130] Wallace RH, Scheffer IE, Barnett S, Richards M, Dibbens L, Desai RR, *et al*. Neuronal sodium-channel alpha1-subunit mutations in generalized epilepsy with febrile seizures plus. Am J Hum Genet 2001; 68: 859-65.

[131] Abou-Khalil B, Ge Q, Desai R, Ryther R, Bazyk A, Bailey R, *et al*. Partial and generalized epilepsy with febrile seizures plus and a novel SCN1A mutation. Neurology 2001; 57: 2265-72.

[132] Sugawara T, Mazaki-Miyazaki E, Ito M, Nagafuji H, Fukuma G, Mitsudome A, *et al*. Nav1.1 mutations cause febrile seizures associated with afebrile partial seizures. Neurology 2001; 57: 703-5.

[133] Ito M, Nagafuji H, Okazawa H, Yamakawa K, Sugawara T, Mazaki-Miyazaki E, *et al*. Autosomal dominant epilepsy with febrile seizures plus with missense mutations of the (Na+)-channel alpha 1 subunit gene, SCN1A. Epilepsy Res 2002; 48: 15-23.

[134] Annesi G, Gambardella A, Carrideo S, Incorpora G, Labate A, Pasqua AA, *et al*. Two novel SCN1A missense mutations in generalized epilepsy with febrile seizures plus. Epilepsia 2003; 44: 1257-8.

[135] Sugawara T, Mazaki-Miyazaki E, Fukushima K, Shimomura J, Fujiwara T, Hamano S, *et al*. Frequent mutations of SCN1A in severe myoclonic epilepsy in infancy. Neurology 2002; 58: 1122-4.

[136] Ohmori I, Ouchida M, Ohtsuka Y, Oka E, Shimizu K. Significant correlation of the SCN1A mutations and severe myoclonic epilepsy in infancy. Biochem Biophys Res Commun 2002; 295: 17-23.

[137] Claes L, Ceulemans B, Audenaert D, Smets K, Lofgren A, Del-Favero J, *et al*. *De novo* SCN1A mutations are a major cause of severe myoclonic epilepsy of infancy. Hum Mutat 2003; 21: 615-21.

[138] Nabbout R, Gennaro E, Dalla Bernardina B, Dulac O, Madia F, Bertini E, *et al*. Spectrum of SCN1A mutations in severe myoclonic epilepsy of infancy. Neurology 2003; 60: 1961-7.

[139] Wallace RH, Hodgson BL, Grinton BE, Gardiner RM, Robinson R, Rodriguez-Casero V, *et al*. Sodium channel alpha1-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. Neurology 2003; 61: 765-9.

[140] Gennaro E, Veggiotti P, Malacarne M, Madia F, Cecconi M, Cardinali S, *et al*. Familial severe myoclonic epilepsy of infancy: truncation of Nav1.1 and genetic heterogeneity. Epileptic Disord 2003; 5: 21-5.

[141] Wallace RH, Berkovic SF, Howell RA, Sutherland GR, Mulley JC. Suggestion of a major gene for familial febrile convulsions mapping to 8q13-21. J Med Genet 1996; 33: 308-12.

[142] Johnson EW, Dubovsky J, Rich SS, O'Donovan CA, Orr HT, Anderson VE, *et al*. Evidence for a novel gene for familial febrile convulsions, FEB2, linked to chromosome 19p in an extended family from the Midwest. Hum Mol Genet 1998; 7: 63-7.

[143] Nakayama J, Hamano K, Iwasaki N, Nakahara S, Horigome Y, Saitoh H, *et al*. Significant evidence for linkage of febrile seizures to chromosome 5q14-q15. Hum Mol Genet 2000; 9: 87-91.

[144] Nakayama J, Fu YH, Clark AM, Nakahara S, Hamano K, Iwasaki N, *et al*. A nonsense mutation of the MASS1 gene in a family with febrile and afebrile seizures. Ann Neurol 2002; 52: 654-7.

[145] Greenberg DA, Delgado-Escueta AV, Widelitz H, Sparkes RS, Treiman L, Maldonado HM, *et al*. Juvenile myoclonic epilepsy (JME) may be linked to the BF and HLA loci on human chromosome 6. Am J Med Genet 1988; 31: 185-92.

[146] Serratosa JM, Delgado-Escueta AV, Medina MT, Zhang Q, Iranmanesh R, Sparkes RS. Clinical and genetic analysis of a large pedigree with juvenile myoclonic epilepsy. Ann Neurol 1996; 39: 187-95.

[147] Sander T, Bockenkamp B, Hildmann T, Blasczyk R, Kretz R, Wienker TF, *et al*. Refined mapping of the epilepsy susceptibility locus EJM1 on chromosome 6. Neurology 1997; 49: 842-7.

[148] Zara F, Bianchi A, Avanzini G, Di Donato S, Castellotti B, Patel PI, *et al*. Mapping of genes predisposing to idiopathic generalized epilepsy. Hum Mol Genet 1995; 4: 1201-7.

[149] Fong GC, Shah PU, Gee MN, Serratosa JM, Castroviejo IP, Khan S, *et al*. Childhood absence epilepsy with tonic-clonic seizures and electroencephalogram 3-4-Hz spike and multispike-slow wave complexes: linkage to chromosome 8q24. Am J Hum Genet 1998; 63: 1117-29.

[150] Sugimoto Y, Morita R, Amano K, Fong CY, Shah PU, Castroviejo IP, *et al*. Childhood absence epilepsy in 8q24: refinement of candidate region and construction of physical map. Genomics 2000; 68: 264-72.

[151] Elmslie FV, Rees M, Williamson MP, Kerr M, Kjeldsen MJ, Pang KA, *et al*. Genetic mapping of a major susceptibility locus for juvenile myoclonic epilepsy on chromosome 15q. Hum Mol Genet 1997; 6: 1329-34.

[152] Mikami M, Yasuda T, Terao A, Nakamura M, Ueno S, Tanabe H, *et al*. Localization of a gene for benign adult familial myoclonic epilepsy to chromosome 8q23.3-q24.1. Am J Hum Genet 1999; 65: 745-51.

[153] de Falco FA, Striano P, de Falco A, Striano S, Santangelo R, Perretti A, *et al*. Benign adult familial myoclonic epilepsy: genetic heterogeneity and allelism with ADCME. Neurology 2003; 60: 1381-5.

[154] Hirose S, Iwata H, Akiyoshi H, Kobayashi K, Ito M, Wada K, *et al*. A novel mutation of CHRNA4 responsible for autosomal dominant nocturnal frontal lobe epilepsy. Neurology 1999; 53: 1749-53.

[155] Rozycka A, Skorupska E, Kostyrko A, Trzeciak WH. Evidence for S284L mutation of the CHRNA4 in a white family with autosomal dominant nocturnal frontal lobe epilepsy. Epilepsia 2003; 44: 1113-7.

[156] Cho YW, Motamedi GK, Laufenberg I, Sohn SI, Lim JG, Lee H, *et al*. A Korean kindred with autosomal dominant nocturnal frontal lobe epilepsy and mental retardation. Arch Neurol 2003; 60: 1625-32.

[157] Phillips HA, Scheffer IE, Crossland KM, Bhatia KP, Fish DR, Marsden CD, *et al*. Autosomal dominant nocturnal frontal-lobe epilepsy: genetic heterogeneity and evidence for a second locus at 15q24. Am J Hum Genet 1998; 63: 1108-16.

[158] Ottman R, Risch N, Hauser WA, Pedley TA, Lee JH, Barker-Cummings C, *et al*. Localization of a gene for partial epilepsy to chromosome 10q. Nat Genet 1995; 10: 56-60.

[159] Kalachikov S, Evgrafov O, Ross B, Winawer M, Barker-Cummings C, Martinelli Boneschi F, *et al*. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. Nat Genet 2002; 30: 335-41.

[160] Xiong L, Labuda M, Li DS, Hudson TJ, Desbiens R, Patry G, *et al*. Mapping of a gene determining familial partial epilepsy with variable foci to chromosome 22q11-q12. Am J Hum Genet 1999; 65: 1698-710.

[161] Dutzler R, Campbell EB, Cadene M, Chait BT, MacKinnon R. X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity. Nature 2002; 415: 287-94.