

# Ion channels and epilepsy

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**ABSTRACT** – Ion channels play a central role in the generation and control of neuronal excitability. Genetic defects in ion channels are associated with several forms of human idiopathic epilepsies. These defects range from non-sense and missense point mutations to insertion, truncation and splice site mutations producing altered, non-functional or negative-dominant channel subunits. To date, 12 mutated genes have been identified. They code for Na<sup>+</sup> (*SNC1A*, *SNC2A*, *SNC1B*), K<sup>+</sup> (*KCNA1*, *KCNQ2*, *KCNQ3*) and Cl<sup>-</sup> (*CLCN2*) channel subunits, as well as neurotransmitter receptor subunits including Cl<sup>-</sup> channel GABAA receptor (*GABRA1*, *GABRG2*) and cationic channel acetylcholine receptor (*CHRNA4*, *CHRN2*). One ion transporter Na<sup>+</sup>/K<sup>+</sup> ATPase gene (*ATP1A2*) has also been identified. The epilepsy syndromes related to these genes are as diverse as benign familial neonatal (BFNC - *KCNQ2* and *3*) and infantile (BFNIC - *SNC2A* and *ATP1A2*) convulsions, episodic ataxia with seizures (AE2 - *KCNA1*), generalized epilepsy with febrile seizure plus (GEFS+ - *SCN2A*, *1A*, *1B* and *GABRG2*), autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE - *CHRNA4* and *B2*), severe myoclonic epilepsy of infancy (SMEI - *SNC1A*), juvenile myoclonic epilepsy (JME - *GABRA1* and *CLCN2*), and childhood and juvenile absence epilepsy (CAE, JAE - *SNC1B*, *GABRG2* and *CLCN2*). Despite the difficulty to correlate genotypes and phenotypes, these studies have increased our understanding of causal mechanisms of epilepsy and open a wide range of possibilities for developing better antiepileptic drugs and treatments.

**Keywords:** ion channels, epilepsy, epilepsy genetics

Epilepsy is a commonly occurring chronic disease affecting at least 1% of the world population. The international classification of epileptic syndromes identifies idiopathic, symptomatic and cryptogenic epilepsies. The main etiological factor related to idiopathic epilepsy is genetic predisposition, either confirmed or suspected (Baraister, 1990).

To date, mendelian transmission of some rare forms of idiopathic epilepsy has been demonstrated by studies of large families in which many family members suffer from epilepsy. Studies of twins also argue for a genetic origin in idiopathic epilepsies (Berkovic *et al.*, 1998, Corey *et al.*, 1991, Harvold and Hauge 1965, Inouye, 1960, Lennox and Lennox 1960, Sillanpaa *et al.*, 1991). However, most idiopathic epi-

lepsies are genetic disorders showing complex mendelian transmission (Kaneko and Wada 1998), which suggests simultaneous involvement of several genes. The diversity of these "susceptible" genes is likely to play a role in determining relative risk (Delgado-Escueta *et al.*, 1994).

In basic terms, an epileptic seizure can be caused by three types of factors: impaired inhibition, excess of excitation, or faulty regulation of the membrane resting potential. Therefore, ion channels expressed in the central nervous system (CNS) are ideal candidates for the study of mutational polymorphisms in a given patient population. Propagation of electrical impulses in the neurons is initiated by the opening of voltage-gated sodium channels, which allows sodium ion

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**Table 1.** Genetic mutations responsible for various forms of idiopathic epilepsy.

	Subunit	Gene	Locus	Mutations	Epileptic syndrome
<b>Inhibitory currents</b>					
Potassium channel	KCNQ2	KCNQ2	20q13	missense, truncation	BFNC (myokymia, FC+, CTE, GIE)
	KCNQ3	KCNQ3	8q24	missense	BFNC
	Kv1.1	KCNA1	12p13	missense	Type 1 episodic ataxia and partial idiopathic seizures
Chloride channel	CLC-2	CLCN2	3q26	missense, truncation, splicing	Childhood and juvenile absence epilepsy (CAE, JAE) juvenile myoclonic epilepsy, matutinal grand mal epilepsy
GABA A receptor (chloride channel)	$\alpha 1$	GABRA1	5q34	missense	myoclonic epilepsy
	$\gamma 2$	GABRG2	5q34	missense, truncation	GEFS+, JAE
<b>Excitatory currents</b>					
Sodium channel	$\alpha 1$	CNS1A	2q24	missense, nonsense, truncation, splicing (skipped phase)	GEFS+, Dravet
	$\alpha 2$	CNS2A	2q24	missense	GEFS+, BFNC
	$\beta 2$	CNS1B	19q13	missense	GEFS+, JAE
Acetylcholine receptor (cationic channel)	$\alpha 4$	CHRNA4	20q13	missense, insertion	ADNFLE
	$\beta 2$	CHRNA2	1p21	missense	ADNFLE
<b>Ion transporter</b>					
Na/k ATPase (active transporter)	$\alpha 2$	ATP1A2	1q21	missense	BFIC

FC+: febrile convulsions +; BFIC: benign familial infantile convulsions; BFNC: benign familial neonatal convulsions; CTE: centrotemporal epilepsy; ADNFLE: autosomal dominant nocturnal frontal lobe epilepsy; GEFS: generalized epilepsy with febrile seizures; GIE: generalized idiopathic epilepsy; Dravet: severe myoclonic epilepsy of infancy.

influx ( $\text{Na}^+$ ) along their concentration gradient. Action potential ends upon simultaneous activation of voltage-gated potassium channels, followed by potassium ion ( $\text{K}^+$ ) outflow, which restores cell membrane resting potential. Voltage-gated calcium channels at axonal endings allow the conversion of electrical signals to chemical signals thanks to the input of calcium ions ( $\text{Ca}^{2+}$ ) which induce the release of neurotransmitters of synaptic vesicles. These neurotransmitters, such as acetylcholine, GABA or glutamate, subsequently stimulate postsynaptic channel receptors. Fixation of these ligands on their receptors generates a new action potential. Mutations in 12 genes encoding channels and channel receptors are responsible for various forms of idiopathic epilepsy (table 1). These mutations and their connection with epilepsy are the subject of this review.

### Acetylcholine receptors, Autosomal Dominant Nocturnal Frontal Lobe Epilepsy, Paroxysmal Rolandic Epilepsy

#### Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)

This epilepsy is characterized by short seizures occurring during slow sleep (stage 2). Syndrome diagnosis is often

made late in the course of the disorders, following diagnostic speculations all the way from paroxysmal nocturnal dyskinesia to hysteria, and including sleep problems such as night terrors and nightmares (Scheffer *et al.*, 1994).

ADNFLE is an autosomal dominant condition with relatively high penetrance (70-80%) (Scheffer *et al.*, 1995, Picard *et al.*, 2000). Great individual variations in the presentation of the disease have been described within the same family in terms of age at onset and severity of symptoms. In most patients, the first seizures appear in the first or second decade of life. Seizures can start as tremors, grunts or vocalizations, and are sometimes preceded by an aura. Secondary generalization is only seen in rare cases. Focal seizures seen in this epilepsy are those which affect motor activity: disorganized hyperkinetic activity or tonic symptoms, sometimes with added clonic manifestations. Intervals of several years without seizures have been reported. Interictal EEG findings tend to be of little help. Therefore, nocturnal polysomnographic video EEG recordings are useful for confirming the diagnosis.

In the last ten years, important discoveries have made it possible to genetically distinguish three types of ADNFLEs. In the case of type 1 ADNFLE, the study of several families made it possible to describe mutations of the gene encod-

ing the  $\alpha 4$  subunit of the neuronal acetylcholine receptor CHRNA4.

These are point mutations by insertion or by transposition in exon 5 of the gene located on chromosome 20 (20q13.2-q13-3) causing point mutations in the second transmembrane domain (M2) (Phillips *et al.*, 1995). The first mutation found in familial epilepsy was discovered in an Australian family, and consists of the transposition of a cytosine into thymidine. This transposition results in the replacement of a serine, a neutral amino acid, by a phenylalanine, an aromatic residue (mutation S248F) (Steinlein *et al.*, 1995). A mutation by insertion was later described in a Norwegian family; the mutation is due to the insertion of three nucleotides encoding for an additional serine at the extracellular tip of M2 (776 ins 3) (Steinlein *et al.*, 1997). A transposition replacing serine 252 with leucine was also found in a Japanese family (Hirose *et al.*, 2000). An S252L *de novo* mutation found in a woman brought into question the initial diagnosis of sporadic nocturnal frontal lobe epilepsy (Phillips *et al.*, 2000). Analysis of other families led to the identification of two mutations by the transposition of S252L and S248F (Phillips *et al.*, 2000, Steinlein, 2000).

A Korean child in a family of patients with ADNFLE associated with the S252L mutation has been described; in addition to ADNFLE, the child presents mental retardation and poor therapeutic response to carbamazepine (Cho *et al.*, 2003), suggesting the existence of other genetic or environmental factors.

The study of other families has uncovered a link with 15q24. This type 2 ADNFLE locus contains the clusters created when genes encode other subunits for neuronal acetylcholine receptor:  $\alpha 3$ ,  $\alpha 5$  and  $\beta 4$  respectively (CHRNA3, CHRNA5, CHRN4). However, no direct evidence of mutation in these proteins has been uncovered to date (Phillips *et al.*, 1998).

A third locus, type 3 ADNFLE, has been discovered in the pericentromeric region of chromosome 1 (Gambardella *et al.*, 2000). The  $\beta 2$  subunit of the nicotinic channel is encoded in this region. Transposition of a guanine to a cytosine in exon 5 of CHRN2 causes the replacement of valine with leucine in position 287 in M2 (De Fusco *et al.*, 2000). A second mutation of this valine to methionine was identified in a Scottish family (Phillips *et al.*, 2001). These two V287L and V287M mutations affect the same residue, suggesting the possibility of a specific mutation site. Patients presenting mutations in one or the other of subunits  $\alpha 4$  and  $\beta 2$  are clinically identical. Thus, two different genotypes express the same phenotype (McLellan *et al.*, 2003).

Neuronal acetylcholine receptors are formed by the association of several different subunits (*figure 1*). Nicotinic receptors are homologous or heterologous pentamers created from 11 known subunits ( $\alpha 2$ - $\alpha 9$ ;  $\beta 2$ - $\beta 4$ ) (Le Novère and Changeux, 1995). The  $\alpha$  subunits form the sites of interaction with acetylcholine and are characterized by

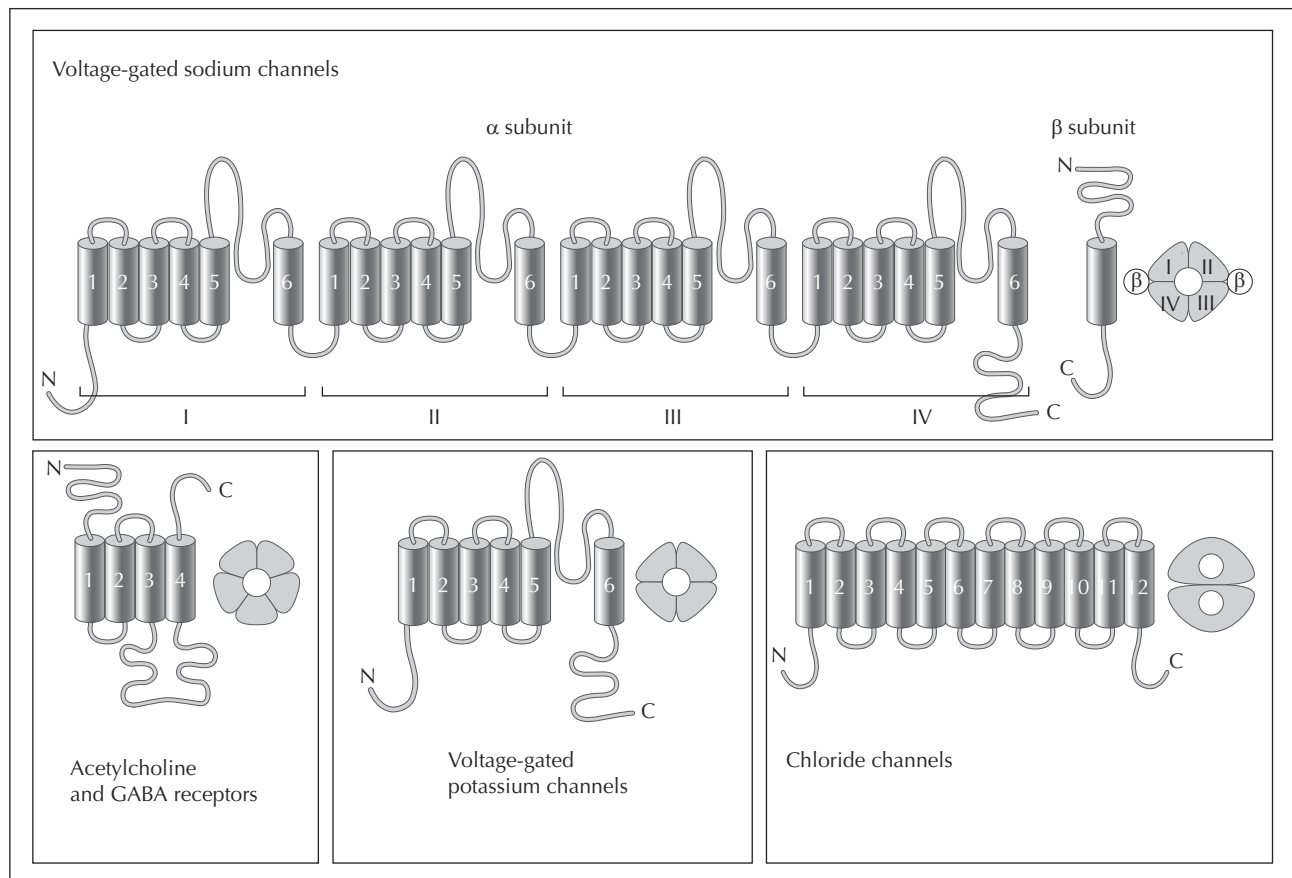
the presence of two adjacent cysteines (positions 192 and 193 in the  $\alpha 1$  subunit of skeletal muscle) (Galzi *et al.*, 1996). Subunit  $\beta$  likely contributes to the pharmacologic specificity of the receptor (Luetje and Patrick, 1991). In the CNS, there are six types of  $\alpha$  subunits ( $\alpha 4$ - $\alpha 7$ ) and three types of  $\beta$  subunits ( $\beta 2$ - $\beta 4$ ) which combine to form receptors with at least two types of different subunits. The predominant combination in the brain is  $\alpha 4/\beta 2$  (Whiting *et al.*, 1991). Cerebral distribution of the subunits of this receptor was described as having numerous variations (Deneris *et al.*, 1989, Duvoisin *et al.*, 1989, Wada *et al.*, 1988, Wada *et al.*, 1989, Park *et al.*, 1997, Zoli *et al.*, 1998). Messenger RNA  $\alpha 4$  and  $\beta 2$  is found in a great number of CNS nuclei, as well as in cholinergic pathways. Although CHRNA4 ( $\alpha 4$ ) and CHRN2 ( $\beta 2$ ) genes are expressed in many regions of the brain, at this time we cannot make a clear correlation between their distribution and electroencephalographic manifestations recorded during critical episodes in the frontal lobe regions and - if the supposition of CHRNA4 in RPE is confirmed (see below) - in the centrotemporal region. The hypothesis of compensation for loss of function of the  $\alpha 4/\beta 2$  receptor by non mutated subunits in regions of the brain other than the frontal lobes is very attractive.

From the physiopathologic point of view, electrophysiological recordings of transfected cells expressing mutated receptors make it possible to define the effects of these mutations on the behaviour of the receptors involved. As far as type 1 ADNFLE is concerned, various *in vitro* studies of these mutations have shown that they produce similar effects:  $\alpha 4$  (S248F),  $\alpha 4$  (776ins3) and  $\beta 2$  (V248F) mutations displace the response curve to acetylcholine to the left (Bertrand *et al.*, 2002). The  $\alpha 4$  (S248F) mutation also increase the potential for receptor activation by acetylcholine (Kuryatov *et al.*, 1997), while the  $\alpha 4$  (77ins3) mutation reduces calcium-dependent activity during receptor activation by acetylcholine (Steinlein *et al.*, 1997).

As for type2 ADNFLEs, mutated receptors  $\beta 2$  (V287L) and (V287M) show great slowing of their desensitization kinetic activities (De Fusco *et al.*, 2000). The V287M mutation also produces an increase in sensitivity to acetylcholine ten times higher than that seen in wild-type receptors (Phillips *et al.*, 2001).

Nicotinic receptors are thought to be located almost exclusively at the presynaptic level, and to regulate the release of neurotransmitters such as glutamate and GABA; the mechanism through which hypoactivity in this channel leads to neuronal hyperactivity is still unknown.

Recently, Pinguet and colleagues (Rodrigues-Pinguet *et al.*, 2003) have formulated the hypothesis that ADNFLE mutations reduce the  $Ca^{2+}$  dependence of the  $\alpha 4/\beta 2$  acetylcholine response. During the synchronic variation phases of light sleep,  $Ca^{2+}$  modulation may present presynaptic acetylcholine receptors from overstimulating glutamate release during repetitive activity. Reducing the  $Ca^{2+}$  dependence of the acetylcholine response could



**Figure 1.** Structure of ion channels and receptor channels associated with epilepsy.  $\text{Na}^+$  channels are formed by the association of the main  $\alpha$  subunits, made up of four repeated regions (I to IV) with six transmembrane helices, and of  $\beta$  subunits with one transmembrane helix. In  $\alpha$  subunits, the N and C terminal endings are cytoplasmic. In  $\beta$  subunits, the N-terminal ending is extracellular. The acetylcholine nicotinic receptors and the GABA receptors are heteropentameric, made up of four-helix transmembrane subunits whose N and C terminal endings are extracellular.  $\text{K}^+$  channels are tetrameric in subunits with six transmembrane helices, and  $\text{Cl}^-$  channels are dimerous in subunits with 12 transmembrane helices. In contrast to other channels, the  $\text{Cl}^-$  channel dimers contain two ionic pores. In the  $\text{K}^+$  and  $\text{Cl}^-$  channels, N and C terminal endings are cytoplasmic.

trigger seizures by increasing  $\alpha 4/\beta 2$ -mediated glutamate release.

From the therapeutic standpoint, the abnormal activity of the identified mutants is blocked by carbamazepine, suggesting that the latter could be used to treat this type of epilepsy. However, the *in vitro* study in question only tested two antiepileptic drugs, sodium valproate and carbamazepine (Picard *et al.*, 1999).

Mice no longer expressing the  $\alpha 4$  subunit (murine KO for  $\alpha 4$ ) do not present epileptic phenotypes spontaneously, but do have a higher epileptogenic threshold than wild-type mice when they are stimulated with proconvulsive agents, and particularly with GABAergic inhibitors like bicuculline (BIC) and pentylenetetrazole (PTZ) (Delgado-Escueta *et al.*, 1994, McColl *et al.*, 2003). Another mouse strain in which a large portion of chromosome 2 is deleted (300kb), including the  $\alpha 4$  encoding gene and the gene encoding the KCNQ2  $\text{K}^+$  channel, which is involved in

another type of familial epilepsy, presents increased susceptibility to electroshocks (Yang *et al.*, 2003).

### Rolandic epilepsy (RE)

This benign epilepsy belongs to the group of partial idiopathic epilepsies. It is the most frequent and the most characteristic of this syndrome group. RPE shows a slight male preponderance; it starts between the ages of three and 13 years, with the average age at onset being 9.9 years; it stops spontaneously before the age of 16. Clinical manifestations include brief motor, clonic, somatomotor or tonic-clonic seizures, hemifacial or affecting the buccopharyngeal-laryngeal area. Seizures are typically sleep-related and are responsible for anarthria and usually without altered consciousness. Secondary generalization is frequent in early progression, with grunt-type vocal manifestations or gurgling related to anarthria and to hy-

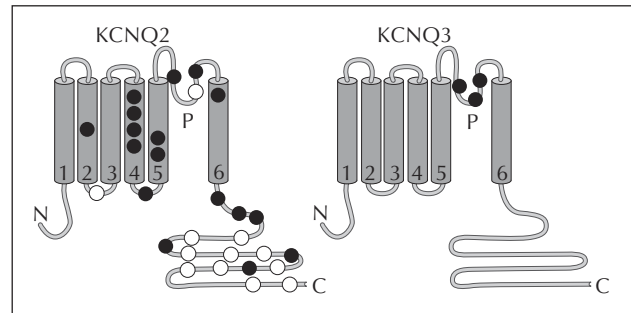
persalivation, which often wake the parents. Symptoms can extend to the ipsilateral upper limb.

A somatosensitive component with unilateral paresthesia is frequent in the regions where motor manifestations occur. The EEG shows slow centroparietal high voltage biphasic spikes, increasing in frequency upon falling asleep and at all stages of sleep, during which they tend to become bilateral. In 1998, Neubauer and his colleagues (Neubauer *et al.*, 1998) demonstrated a link between this epileptic syndrome and the q14 region of chromosome 15, after studying 22 small families. The  $\alpha 7$  subunit of the neuronal acetylcholine receptor (CHRNA7) is located in this region. In addition, in 1996 Vaughn and his colleagues (Vaughn *et al.*, 1996) found that children presenting complex REs had other anomalies, including large deletion of the long arm of chromosome 1.

## Voltage-gated K<sup>+</sup> channels and benign familial neonatal convulsions

Benign familial neonatal convulsions (BFNC) syndrome is rare, and is transmitted in an autosomal dominant mode. Gene penetrance is 85% (Plouin, 1994). Clinically, convulsions occur on the third day of life and manifest as tonic-clonic seizures, generalized or multifocal, which disappear spontaneously after a few weeks of life. There are great intrafamilial and interfamilial variations in age at onset (from the second day to the fourth month of life), as well as in the duration of manifestations of the disease (from a few days to a few months). Seizures last from a few seconds to several minutes; exceptionally progressing to a status epilepticus (Ronen *et al.*, 1993, Wakai *et al.*, 1994). Initial symptoms include tonic postures of the trunk and limbs, apnea, moaning, oculomotor manifestations and changes in skin colour. Interictal EEGs are normal. Ictal activity often starts by desynchronisation of the EEG. This condition does not usually alter the neurocognitive development of subjects, contrary to most epileptic syndromes starting in the neonatal period. Learning problems or minor cognitive disorders have been reported in a small number of patients (Ronen *et al.*, 1993); 11 to 15% of patients present convulsions (Plouin, 1997) or focal EEG anomalies (Maihara *et al.*, 1999, Neubauer *et al.*, 1997, Coppola *et al.*, 2003). These "tardive convulsions", often occur in response to particular stimuli such as noise or emotional stress (Ronen *et al.*, 1993). In addition, 5% of children with BFNC develop febrile convulsive seizures in childhood (Plouin, 1994).

One of the major loci of BFNC is chromosome 20q13.3 (Leppert *et al.*, 1989, Lewis *et al.*, 1993, Malafosse *et al.*, 1992). A link with another locus on chromosome 8q24 has been discovered (Steinlein *et al.*, 1995, Lewis *et al.*, 1993). It is very likely that other loci have not yet been found. The first two genes responsible for this syndrome have been identified recently. They both encode voltage-



**Figure 2.** Schematic localisation of mutations associated with epilepsy in subunits KCNQ2 and KCNQ3 of K<sup>+</sup> channels. The full circles represent missense mutations and empty circles represent other types of mutations, including nonsense mutations, as well as insertions and deletions producing truncated proteins. In KCNQ2, the 29 identified mutations are preferentially located in the fourth transmembrane segment (voltage sensor), in the P domain that contributes to the creation of the channel pore, and in the C-terminal cytoplasmic ending. In KCNQ3, the three identified mutations are located in the P domain.

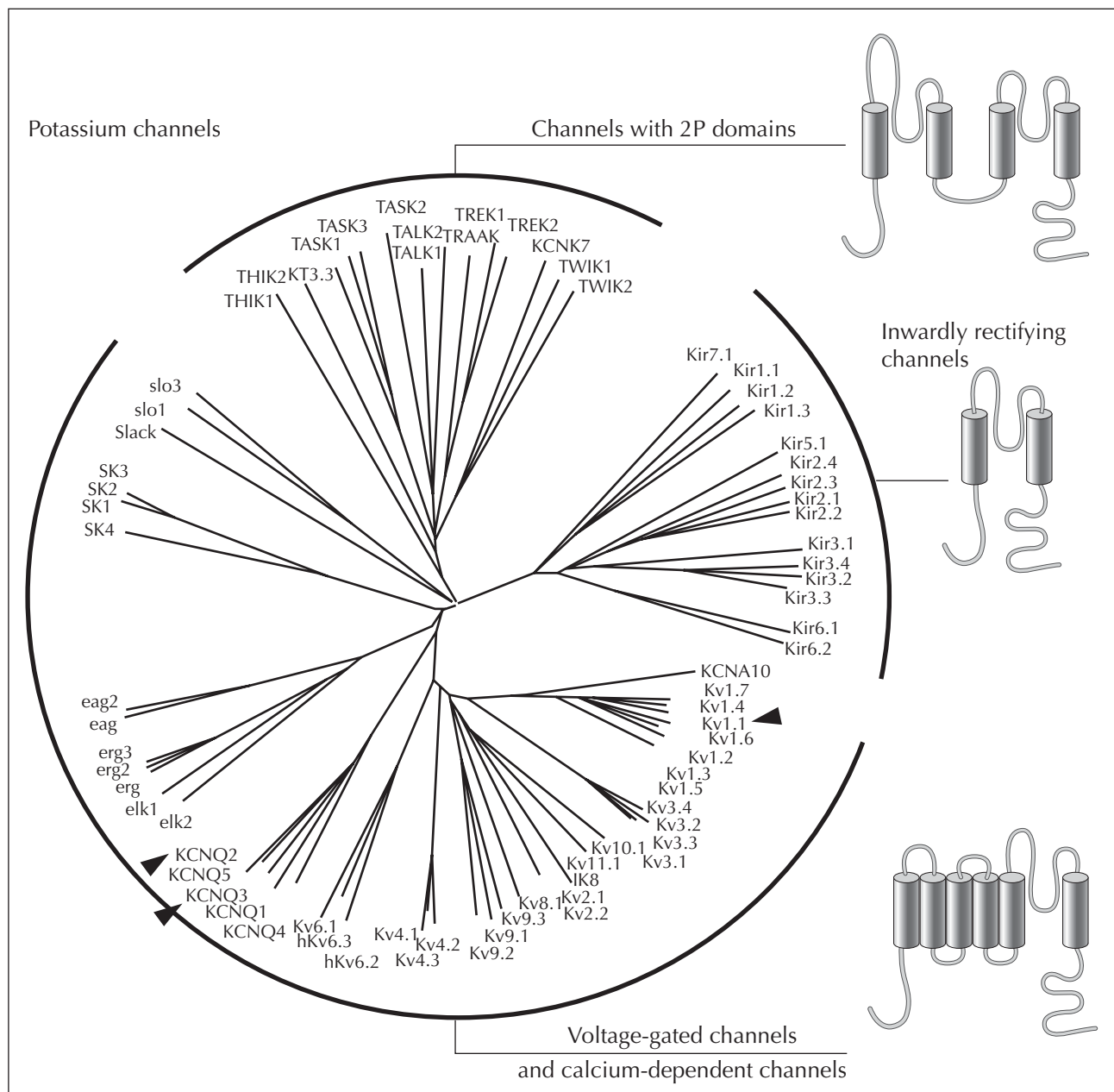
gated K<sup>+</sup> channels, KCNQ2 on chromosome 20q (Biervert *et al.*, 1998, Singh *et al.*, 1998) and another similar gene, KCNQ3, on 8q24 (Charlier *et al.*, 1998). These two genes are transcribed in practically the whole CNS (Biervert *et al.*, 1998).

KCNQ channels are expressed in the myocardium (KCNQ1), in the central nervous system (KCNQ2-5), in the inner ear (KCNQ4) and in skeletal muscle (KCNQ5) (Jentsch, 2000). These channels belong to a family of proteins with many similarities, an intracellular aminoterminal portion, six transmembrane domains (S1 to S6), a P loop between S5 and S6 with a GYG domain which confers potassium selectivity to the pore, and a long carboxy-terminal cytoplasmic chain (figures 1 and 2).

A subgroup of this channel family includes KCNQ2 and KCNQ3, and three other subunits: KCNQ1, KCNQ4 (Kubisch *et al.*, 1999) and KCNQ5 (figures 2 and 3). Genomic analysis of KCNQ2 shows it to be encoded by 18 exons between 30 pb (exon 8 and 10) and a 4-6 kbp (exon 17). It is also interesting to note that genes KCNQ2 and CHRNA4 are only 30 kb apart (see above).

The first identified and described KCNQ2 mutation at the functional level was discovered in an Australian family and consists of a 5pb insertion in the sequence encoding the carboxy-terminal ending (Biervert *et al.*, 1998), which produces a truncated protein. Expressed in the *Xenopus* oocyte, this truncated protein does not express discernable current, indicating total loss of function associated with this mutation. Expression of both mutant and wild-type proteins causes a reduction of expected currents without a negative dominance effect. It is clear that seizures in these patients are due to haploinsufficiency of KCNQ2 (Biervert *et al.*, 1998). Recently, the first dominant negative mutation has been identified (Singh *et al.*, 2003).





**Figure 3.** Dendrogram of human K<sup>+</sup> channels. The 75 channels fall into three large categories according to their sequence homology and their membrane topology. Mutations in KCNQ2, KCNQ3 and Kv1.1 subunits, indicated by a black arrow, are associated with epilepsy.

Expression of both KCNQ2 and KCNQ3 in *Xenopus* oocytes results in heterotetrameric channels whose electrophysiologic properties are different than those of either one of the subunits expressed alone. The heteromeric channels KCNQ2/3 show a current amplitude 11 times greater than that of homomeric channels (Yang *et al.*, 1998). Immunohistochemistry has shown that these KCNQ2 and KCNQ3 subunits immunoprecipitate together from a lysate of the human brain (Cooper *et al.*, 2000). Wang and his colleagues have shown that the M

current is produced by the assembly of the two proteins, KCNQ2 and KCNQ3 (Wang *et al.*, 1998). Given the extensive expression of these two subunits in the CNS, these observations support KCNQ2 and KCNQ3 heteromerisation in the brain. As is the case for ADFLEs, BFNCs are caused by mutations which affect different subunits of the same channel.

M current is a K<sup>+</sup> current characterized by a slow activation and inactivation conductance. It is expressed in the periphery of sympathetic neurons, as well as in many CNS

structures. It reduces neuronal excitability by causing an efflux of  $K^+$  ions during the formation of action potential; by so doing, it plays a crucial role in neuronal excitability regulation (Jentsch, 2000, Marrion, 1997, Wang *et al.*, 2000, Cooper and Jan, 2003). KCNQ2 mutations responsible for BFNC alter control of excitability by modifying the M current (Schroeder *et al.*, 1998).

Most KCNQ2 mutations are located in the sixth S6 transmembrane domain, at the level of the P pore domain or, in most cases (57%), on the carboxy-terminal loop (Biervert *et al.*, 1998, Singh *et al.*, 1998, Charlier *et al.*, 1998, Singh *et al.*, 2003, Lerche *et al.*, 2000, Lee *et al.*, 2000) (figure 2). Mutations affecting the pore reduce  $K^+$  current by modifying channel conductance, but do not affect channel expression to the cell surface (Schwake *et al.*, 2000). Mutations in the carboxy-terminal portion could alter assembly of subunits and reduce the number of functional channels expressed to the cell surface (Lerche *et al.*, 2000, Schwake *et al.*, 2003, Maljevic *et al.*, 2003).

Among all the hereditary epilepsies studied to date, four present KCNQ2 mutations and express a different phenotype than that seen in BFNCs. A familial KCNQ2 mutation located in the voltage sensor domain (S4) results in the BFNC syndrome and, in adulthood, is accompanied by myokymia (involuntary muscle contractions) (Dedek *et al.*, 2001). The other three mutations manifest in seizures that start after the neonatal period, with no remission after the fourth month (Singh *et al.*, 1998, Singh *et al.*, 2003). These observations reveal the function of these voltage-gated  $K^+$  channels, both in the central and the peripheral nervous system. In addition, some patients from families where KCNQ2 mutations were found also present neonatal convulsions, generalized infantile or adult epilepsies, febrile convulsions plus (generalized tonic-clonic febrile convulsions occurring in patients after the age of six years), or centrottemporal epilepsies. These phenotypes seem particular to each family and are expressed with variable penetrance, which leaves us to suppose that other environmental or genetic factors could be involved in these cases.

There is no clear explanation for BFNC onset solely in the first weeks of life *ex utero*. The only attractive hypothesis is that the immature brain is more vulnerable to small variations caused by mutations in KCNQ2 and KCNQ3 channels. Another hypothesis is that the human brain only expresses these channels in a limited timeframe and that, subsequently, the overexpression of another  $K^+$  channel compensates for the loss of function caused by these mutations. Studies of rat hippocampus during the postnatal period have shown that these KCNQ channels play a predominant inhibitive role in the first week of life, when GABAergic transmission loses its excitatory activity (Okada *et al.*, 2003).

Moreover, two mutations of the gene encoding for a sodium channel subunit, SCN2A, have been found in two families of patients with BFNC, widening the genotypic

spectrum of this condition (Heron *et al.*, 2002). Given the onset of this syndrome during the neonatal period, it is also interesting to study its occurrence in premature infants. In fact, convulsions seem to start at the expected corrected age (Singh *et al.*, 2003), making it possible to suppose that phenomena involved in the production of these seizures are conditioned by brain maturation, and that the latter evolves in the same manner regardless of the milieu in which it takes place.

KO heterozygous KCNQ2<sup>+/-</sup> mice present neuronal hyperexcitability to PTZ, while KCNQ2<sup>-/-</sup> homozygous mice die spontaneously in the first 48 hours of life (Watanabe *et al.*, 2000). At present, 29 mutations have been identified in KCNQ2 (Biervert *et al.*, 1998, Singh *et al.*, 2003, Lee *et al.*, 2000, Dedek *et al.*, 2001, Moulard *et al.*, 2001, Lerche *et al.*, 2001, Lerche *et al.*, 1999) (figure 2). The three mutations in KCNQ3 are missense mutations in the sequence encoding the pore (Hirose *et al.*, 2000, Charlier *et al.*, 1998, Singh *et al.*, 2003) (figure 2).

Mutations in other subunits of these channels have been found; they apply to KCNQ1 and KCNQ4. Mutations in KCNQ1 result in cardiac arrhythmia due to Torsade de Pointes as part of long QT syndrome (Romano-Ward syndrome), as well as in long QT syndrome associated with perceptive deafness, Jervell, Lange-Nielsen syndrome (Neyroud *et al.*, 1997, Sanguinetti, 1999, Splawski *et al.*, 2000), or atrial fibrillation (Chen *et al.*, 2003).

Thus, different mutations in KCNQ1 are responsible for different pathologies. Mutations in KCNQ1 responsible for the Jervell, Lange-Nielsen syndrome involve the carboxy-terminal loop and impair assembly of channel subunits (Schmitt *et al.*, 2000).

Mutations in KCNQ4 cause congenital progressive deafness (DFNA2) (Kubisch *et al.*, 1999, Coucke *et al.*, 1999, Talebizadeh *et al.*, 1999, Akita *et al.*, 2001). These mutant subunits act, in all three cases, as negative dominant genes (Kubisch *et al.*, 1999, Chouabe *et al.*, 1997). The latest member of this gene family, KCNQ5, whose protein is expressed both in the CNS and in skeletal muscle, has not yet been linked to any disease (Lerche *et al.*, 2000, Kananura *et al.*, 2000).

From a therapeutic standpoint, retigabine, a new anti-epileptic drug, activates KCNQ2/3 currents by producing displacement to the left of voltage-dependent activation in these channels (Rundfeldt and Netzer, 2000, Main *et al.*, 2000, Wickenden, 2002). The anticonvulsant effects of retigabine have already been demonstrated on several animal models of epileptic seizures (Rostock *et al.*, 1996, Tober *et al.*, 1996). In a phase II clinical trial, 12 out of 35 patients with pharmacoresistant epilepsies experienced fewer seizures when treated with retigabine (Bialer *et al.*, 2001). It is important to note that this molecule could have an anticonvulsant effect not only by affecting KCNQ2/3, but also by activating GABAergic transmission in the CNS (Kapetanovic *et al.*, 1995).

## Voltage-gated K<sup>+</sup> channels and episodic familial ataxia

*Type 2 episodic ataxia* (EA2), is a rare autosomic dominant disorder characterized by intermittent episodes of balance problems and myokymia. It is associated with KCNA1 (also called Kv1.1) mutations in a K<sup>+</sup> voltage-gated channel (*figures 1 and 3*).

Of five patients identified in a Scottish family, two also present a partial epilepsy, indicating that the epilepsy could be secondary to the KCNA1 mutation. Functional *in vitro* studies have shown that the mutant protein produces a negative dominant effect which likely impairs neuronal repolarization (Zuberi *et al.*, 1999).

Over 75 genes encoding the  $\alpha$  subunits of K<sup>+</sup> channels have been cloned in humans. It is the largest ion channel family. Despite this great diversity, these subunits constitute only three structural families (*figure 3*). KCNQ2, KCNQ3 and KCNA1 belong to the category of voltage-gated channels with six transmembrane segments. There is also a category of so-called inwardly rectifying channels, with two transmembrane segments. Many of these voltage-gated and input rectification channels are expressed in the CNS. The latest category of K<sup>+</sup> channels that has been identified corresponds to subunits with four transmembrane segments and two P domains (Lesage, 2003, Lesage and Lazdunski, 2000). These channels are expressed in the CNS, where they contribute to excitability control. Some are opened by unsaturated fatty acids, by internal acidification or by general volatile anesthetics. Opening the channels with anesthetics causes neuronal hyperpolarization, which no doubt contributes to the general depression of the CNS associated with anesthesia. These channels are excellent targets in the search for new mutant genes in idiopathic epilepsies.

## Ca<sup>2+</sup> voltage-gated channels and epilepsy

The importance of calcium channels in epilepsy has been demonstrated in a number of mouse models of generalized epilepsy (Meisler *et al.*, 2001). These models involve to a great extent absence epilepsy with ataxia, a rare combination in human epileptology. Two publications describe mutations in genes encoding for Ca<sup>2+</sup> channel subunits in a few patients with generalized epilepsies (Escayg *et al.*, 2000, Jouvenceau *et al.*, 2001), but no functional study of mutations has been carried out to date; nor has there been any other wider genetic study on the subject. These important data are needed to confirm the role of mutations of this type in these forms of epilepsy. Ca<sup>2+</sup> channels are heteropolymers with one main  $\alpha 1$  subunit whose structure is close to that of the  $\alpha$  subunits of voltage-gated Na<sup>+</sup> channels with four repeated homologous domains (*figure 1*), associated with auxiliary  $\beta$ ,  $\alpha 2\delta$  and  $\gamma$  subunits. There are ten genes encoding  $\alpha 1$  subunits in humans; practically all of these subunits are expressed in the CNS.

## Na<sup>+</sup> channels GABAA receptor, generalized epilepsy with febrile seizures *plus* and severe myoclonic epilepsy of infancy

### Generalized epilepsy with febrile seizures plus (GEFS+)

Generalized epilepsy with febrile seizures plus (GEFS+) is one of the most recently identified epileptic "syndromes" (Scheffer and Berkovic, 1997). It is characterized by a wide range of associated seizures (Scheffer and Berkovic, 1997, Wallace *et al.*, 1998, Singh *et al.*, 1999). The most affected members of a family present a phenotype generally known as "febrile convulsions plus", a disorder involving numerous generalized tonic-clonic seizures often associated with fever spikes.

In contrast to the clearly defined syndrome of febrile convulsions (FC), the FC+ "syndrome" manifests in children over six years of age and is accompanied by generalized tonic-clonic convulsive seizures with no associated fever. Two thirds of GEFS+ patients present an FC or FC+ phenotype. The remaining third present various types of seizures (for example, tonic-clonic, myoclonic, myoclonic-astatic seizures, absences or atonic seizures).

## Voltage-gated Na<sup>+</sup> channels SCN1A, SCN2A and GEFS plus

Given the wide spectrum of phenotypes, bilinear transmission and a large number of phenocopies (febrile convulsions have an age-dependent frequency of 3 to 5%), the mode of transmission of GEFS+ is still poorly understood. It appears to be complex, and monogenic transmission seems to be the exception. But, as is the case for the two epileptic syndromes described above, the first mutations were identified by studying large families. Regarding a large Tasmanian family, it was strongly argued that a major gene responsible for febrile convulsions is located in 19q13. Further examination of this locus made it possible to identify a mutation in the  $\beta$  SCN1B subunit of the voltage-gated Na<sup>+</sup> channel (Wallace *et al.*, 1998). Although this subunit is also expressed in skeletal muscle, none of the patients who are carriers of this mutation present signs of myotonia, as it is described in other patients presenting mutations of the SCN4A gene encoding the  $\alpha$  subunit of the same Na<sup>+</sup> channel. A second locus was identified in 2q24-q33 in two French families (Moulard *et al.*, 1999, Baulac *et al.*, 1999).

This locus corresponds to a gene cluster encoding three  $\alpha$  subunits of the Na<sup>+</sup> channels (SCN1A, SCN2A, SCN3A). Mutations of two amino acids have been found in this region of the SCN1A (T875M and R1648H) gene, both located in S4 transmembrane segments corresponding to the voltage sensor of the  $\alpha$  subunit (Escayg *et al.*, 2000). Another R187W mutation located in the intracellular loop between domain II and domain III of the SCN2A subunit was also identified (Sugawara *et al.*, 2001).



The voltage-gated Na<sup>+</sup> channels are made up of one main 260 kDa  $\alpha$  subunit that forms a channel capable of generating sodium current in response to membrane depolarization, and two to three small 35 kDa  $\beta$  subunits (Krafte *et al.*, 1988, Krafte *et al.*, 1990, Makita *et al.*, 1994, Isom *et al.*, 1992, Isom *et al.*, 1995, Catterall, 1999) (*figure 1*). Four of the ten  $\alpha$  subunits of the human genome are expressed largely in the CNS: SCN1A, SCN2A, SCN3A and SCN8A (Meisler *et al.*, 2001). Channels containing the SCN1A subunit are expressed at the level of inhibitor neurons (Whitaker *et al.*, 2000).

The  $\alpha$  subunit is composed of four domains (I to IV), each one containing six transmembrane helices (S1 to S6), as well as a pore P loop. Positively charged amino acids at the S4 helices probably act as the channel's voltage sensor.  $\beta$  subunits seem to modulate inactivation of the channel. At the structural level, the C121W mutation in the SCN1B subunit results in the loss of a disulfide bridge involved in the formation of the immunoglobulin-type motif of the extracellular loop in subunit  $\beta 1$ .

At the functional level, this mutation leads to slowing of the inactivation kinetics of the sodium current produced by the mutated channel expressed in a heterologous system (Wallace *et al.*, 1998). Concerning SCN1A subunit (Escayg *et al.*, 2000, Escayg *et al.*, 2001, Wallace *et al.*, 2001), functional expression of SCN1A mutations at the level of segments II/S4 and IV/S4, reveals more rapid activation (opening triggered by depolarization) and inactivation (spontaneous reclosing of the activated channel), and absence of residual current (Alekov *et al.*, 2000, Alekov *et al.*, 2001, Spanpanato *et al.*, 2001). The most important modification observed for the mutant IV/S4 is an acceleration of the time of return to the inactive state (Lerche *et al.*, 2001). This observation was made in preliminary studies (Escayg *et al.*, 2001). Moreover, for the two mutations discussed, Lerche and his colleagues found an acceleration of activation time for negative imposed potentials ( $\leq 20$  mV) (Lerche *et al.*, 2001).

## GABAergic receptors and GEFS plus

Mutations in a  $\gamma 2$  subunit of gabaergic receptor GABA<sup>A</sup> have been identified in two families of patients with GEFC+. One of these families shows a typical GEFC+ phenotype (Baulac *et al.*, 2001), while in the other family patients present a combination of febrile convulsions and absence epilepsy (Wallace *et al.*, 2001). The two mutations are located in different regions of the channel, one on the GABA connection site of the extracellular loop (Wallace *et al.*, 2001), the other on the M2 and M3 membrane segment connection loop (Baulac *et al.*, 2001) (*figure 1*). Functional expression of these mutated subunits assembled with subunits  $\alpha$  and  $\beta 2$  reveals two different kinds of losses of function. The first mutation involving the GABA connection site reduces activation current in the

channel by a factor of ten, while the second expresses current normally activated by the GABA, but is insensitive to benzodiazepines. Regarding the second mutation, Wallace and his colleagues suggest that the receptor's loss of reactivity to "endozepines" leads to neuronal hyperexcitability.

## Na<sup>+</sup> channels and severe myoclonic epilepsy of infancy (Dravet syndrome)

Severe myoclonic epilepsy of infancy (SMEI) (Dravet's syndrome) is characterized by clonic and tonic-clonic seizures, generalized or unilateral, often prolonged and initially occurring in a febrile context in the first year of life. Secondly, these children present generalized non-febrile seizures such as myoclonia, absences or tonic-clonic seizures, as well as simple or complex partial seizures. The epilepsy is typically pharmacoresistant. The children also present arrest of psychomotor development with major intellectual retardation.

The recent discovery of mutations in the SCN1A gene leading to the production of truncated proteins in patients with this syndrome allows a better understanding of the mechanisms involved in this serious disease. These mutations are spontaneous (*de novo*) (Claes *et al.*, 2001), contrary to familial mutations found in the GEFC+ syndrome. These missense mutations are most often non functional, or are responsible for drastic reductions in sodium currents. The mutations cause functional modifications opposed to those observed in GEFC+ (Sugawara *et al.*, 2003). Mutations of this gene have been found in 33 to 100% of patients with SMEI (Sugawara *et al.*, 2002, Ohmori *et al.*, 2002, Claes *et al.*, 2003, Wallace *et al.*, 2003, Nabbout *et al.*, 2003). However, the fact that about 50% of patients with SMEI belonged to GEFC+ families remains unexplained (Singh *et al.*, 2001, Fujiwara *et al.*, 2003).

The phenotypic spectrum of SCN1A mutations was extended to other severe infantile epilepsies (Fujiwara *et al.*, 2003). In families of patients presenting both GEFC+ and SMEI, it appears, once again, that other environmental or genetic factors determine the seriousness of the phenotype (Fujiwara *et al.*, 2003, Scheffer and Berkovic, 2003).

## Mutations of the CLCN2 chloride channel and absence epilepsy in children and adolescents, juvenile myoclonic epilepsy and epilepsy with grand mal seizures on awakening

### Juvenile myoclonic epilepsy

Juvenile myoclonic epilepsy (JME) is one of the best defined epileptic syndromes. Onset is between the ages of six and 25 years, with a peak in frequency at adolescence, between the ages of 12 and 17. The clinical picture reveals

the presence of myoclonic, spontaneous, bilateral tremors, generally symmetrical, isolated or repetitive, involving preferentially the upper limbs and the face, sometimes responsible for falls when they extend to the lower limbs. Myoclonia occur in a fully conscious state, shortly after waking, and they interfere greatly with activities upon rising. Tonic-clonic generalized seizures which usually start with a crescendo of massive, bilateral myoclonia and become clonic-tonicoclonic seizures are associated in 90% of cases with morning myoclonia, and are the most frequent reason for the initial consultation. At that time, myoclonia have been present for months, sometimes years. The intercritical EEG shows generalized spikes waves and polyspikes waves, often asymmetrical, with a frequency of over 3 Hz. Myoclonia, always preceded by a polyspike-and-wave complex on the scalp EEG, can be recorded by means of a polygraphic video EEG exam in the morning upon waking, after sleep deprivation. Response to treatment is spectacular (valproate, benzodiazepines), but JME is pharmaco-dependent: stopping treatment causes a recurrence of clinical symptoms in 90% of cases. Therefore, treatment must continue for a very long time, sometimes for life.

### Absence epilepsy in children and adolescents

Childhood absence epilepsy (CAE) is a common form of idiopathic epilepsy occurring in normal, school age children. Peak frequency is at about age seven years. Typical absences are inaugural, very frequent (between 10 and 200 per day), easily triggered by hyperpnea, and taking various forms: simple absences or absences accompanied by discrete tonic, clonic, atonic, automatic or vegetative components. The EEG shows normal background activity, with regular, bilateral, synchronous, symmetrical 3 Hz spike waves discharges, accompanied by clinical absence when their duration exceeds 4 to 5 seconds.

Rhythmic, intercritical posterior delta activity is sometimes detected. Evolution is variable. Absences are easily controlled with treatment (valproate, ethosuximide, lamotrigine) and rarely persist in isolation in adulthood. Juvenile absence epilepsy (JAE) starts later than CAE, around puberty. Absences are much rarer and usually occur in clusters, in the morning upon waking. Critical EEG anomalies correspond to 3Hz spikes sharp wave discharges, or more rapid (4-5 Hz) discharges. Tonic-clonic generalized seizures are associated with absences in 80% of cases. Differential diagnosis with matutinal epilepsy with grand mal seizures can be difficult to make.

### Epilepsy with grand mal seizures on awakening

This epilepsy starts in adolescence. Seizures occur exclusively or predominantly shortly after waking in the morning, after a nap or during an evening rest. Factors triggering seizures include sleep deprivation, excessive alcohol consumption and forced waking. EEG tracing can be normal

or can show generalized spikes sharp wave discharges or rapid polyspikes sharp wave discharges, particularly visible in the morning upon waking. Passing forms with rare morning myoclonia and/or rare absences are likely and reinforce the hypothesis of a neurobiological continuum between CAE, JAE, JME and MEGMS (Thomas and Arzi-manoglou, 2003).

### CLCN2 Cl<sup>-</sup> channel

Three different mutations in the CLCN2 chloride channel (Cl<sup>-</sup>) have been discovered in three families where patients present these forms of idiopathic epilepsies (Haug *et al.*, 2003). The CLCN2 channel is abundantly expressed in the CNS, particularly in neurons inhibited by GABA (Sik *et al.*, 2000). It is believed that this channel plays an essential role in maintaining the low intracellular chloride concentrations needed to produce an inhibitive response to GABAergic stimulation (Staley, 1994, Staley *et al.*, 1996, Mladnic *et al.*, 1999). The CLCN2 channel is formed by the assembly of two 898 amino acid polypeptides, having a cytoplasmic amino-terminal portion, 20 different domains (labeled A to R), including 12 potential transmembrane segments, as well as two cystathionine- $\beta$ -synthetase (CBS) domains at the level of its intracytoplasmic carboxy-terminal (*figure 1*). The opening of this channel is voltage-gated and modulated by variations in intracellular Cl<sup>-</sup> (Cui *et al.*, 1997).

A drop in Cl<sup>-</sup> intracellular concentration produces displacement of the activation curve toward more hyperpolarizing potentials. Given this particularity, channel opening occurs only when membrane potential drop below the chloride reversion point, this being the exclusive output of these ions into the extracellular milieu. The channel is encoded with 24 exons at chromosome 3q26 (Thiemann *et al.*, 1992, Cid *et al.*, 1995).

In the first family where four members were epileptic over four generations, three individuals presented JME, one presented MEGMS, and one had electroencephalographic anomalies of the spike sharp wave and polyspike sharp wave type, with no critical symptoms. A 597insG mutation in the CLCN2 channel appears to be responsible for these findings. This insertion introduces an early stop codon (M200fsx231) and produces a truncated protein in which major pore determinants are missing. When expressed alone in tsA201 cells, the mutant protein makes it impossible to observe Cl<sup>-</sup> current. When expressed with wild-type protein, it produces low intensity Cl<sup>-</sup> current compared to wild-type current.

In the second family, eight members presented epileptic syndromes: five had MEGMS, one CAE, one had electroencephalographic anomalies of the spike sharp wave type with no paroxystic manifestations; one was a carrier of the mutation identified in the family, but had neither seizures nor EEG anomalies. A del 74-117 mutation was

identified; this variant was found in a healthy population, but in affected individuals, a much greater expression of the variant was noted. The resulting protein is amputated of part of an  $\alpha$  helix in the B domain. The truncated protein is properly expressed to the cytoplasmic membrane of the neurons. Expression of this variant in a heterologous system does not produce  $\text{Cl}^-$  current. Its co-expression with the wild-type protein reveals a negative dominant effect (Haug *et al.*, 2003).

These two mutations, 597insG and del74-117, could lead to an accumulation of intracellular  $\text{Cl}^-$ , and therefore to a reduction of the GABAergic inhibitive response, and even to an excitatory response responsible for neuronal hyperexcitability causing epileptic seizures.

Of the five individuals in the third family, one is a healthy progenitor, carrier of the mutation; of the three other carriers of the mutations, two presented CAE and one had no clinical symptoms but presented spike sharp wave type anomalies at EEG. The mutation involving the CLCN2 channel is probably G715E. This mutation affects the carboxy-terminal portion located between the two CBS domains. These mutated channels, contrary to the previous two, express normal amplitude current, but their voltage-dependent opening differs from that of wild-type channels. In fact, these mutants are activated at much higher membrane potentials than those observed in wild-type channels. Heterodimers (mutated and wild-type channels) open at intermediary potentials.

The mechanism responsible for hyperexcitability is different in the case of this mutation. After a period of intense synaptic activity responsible for membrane depolarization by the activation of glutamatergic receptors and by the influx of  $\text{Cl}^-$  ions through the GABAergic receptors, the G715E mutation leads to an increase of  $\text{Cl}^-$  current during the repolarization phase. This increase in function is believed to make the post-synaptic membrane of GABAergic neurons hyperexcitable, and could therefore be responsible for epileptic seizures. A strain of mice KO for the CLCN2 channel does not develop spontaneous epileptic seizures. Different compensatory mechanisms than those of humans, or anatomic or physiological variations between the two species, could explain this difference.

Just as mutations of the GABA<sup>A</sup> receptor are involved in the pathogenesis of idiopathic epilepsies, mutations in the CLCN2 channel cause similar paroxysmic manifestations, demonstrating the importance of the GABAergic system in epileptogenesis. Moreover, identification of these genetic mutations both in the CLCN2 channel and in the GABA<sup>A</sup> receptor confirms the clinical hypothesis of etiopathogenic connections between the three syndromes described above. In the near future, these findings could lead to a clearer understanding of the mechanisms involved in these idiopathic epilepsies.

## Na<sup>+</sup>/K<sup>+</sup>-ATPase and benign familial infantile convulsions (BFIC)

In the past few years, many publications described a focal epilepsy starting in the first months of life and having an idiopathic etiology and favorable evolution (Okumura *et al.*, 2000, Vigevano *et al.*, 1992).

Vigevano *et al.* pointed out the existence of cases with a family history of convulsions, benign evolution in childhood, and autosomic dominant heredity; they proposed the term "benign familial infantile convulsions" to designate this syndrome.

Vanmolkot *et al.* (Vanmolkot *et al.*, 2003) described a genetic connection between hemiplegic familial migraine (HFM) and BFIC, on the basis of mutations in the Na<sup>+</sup>/K<sup>+</sup> type 2 ATPase pump (ATP1A2), found in a family in which patients presented either episodes of migraine of which some were hemiplegic, or generalized convulsive seizures between the ages of 1.5 and 5 months (no critical electroencephalograms are available) or, in the case of four patients, both migraines and infantile spasms.

## Conclusion

Ion channels are responsible for generating and controlling neuronal excitability. Mutations in 12 genes encoding channels, receptors and ion transporters have already been linked with different forms of idiopathic epilepsy. Heterologous expression of mutated channels has made it possible to study the electrophysiologic modifications produced by the mutations. These modifications generally fall into three major categories: loss of function, negative dominance, or more subtle modifications of functional properties.

Despite these findings and knowledge about distribution of these channels in the CNS, a correlation between mutations and electro-clinical data remains difficult to establish. Mutations in the same gene can produce different clinical syndromes, while certain mutations in different genes lead to the same clinical manifestations. Phenotypic variability within the same family suggests the existence of polymorphisms in other genes likely to influence phenotypic expression, such as environmental or developmental factors. New techniques of molecular analysis should facilitate future study of the majority of idiopathic epilepsies whose transmission is complex and involves multiple genes. Despite the questions they continue to elicit, the studies already completed open important perspectives in terms of genetic diagnosis and improved anti-epileptic treatments. □

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