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Title of Dissertation: **"Epilepsy: The Role of the Super Family of Voltage Gated Ion Channels. "**

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Abstract

Title of Thesis: Epilepsy! The Role of the Superfamily of Voltage Gated Ion Channels!

Edward L. Jones, Masters of Neuroscience 2010

Thesis directed by Aviva Symes, Maria Braga, Department of Neuroscience, and William Watson Department of Neurology.

The superfamily of voltage gated ion channels is composed of channels regulating sodium, calcium or potassium entry into cells, regulated by alterations in the membrane potential. Dysregulation of members of this family often leads to the development of epilepsy. This review focuses on different mutations in this superfamily that are linked to the development of epilepsy and details the known mutations, the role of the specific mutated ion channel and the clinical consequences of each mutation. Disruption of voltage gated ion channel function can lead to increased excitability and contribute towards the development of epilepsy. The position of the mutation within the ion channel gene often influences the clinical presentation of symtoms, even for mutations within the same gene. A common feature of the superfamily of voltage gated ion channels is an α selective pore with augmentation by pore modifying protein subunits. Mutation of either the α pore or augmenting auxiliary proteins significantly alters ion channel function. An understanding of the molecular mechanisms of inherited epilepsy, will assist in establishing the etiology of all types of epilepsy and thus aid in the development of targeted therapeutics.

Epilepsy!

The Roll of the Superfamily of Voltage Gated Ion Channels!

By

Edward L. Jones

Thesis Submitted to the Faculty Department of Neuroscience

Graduate Program of the Uniformed Service University of

the Health Sciences in partial fulfillment of the

requirements of the degree of

Masters in Neuroscience 2011

Dedication:

There once was a boy born into a world of chaos, abandonment, violence, and abuse. In a desperate but calculated choice, the boy joined the Army in order to gain his chance at freedom - freedom from prison, drugs, alcohol, and violence that had plagued his siblings and parents alike. The Army told the boy, in not so many words, that freedom was not free. The Army through its action showed the boy that dedication and hard work was to be the beginning of his sacrifice to earn freedom.

Mother Army took this boy under her wings protected him from the world's temptations and himself. Mother Army smacked him when he was wrong and praised him when he was right.

As the boy grew and started to spread his wings, she still kept her eye on him. Now a loving and loyal son would do the same for her.

The boy was to be struck down by disease, but always there, mostly silent, was Mother Army. Like any good mother she could not abandon her child when he was week. Mother Army would care for her boy and his young wife and child. When Mother Army did not know how to care for him herself, she sought out the best the world had to offer.

The boy recovered and began to live life anew. Dedicated, like he had been taught by his mother, he would begin to serve others.

Out of nowhere, the boy became sick again, and as you know without even reading further, Mother Army was there once again.

Time would pass and the boy once again recovered only to be struck down by a new disease. Physically and emotionally spent, exhausted, desperate, angry, and sad, the boy began to cry like he had never cried before. He did not know how he, now a father, would care for his boy!

Unable to work like he had before, he once again was shielded by the wings of Mother Army. She would see that her boy would be given the best care and rehabilitation available. She would make sure that the boy would have resources to take care of himself and his new family.

Here's to Mother Army. She is the best social program ever invented! Thank you for my chance at freedom and at life! As my days under your wings draw closer to an end, I will always remember you and do whatever I can to help you and your other children!

Forever your Child!

Hooah

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Epilepsy! The Roll of the Superfamily of Voltage Gated Ion Channel!

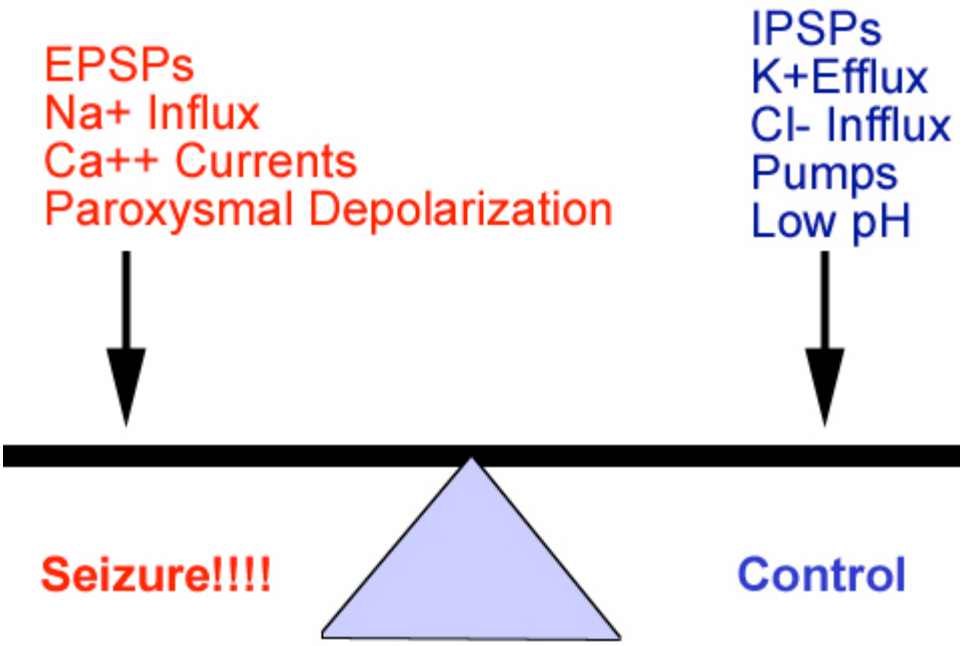
1. Epilepsy is a balancing act

A modern approach to a seizure is an abnormal period of excessive synchronized neuronal excitation in an area of the brain that has become hyperexcitable, an epileptogenic center which is encouraged to spontaneously discharge/depolarize. Epilepsy is a chronic disorder of repeated seizure like states of excessive neuronal discharge described above.

Every living being can have a seizure or seizures, as neurons are held close to threshold thus allowing rapid firing. It is the balance between neuronal inhibitory and excitatory triggers, the equilibrium, which determines under what conditions resting neurons will depolarize. Those with epilepsy have either impaired inhibitory or enhanced excitatory triggers and hence are more inclined to have epileptic discharges. Resting membrane potential is the dormant, non-depolarizing, stable period before communication between neurons or neuron and effecter organ, at this point the membrane voltage matches the concentration gradient of ions, see figure 1 .

Electrical signals control the contraction of muscle, secretion of hormones, relay and processing of information in the nervous system and more. The transmission of depolarizing impulses throughout the nervous system is due to its network of neurons. The neurons operate through a series of voltage gated ion and ligand gated ion channels. Voltage gated ion channels (VGIC) or the ion channel super family are transmembrane proteins that mediate ion flux between the extracellular and intracellular environments. The electrical charge difference

between these two environments allows there to be an electrochemical gradient that then rapidly transmits electrical impulses throughout the nervous system all while maintaining homeostasis.



Em = Px/Ptotal (Ex) + Pxi/Ptotal (Exi) + Pxii/Ptotal (Exii) + etc...

Figure 1 Epilepsy is a balance of inhibitory and excitatory factors. The influxes of sodium, or calcium for example push cells to depolarize where as efflux of potassium or influx of chloride drive cells to hyperpolarize. The membrane potential is the weighted average of each contributing ion's equilibrium potential. The size of each weight is the relative permeability of each ion.

Em = Membrane Equilibrium

Px, xi, xii is the relative permeability of ion x, xi, and xii

Ex, xi, xii is the equilibrium potential for x, xi, and xii

Ptotal is the total permeability of all permeant ions

Taken and modified from Emedicine Antiepileptic Drugs by Juan G Ochoa, MD http://emedicine.medscape.com/article/1187334-overview.

Evolution has taken this electrochemical gradient and exploited it into a means to transmit information rapidly throughout the organism. In higher organisms we think of these channels carrying the impulse that ultimately results in intercellular signals. Disruption of this evolutionary evolved system can and does have a wide variety of consequences, of importance in this review epilepsy.

Successive neuronal conduction is mediated by action potentials and signal transduction through synaptic discharges. Ion channels are the basis for conduction therefore; any channel disruption may alter inhibitory or excitatory signals leading to the development of abnormal discharge seen in seizures.

Ion channels involved in epilepsy are membrane spanning, pore forming, ion selective proteins. These ion channels, that result in channelopathies, are either of two types, ligand, or of concern in this review, voltage gated channels. These ion channels are specifically tailored to regulate transmembrane ion fluxes that are activated by changes in membrane voltage.

i. **Controlling resting potential is the key to controlling seizures.**

Normal neurons via inhibition are held close to threshold thus allowing their rapid firing when called upon. Loss of inhibition can cause a shift to fire, induced action potential, more easily. Action potentials/Neuronal excitability can cause a hyper-excitable state that can result from any of the mechanisms responsible for epilepsy. This makes the control of resting potential critical if we are to control seizures.

2. **Structure and Function of Voltage Gated Ion Channels (VGIC)**

Voltage gated Ion channels specifically, the "Super Family" of voltage gated ion channels Na^{+},Ca^{++}, and K^{+} are defined by their high sequence homology due to evolutionary conserved genes. Rodents Voltage gated sodium channels show an extremely high homology to humans and this why the rat and mouse provides much of the initial data with which human correlates have been made.

The defining units of a VGIC are the membrane spanning, ion conduction, aqueous α/α1 pore. The pore is added by modifying auxiliary subunits, differential subunit composition and modulation lead to the formation of channels with different ion permeabilities, gating properties, and kinetics, this will be reviewed throughout the paper. A single α subunit of sodium or calcium channel is composed of four continues homologous domomains from a single polypeptide forming a single ion channel, each domain consists of six transmembrane alpha helical regions. In the case of potassium four homologous or heterologous α1 subunits combine to be the equivalent of a single sodium or calcium α subunit. Interestingly it is believed that the sodium and calcium are the off spring of the potassium channel (Choe, 2002). The α subunits of Na^{+} and Ca^{++}, or four distinct α1 subunits of K^{+}, are the only subunits that are essential to carry the voltage dependent electrically gating signal, they are selective for a particular ion and are each tissue specific.

Voltage gated sodium channels (VGSC) are approximately 260kDa, and found in two chromosomal location, 2q24 and 3q21-24, that encode the SCN genes, that produce the α subunit that are selective for sodium (Yu et al., 2006). There are approximately ten sodium α subunits identified thus far in humans, seven of which are found in the nervous system, each

with unique roles with in the organism and varying locations, (Meisler and Kearney, 2005). The ten α subunits have greater than 50% homology between them (Marban et al., 1998).

For calcium there are 3 chromosomal locations, 16p13.3, 17q22, 19p13 that encode the CACNA1 G, H, and I genes respectively. These genes encode approximately 11 α subunits each with unique roles and locations.

For VGKC there over 40 genes in various loci for the Kv channel alone. This helps to explain why potassium channels are the most diverse subset of all ion channels.

The α subunits have a common tetrameric structure of four homologous membrane spanning domains (I-IV) each with six transmembrane segments (S1-S6). One domain of a sodium or calcium channel is equivalent to one of the four α1 subunits of potassium.

3. Gating of the alpha pore. Changing from non-conducting to conducting.

S1-S4 are voltage sensors of depolarization and S4 helical segments are the voltage responders, due to its high charge density. They produce conformational changes of the ion channels, initiating activation known as gating. Every third position of the S4 is a repeated positively charged aa motif, arginine or lysine, followed by two hydrophobic aa, this serves as the gating charge (Shalini Arora, 2005). Potentially, the S4 gatting charge creates a down stream conformational helical arrangement for the ion to travel down essentially, creating the ion pore via S5 and S6 changes (Shalini Arora, 2005). Neutralization of the argine/lysine position, of the S4, greatly decreases the voltage dependence of gating (Stuhmer et al., 1989). The relatively negative charge of the interior of the cell pull on the positively charged amino acids of S5 and S6, then triggering conformational change of the VGIC that is quickly reversed, stopping the inward flow of ions (Lossin, 2009). The VGNC and VGCC receive their ion hydrated while the VGKC receives its ion dehydrated, a process that was not conserved through evolution. The exact reason for the change has not been determined.

S4 in conjuction with S5 is the nidus for conformational change in the S5-S6 pore helix. Between the S5 to S6 segment there is a hairpin like reentrant pore loop that is highly conserved within a channel family (Catterall, 2000a) (Marban et al., 1998), no two P-segements are the same within a single VGSC (Marban et al., 1998). Each pore segment from the four domains come together to form the selective aqueous ion pore (Catterall, 2000a). Mutations of the pore have devastating effects on ion selectivity and are thought to be potential sites for disease (Sun

et al., 1997). The α peptide domains ultimately fold upon themselves to form the ion specific pore via the III and IV domains. The opening of VGIC allows ions to move rapidly although passively across the cellular membrane while maintaining selectivity in an environment filled with ions of similar size and charge (Choe, 2002).

Inactivation of the ion channel occurs within milliseconds of opening via closing the intracellular inactivation gate by an unknown mechanism at this time. The inactivation gate is formed by a conserved loop between domains III and IV, and mutations in this loop or antibodies directed to it experimentally prevent rapid inactivation and thus allow a greater influx through the channel (Catterall, 2000b).

Auxiliary subunits modify the voltage dependents and kinetics of gating and will be addressed during review of the specific auxiliary subunit.

4. The selective filters: Discriminating between ions

The VGICs discussed in this review have different means of ion selectivity yet, all are quite efficient as to allow ions to flow fast and passively at rates close to the diffusion limit through the plasma membrane. Selectivity is accomplished at the narrowest part of the pore referred to as the selective filter (Choe, 2002).

All Potassium channels, not just Voltage gated channels have a five amino acid highly conserved sequence, TXXXG, forming the electro-negative, cation attractive, via interaction of carbonyl oxygens from the four P loops (Lipkind and Fozzard, 2008). The TXXXG sequence is thought to be the main determinate of the selective filter. The precise arrangement of carbonyl oxygens are thought to strip potassium of its hydration shell. There by functioning to compensate for the electro-negative charge of the oxygen from water molecules and allow the potassium ions to enter the neuron dehydrated (Lipkind and Fozzard, 2008). Other cations fail to meet the precise size requirements to interact with the carbonyl oxygen's and are excluded due to their smaller size.

In VGSC the DEKA locus made from one aa residue from each P-loop region of the four domains (Aspartate 400)-(Glutamate 755)-(Lysine 1237)-(Alanine 1529) form the selective, ring like, filter allowing a hydrated sodium ion to pass. The VGCC functions like the VGNC only replacing the DEKA locus with an EEEE locus, made of four glutamate aa. The four glutamates have a more negative charge and hence have a greater pull on the divalent Ca^{++}. Mutations of the DEKA locus residues to more negative residues alter ion permeation and selectivity toward calcium over sodium (Lipkind and Fozzard, 2008).

Opening of the ion selective filters activation gate is driven by the conformation changes in the S4 voltage sensor.

a. The role of activation and inactivation gates

Note: the flow rate through VGICs is close to the diffusion limit of the cation, and it is driven solely by its electrochemical potential.

The opening of VGIC allows ions to move rapidly although passively across the cellular membrane while maintaining selectivity in an environment filled with ions of similar size and charge (Choe, 2002). Ion channels open/activate when driven by a stimulus initiating a local reversal in membrane potential. Inactivation of the ion channel occurs spontaneously within milliseconds of opening via the inactivation gate made by the conserved loop between domains III and IV. The inactivation gate closes before the activation gate and there by limits the channel availability, mutations in this loop or antibodies directed to it prevent fast inactivation and may lead to neuronal hyper-excitability (Catterall, 2000a).

There are three main conformation states of VGIC an open, closed, and inactivated.

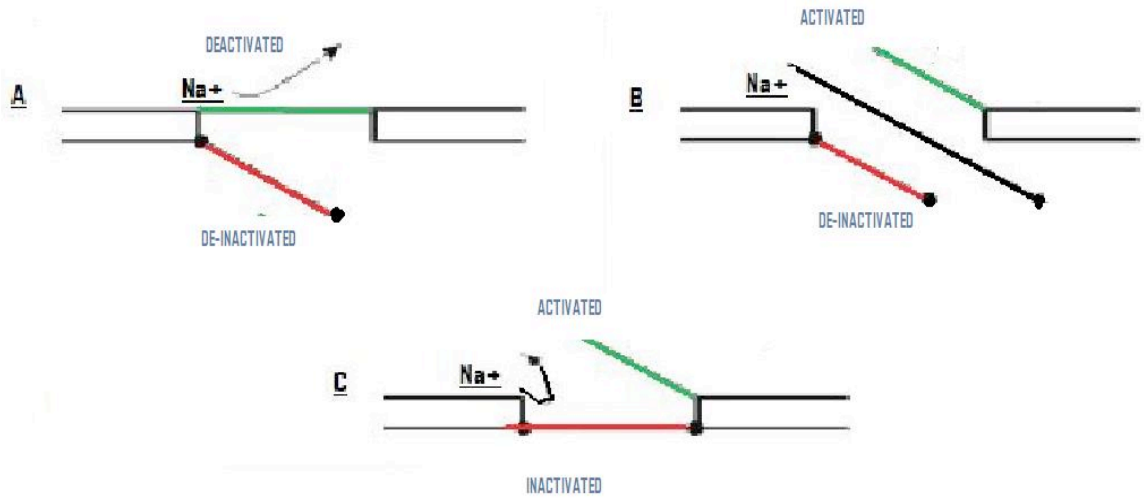


Figure 2. The sodium channel can be in many conformations. Normally at resting membrane potential the activation gate, represented as green lines, is deactivated and the inactivation gate is open or de-inactivated, represented as red (A). Upon reaching threshold potential the deactivated channel activates and the flow of ions begins (B). Within ms the the de-inactivation gate quickly inactivates stopping the flow of ions. Figure modified from Voltage-gated Sodium Channels: Physilogoy, Pathology, and Therapeutic Potential.

5. Voltage Gated Sodium channels:

Role in the nervous system, region specificity, and augmentation by supporting β proteins.

Crucial in the Initiation and propagation of action potentials throughout the nervous system is the VGSC. This in turn makes the voltage gated sodium channel critical in neuronal excitability as it controls the rising phase of the action potential.

There are ten different VGSC. Of the VGSC NaV1.1-1.9 have functional roles defined and NaV(X) does not at this time.

Table 1. VGSC Isoforms and Tissue Expression

(A) The α Subunits		
Protein	**Gene Symbol (Human)**	**Tissue Location**
$Na_v1.1$	*SCN1A*	CNS, PNS, heart
$Na_v1.2$	*SCN2A*	CNS, PNS
$Na_v1.3$	*SCN3A*	CNS, PNS
$Na_v1.4$	*SCN4A*	Skeletal muscle
$Na_v1.5$	*SCN5A*	Uninnervated skeletal muscle, heart, brain
$Na_v1.6$	*SCN8A*	CNS, PNS, heart
$Na_v1.7$	*SCN9A*	PNS, neuroendocrine cells, sensory neurons
$Na_v1.8$	*SCN10A*	Sensory neurons
$Na_v1.9$	*SCN11A*	Sensory neurons
Na_x	*SCN6A, SCN7A*	Heart, uterus, skeletal muscle, astrocytes, DRG
(B) The β Subunits		
Protein	**Gene Symbol (Human)**	**Tissue Location**
β1	*SCN1B*	Heart, skeletal muscle, CNS, glia, PNS
β1A (β1B)	*SCN1B*	Heart, skeletal muscle, adrenal gland, PNS
β2	*SCN2B*	CNS, PNS, heart, glia
β3	*SCN3B*	CNS, adrenal gland, kidney, PNS
β4	*SCN4B*	Heart, skeletal muscle, CNS, PNS

Abbreviations: VGSC = voltage-gated Na^+ channel; PNS = peripheral nervous system; DRG = dorsal root ganglia.

William J. Brackenbury, Mustafa B.A. Djamgoz, and Lori L. Isom *Neuroscientist, December 2008; vol. 14, 6: pp. 571-583.*

a. Sodium α subunit

In the central nervous system the genes SCN1A, SCN2A, SCN3A, and SCN8A encode the α portion of the proteins NaV1.1, Nav1.2, NaV1.3, and NaV1.6 respectively (Goldin, 2001) (Lai and Jan, 2006). Within each neuron the various sodium channels have unique locations. (SCNXA = Sodium channel, Voltage gated, type X alpha subunit)

In rodents NaV1.3 is highly expressed in fetal nervous tissue and ultimately replaced by NaV1.1, NaV1.2 and NaV1.6 in the adult (Westenbroek et al., 1989). NaV1.1, NaV1.3 are predominately expressed in the soma of the neurons (Westenbroek et al., 1989). NaV1.2 in unmylinated portions of axons where it is important in conduction (Westenbroek et al., 1989). NaV1.6 can be found in myelinated axons and in dendrites (Caldwell et al., 2000).

There is slight expression variation in different neurons throughout the nervous system, the main change being a change in the density of the channels. To summarize the NaV channel, it can be thought of as being responsible for action potential initiation at axon hillock site and propagation at nodes of Ranvier.

b. Augmentation: Sodium channel α subunits rarely act independently.

The Beta subunits (β) modulate VGSC surface expression, voltage dependence of activation, location and the gating of the sodium channel (Patino et al., 2009). There are four different Beta subunits (β1-β4) that are between 30-40kDa, the genes for the β subunit proteins are SCNB1-SCNB4 (Uebachs et al., 2010) (Catterall, 2000b) are located on 19q13 and 11q22-23 (Goldin, 2001). β subunits have an extra cellular Ig domain via the N terminus portion they act in cell to cell interaction via cellular adhesion molecules (CAMs) (Meadows et al., 2002a) (Meadows et al., 2002b)

The Ig domain of the β subunits also interacts with the α subunit extracellular P-loops of domain IV either non-covalently (β1 and β3) or by disulphide bonds (β2 and β4) (Uebachs et al., 2010). They work in neural migration that is critical for channel density and hence conduction. The β subunits also have an α helix that represents its trasnmembrane portion and the intracellular portion is represented by the C terminus (Merrick et al., 2009). Each β subunit has redundant and unique features, "β1 increases fast inactivation (Chen and Cannon, 1995), β2 increases fast inactivation and persistant current, β3 increases persistant current, β4 deals with resurgent currents" (Aman et al., 2009).

Ultimately the β subunit helps to regulate the VGSCs activation and inactivation gating, disruption leads to disease states from changes in neuronal excitability (Merrick et al., 2009). There are other functions for the β subunit but they are outside the scope of this review as it pertains to the central nervous system.

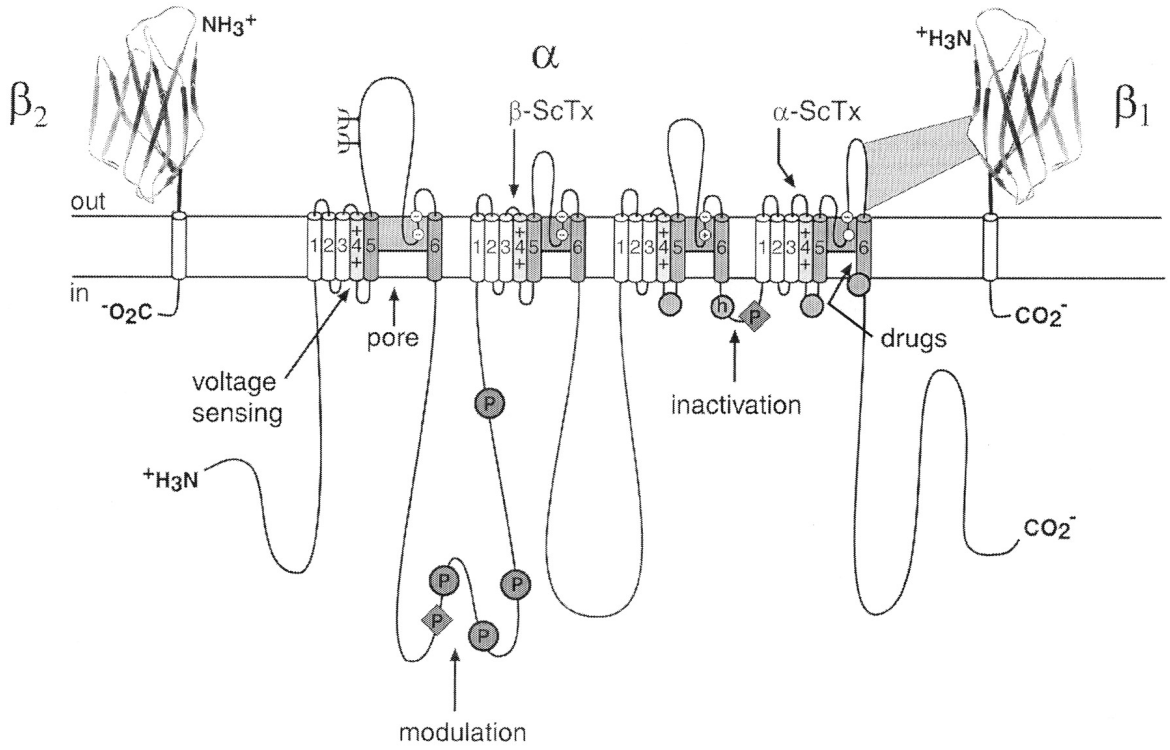


Figure 3 Voltage Gated Sodium Channel from: International Union of Pharmacology. XXXIX. Compendium of Voltage-Gated Ion Channels: Sodium Channels. IUPC W.A. Catterall 2000

6. The Voltage Gated Potassium Channel Family

Role in the nervous system, region specificity, and augmentation by supporting proteins.

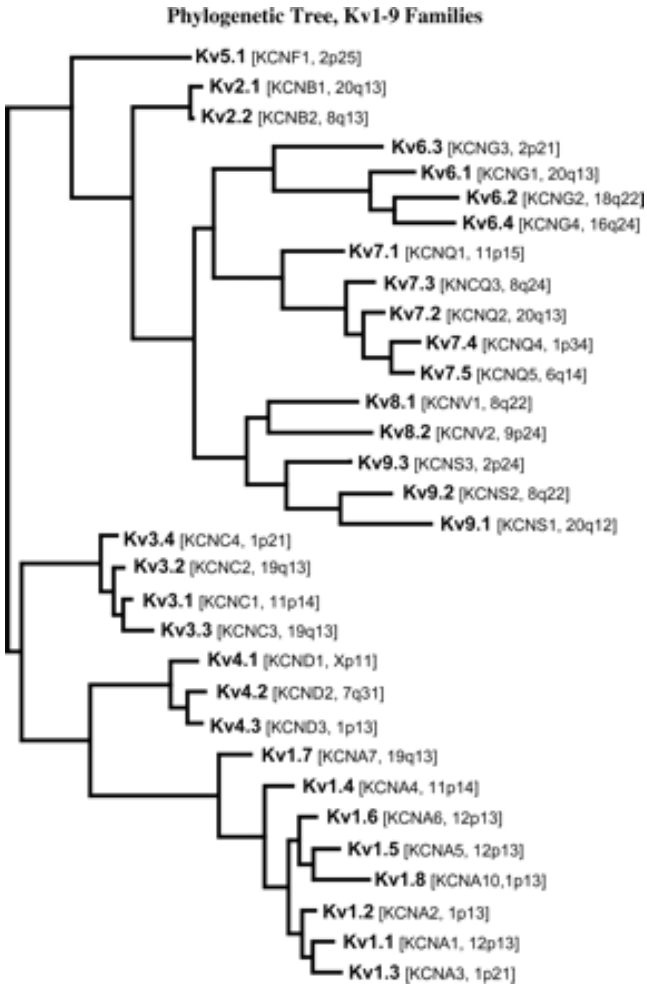


Figure 4 Phylogenetic tree of the K_v1–9 families.
International Union of Pharmacology. LIII. Nomenclature and Molecular Relationships of Voltage-Gated Potassium Channels.

a. Role in the Nervous system.

Voltage gated potassium channels are crucial in reestablishing resting membrane potential control by limiting action potential duration.

b. Specificity

Kv channels comprise the largest and most diverse class of voltage gated ion channels there are a total of 26 genes in this family. The Kv family is made of nine subfamilies, Kv1-Kv9, each subfamily has a homology of 70% amoung members. There are slight distinctions based on function within the VGKC, Kv1 through Kv4 can form functional homotetrameric VGKC whereas Kv5 through Kv9 require heteromeric assembly to be functional.

c. Augmentation

Like other members of the Superfamily, VGKC are not solely controlled and composed by poreforming α1 subunits but they are also composed of auxiliary subunits Kvβ, ChIps, MinK and others.

Kvβ is a cytoplasmicly located auxiliary protein that promotes cell surfaces expression of the α1 subunit and modulates the gating stability (Campomanes, 2002). Kvβ affects the kinetics of the α1 channel, Accili has shown that more dendrotoxin is taken up by the neuron in the presence of neurons expressing Kvβ ((Eric A. Accili, 1997). The Kvβ auxiliary subunit interacts with the amino terminus end of the protein, T1 domain, and serves as an inactivation gate on Kv1 family (Pongs, 1999). It, acts only during sustained opening of the channel (Pongs, 1999). ChIps enhance Kv4 expression and modifies its functional properties by binding in a similar

fashion as the β subunit. The minK-like subunits are associated with Long QT syndrome type 5 as they interact with the Kv7 α subunit (McCrossan and Abbott, 2004).(Catterall, 2000b)

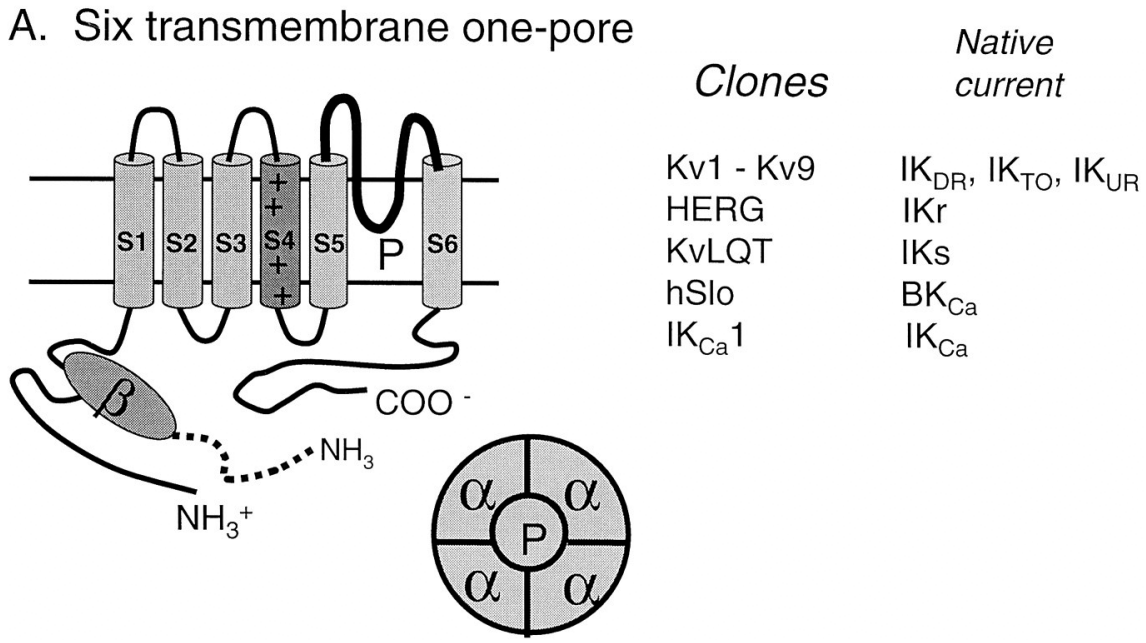


Figure 5 Classic example of α peptide subunit from voltage gated potassium channel. Four α peptide subunit join together to form one function Voltage Gated Ion Channel. The α peptide subunits can be homogenous or heterorgenous and different kinetics of the channel are displayed depending on the α peptide subunits that coassemble. From Potassium Channels: Molecular Defects, Diseases, and Therapeutic Opportunities (Shieh et al., 2000)

7. Voltage Gated Calcium Channels Family: Role of the Voltage Gated Calcium Channel.

Voltage gated calcium channels (VGCC) are the key signal transducers of sodium's electrical signaling. They convert the cell membrane depolarization in to an influx of calcium ions that initiate neurotransmition, secretion, contraction, and more.

Through action potentials they allow calcium influx ultimately triggering synaptic transmition/neurotransmitter release, a perfect example of second messenger system (Catterall, 2000b). All calcium channels that are voltage gated contain a central α1 pore that is essential in their pharmacological and physical profiles (Catterall, 2000b). Much like the sodium channel α subunit the calcium channel α1 subunit is all that is need to carry out the basic function of the channel, interestingly they carry around 25% amino acid homology in the transmembrane region.

a. **Divisions among Voltage Gated Calcium Channels!**

The calcium channels can be divided into low voltage activated channels (LVA) CaV3 channels and more complex high voltage activated channels (HVA) CaV1 and CaV2 channels (Benarroch, 2010). One distinction between these two channel types, LVA and HVA, is made by the membrane potentials at which action potentials are triggered (Zhang et al., 2002). Subcategories of these channels have also been identified and they have unique roles, locations, and pharmacologic interactions (Benarroch, 2010).

High voltage activated channels (HAV) are made up of a heteromultimers of α1 central pore that control the channel/pore subtype and a combination of possibly three ancillary subunits $\alpha_2\delta$, β, and or γ that are unrelated to the VGSC ancillary subunits (Zamponi et al., 2009). Low voltage activated channels (LVA) are only made up of α1 subunit.

There are a total of 10 different α1 central proteins in vertebrates belonging to gene families Ca_v1, Ca_v2, and Ca_v3.

Ca_v1 gene has four different α1 proteins ($Ca_v1.1$, to $Ca_v1.4$). Each of these proteins is a part of the L type ("long lasting") calcium channel current which has different distribution and function (Benarroch, 2010). They are defined by their interaction with drugs in the dihydropyridine family such as nifedipine (Benarroch, 2010). These channels have the property of slow activation and inactivation as relatively compared to their counter parts Ca_v2 (Venance et al., 2006). $Ca_v1.2$ subunit allows large influxes of calcium to enter the soma or dendrites of the neurons (Benarroch, 2010). This activates protein kinase downstream pathways that promote transcription of genes for synaptic plasticity in the cerebral cortex and hippocampus (Benarroch, 2010).

CACNL1A4 genes comprise the Ca_v2 subfamily which has three different α1 proteins $Ca_v2.1$, $Ca_v2.2$. and $Ca_v2.3$ that respectively are a part of the P/Q, N, and R type of calcium channels (Benarroach, 2010) and are known for fast synaptic transmission in the nervous system. The $Ca_v2.2$ are defined by their sensitivity to biologic toxins from arachnids and gastropodia that act as blockades (Catterall, 2000b). Found primarily in

soma and dendrites of neurons, all three sub groups of the Ca_v2 family are involved in presynaptic terminal excitation and neurotransmitter release (Catterall, 2000b). Spontaneous or lab induced $Ca_v2.1$ mutations are known to cause absence epilepsy and ataxia.

CACNA1 genes G, H, and I encode the α1 proteins $Ca_v3.1$, $Ca_v3.2$. and $Ca_v3.3$ respectively. Each of these proteins is part of the T-type (transient) calcium channel, a low voltage activated channel that, as the name states, is transiently active. Ca_v3s are located at distal dendritic sites as well as close to the cell body (Molineux et al., 2006). While the Ca_v3x can be found throughout the brain, thalamic neurons expressing Cav 3x are most uniquely defined by their two patterns of discharge, rebound burst and tonic firing mode (Benarroch, 2010). These low voltage activated channels (LVA) activate at hyperpolarized membrane potentials (~ -70mv) where the previously mentioned high voltage activated channels (HVA) are inactivated. HVAs are the Ca_V1 and Ca_V2 channels described previously.

The LVA or T-type calcium channels are monomers of the α_1 subunit but there is some debate as to their overall structure and composition (Catterall, 2000a). The α_1 subunit defines the channel subtype for both LVA and HVA (Catterall, 2000a). Of note LVAs also lack the conserved region within the I-II loop called the alpha interaction domain (AID) that is present in the HVA and allows binding of the β subunit (Pragnell et al., 1994). Thus, even if there are β auxiliary subunits in the cytoplasm of neurons they would are not able to bind the LVA α1 channels.

As the name states, high voltage activated channels HAVs have a higher threshold of activation and thus require a larger depolarization to achieve activation compared the LVAs. The LVAs inactivate and activate extremely fast compared to the HVAs that have a slight delay in deactivation (Perez-Reyes, 2003).

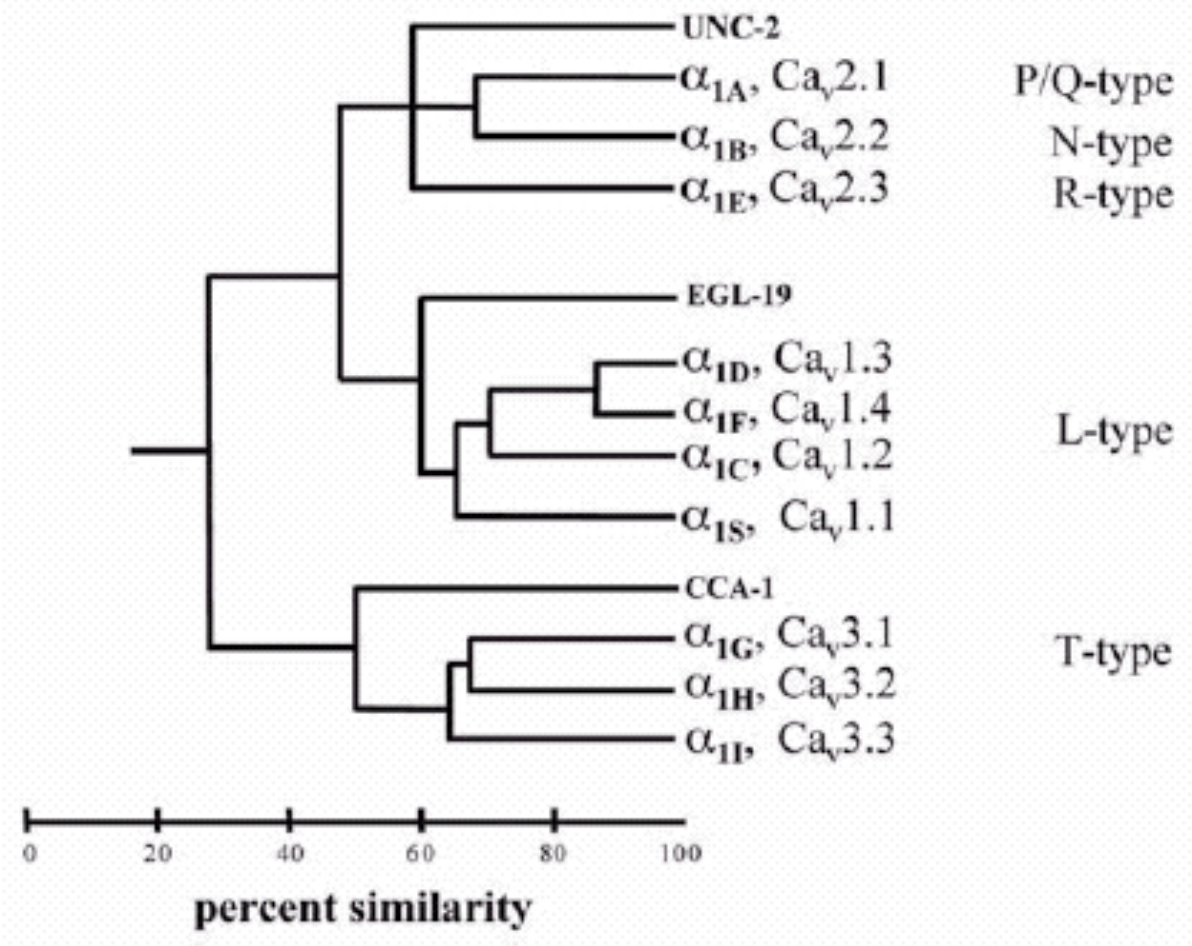


Figure 6 Phylogenetic tree of known Voltage Gated Calcium Channels.
(Snutch, 2005). Targeting chronic and neuropathic pain: the N-type calcium channel comes of age. NeuroRx 2, 662–670.

b. Extrinsic modification of α1 subunit: Defining the function of auxiliary subunits β, α2δ, CaM, and γ.

While the α1 subunit are charged with the duty of calcium ion selective conductance and voltage sensing, the auxiliary subunits are charged with modulation of the α1 channel as well as membrane targeting (Dolphin, 2009).

In Mammalian species there are at least four types of auxiliary subunit families that attach to the α1 pore of HVA calcium channels. These include α2δ, β, CaM, and γ each with its own respective subtypes (Bichet et al., 2000). Each HVA calcium channel has at least one β, and one α2δ the role of the γ auxiliary unit is in debate (Dolphin, 2006). The primary structure of the auxiliary subunits was devised using cDNA cloning, sequencing, and protein chemistry (Tanabe et al., 1987).

Just as in the case of the sodium channels the ancillary subunits control modulation of the overall voltage gated calcium channel complex but they have little effect on the basic over all function of the channel (Benarroch, 2010).

i. β subunits

The β subunits (55 kDa) are the only calcium channel subunits that are completely intracellular (Opatowsky et al., 2003). In vertebrates there are four β subunits (β1-β4) each encoded by its own gene (Richards et al., 2004). The β subunit is integral in enhancing the localization of the α1 calcium channel to the cell membrane from there subcellular location (Bichet et al., 2000; Catterall, 2000b). Obermair et al showed that

mutations at the β α1 interaction site or linker, the guanylate kinase domain, decreased membrane expression and targeting of the α1 subunit (Obermair et al., 2010). This is most likely due to the fact that the β protein hides the endoplasmic retention signal that is on the α1 protein (Bichet et al., 2000). As stated earlier the β subunit binds to a highly conserved portion of the α1 HVA channel, the α1 interaction domain (AID) (Obermair et al., 2010) (Pragnell et al., 1994).

Further examination also shows that all of the beta isoforms are expressed in similar levels with similar distributions in presynaptic and postsynaptic calcium channels of hippocampal neurons (Obermair et al., 2010). Obermair further suggest the amount of β subunit is a limiting factor for α1 subunit surface expression (Obermair et al., 2010).

Overall the β subunit modifies the kinetics of the α1 channel adding to the channels diversity and increasing its membrane surface expression (Benarroch, 2010) (Opatowsky et al., 2003).

ii. γ Subunit

The γ subunit (33kDa) a glycol protein with four transmembrane segments and an intracellular N and C terminus, was originally thought to have no neuronal component (Jay et al 1990) (Catterall, 2000b). The Stargazer mouse model revealed its neuronal significance, it is a mutation in the second intron of the *CACNG2* gene on chromosome 15, by limiting surface expression of the γ2 subunit (Letts et al., 1998). There have been a total of eight γ subunits (γ1-γ8) identified to date and each has a slightly different

extracellular function. There molecular interactive role with the α1 subunit remain mostly elusive thus far. However, γ2 appears to associate with the α1 P/Q channel and the γ3 with the N type channel (Arikkath and Campbell, 2003). Premature truncation of the γ2 portion P/Q calcium channel produces the, "stargazing mouse" absence seizure model which will be covered later. The Role of the CAM subunit will not be reviewed here.

iii. α2δ subunit

The α2δ subunit (170 kDa) is actually two peptides that are dependent upon each other for their affect on the α1 pore. In vertebrates there are four α2δ subunits encoded by four separate genes some with varying locations (Arikkath and Campbell, 2003). The single gene single protein rule of molecular biology means that in order to get an α2δ there must be a post translational modification giving the extracellular N terminal α2 (147kDa) and transmembrane δ (27kDa) portions of the α2δ subunit (De Jongh KS, 1990). The two peptides from one gene are linked together via a disulfide bond (Dolphin, 2006). The α2δ subunit alters the HVA channel pharmacology, gating, and peak current amplitude (De Jongh KS, 1990). How this is accomplished is not known or investigated at this time.

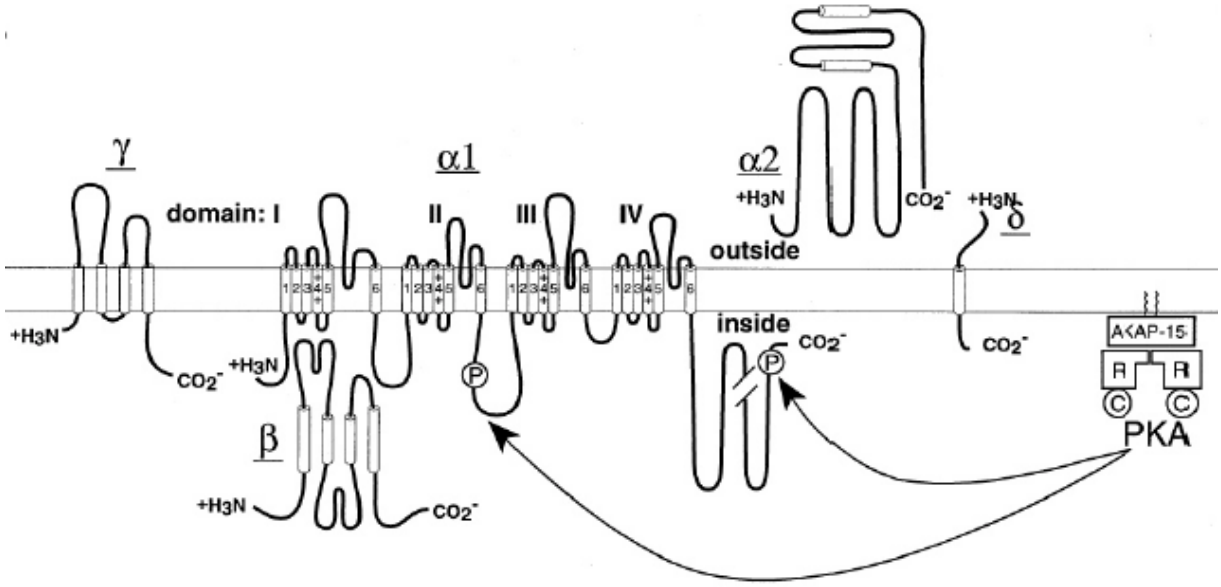


Figure 7. Subunit structure of Ca_V1 channels. The subunit composition and structure of calcium channels purified from skeletal muscle are illustrated. This model fits available biochemical and molecular biological results for other Ca_V1 channels and for Ca_V2 channels. Predicted α-helices are depicted as cylinders. The lengths of lines correspond approximately to the lengths of the polypeptide segments represented.. Ann. Rev. Cell Dev Biol. 2000 (Catterall, 2000b)

iv. T-type Ca Channels Currents: The Pacemaker produced by LVA Ca Channels

Although LVA (T-type ca^{++} channel) do not have the auxiliary subunits they produce a transient, T current that has unique biophysical features compared to the HVA channels and their auxiliary subunits. T - type ca channels activate and inactivate near resting membrane potential ~-60mV. The current trend of thought is that T-type Ca^{2+} channel is a pacemaker type cell that serves to keep oscillatory rhythms in cardiac and neuronal tissue. The activation kinetics of T channel produces a window current with overlapping open and closed channels. This allows a “one foot in the door approach” for rapid activation by utilizing unclosed calcium channels and quickly opening others.

8. Voltage Gated Sodium channels and the Hyperexcitability of epilepsy!

SCN1A (2q24.3) belongs to the gene family that encodes the VGSCs that initiate electrical excitability signals, in some models it ultimately results in presynaptic release of the inhibitory neurotransmitter GABA through action potentials. Sequence analysis is used to find mutations in the SCN1A gene, approximately 330 known mutations with varying levels of impact ranging from no effect to severe myoclonic epilepsy of infancy (Dravet syndrome) have been found using sequence analysis or deletion testing (Lossin, 2009). SCN1A mutations are dominate heterozygous mutations that result in a variety of changes in the NaV1A protein ultimately leading to epilepsy, neuropathic pain, and other conditions.

Many mutations are single amino acid mutations such as insertion, deletion, or missence. Many of these mutations result in mild simple change in the protein structure and therefore possibly change the gating, translocation, or auxiliary subunit attachment activity of the channel. Changes that result in nonfunctional versions of the NaV1.1 protien are obviously worse and possibly incompletely compensated for by the nervous systems remaining normal SCN1A gene, they have been known to result from frame shift mutation, missence mutations, and a host of other mutations (Claes et al., 2001). The ultimate result of many of the mutations is altered sodium ion translocation across the membrane spanning channel, the degree to which seems to underlie the severity of the mutation (Escayg and Goldin, 2010). This predisposes the organism to neurologic diseases, of importance in this review, epilepsy.

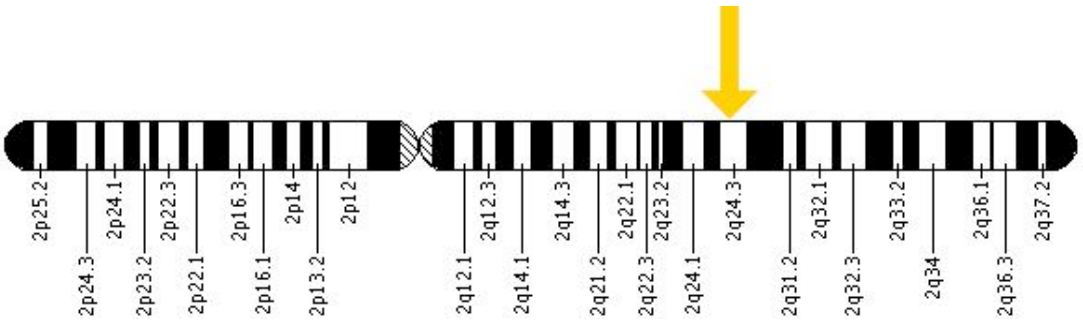


Figure 8 SCN1A gene location on chromosome 2q24.3. Obtained from US National Library of Medicine via link http://ghr.nlm.nih.gov/gene=scn1a

9. **SCN1A mutations are a continuum of related epileptic disorders that present with variation in phenotype even among family members.**

(FS = febrile seizure, GEFS = generalized epilepsy febrile seizure, ICEGCT = intractable childhood epilepsy with generalized tonic-clonic seizures, SMEI/Dravet = sever myoclonic epilepsy of infancy)

The range of SCN1A mutations leads to a host of disease states, these disease states mainly vary based on the severity and or location of mutation. Along the continuum of disease there is FS, GEFS+, ICEGCT, SMEIB, and SMEI. There are less common disease states, Lennox-Gastaut, vaccine related encephalopathy and seizure, etc, that maybe allowed in this spectrum of disease but they will not be considered here.

Of note, the following epilepsy/seizure conditions described for SCN1A can be thought of as stacked building blocks, a continuum. Each successive condition encompasses the previous with increases in ionic conductance disturbance and phenotypic severity!

a. **Febrile Seizures**

Febrile Seizures (FS) are the most common type of seizures in children occurring in 3 to 5% of children less than 6yrs old (Escayg and Goldin, 2010). FS can be simple or complex, complex last longer than 15 minutes, and may occur in multiples per 24 hour period. FS occur only in the setting of fever usually greater than 38 °C, they typically occur after 6mo of age and resolve by age 5 or 6 (Hemal et al., 2010

). Despite investigation these seizure appear to have no clinical cause in the majority of cases. FS**+** (plus) are known to persist beyond the age of 6yrs and may have status epilepticus presentation (Hemal et al., 2010

). The loci for Febrile seizure (FS) are on several genes, of interest in this review are the autosomal dominantly inherited FEB3 gene located on 2q 23-24 the same region as SCN1A (Mantegazza et al., 2005). Mantegazza demonstrated that M145T mutation causes a 60% decrease in current and hence decrease in NaV1.1 channel function (Mantegazza et al., 2005).

FS resolve in early childhood in the vast majority of cases, when they fail to resolve in early childhood they gain the (+) designation (Scheffer and Berkovic, 1997).

b. Generalized Epilepsy Febrile Seizure Plus

Generalized (genetic) **Epilepsy Febrile Seizure Plus** (GEFS+) a genetic epilepsy with strong familial ties and variable expressivity is thought to be based on the position of mutation and the small changes due to the relatively high degree of missence mutations and possibly environmental cues, it began to first unravel to Scheffer as he discovered multiple mutations in the SCN1A gene (Scheffer et al., 2009) (Escayg and Goldin, 2010). GEFS+ is an expansion on FS+ but it seizure type is not limited to febrile seizures, although they are the most common. In contrast these patients also experience afebrile tonic-clonic, absence epilepsy, myoclonic and atonic seizures thus it is a spectrum of disease states with ever changing severity (Scheffer and Berkovic, 1997) (Gambardella and Marini, 2009). This form of epilepsy and the remaining epilepsies discussed pertaining to SCN1A are autosomal dominantly inherited and have varying degrees of haploinsuffiency thought to be tied to the severity level of mutations (Scheffer et al., 2009)

c. Dravet syndrome

Dravet syndrome (SMEI and SMEIB) is the most severe aggressive epilepleptic disease phenotype associated with mutation in the SCN1A gene. Unlike the previously mentioned SCN1A disease states, Dravet syndrome is refractory to many common epileptic drugs further; many drugs exacerbate the disease state (Mullen and Scheffer, 2009). Like its preceding counter parts Dravet syndrome is triggered by elevated temperatures, but these seizures seem to be triggered more easily, perhaps at lower temperatures or shorter length of exposure, such as exercising and hot bathes (Catterall et al., 2010). The time frame for initiation of the seizures, 6mo, holds for Dravet syndrome but the seizure severity and type are not as limited as the previous SCN1A seizure types (Scheffer et al., 2009). In fact the initial seizure maybe generalized tonic clonic (Gambardella and Marini, 2009). The second year of life is marked by greater developmental delay and possibly greater motor impairment than what is expected in GEFS+, the seizure begin to progress in length and status epilepticus is not rare (Scheffer et al., 2009) (Catterall et al., 2010). With the combination of all the above, the long term outlook is bleak with developmental regression (Kullmann and Waxman, 2010). When all of the subtypes of Dravet syndrome are combined (SMEI, SMEIB, ICEGTC) it encompases ~ 85-90% of phenotypes related to SCN1A gene.

Of note “The full phenotypic spectrum of SCN1A mutations is still undefined” (Gambardella and Marini, 2009).

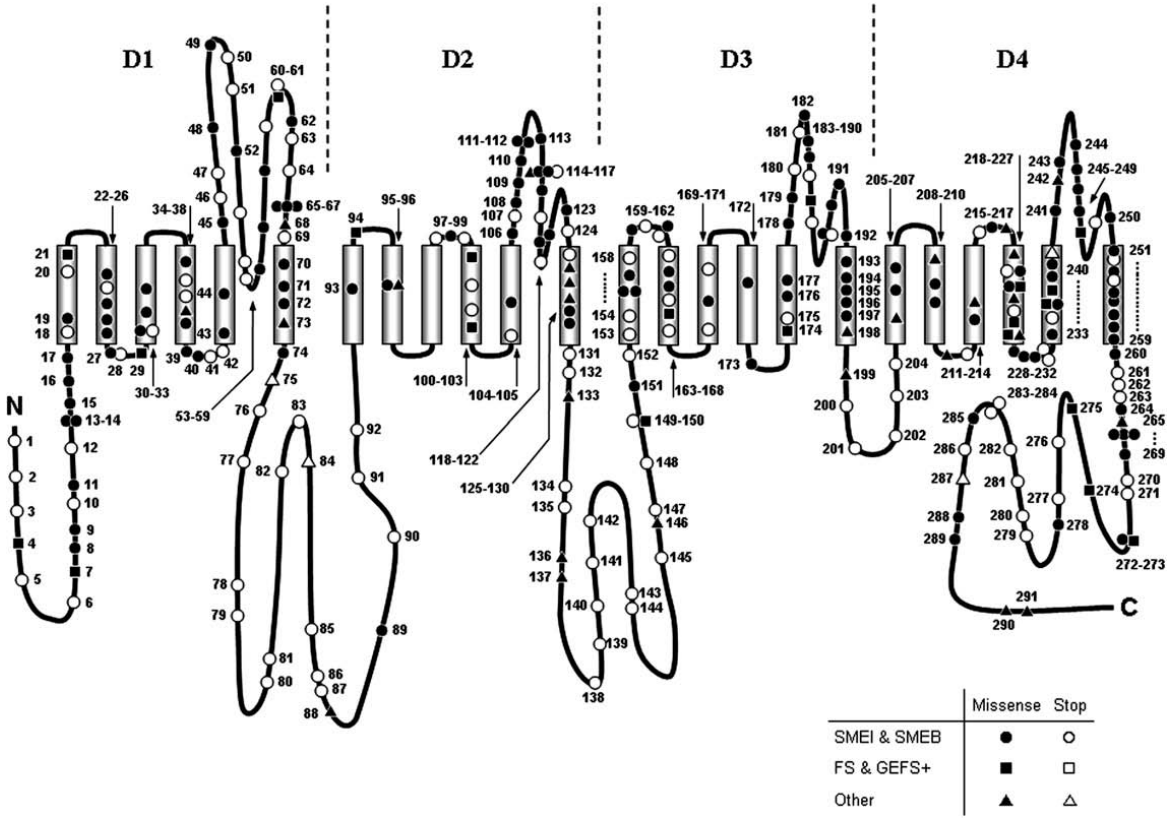


Figure 9 Mutation map for Nav 1.1. Two dimensional rendering of the Nav1.1 protein with all currently reported exonic mutations at their approximate location. Topology based on Escayg and colleagues. For the sake of clarity, SMEI and SMEB phenotypes (e.g., SMEB-O, SMEBM, aSMEI, etc.) were combined and drawn as circles on the basis of their phenotypic similarity. Benign febrile epilepsy types, GEFS+ and FS, are depicted as squares. Mutations causing epilepsy or neurological disorders not falling into the previous two categories are shown as triangles. The numbering of the mutations corresponds to Table 2. Open symbols highlight mutations that generate non-sense truncated Nav1.1 (Lossin, 2009).

Approimately ten percent GEFS+ is related to the SCN1A gene and eighty five percent to 90 percent of Dravet syndrome is related to SCN1A (Lossin, 2009). All of the GEFS+ mutations associated with SCN1A, of which there are approximately 30, have been missence mutations (Escayg and Goldin, 2010). Due to the milder nature of the GEFS+ phenotype as compared to the Dravet syndrome these mutations are speculated to be milder perhaps leaving some residual function of the NaV1.1 channel (Escayg and Goldin, 2010). In Dravet syndrome somewhere in the neighborhood of 300 mutations, ~ 50% of the total mutations, are linked to frame-shift, nonsense, and splice site mutations (Meisler and Kearney, 2005) (Escayg and Goldin, 2010). As one might imagine the earlier the disruption in protein structure, or increase in the severity of disruption caused by some missence mutations, the greater the loss of function from that protein (Meisler and Kearney, 2005). This seems to be the case in many of the mutations involved in Dravet syndrome. This may be explained by looking at knockout models that would show haploinsuffiency of the NaV1.1 protein (Yu et al., 2006). This is further supported by the premise that most Dravet patient's mutations are de novo versus the familial nature of the GEFS+ patients. The Dravet patients are so affected that they rarely are able to reproduce as compared to the GEFS+ patients.

Although both Dravet and GEFS+ have missence mutations it is postulated that the position of Dravet mutations are likely to be in the pore region therefore having a greater effect on ion transport and therefore increase the disease severity (Meisler and Kearney, 2005).

d. **"Exonic mutations of the voltage-gated sodium channel Nav1.1-epilepsies and other neurological disorders." The table 2 was adopted from "A catalog of SCN1A variants" by Christopher Loosin.**

Table 2

AA Level	NA Level	Exon	Topology	Phenotype	Reference
Q3X	C7T	1	N-terminus	SMEI	(Claes et al., 2003)
F14fsX91	[T]41del	1	N-terminus	SMEI	(Harkin et al., 2007)
E20X	G58T	1	N-terminus	SMEI	(Ceulemans et al., 2004)
R28C	C82T	1	N-terminus	GEFS+	unpublished data
P37fsX91	[C]111del	1	N-terminus	SMEI	(Wallace et al., 2003)
Y65X	T195A	1	N-terminus	SMEI	(Zucca et al., 2008)
S74P	T220C	1	N-terminus	FS	(Marini et al., 2007)
E78D	G234T	1	N-terminus	SMEI	(Nabbout et al., 2003)
D79H	G235C	1	N-terminus	SMEB-O	(Harkin et al., 2007)
Y83X	C249G	1	N-terminus	SMEI	(Nabbout et al., 2003)
Y84C	A251G	1	N-terminus	SMEI	(Harkin et al., 2007)
N94X	[TT]277-278del	2	N-terminus	SMEI	(Mancardi et al., 2006)
R101W	C301T	2	N-terminus	SMEI	(Harkin et al., 2007)
R101Q	G302A	2	N-terminus	SMEB	(Fukuma et al., 2004)
S103G	A307G	2	N-terminus	SMEI	(Fujiwara et al., 2003)
T112I	C335T	2	N-terminus	SMEI	(Fujiwara et al., 2003)
R118S	G354C	2	N-terminus	SMEI	(Zucca et al., 2008)
S128X	C383A	2	D1/S1	SMEI	(Nabbout et al., 2003)
[L]129del	[ATT]387-389del	3	D1/S1	SMEI	(Mancardi et al., 2006)
V143fsX148	[GT]429-430del	3	D1/S1	SMEI	(Fukuma et al., 2004)
M145T	T434C	3	D1/S1	FS	(Mantegazza et al., 2005)

T162P	A484C	4	D1/S2	SMEI	(Mancardi et al., 2006)
Y165X	T495A	4	D1/S2	SMEI	(Nabbout et al., 2003)
T166fsX170 495[GTGAATC]	496ins	4	D1/S2	SMEI	(Harkin et al., 2007)
I171K	T512A	4	D1/S2	SMEB-SW	(Harkin et al., 2007)
A175T	G523A	4	D1/S2	SMEB-O	(Harkin et al., 2007)
G177E	G530A	4	D1/S2-S3in	SMEI	(Nabbout et al., 2003)
G177fsX180	[G]530del	4	D1/S2-S3in	SMEI	(Fujiwara et al., 2003)
D188V	A563T	4	D1/S2-S3in	GEFS+	(Wallace et al., 2001)
W190R	T568C	4	D1/S3	SMEI	(Oguni et al., 2005)
W190X	G570A	4	D1/S3	SMEI	(Marini et al., 2007)
D194N	G580A	4	D1/S3	SMEI	(Mancardi et al., 2006)
T199R	C596G	4	D1/S3	SMEB-SW	(Harkin et al., 2007)
T217K	C650A	5	D1/S4	SMEI	(Mancardi et al., 2006)
S219fsX275	[AG]657-658del	5	D1/S4	SMEI	(Claes et al., 2001)
R222X	C664T	5	D1/S4	SMEI	(Claes et al., 2001)
T226M	C677T	5	D1/S4	CGE	(Harkin et al., 2007)
I227S	T680G	5	D1/S4	SMEI	(Nabbout et al., 2003)
A239T	G715A	6	D1/S4-S5in	SMEB-SW	(Harkin et al., 2007)
V244L	G730T or G730C	6	D1/S4-S5in	SMEI	(Morimoto et al., 2006)
V244fsX275	[GT]731-732 del	6	D1/S4-S5in	SMEI	(Marini et al., 2007)
K246X	A736T	6	D1/S4-S5in	SMEI	(Morimoto et al., 2006)
I252N	T755A	6	D1/S5	SMEI	(Ceulemans et al., 2004)
G265W	G793T	6	D1/S5	SMEI	(Fujiwara et al., 2003)
W280R	T838C	6	P1	SMEI	(Nabbout et al., 2003)
A285fsX290	[CTTC]854-857del	6	P1	SMEI	(Nabbout et al., 2003)
E289X	G865T	6	P1	SMEI	(Nabbout et al., 2003)
T297I	C890T	6	P1	SMEI	(Nabbout et al., 2003)
R322I	G965T	6	P1	SMEI	(Marini et al., 2007)
Y323X	T969A	7	P1	SMEI	(Mancardi et al., 2006)
L331fsX339	992[T]993ins	7	P1	SMEI	(Mancardi et al., 2006)
G343E	G1028A	7	P1	aSMEI	(Fujiwara et al., 2003)
G348X	G1042T	8	P1	SMEI	(Mancardi et al., 2006)
M350fsX355	[AT]1048-1049del	8	P1	SMEI	(Harkin et al., 2007)
V352fsX355	[TG]1055-1056del	8	P1	SMEI	(Harkin et al., 2007)
R356G	A1066G	8	P1	SMEB	(Marini et al., 2007)
P358T	C1072A	8	P1	SMEB	(Marini et al., 2007)

D366E	T1098A	8	P1	SMEI	(Zucca et al., 2008)
S374fsX378	[C]1121del	8	P1	SMEI	(Nabbout et al., 2003)
R377X	C1129T	8	P1	SMEI	(Mancardi et al., 2006)
R377Q	G1130A	8	P1	GEFS+	(Zucca et al., 2008)
F383L	C1149G	8	P1	SMEI	(Mancardi et al., 2006)
W384X	G1152A	8	P1	SMEB	(Berkovic et al., 2006)
Q389X	C1165T	8	P1	SMEI	(Nabbout et al., 2003)
R393S	C1177A	9	P1	SMEI	(Mancardi et al., 2006)
R393C	C1177T	9	P1	SMEI	(Mancardi et al., 2006)
R393H	G1178A	9	P1	SMEI	(Claes et al., 2003)
A395P	G1183C	9	P1	CGE	(Harkin et al., 2007)
Y399X	C1197A	9	P1	SMEI	(Harkin et al., 2007)
[M]400del	[ATG]1198-120del	9	D1/S6	SMEI	(Ceulemans et al., 2004)
F403L	T1207C	9	D1/S6	SMEI	(Berkovic et al., 2006)
Y413N	T1237A	9	D1/S6	SMEI	(Berkovic et al., 2006)
V422E	T1265A	9	D1/S6	CGE	(Harkin et al., 2007)
Y426N	T1276A	9	L1	SMEI	(Nabbout et al., 2003)
L433fsX449	1299[C]1300ins	9	L1	MAE	(Ebach et al., 2005)
E435X	G1303T	9	L1	SMEI	(Fukuma et al., 2004)
I448X	1342-1352del	9	L1	SMEI	(Wallace et al., 2003)
R501fsX543	[G]1502del	10	L1	SMEI	(Ohmori et al., 2002)
R542X	C1624T	10	L1	SMEI	(Mancardi et al., 2006)
K547fsX570	1640[A]1641ins	10	L1	SMEI	(Ohmori et al., 2002)
K547fsX569	[AA]1639-1640del	10	L1	SMEI	(Harkin et al., 2007)
F563fsX622	[C]1687del	11	L1	SMEI	(Harkin et al., 2007)
R568X	C1702T	11	L1	SMEI	(Ohmori et al., 2002)
F575fsX622	[T]1724del	11	L1	CFE	(Harkin et al., 2007)
S607fsX622	[C]1820del	11	L1	SMEI	(Ohmori et al., 2002)
R613X	C1837T	11	L1	SMEI	(Mancardi et al., 2006)
S620fsX624	1857[GCAAC]1858ins	11	L1	SMEB	(Marini et al., 2007)
S626G	A1876G	11	L1	CGE	(Harkin et al., 2007)
D674G	A2021G	11	L1	SMEI	(Harkin et al., 2007)
P707fsX715	2118[AA]2119ins	12	L1	SMEI	(Ohmori et al., 2002)
R712X	C2134T	12	L1	SMEI	(Sugawara et al., 2002)
Q732fsX749	2196[CACCCGT]2197ins	13	L1	SMEI	(Fujiwara et al., 2003)
L783P	T2348C	13	L1	SMEI	(Harkin et al., 2007)
Y790C	A2369G	13	D2/S1-S2ex	GEFS+, PS	(Annesi et al., 2003)
T808S	A2422T	14	D2/S2	ICEGTC	(Fujiwara et al., 2003)
T808R	C2435G	14	D2/S2	SMEI	(Mancardi et al., 2006)
G854fs876X	[G]2561del	14	D2/S3-S4ex	SMEI	(Buoni et al., 2006)

E853K	G2536A	14	D2/S3-S4ex	SMEI	(Mancardi et al., 2006)
G854fsX876	[A]2562del	14	D2/S3-S4ex	SMEI	(Harkin et al., 2007)
R859C	C2575T	14	D2/S4	GEFS+	(Barela et al., 2006)
R865X	C2593T	15	D2/S4	SMEI	(Ohmori et al., 2003)
A870fsX874	[GCAAAAT]2608-2614del	15	D2/S4	SMEI	(Marini et al., 2007)
T875M	C2624T	15	D2/S4	GEFS+	(Escayg et al., 2001)
L893X	T2678A	15	D2/S5	SMEI	(Mancardi et al., 2006)
F902C	T2705G	15	D2/S5	SMEI	(Ohmori et al., 2002)
R931C	C2791T	15	P2	SMEI	(Ohmori et al., 2002)
W932X	G2796A	15	P2	SMEI	(Claes et al., 2003)
M934I	G2802C or G2802A	15	P2	SMEB	(Fukuma et al., 2004)
H939Q	C2817G	15	P2	SMEI	(Claes et al., 2003)
L942P	T2825C	15	P2	SMEI	(Mancardi et al., 2006)
V944A	T2831C	15	P2	SMEB	(Fukuma et al., 2004)
V944E	T2831A	15	P2	SMEI	(Harkin et al., 2007)
F945L	T2833C	15	P2	SMEI	(Harkin et al., 2007)
R946C	C2836T	15	P2	SMEB	(Fukuma et al., 2004)
R946H	G2837A	15	P2	SMEB	(Fukuma et al., 2004)
R946S	C2836A	15	P2	SIGEI	(Ebach et al., 2005)
R946fsX953	[C]2835del	15	P2	SMEI	(Fujiwara et al., 2003)
G950E	G2849A	15	P2	SMEI	(Harkin et al., 2007)
W952X	G2855A	15	P2	SMEI	(Fujiwara et al., 2003)
W957L	G2870T	15	P2	SMEI	(Marini et al., 2007)
D958fsX973	[C]2874del	15	P2	SMEI	(Sugawara et al., 2002)
C959R	T2875C	15	P2	SMEI	(Claes et al., 2003)
M960V	A2878G	15	P2	SMEI	(Fujiwara et al., 2003)
Q965X	C2893T	15	P2	SMEI	(Harkin et al., 2007)
T970fsX972	[CATG]2916-2919del	15	D2/S6	SMEI	(Mancardi et al., 2006)
M973V	A2917G	15	D2/S6	CGE	(Harkin et al., 2007)
G979R	G2935A	15	D2/S6	ICEGTC	(Fujiwara et al., 2003)
V983A	T2948C	16	D2/S6	ICEGTC	(Fujiwara et al., 2003)
N985I	A2954T	16	D2/S6	SMEI	(Fujiwara et al., 2003)
L986F	C2956T	16	D2/S6	SMEI	(Claes et al., 2001)
A1002fsX1009	[C]3006del	16	L2	SMEI	(Ohmori et al., 2002)
E1008X	G3022T	16	L2	SMEB-SW	(Harkin et al., 2007)
N1011I	A3032T	16	L2	ICEGTC	(Fujiwara et al., 2003)
K1027X	A3079T	16	L2	SMEI	(Ohmori et al., 2002)
E1032fsX1045	[A]3096del	16	L2	SMEI	(Harkin et al., 2007)
I1034T	T3101C	16	L2	FA	(Weiss et al., 2003)

F1038L	C3114	G or A	L2	FA	(Weiss et al., 2003)
K1058fs1078X	[AAGA]3173-3176del	16	1	SMEI	(Mancardi et al., 2006)
H1065fsX1073	[TA]3195-3196del	16	L2	SMEI	(Nabbout et al., 2003)
K1077fsX1079	[A]3231del	16	L2	SMEB	(Berkovic et al., 2006)
T1082fsX1086	[C]3245del	16	L2	SMEI	(Ohmori et al., 2002)
E1099X	G3295T	16	L2	SMEI	(Mancardi et al., 2006)
S1100fsX1107	3299[AA]3300ins	16	L2	SMEI	(Claes et al., 2001)
P1116fsX1119	[C]3347del	16	L2	SMEI	(Marini et al., 2007)
G1154fsX1163	[T]3462del	17	L2	SMEI	(Harkin et al., 2007)
T1174S	C3521G	17	L2	FHM	(Gargus and Tournay, 2007)
L1175fsX1182	[TT]3524-3525del	17	L2	SMEI	(Fukuma et al., 2004)
Q1187fsX1215	[AA]3561-3562del	18	L2	SMEI	(Harkin et al., 2007)
W1204X	G3611A	18	L2	SMEI	(Sugawara et al., 2003)
W1204R	T3610C	18	L2	GEFS+	(Escayg et al., 2001)
L1207P	T3620C	18	L2	SMEI	(Zucca et al., 2008)
R1213X	C3637T	18	L2	SMEI	(Fujiwara et al., 2003)
I1214X	[TA]3641-3642del	18	D3/S1	SMEI	(Nabbout et al., 2003)
V1215X	3642[TA]3643ins	18	D3/S1	SMEI	(Nabbout et al., 2003)
S1231R	T3693A	18	D3/S1	SMEI	(Fujiwara et al., 2003)
S1231T	G3692C	18	D3/S1	SMEI	(Kearney et al., 2006)
G1233R	G3697C	18	D3/S1	SMEI	(Nabbout et al., 2003)
L1235fsX1244	[G]3705del,3704 [20-bp]3705ins	18	D3/S1	SMEI	(Mancardi et al., 2006)
E1238D	A3714C	19	D3/S1-S2ex	SMEI	(Berkovic et al., 2006)
I1242fsX1270	3726[AT]3727ins	19	D3/S1-S2ex	SMEI	(Marini et al., 2007)
R1245Q	G3734A	19	D3/S1-S2ex	SMEI	(Mancardi et al., 2006)
R1245X	C3733T	19	D3/S1-S2ex	SMEI	(Nabbout et al., 2003)
V1257fsX1269	[T]3774del	19	D3/S2	SMEI	(Zucca et al., 2008)
F1263L	C3789G	19	D3/S2	aSMEI	(Fujiwara et al., 2003)
L1265P	T3794C	19	D3/S2	SMEI	(Ohmori et al., 2002)
L1269fsX1292	[A]3774del	19	D3/S2	SMEI	(Zucca et al., 2008)
K1270T	A3809C	19	D3/S2	GEFS+	(Abou-Khalil et al., 2001)
W1271X	G3812A	19	D3/S2	SMEI	(Ohmori et al., 2002)
W1284X	G3852A	19	D3/S3	SMEI	(Fujiwara et al., 2003)
[F]1289del	[CTT]3867-3869del	19	D3/S3	SMEI	(Ohmori et al., 2002)
D1293fsX1299	[A]3878del	19	D3/S3	SMEI	(Marini et al., 2007)
A1326P	G3976C	20	D3/S4	SMEI	(Wallace et al., 2003)

V1335M	G4003A	21	D3/S4-S5in	SMEI	(Zucca et al., 2008)
V1353L	G4057C	21	D3/S5	GEFS+	(Wallace et al., 2001)
C1354fsX1359	[T]4062del	21	D3/S5	SMEI	(Berkovic et al., 2006)
L1355P	T4064C	21	D3/S5	SMEB	(Fukuma et al., 2004)
W1358S	G4073C	21	D3/S5	SMEI	(Zucca et al., 2008)
V1390M	G4168A	21	P3	SMEI	(Ohmori et al., 2002)
C1396G	T4186G	21	P3	SMEB	(Berkovic et al., 2006)
R1407X	C4219T	21	P3	SMEI	(Sugawara et al., 2002)
W1408X	G4223A	21	P3	SMEI	(Fujiwara et al., 2003)
N1414Y	A4240T	21	P3	SMEI	(Marini et al., 2007)
Y1422C	A4265G	21	P3	SMEI	(Mancardi et al., 2006)
L1426R	T4277G	21	P3	SMEI	(Mancardi et al., 2006)
Q1427X	C4279T	21	P3	SMEB-SW	(Harkin et al., 2007)
V1428A	C4283T	21	P3	GEFS+	(Sugawara et al., 2001)
A1429fsX1443	4286-4290del,[ATGTCC]ins	22	P3	SMEI	(Ohmori et al., 2002)
W1434R	T4300C	22	P3	SMEI	(Ohmori et al., 2002)
W1434X	G4301A	22	P3	SMEI	(Zucca et al., 2008)
A1441P	G4321C	22	P3	SMEI	(Harkin et al., 2007)
Q1450R	A4349G	23	P3	SMEI	(Ohmori et al., 2002)
P1451L	C4352T	23	P3	SMEI	(Mancardi et al., 2006)
L1461I	C4381A	23	D3/S6	SMEI	(Nabbout et al., 2003)
Y1462C	A4385G	23	D3/S6	SMEI	(Zucca et al., 2008)
F1463S	T4388C	23	D3/S6	SMEI	(Nabbout et al., 2003)
G1470W	G4408T	23	D3/S6	SMEI	(Marini et al., 2007)
L1475S	T4424C	23	D3/S6	SMEI	(Mancardi et al., 2006)
G1480V	G4439T	23	D3/S6	MAE	(Harkin et al., 2007)
Q1489K	C4465A	23	L3	FHM	(Dichgans et al., 2005)
G1495fsX1500	[G]4484del	24	L3	SMEI	(Mancardi et al., 2006)
N1509fsX1511	[A]4526del	24	L3	SMEI	(Berkovic et al., 2006)
S1516X	C4546T	24	L3	SMEI	(Sugawara et al., 2002)
K1517fsX1536	4589[A]4590ins	24	L3	SMEI	(Marini et al., 2007)
R1525X	C4573T	24	L3	SMEI	(Kearney et al., 2006)
F1543S	T4628C	25	D4/S1	CFE	(Harkin et al., 2007)
I1545V	A4633G	25	D4/S1	SMEI	(Harkin et al., 2007)
[M]1559del	[ATG]4675-46774del	25	D4/S1	SMEI	(Fukuma et al., 2004)
R1575C	C4723T	25	D4/S2	RE	(Ohmori et al., 2008)
[F]1584del	[TTT]4750-4752del	25	D4/S2	SMEB	(Fukuma et al., 2004)
C1588R	T4762C	25	D4/S2	SMEI	(Marini et al., 2007)
R1596C	C4786T	25	D4/S2-S3in	CFE	(Harkin et al., 2007)

Y1598X	T4794A	25	D4/S2-S3in	SMEI	(Harkin et al., 2007)
D1608Y	G4822T	25	D4/S3	SMEI	(Marini et al., 2007)
V1611F	G4831T	25	D4/S3	ICEGTC	(Fujiwara et al., 2003)
Y1628X	T4884A	26	D4/S3-S4ex	SMEI	(Nabbout et al., 2003)
V1630M	G4888A	26	D4/S3-S4ex	SMEI	(Marini et al., 2007)
P1632S	C4894T	26	D4/S3-S4ex	ICEGTC	(Fujiwara et al., 2003)
R1636Q	G4907A	26	D4/S4	LGS	(Harkin et al., 2007)
R1645X	C4933T	26	D4/S4	SMEI	(Fukuma et al., 2004)
R1645Q	G4934A	26	D4/S4	SMEI	(Berkovic et al., 2006)
R1648C	C4942T	26	D4/S4	SMEI	(Ohmori et al., 2002)
R1648H	G4943A	26	D4/S4	GEFS+	(Escayg et al., 2001)
L1649Q	T4946A	26	D4/S4	FHM	(Vanmolkot et al., 2007)
I1650fsX1672	4949-4950insT	26	D4/S4	SMEB-M	(Harkin et al., 2007)
I1656M	C4968G	26	D4/S4	GEFS+	(Wallace et al., 2001)
R1657C	C4969T	26	D4/S4	GEFS+	(Lossin et al., 2003)
R1657H	G4970A	26	D4/S4	CFE	(Harkin et al., 2007)
T1658R	C4973G	26	D4/S4-S5in	SMEB	(Harkin et al., 2007)
F1661S	T4982C	26	D4/S4-S5in	SMEI	(Claes et al., 2003)
P1668A	C5002G	26	D4/S4-S5in	SMEI	(Nabbout et al., 2003)
F1671fsX1678	[TGTT]5008-5011del	26	D4/S4-S5in	SMEI	(Mancardi et al., 2006)
F1671fsX1678	[GTTT]5010-5013del	26	D4/S4-S5in	SMEI	(Claes et al., 2001)
G1674R	G5020C	26	D4/S5	SMEI	(Ohmori et al., 2002)
V1680fsX1715	5040[AA]5041ins	26	D4/S5	SMEI	(Mancardi et al., 2006)
A1685D	C5054A	26	D4/S5	SMEI	(Fujiwara et al., 2003)
A1685V	C5054T	26	D4/S5	GEFS+	(Sugawara et al., 2001)
F1687S	T5060C	26	D4/S5	GEFS+	(Marini et al., 2007)
F1692S	T5075C	26	D4/S5	SMEI	(Fukuma et al., 2004)
Y1694C	A5081G	26	D4/S5	SMEI	(Fukuma et al., 2004)
V1695fsX1714X	[G]5083del	26	D4/S5	CGE	(Rudolf G. 2007)
F1707V	T5119G	26	P4	SMEI	(Harkin et al., 2007)
T1709I	C5126T	26	P4	ICEGTC	(Fujiwara et al., 2003)
S1713N	G5138A	26	P4	SMEI/FS	(Kimura et al., 2005)
M1714R	T5141G	26	P4	SMEI	(Mancardi et al., 2006)
C1716R	T5146C	26	P4	SMEB	(Marini et al., 2007)
T1721R	C5162G	26	P4	SMEI	(Harkin et al., 2007)

W1726R	T5176C	26	P4	SMEI	(Harkin et al., 2007)
D1742E	C5226A or G	26	P4	GEFS+	(Pineda-Trujillo et al., 2005)
L1747fsX1779	5240[AA]5241ins	26	P4	SMEI	(Gennaro et al., 2003)
G1749E	G5246A	26	P4	SMEI	(Claes et al., 2003)
G1762E	G5285A	26	D4/S6	SMEI	(Mancardi et al., 2006)
F1765fsX1794	5292[T]5293ins	26	D4/S6	SMEI	(Fujiwara et al., 2003)
[F]1766del	[TTT]5296-5298del	26	D4/S6	SMEB	(Fukuma et al., 2004)
F1765fsX1777	[TTTT]5295del	26	D4/S6	SMEI	(Marini et al., 2007)
Y1781C	A5342G	26	D4/S6	SMEI	(Fukuma et al., 2004)
S1773F	C5318T	26	D4/S6	SMEI	(Mancardi et al., 2006)
M1780T	T5339C	26	D4/S6	SMEI	(Nabbout et al., 2003)
A1783T	G4347A	26	D4/S6	SMEI	(Harkin et al., 2007)
A1783V	C5348T	26	D4/S6	SMEI	(Marini et al., 2007)
E1787K	G5359A	26	C-terminus	SMEI	(Marini et al., 2007)
N1788fsX1796	5363[TGACTTT]5364ins	26	C-terminus	SMEI	(Wallace et al., 2003)
F1789fsX1793	[CA]5367-5368del	26	C-terminus	SMEI	(Marini et al., 2007)
F1805X	[TT]5414-5415del	26	C-terminus	SMEI	(Nabbout et al., 2003)
[MFYE]1807-1810del	5419-5430del	26	C-terminus	SMEI	(Fujiwara et al., 2003)
F1808L	T5422C	26	C-terminus	ICEGTC	(Fujiwara et al., 2003)
W1812G	T5434G	26	C-terminus	SMEI	(Fujiwara et al., 2003)
W1812X	G5436A	26	C-terminus	SMEI	(Harkin et al., 2007)
W1812C,1813-1815del	5436-5444del	26	C-terminus	SMEI	(Nabbout et al., 2003)
F1831S	T5492C	26	C-terminus	SMEI	(Fujiwara et al., 2003)
N1845fsX1856	[CAAA]5531-5534del	26	C-terminus	SMEI	(Mancardi et al., 2006)
K1846fsX1856	[AAAC]5536-5539del	26	C-terminus	SMEI	(Claes et al., 2001)
M1852R	T5555G	26	C-terminus	SMEI	(Annesi et al., 2003)
M1852T	T5555C	26	C-terminus	GEFS+	(Annesi et al., 2003)
V1857L	G5569C	26	C-terminus	GEFS+	(Nagao et al., 2005)
D1866Y	G5596T	26	C-terminus	GEFS+	(Spampanato et al., 2004)
R1874fsX1941	[CGGGTTCT]5620del	26	C-	SMEI	(Marini et al., 2007)

			terminus		
G1880fsX1881	5640-5645del,[CTAGAGTA]ins	26	C-terminus	SMEI	(Ohmori et al., 2002)
E1881D	G5643T or G5643C	26	C-terminus	SMEI	(Wallace et al., 2003)
R1886X	C5656T	26	C-terminus	SMEI	(Mancardi et al., 2006)
L1885fsX1910	[G]5657del	26	C-terminus	SMEI	(Mancardi et al., 2006)
M1889fsX1910	[G]5668del	26	C-terminus	SMEI	(Nabbout et al., 2003)
R1892X	C5674T	26	C-terminus	SMEI	(Sugawara et al., 2002)
Q1904X	C5710T	26	C-terminus	SMEI	(Harkin et al., 2007)
Q1904fsX1945	5712[ATCA]5723ins	26	C-terminus	SMEI	(Sugawara et al., 2002)
T1909I	C5726T	26	C-terminus	SMEI	(Ohmori et al., 2002)
R1912X	C5734T	26	C-terminus	SMEI	(Fukuma et al., 2004)
Q1914fsX1943	[AA]5741-5742del	26	C-terminus	ICEGTC	(Harkin et al., 2007)
I1922T	T5765C	26	C-terminus	SMEI	(Harkin et al., 2007)
R1928G	C5782G	26	C-terminus	SMEI	(Zucca et al., 2008)
I1955T	T5864C	26	C-terminus	FA	(Weiss et al., 2003)
E1957G	A5870G	26	C-terminus	IS	(Wallace et al., 2003)

The nomenclature of the amino acid changes uses the single-letter amino acid code with the original residue at the beginning, followed by the open reading frame position and the residue present in the mutant. Exon–intron boundaries were deduced from rat Scn1a using NCBI's Basic Local Alignment Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi). An effort was made to reference the first, original report of the mutation. Topological data are taken from the secondary structure suggested by Escayg and colleagues [14]. Non-standard denominations: X – termination codon, del – deletion of residue/base, P37fsX91 (example) – frameshift with proline 37 being the last wild-type residue, followed by non-sense residues until ORF position 91, where the protein is prematurely terminated, AA: amino acid, D1/S2 (example): domain 1/transmembrane region 2 (topology code indexes ''in" and ''ex" denote internal and external loop, respectively), NA: nucleic acid. For individual references pleases see origingal article by Lossin (Lossin, 2009).

e. Uncovering the Mechanism of SCN1A mutations in Dravet and GEFS+.

The question one must ask is how can loss of an excitatory ion channel, sodium, cause excitatory signals leading to epilepsy? The answer derived by Yu and Ogiwara is simple, "location" (Yu et al., 2006) (Ogiwara et al., 2007)! Ogiwara created knock-in mouse model that was identical to three unrelated human cases of Dravet syndrome (Ogiwara et al., 2007) models of SCN1A gene that produced NaV1.1-/- and NaV1.1+/- proteins in separate systems (Yu et al., 2006). The density of sodium current was dramatically reduced in inhibitory (GABA) interneurons but not excitatory (glutamate) pyramidal neurons. Test on wild type proved that inhibitory interneurons poses more NaV1.1 channels than pyramidal cell and thus are effected to a larger degree than interneurons, this determination was based on sodium currents in the preceding cell lines (Ogiwara et al., 2007). Simply put, loss of the excititatory ion channels, inhibitory interneurons, leads to decreased pyramidal inhibition; in other words, excitatory pyramidal cells are disinhibited. Thus, Dravet is not simply loss of function by interneurons, it is a gain of function by pyramidal neurons that are now uninhibited (Yu et al., 2006).

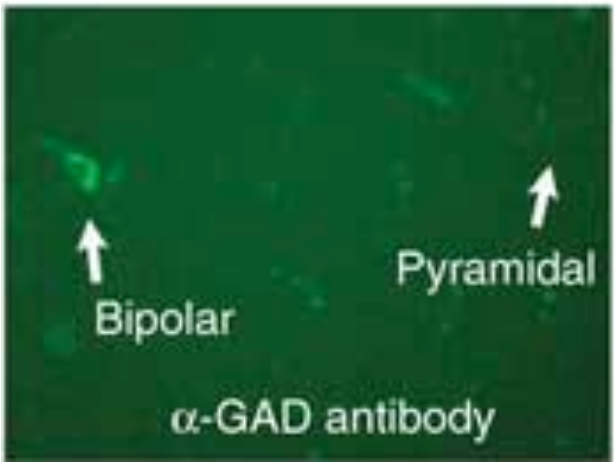


Figure 10 Hippocampal neurons that were acutely dissociated from P14 wild-type mice. The pyramidal-shaped and bipolar-shaped neurons are indicated with arrows. After immunocytochemical processing and staining with anti-GAD. The bipolar-shaped cells, but not the pyramidal-shaped cells, were strongly labeled, which indicates that they are GABAergic inhibitory interneurons. (Yu et al., 2006)

10. SCN1B mutation

The original model for GEFS+ was based on the theory of a mutation in the β1 subunit of the VGSC not the infamous α subunit that is the main pore forming portion of the voltage gated sodium channel. C121W is a mutation in the extracellular Ig domain portion of the β1 subunit derived from a C to G point mutation substituting a tryptophan from a cysteine on chromosome 19q13.1 (Wallace et al., 1998). The complete mechanism of how this mutation causes hyperexcitability and epilepsy is not completely understood but, it is characterized as a loss of function mutation by disrupting a disulfide bond and thereby likely disrupting trafficking to neuronal membrane) (Meadows et al., 2002b) (Wallace RH and GR, 2002). The wild type β1 accelerates current decay and the C121W mutant does not affect it thus increasing the duration of sodium current (Chen et al., 2004). This proposes a loss of function or reduction of function mutation with the production of a SCN1B null mouse model (Chen et al., 2004). Some insight to the mechanism might be gained by the fact that β1 subunits are known to speed up channel inactivation by interacting with the C terminus of the α subunit III-IV domain (Annesi et al., 2003), yet in the presence of C121W mutation the inactivation is delayed possibly allowing for further activation of (excitatory) pyramidal neurons to the cortex and hence hyperexcitability that then causes GEFS+ (Meadows et al., 2002b). It is plausible but unproven to date that this is the mechanism of excitation of pyramidal neurons in the C121W mutated patients. Another, yet unproven, possible mechanism that may account for GEFS+ phenotype in C121W mutated patients is that β1 in theory acts as an inhibitor to β4 which promotes channel opening and thus promoting hyper-excitability (Aman et al., 2009).

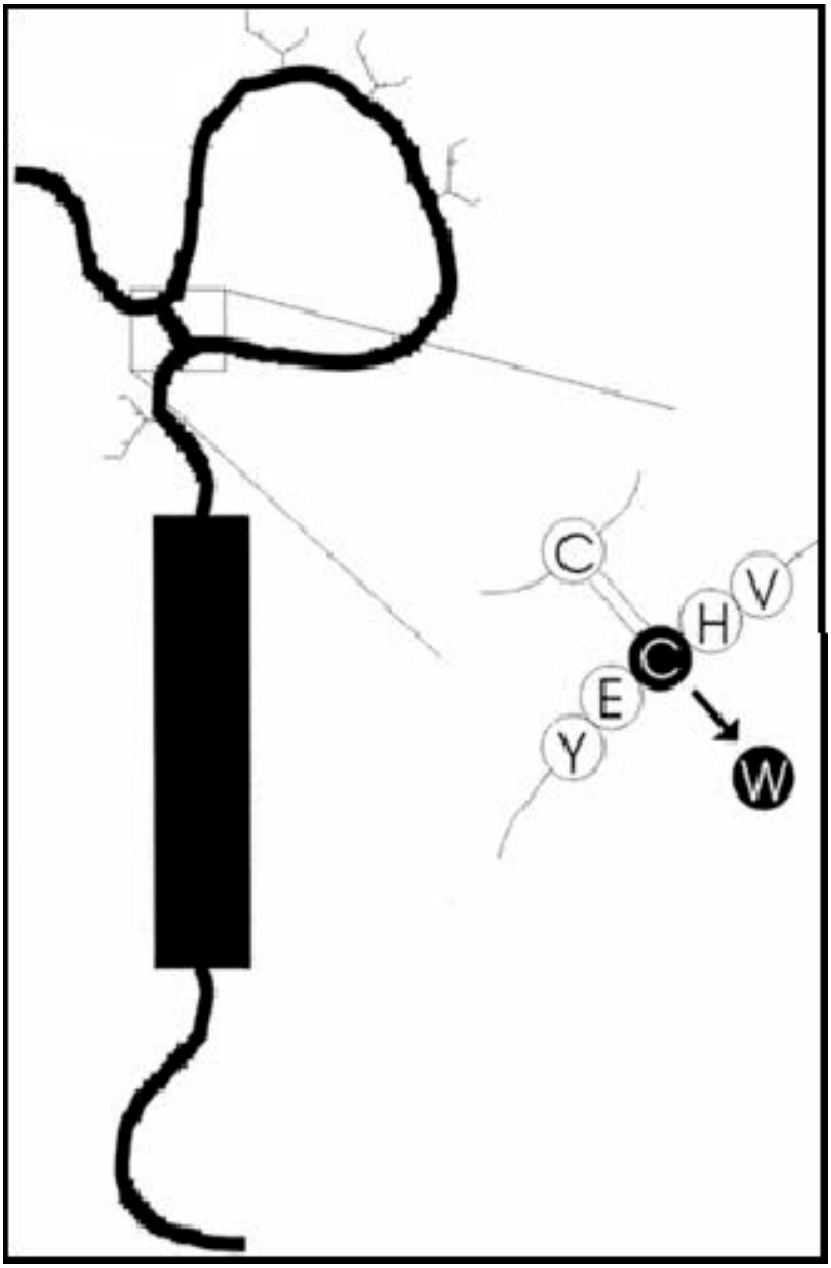


Figure 11 Schematic representation of the *SCN1B* gene product showing the location of the C121W mutations associated with generalized epilepsy with febrile seizures plus. The black box denotes the transmembrane segment of the SCN1B (Audenaert et al., 2003).

Dravet syndrome from mutation of the SCN1B.

Single nucleotide mutation of SCN1B at residue 125, a change from arginine to a cystine, leads to complete failure of the β alpha interaction in immunoglobulin like extracellular region of the mutated β subunit p.R125 of both human and mouse mutants (Patino et al., 2009). The mutation leads to trafficking problem for the NaV1.1 channel at mammalian body temperatures. Function is maintained in the heterozygotes without the presence of seizures in the limited population group studied. However, with the homozygous mutation the result is similar to a null mutation of the β subunit (Patino et al., 2009) (Chen et al., 2004).

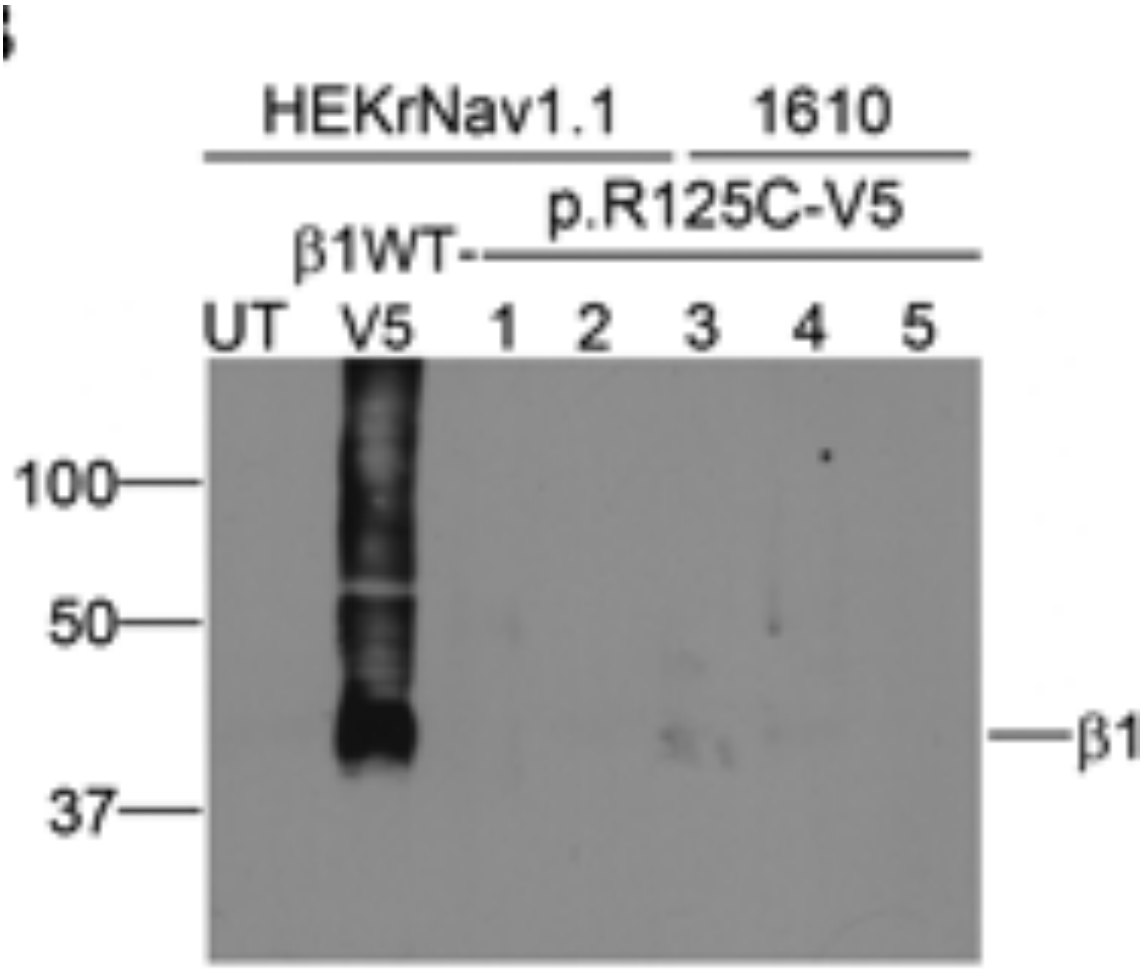


Figure 12 p.R125C is poorly expressed at the cell surface at physiological temperatures. Cell surface expression of β1WT versus p.R125C. HEKrNav1.1 or 1610 cells stably transfected with V5-tagged β1WT or p.R125C were surface biotinylated, and the biotinylated proteins were probed as described in Materials and Methods. Untransfected cells show no anti-V5 immunoreactivity (UT; lane 1). Cells transfected with β1WT show robust cell surface expression (lane 2). Faint or no cell surface expression was detected in multiple clones of cells transfected with the mutant p.R125C (HEKrNa$_v$1.1 cells, samples 1 and 2; 1610 cells, samples 3–5). (modified from figure 4B Patino et al., 2009).

The one reported human case of homozygous mutation in SCN1B demonstrated the phenotypic picture of Dravet syndrome (Patino et al., 2009).

Again the question arises how does loss of the sodium channel lead to hyperexcitability? The answer for both SCN1A (mutations) and SCN1B (p.R125) mutation seems to be that the loss of inhibition leads to a gain of excitation!

It should be noted that our current understanding limits us to only mutations in the β1 subunits and that any reference above not specifically sub noted were referring to the β1 subunit.

11. SCN2A Mutations : Diseases caused by SCN2A mutation, specifically Benign Familial Neonatal Infantile Seizures.

SCN2A encodes the NaV1.2 protein α subunit and is expressed throughout the CNS especially at axonal initiation sites for the purpose of initiation of action potentials, it is also found at nodes of Ranvier for saltitory conduction (Shi et al., 2009). The vast majority of clinically significant mutations in SCN2A gene (2q21-33) have proven to be inherited missence mutation in origin (Litt et al., 1989; Ogiwara et al., 2009). The mechanism of the various mutation seems to be a change in the kinetics that slows the closure of the inactivation gate that then allows greater influx of sodium ions and there for hyper-excitability (Sugawara et al., 2001). The phenotypes range from the classic Benign Familial Neonatal Infantile seizure (BFNIS), described below, and GEFS+ to Dravet syndrome (Kamiya et al., 2004).

GEFS+ and Dravet syndrome (SMEI) are describe in the previous SCN1A section above.

a. Benign Familial Neonatal Infantile seizure (BFNIS)

BFNIS can be caused by autosomal dominate mutation in the SCN2A gene effecting the NaV1.2 protein (Herlenius et al., 2007). The typical onset ranges from the first week of life to the fourth month with some variation reported (Misra et al., 2008). It is characterized by afebrile seizures that spontaneously remit within the first year of life and is highly responsive to Anti Epileptic Drugs (Herlenius et al., 2007). In comparison to the other SCN2A mutations there is considerably less long term neurologic manifestation associated with this disease phenotype (Berkovic et al.,2004).

In BFNIS there are functional sodium channels transcribed but their peak current is significantly lower than that of the wild type, which points to decreased channel activity in the

plasma membrane (Misra et al., 2008). This can be explained by the decreased level of expression of NaV1.2 channels at the neuronal membrane without a significant change in the amount of total protein transcribed (Misra et al., 2008) or a change in the gating kinetics of proteins that are transcribed (Scalmani et al., 2006). The question that is asked next is what is the mechanism that causes retention of mutated NaV1.2 protein?

Although inherited missence mutation are the predominate cause of disease of the SCN2A mutations, inferring only a partial loss of function, recently there are some de novo mutation that can give a Dravet phenotypic picture that differ due to eletrophysiologic alteration of mutated Nav1.2 channels (Ogiwara et al., 2009) (Shi et al., 2009). A nonsense mutation, in the early N terminus gives intractable epilepsy condition similar to Dravet syndrome (Kamiya et al., 2004). The early position of the mutation, before the first transmembrane domain, destroys the vast majority of the function compared to that of the WT channel and therefore may explain the severity of this and other mutations (Kamiya et al., 2004). Interestingly, this mutated R102X channel causes a “dominate negative” effect taking out the non-mutated SCN2A gene (Kamiya et al., 2004). This is much different than the proposed haploinsufficiency (-/+) of SCN1A and SCN2A (Kamiya et al., 2004).

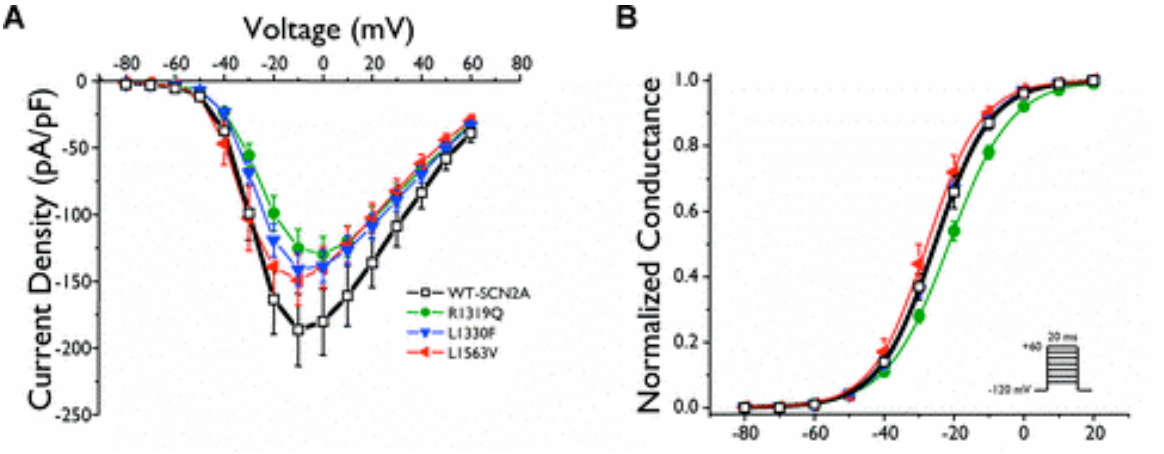


Figure 13 Biophysical properties of human WT, R1319Q, L1330F, and L1563V expressed in human tsA201 cells. (A**) Peak current density elicited by test pulses to various potentials and normalized to cell capacitance. (**B**) Voltage dependence of channel activation measured during voltage steps between –80 and +20 mV. R1319Q displayed a significant depolarizing shift in activation compared to WT (Misra et al., 2008).**

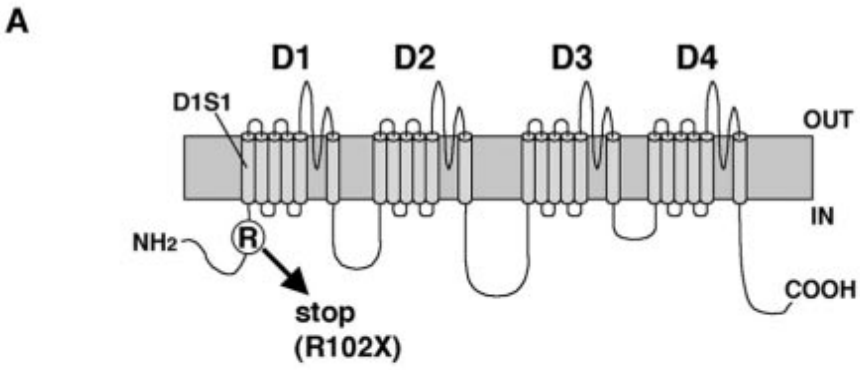


Figure 14 Schematic representation of the predicted folding topology of Nav1.2. R102X nonsense mutation identified in a patient with intractable childhood epilepsy and severe mental decline locates at the N terminus [The Journal of neuroscience March 17, 2004; A Non-Sense mutation of the Sodium Channel gene SCN2A a Patient with Intractable Epilepsy and Mental Decline.] (Kamiya et al., 2004)

12. Absence epilepsy (pycnolepsy) and the Voltage Gated Calcium Channels:

a. Classification and clinical presentation:

Absence seizures account for around 4 percent of all seizure disorders (Epilepsyfoundation.org). Absence epilepsy spans a spectrum ranging from Typical absence epilepsy (CAE), Juvenile absence epilepsy, and Juvenile myoclonic epilepsy. Typical absence epilepsy is are characterized by "Staring Spells" or eyelid blinking that last less than ~30 seconds and have a peak occurrence around 5-7years of age. These are sudden but brief disruptions in the level of consciousness that can be repeated up to 50 times per day and are exacerbated by exercise or drowsiness. Phenotypically, the seizures can be seen as completed when the patient stops starting, generally carrying on with their previous task unaware of the event, there is no postictal, confusion state.

The EEG reveals bilateral, synchronous symmetrical ~3 Hz spike and wave discharges that are brief (~ 10sec) with normal background activity. The EEG show maximal intensity over the frontal and minimal over the posterior brain regions. The freqeuency may be between 10 to 100 seizures daily.

Failure of the patient to show a change in level of consciousness ie an absence period or a failure of myoclonic jerking just prior to or during the absence episode makes the diagnosis of absence epilepsy unlikely (Epilepsy.org).

Normal neurological development is expected and greater than 9 out of 10 children suffering from absence epilepsy are seizure free by puberty.

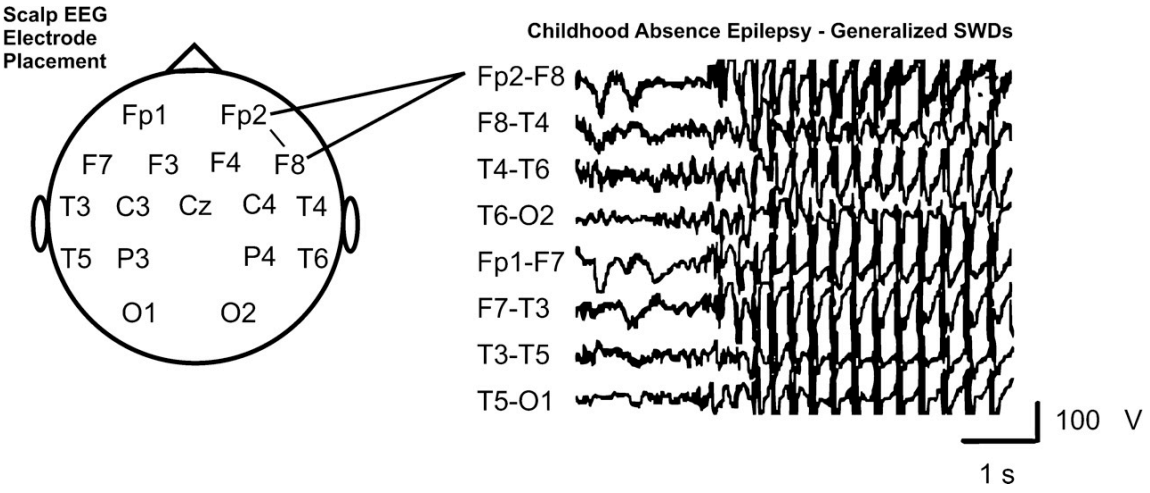


Figure 15 EEG shows a normal background and 3-Hz generalized spike and wave discharges. Frequency of the spike-wave complexes is usually 4 Hz at the onset of the absence seizures and may slow to 2.5 Hz at the end of a seizure Epilepsy.org

b. Pathogenesis of absence epilepsy via T type calcium channels: hyper-synchronized excitatory activity in the thalamic relay cells of the thalamus the gate way to the cortex.

i. Physiology of T-type (LVA) Calcium channels in the Thalamus.

Only little bits of information are directly sent to the cortex. The thalamus via the thalamocortical relay circuit is the bottle neck link between cortex and peripheral sensory system regulating brain states such as slow wave sleep and arousal (Khosravani and Zamponi, 2006).

The thalamocortical relay circuit has three functional firing modes that vary with the level of consciousness and disease state. The first, tonic mode, is associated with awake states and rapid eye movement from a slightly depolarized membrane potential (~-55mv), intermediate mode (~-60mv), and burst mode (~ -70mv) that is associated with decreasing activity from deeper activating brain regions that result in deep sleep and dysrhythmias seen in epilepsy (Weiergraber et al., 2010).

LVA T-type calcium channel act as pacemakers inducing Low Threshold Spikes (LTS) seen in burst mode firing in the thalamocortical relay circuit.

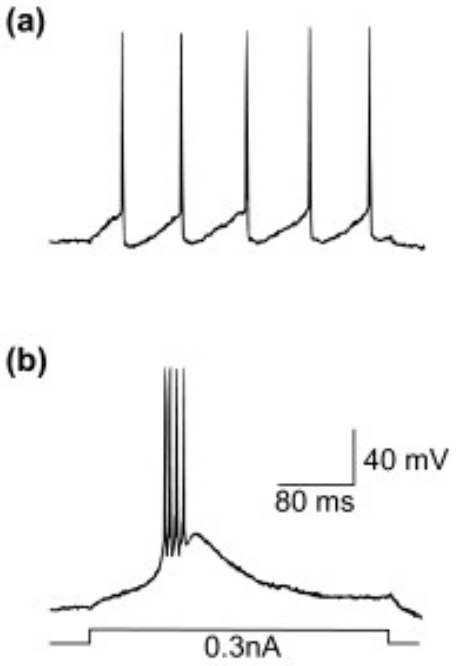


Figure 16 Intracellular recording from relay cell of lateral geniculate nucleus of a cat in vitro. At initial membrane potentials ~-59mv, T-type calcium current is inactivated and thus the cell responds in a tonic mode (a). At membrane potential of ~-70mv T-type calcium channels de-inactivate and respond with burst mode upon depolarization of conventional VGSC driven action poentials(b). Recreated from: Molecular Physiology of Low-Voltage-Activated T-type Calcium Channels (Perez-Reyes, 2003). The thalamcortical relay neurons undergo a conformational change that initiates a transient depolarization from hyperpolarized membrane potentials (~-70mv) with LVA channels that are normally closed at resting membrane potentials. Do to the density of LVA T-type currents in the thalamocortical relay cells soma and dendrites, the LTS generated are sufficient to bring the resting membrane potential to ~-40mv allowing sodium voltage gated ion channels to open and thus depolarize the neuron. LVA quickly recover, within the "After Hyperpolerization" window, and are capable of inducing successive sodium channel spikes seen as bursting. Normally the LTS serve as a pacemaker system within the thalmocortical system however, in disease states such as absence epilepsy SWDs are generated. It is the CaV3.1 T-type Calcium channels that are predominately associated with thalamocortical relay neurons and CaV3.2 and CaV3.3 are associated with Reticulotholamic neurons (Weiergraber et al., 2010).

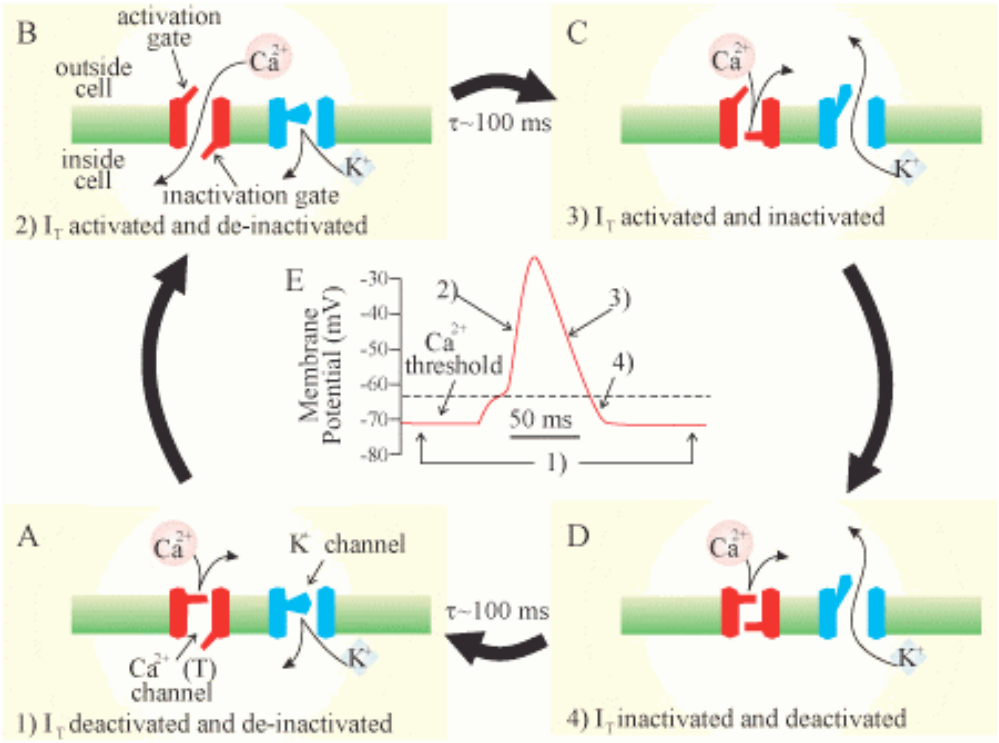


Figure 17 T-type Ca++ channels open at hyperpolarized potentials and are closed at normal resting membrane potential. The LVA activation further triggers VGSC to open by bring the cell to threshold potential. (Sherman SM and RW, 2006).

Normally in the awake state the thalamus keeps the thalamocortical relay and reticular neuron's T-type calcium channels closed as the neuronal membranes are kept slightly depolarized around -55mv. That is to say that the activation gate is closed or deactivated as well as the inactivation gate being closed or de-inactivated. The same is true of normal neuronal resting membrane potential of -60 to -65mv and hyperpolerizations ~-70ms lasting less than 100ms.

Hyperpolerization lasting longer than 100ms as is seen in deep sleep and spike and wave disorders (SWD) causing the inactivated gate of the T-type channel to de-inactivate. If a depolarization is brought the activation gate of the T-type channel will then open. Together the two channels now being open allow a surge/spike of calcium to flow down its concentration gradient through the T-type calcium channel. The spike of calcium, an IT current (T current), triggers conventional sodium action potentials that superimposes itself on the calcium spike and ultimately leads to neuronal depolarization, seen as burst firing, and propagation of information to the cortex.

After approximately another 100ms the de-inactivation gate closes/inactivates, stopping the flow of calcium ions. At this time the rectifier potassium channel opens to reestablish resting membrane potential and potentially priming the T-type channel for another round via hyperpolarization.

c. Pathology of absence epilepsy related to VGCC: an evolution of our understanding.

CANA1A, 19q13, $Ca_V2.1$ (P/Q) a form of HVA:

CaV2.1 are important for synaptic transmition as they are located predominately at presynaptic terminals, reduced channel function is mirrored by reduced neurotransmitter releases from cortical neurons. Evidence implicating the CaV2.1 channel in absence epilepsy comes from several mutant knockout mice. The first mouse model of absence epilepsy, via SWD, with ataxia was Tottering (CACNA1A-Tg). Tottering encodes a mutated α1A central pore of the P/Q channel a form of voltage gated calcium channel. There are other offshoots of the Cacna1a-tg model, i.e. Cacna1a-tg^{la}, but comparison of these mutations, in a single gene, still give rise to the spike and wave model of absence epilepsy with ataxia due to a decrease in function of the P/Q channel either due directly to the α subunit or an auxiliary subunit (Catterall, 2000b).

Do to the fact that the distribution of the CaV2.1 channel of the human mirrors that of the mouse, ataxia is common. Non-functional channels arising from truncation mutation in the C terminal region give rise to patients with CAE combined with ataxia or generalized seizures combined with episodic ataxia type 2 (Zamponi et al., 2009).

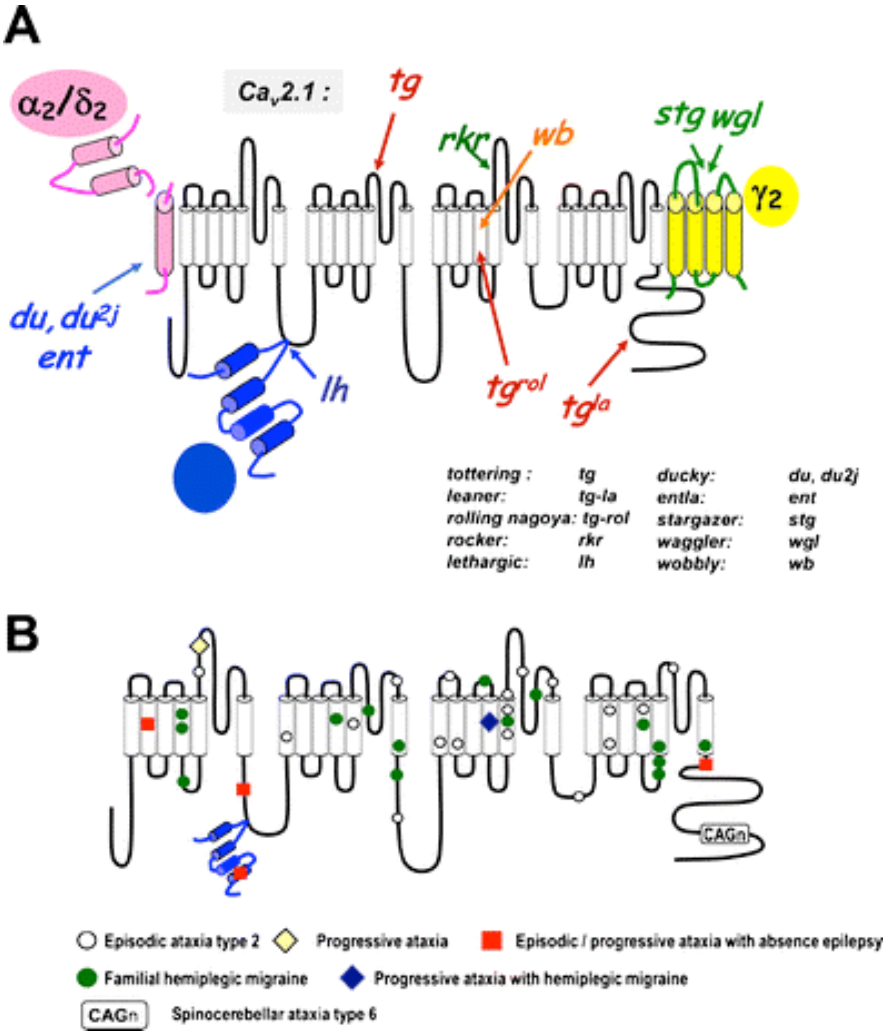


Figure 18 Common mutations in P/Q type Calcium channels in mouse (A) and humans (B). a Dendrogram illustrating the three subfamilies of VGCCs: the Cav1 (L-types), the Cav2 (neuronal types), and the Cav3 (T-types). Cav1 and Cav2 channels are high-voltage-activated (HVA), in contrast to Cav3 channels (T-types) which are low-voltage-activated (LVA). B The molecular structure of HVA channels comprises ancillary subunits α2δ, β, and γ each encoded by several subunits. In contrast, the subunit composition of LVA/T-type channels is not yet resolved (Zamponi, Lory, Perez-Reyes 09).

d. Accesory subunit disease models of absence epilepsy with ataxia.

As with all the ancillary/accessory subunits of the voltage gated pore forming units their modification can disrupt the entire ion gated pore. The lethargic mouse, a frameshift mutation of the β4 calcium channel subunit, effectively knocks out the voltage gated calcium channel. Even reductions in the amount of current seen in the ducky and mouse experiments cause the absence epilepsy with ataxia by disrupting the α2δ2 subunit. Stargazer, a γ2 mutation, causing a hyperpolarizing shift in activation of the VGCC.

Indeed P/Q type calcium channel KO mice may experience an absence epilepsy but the condition may be rescued by knocking out the T-type channels (Song et al., 2004).

The reticular thalamic neurons show an increase in calcium channel current in upwards of 50% (song 04). Further studies would show that the increase in calcium current in the thalamus was not directly due to the P/Q channel (Zamponi et al., 2009). A T-type calcium current, was responsible for increase in calcium current and this was supported by a slight increase in T-type channel Cacna1g mRNA (Zamponi et al., 2009). (Reader is Refered to introduction for description of T channels)

Evidence that the CaV3.2/T-type channels are responsible for absence epilepsy phenotype comes from Zang's models were their team took a Tottering mutated mouse, loss of CaV2.1 function, and crossed breed it with a CACNA1H knockout mouse (Zhang et al., 2002) (Song et al., 2004). The result was a cure of the Tottering mouse absence like seizures due to an apparent double loss of the CaV2.1 and CaV3.2 calcium channels.

In this experiment the loss of CaV2.1 was unable to induce an increase in LVA current from the CaV3.2 channel because both genes were knocked out (Zhang et al., 2002). Note: ataxia was

still present in this mouse model. Exactly how P/Q calcium channel mutations, a loss of function mutation, could increase the T-type calcium channel in different populations of neurons is unknown! What is known is when the T-type calcium channel is completely knocked out along with the corresponding P/Q channel or a P/Q channel loss of function model, absence seizures are not seen (Zamponi et al., 2009) (Ernst et al., 2009). Interestingly a CaV2.1 -/- and CaV 3.2 -/+ cross show a 25% reduction in current from wild type mouse and still cause an absence phenotype pointing to the fact that current cannot be the complete answer to this complicated puzzle (Khosravani and Zamponi, 2006).

Ernst took the idea of the T-type channel being associated with absence epilepsy, and possibly ataxia, a step further. Before his experiment there had always been mutations in the P/Q or a combination of P/Q and T-type mutation (Ernst et al., 2009). Ernst needed to find out if Spike and wave, absence like, disorders with ataxia could be generated by the T-type channels alone. Ernst induced mutated mouse lines to over express Cacna1h mRNA that codes for the neuronal T-type (H) channels. He was able to induce the neurons to over express T-type channels and T-type currents while maintaining normal P/Q channels . Using this model he was able to generate the first model that only reproduced absence epilepsy.

e. CACNA1H the Main Source of Human T currents

Chen has sequenced the CACNA1H gene encoding the α1H calcium channel and found it to be significantly envolved in human cases of absence epilepsy of Han Chinese in comparison to earlier thoughts that it was possibly the CACNA1G gene responsible for the abnormal T currents (Chen et al., 2003).

f. Summary

Burst mode of firing in thalamic neurons is a common in many absence models. Genetic knockouts of α1G have decreased T currents. Genetic knockouts of 1G T currents cannot be induced to spike by γ aminobutyric acid type B. Increased over expression of either α1G or α1H in a mouse model induces absence epilepsy. Increased over expression leading to altered T currents can be blocked by ethosuximide.

While mutations in the classic Tottering and Stargazin models appear to create an absence like picture the model must be manipulated to achieve our current understanding of the human disease. Low voltage activated T–type Calcium current must be enhanced in the pathogenesis of absence epilepsy as we currently understand it.

13. Benign Familial Neonatal Convulsions.

Description, presentation, and resolution.

Benign Familial Neonatal Convulsions (BFNC), originally eluted to in the 1960s, is a rare hereditary idiopathic generalized epilepsy disorder that presents heterogeneously with daily, focal or generalized tonic clonic seizures during wakefulness or sleep (Rett 64). The seizures increase in frequency until the infant goes into status epilepticus usually lasting less than 24hrs. The seizures occur between the second and eighth day after birth without an apparent cause (normal labs) in otherwise healthy newborns with APGAR scores of nine or above at five minutes. The infant has normal physical exam, laboratory test, and radiologic scans prior to development of seizures, between seizures, and after seizures remit spontaneously around the six week mark.

The EEG is generally normal, 50 to 70% of the time, when preformed interictally, 25% of BFNC show "theta pointu alternant" pattern and a small percent have focal, often, rolandic, discharges. Ictal seizures present with flattening of the EEG during apenic phase and generalized spike and wave in the clonic phase. There is no specific EEG pattern to this seizure type and EEG abnormalities generally resolve by two years of age. Seizures typically become less severe until they disappear around the 15th week.

The seizures are generally treatable with Phenobarbital but resolution and overall outcome does not seem to be effected. Interestingly, in the majority of cases, there does not seem to be any long term sequelae from this seizure disorder, hence the term benign, this is odd in comparison to many other seizure disorders. Other seizure forms and neuropsychiatric

abnormalities do occur at a higher rate than in the general population, approximately 15% of the BFNC, which brings the term benign in question (Steinlein et al., 2007). Yet, these neurologic manifestations seem to be unlinked to the initial mechanism of BFNC. Rarely are the seizures drug resistant and of those that are, a small percentage, present with epileptic encephalopathy.

An interesting question yet to be uncovered is why the BFNC preferentially occur in the neonates?

14. Benign Fetal Neonatal Convulsions and the KNCQ gene family.

The search for a cause has lead to the discovery of the KNCQ gene family, a member of the Kv channels. KNCQ genes account for aporximately 70% of BFNS all of which are inherited autosomal dominantly, the remaining 30% of cases being of unknown origin suggesting the possible existence of addition loci yet to be discovered (Herlenius et al., 2007).

a. KNCQ GENE FAMILY Background into KV7 pro / KNCQ gene!

There are five KNCQ genes (KCNQ1-5) that code for the Kv7 α subunit protein family (Kv7.1-Kv7.5), which are voltage gated delayed rectifying potassium channels. They are predominantly located on axons, although there is somato-dendritic distribution in K_v7.2 and Kv7.3 channels in some neurons. Four of the five Kv7 channels are associated with inherited neurologic diseases (Maljevic et al.).

KCNQ1 is expressed in epithelial cells and more importantly cardiac myocytes. KCNQ1 co-assembles with KCNE1 forming a VGKC complex that makes up the slow component of the delayed rectifier current $I_{KS,}$ this current controls repolarization of cardiac action potentials and is altered in the arrhythmia Long QT syndrome caused by a dominate negative mutation (Maljevic et al.) and (Peroz et al., 2008).

KCNQ2 and KCNQ3 is expressed in CNS particularly in the hippocampus and neo-cortex. Together they are responsible for, a haplo-insufficiency mutation, BFNC (see sections below). K_v7.3 mRNAs is also expressed at high levels in the amygdala and the thalamus (Saganich et al.,

2001). Expression patterns of Kv7.2 and Kv7.3 change during but stay primarily localized to the axonal initiation segement.

KCNQ4 is expressed in choclear neuronal cells of the inner ear and is responsible for and autosomal dominate missense mutation, dominate negative effect, that leads to congenital deafness, nonsyndromic progressive hearing loss (DFNA2) (Jentsch, 2004).

KCNQ5 is widely expressed in the nervous system a high signal has been detected in the hippocampus (in particular in the CA3 pyramidal neurons), caudate putamen, and neocortex (Schroeder et al., 2000). Immunohistochemical studies have shown that immunoreactivity for $K_v7.5$ is especially strong in the auditory brainstem nuclei, including the cochlear nucleus, superior olivary complex, nuclei of the lateral lemniscus, and inferior colliculus (Caminos et al., 2007). There are currently no known diseases linked to the KCNQ5 gene.

Expression of the mutated channels listed above reveals a reduction in the current of VGKC of the KCNQ family here on referred to as the slowly activating, non-inactivating M current.

b. Background description of KCNQ2-3 leading into physiology

Found first in an Australian family KCNQ2 was originally mapped to chromosome 20q13.3 and KCNQ3 on loci 8q24 form the proteins Kv7.2 and Kv7.3 respectively (Leppert 89) (Lewis 93). Together or separately Kv7.2 and Kv7.3 channels form functional tetramers that create a VGKC that attempt to maintain resting membrane potential and to repolarize neuronal cell membranes after depolarization.

As covered previously VGIC and now voltage gated potassium channels pass millions of ions per second and are fundamental in regulating neuronal excitability in the nervous system. As

with other Voltage gated ion channels, modulation of their normal function cause a fundamental change in cell excitability and can lead to many disease states where lack of control leads to conditions such as epilepsy. The link of VGKC disruption and epilepsy has been made by disruption of outward rectifying potassium channel current by chemical blockade and then enhancement of the channel current by the drugs XE99 and Retigabine respectively (Boehlen et al., 2009). This has heightened our understanding of the pathogensis of BFNCs due to its impairment of repolarization and hence hyperexcitability of affected neurons.

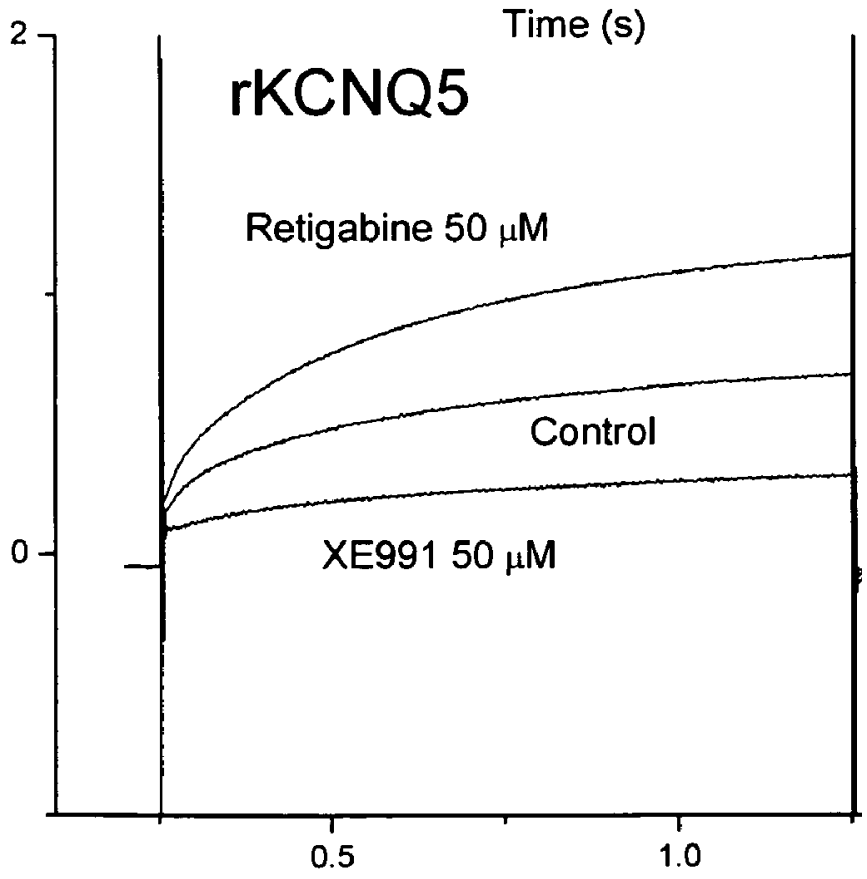


Figure 19 Retigabine VS XE991: Retigabine is an activator of Voltage Gated Potassium Channels and function to restore resting membrane potential. Xe991 is a biological toxin to slow Voltage Gated Potassium Channels from rectifying the polarization of the cell. See text for further description (Wang et al., 2006)

Heteromeric tetramerization of VGKC α subunits Kv7.x, more specifically the combination of Kv7.2 and Kv7.3, is thought to underlie the majority of the M current that repolarizes neurons (Soldovieri et al., 2007a). The combination Kv7.2 and Kv7.3 increase potassium conductance ten fold as compared to the either ion channel alone (Pena and Alavez-Perez, 2006). The M current controls hyperexcitability by counteracting subthreshold membrane depolarizations and limiting repetitive firing.

a. Physiology of M current Stabilizing membrane potential:

The M (K^+) current is a low threshold, slowly activating, non-inactivating K^+ current that is involved in controlling neuronal excitability, at the post synaptic level, by tonicly activating in the range of resting membrane potential thereby, maintain membrane potential below sodium channel activation threshold and hence controlling neuronal excitability and early spike frequency adaptation (marrion 97). The ability to activate at or near resting membrane potential is what sets M channel apart from other VGKCs in fact, it is the only VGIC to be active near threshold potential. Its activity of activation increases as the neuronal membrane progressively depolarize and hence it acts to help restore membrane potential. Normal regulation of the M channel is generated by $G_{q/11}$-coupled receptors. The G proteins function to decrease channel function and reversibly mirror the effects of inhibitory drugs such as XE99 (Pena and Alavez-Perez, 2006).

c. **Pathology of M current reduction:** Haploinsufficiency leading to hyperexcitability.

Mutations most often in the KCNQ2 gene come from a variety of types such as frame shift, missense, nonsense, and more. These mutations have a variety of effects on the KCNQ protein such as extending or more likely truncating the protein. As with the original discovered mutation by Biervert, many of the mutations give no discernable current (Biervert et al., 1998). Homozygous knock-in mice for KCNQ2 or KCNQ3 show no current and spontaneous seizures while heterozygotes show lowered threshold (Singh et al., 2008). Notably, greater than 50% of all mutations are in the C terminus region of the protein and most show only a modest 25% reduction in M current when expressed in a 1:1:2 ratio in comparison to wild type 2:2 ratio thus, even relatively small changes of M current seem to be effective in causing seizures. The C terminus is responsible for heteromeric channel assembly and hence reduction heteromeric assembly leads to reduction in M current. C terminus mutations have also been shown to decrease surface expression, again this would decrease M current (Schwake et al., 2000).

Reduction or suppression of M current, the critical regulator of action potential firing and neuronal excitability in the hippocampal CA1 pyramidal neurons of the neonate, either by KNCQ mutation or drug induced (XE991) blockade leads to failure of controlling/maintaining resting membrane potential. The slightly depolarized neurons lead to hyperexcitability and multiple rhythmic firing upon excitatory signaling do to failure to restore resting membrane potential.

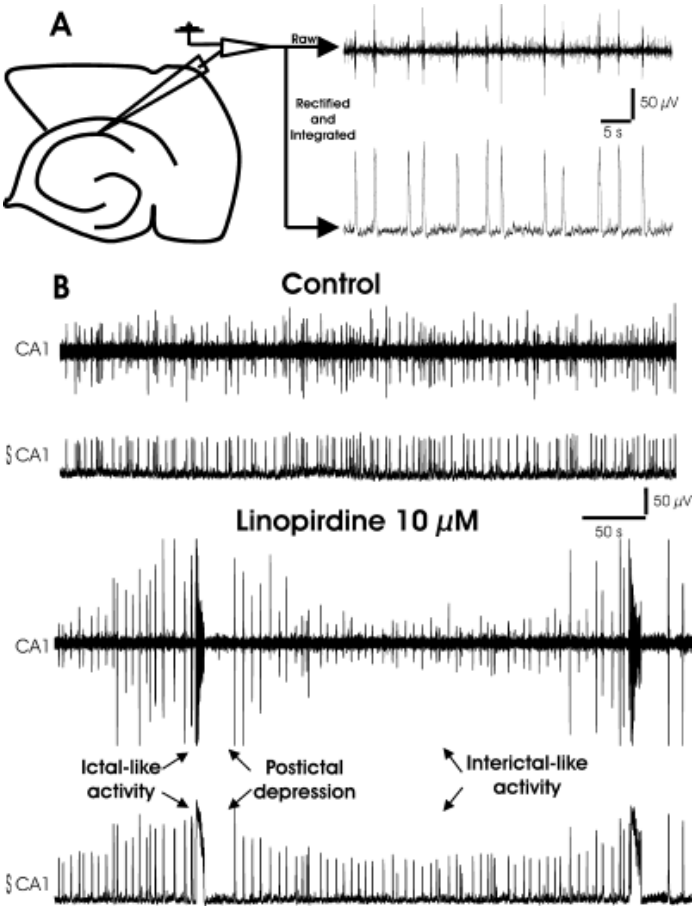


Figure 20 Generation of spontaneous basal and epileptiform activity in hippocampus–entorhinal cortex (H-EC) slices. A: Scheme showing the H-EC slice and the site for CA1 field raw recording (**upper trace**), **which is simultaneously rectified and integrated** (**lower trace**). **Note that the H-EC slice, which was obtained from a neonatal rat, produces spontaneous burst of population activity in control conditions. B: Recording of control raw (CA1**,**upper trace) and integrated (∫CA1) activity (upper traces) and the epileptiform activity induced in a H-EC slice after applying linopirdine, 10 µM** (**lower traces**). **Linopirdine-induced epileptiform activity is characterized by successive periods of ictal-like activity followed by postictal-like depression and interictal-like activity (arrows). From Epileptiform Activity Induced by Pharmacologic Reduction of M-Current in the Developing Hippocampus in Vitro by Fernando pena and Noe Alavez-Perez.**

The functions of the various section of the VGIC have been elucidated in the opening of this paper. The reader is directed back to this section for correlation of the Pathophysiology.

Due to our gain of insight on the Pathophysiology of KNCQ2 or KNCQ3 mutations, the M current has emerged as a therapeutic target in BFNC. Currently activators of the M-channel such as Retigabine hold a key to controlling BFNC and possibly other forms of epilepsy! As one can see from the chart above the precise genetic location of the mutation can be critical in determining the prognosis and severity of BFNC . Ultimately optimal treatment and counseling can only come from combined treatment of the genotype phenotype and this is the next step in managing BFNC.

Table 3 Updated list of KCNQ2 and KCNQ3 mutations.

Overview of the available genetic, clinical and functional data from BFNS families. Table modified from Correlating the Clinical and Genetic Features of Benign Familial Neonatal Seizures (BFNS) with the Functional Consequences of Underlying Mutations. (Soldovieri et al., 2007b).

Kv7.2

Amino Acid Change	**Localization**	**Clinical Data (Beside BFNS)**	**Functional Effects**	**Reference**
M1V	N-terminus	—	—	(Richards et al., 2004)
M1T	N-terminus	—	—	(Richards et al., 2004)
Frameshift at K69	N-terminus	—	—	(Richards et al., 2004)
Frameshift at Q78	N-terminus	LCS+GCS	—	(Claes et al., 2004)
In-frame deletion of S105	S1	—	—	(Claes et al., 2004)
S122L	S2	FS later in life; ~ 7 years	Right shift in current voltage dependence in the subthreshold region; decrease in current activation kinetics	(Hunter et al., 2006)
Splice site mutation at L129	S2	—	—	(Singh et al., 2003)
Frameshift at S195	S2-S3 linker	—	—	(Moulard et al., 2001)
A196V	S4	—	Rightward shift in current voltage-dependence; decrease in current activation kinetics; novel prepulse-dependence of current activation kinetics	(Soldovieri et al., 2007a)
A196V/L197P	S4	—	Rightward shift in current voltage-dependence; decrease in current activation kinetics	(Soldovieri et al., 2007a), (Moulard et al., 2001)

R207W	S4	Myokymia	Marked rightward shift in current voltage-dependence; dramatic decrease in current activation kinetics	(Dedek et al., 2001)
R207Q	S4	Myokymia	Rightward shift in current voltage-dependence; dramatic decrease in current	(Wuttke T, 2007)
M208V	S4	GS between 4 and 7 years	Small decrease in maximal current; increased rate of channel deactivation	(Singh et al., 2003)
R214W	S4	—	Slight rightward shift in current voltage-dependence; no effect on maximal current amplitude	(Castaldo et al., 2002), Giudice E 00
H228Q	S4-S5 linker	—	—	(Singh et al., 2003)
L243F	S5	—	—	(Singh et al., 2003)
S247W	S5	Therapy-resistant seizures; epileptic enecphalpathy	Markedly reduced maximal current amplitude	(Dedek et al., 2001)
S247X	S5	CS; FS at three years	—	(Hunter et al., 2006)
V250G	S5	—	—	(Moulard et al., 2001)
E254X	S5	Mild mental retardation; West syndrome	Lack of functional homomeric channels; markedly reduced current amplitude in heteromeric channels	(Bassi et al., 2005)
W269X	S5-S6 linker	1/7 late onset; 2/7 FS+GS in adulthood	—	(Singh et al., 2003)
G271V	Pore	BFIC	—	(Zhou XH 2006)
Frameshift at K283	Pore	5/19 GS between 21 and 45 years	—	(Singh et al., 1998)
Y284C	Pore	—	Markedly reduced current amplitude 6,	(Castaldo et al., 2002)
A306T	S6	11/69 FS; GS between 1 and 16 years	—	(Singh et al., 1998)

Q323X	C-terminus	2/6 BECTS at 2 and 4 years	Lack of functional homomeric channels; marked reduction in current amplitude in heteromeric channels	(Singh et al., 2003)
R333Q	C-terminus	—	Reduction in current amplitude; Faster rate of current deactivation	(Singh et al., 2003)
L339R	C-terminus	—	—	(Moulard et al., 2001)
R353G	C-terminus	—	Reduced interaction with CaM	(Richards et al., 2004)
Splice site mutation at S373	C-terminus	LCS+GCS; 1 FS	—	(Claes et al., 2004)
S382X	C-terminus	late onset BFNS; 4/11 FS, GS until 10 years	—	(Singh et al., 1998)
Splice site mutation at N396	C-terminus	wide range of clinical manifestations, with partial seizures later in life	—	(Lee et al., 2000)
Splice site mutation at L397	C-terminus	—	—	(Lerche et al., 1999)
Frameshift at K398	C-terminus	GTCS	—	(Pereira et al., 2004)
Splice site mutation at R406	C-terminus	—	—	(Lerche et al., 1999)
R448X	C-terminus	—	30% reduction in maximal current amplitude in heteromeric channels	(Singh et al., 2003), (Richards et al., 2004), Moulard B 01
Splice site mutation at E509	C-terminus	—	—	(Richards et al., 2004)
Frameshift at Q494	C-terminus	—	—	(Lerche et al., 1999)
Splice site mutation at D516	C-terminus	—	—	(Lerche et al., 1999)
Frameshift at S522	C-terminus	Late onset BFNS; 1/6 FS at 2 years	—	(Singh et al., 1998)

Frameshift at P534	C-terminus	—	Lack of functional homomeric channels; reduction maximal current amplitude of heteromeric channels	(Biervert et al., 1998)
Splice site mutation at C544	C-terminus	late onset BFNS	—	(Singh et al., 1998)
R553Q	C-terminus	—	—	(Moulard et al., 2001)
K554N	C-terminus	2/4 therapy-resistant seizures and mental retardation	Slight rightward shift in current voltage-dependence; no effect on maximal current amplitude	(Borgatti et al., 2004)
R581X	C-terminus	—	—	(Singh et al., 2003)
Splice site mutation at R588	C- terminus	—	—	(Richards et al., 2004)
R588X (intronic mutation)	C- terminus	1/11 seizures continued until 14 months of age; photosensitive myoclonic epilepsy at age 13 years; 1/11 mental retardation	—	(de Haan et al., 2006)
L637R	C-terminus	—	Increased interaction with CaM	(Richards et al., 2004)
Frameshift at Y644 (+ 56aa)	C-terminus	In all patients, seizures persisted until 12-18 mo	—	(Tang et al., 2004), (Biervert and Steinlein, 1999)
Frameshift at T653	C-terminus	—	—	(Singh et al., 2003)
Frameshift at P709 (+ 56aa)	C-terminus	1/3 CTS at 3 years; 1/3 GS later in life	Lack of functional homomeric channels; reduction in current amplitude of heteromeric channels; decreased protein stability and enhanced degradation	(Coppola et al., 2003), (Zimprich et al., 2006), (Soldovieri et al., 2006)
Frameshift at G866 (+ 56aa)	C-terminus	—	Reduction of current amplitude in homomeric and heteromeric channels	(Lerche et al., 1999)

Frameshift at W867 (+ 57aa)	C-terminus	3/12 seizures continued until age 2, 3, 7 years	Reduction in current amplitude; Slight shift in activation voltage-dependence; slight acceleration of current deactivation	(Singh et al., 2003)
Kv3 mutations				
Amino Acid Change	**Localization**	**Additional Clinical Data (Beside BFNS)**	**Functional Effects**	
D305G	Pore	—	Reduced current amplitude of heteromeric channels	(Singh et al., 2003)
W309R	Pore	—	—	(Hirose et al., 2000)
G310V	Pore	—	Slight (20%) reduction in heteromeric channels current	(Charlier et al., 1998)
N468S	C-terminus	Three siblings affected by BFIC	No effect; possibly a polymorphism	(Singh et al., 2003)
N821S	C-terminus	Does not cosegregate with the disease	No difference versus wt; possibly a variant of unknown significance	(Bassi et al., 2005)
nt c988t	?	—	—	(Li et al., 2006)

15. Conclusion

The knowledge gained from studying and understanding the mechanism that are involved in the disruption of voltage gated ion channels has allowed, and will evolve to allow, more specific therapeutic designs. As our grasp of how these ion channels are disrupted becomes more clear so will the specific treatments of the various epilepsies. We have begun to realize how various disease presentations can be found with different mutation within the same gene. As is pointed out, throughout the thesis, it appears, at least in part, to be due to different levels of disruption within the ion channel be it, gain or loss of function.

While review of the disease states presented here set the stage for understanding single channel/sing gene dysfunctions we know that epilepsy is not that simple due to the varied presentations within the same family. Channelopathies may be responsible or may increase the susceptibility of the patients suffering from epilepsies with polygenic inheritance and thus lead to seizures as they tip the balance toward excitation but, not every person in the same family with the same mutation express the same epileptic phenotype.

As any scientist-physician can tell you there is no single answer to one problem. Never can simply fixing one channel fix the life of the patient or in this case epilepsy. Patients go through remodeling which causes there channel makeup to constantly change depending on the channel environment. As the patient experiences more seizures, exposed to more antiepileptic drugs, or just the normal aging process, remodeling of the nervous system takes place.

As pointed out throughout this paper it does not seem surprising that disturbances in the "Super Family" of voltage gated ion channels plays a critical role in disturbing neuronal excitability culminating in many cases with epilepsy. We must continue to broaden the overall

definition of “epilepsy and the role of the super family of VGIC by continued simultaneous review of the various ion channels and their predisposition toward seizures.

The Human Genome Project has accelerated the study of epilepsy. While candidate genes are the bases for researchers today whole genome searches are the future! We are ready to throw down our canes and put on our track shoes.

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