

Gating pore channelopathies

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After academic research as a neuroscientist and 20 years of commercial ion channel drug discovery, Marc is now a freelance blogger, advisor and consultant for clients worldwide, where he shares his expertise and enthusiasm for all aspects of ion channel screening. He is particularly interested in automated patch clamp, and exploiting the potential of human iPS stem cell assays to facilitate the successful translation of new drugs into the clinic.

I've written this blog following the recent Biophysics Society meeting in San Francisco (February 2026), collating a series of historical and more recent publications that explore the occurrence of mutations in ion channel voltage sensor gating charges and how the resultant 'gating pore' currents have been linked to human pathologies in muscle and neuronal cells. This is also an opportunity to link so-called 'basic' biophysical experiments to translational disease models and human patients, exemplifying the continued need to support all types of academic and industrial ion channel R&D to yield meaningful understanding and potential treatments for both rare and common human diseases.

Biophysics of gating pore currents

Its probably best to start by defining the 3 different electrophysiological measurements that are used to understand the structure-function of voltage-gated ion channels:

- Ionic current through the main permeant pore within the S5-S6 pore loop domain (α)
- Gating charge movements as charged residues in the S4 helix of the voltage sensing domain (VSD) move across the membrane electric field (Q)
- Gating pore currents that flow through the VSD when charged residues are mutated (ω)

While voltage clamp recordings of ionic current (or indirect fluorescent indicator measurements of permeant ions) are

the major parameter used in ion channel biophysics and drug discovery, the more recent advent of detailed high resolution cryo-EM structures have brought attention back to the fundamental mechanisms of ion channel gating and permeation and the role of specific domains and amino acid residues in controlling these functions. We are also seeing greater interest in deciphering how specific disease-related mutations affect ion channel function, greatly facilitated by automated patch clamp (APC) profiling of patient variants and corresponding insights from computational and structural models and iPSC translational studies. This blog will highlight such work in relation to the growing body of evidence that pathological mutations in VSD S4 helix charged residues can produce anomalous gating pore (omega) currents, in addition to altered ion channel biophysics, which contribute in complex and subtle ways to human disease progression and severity.

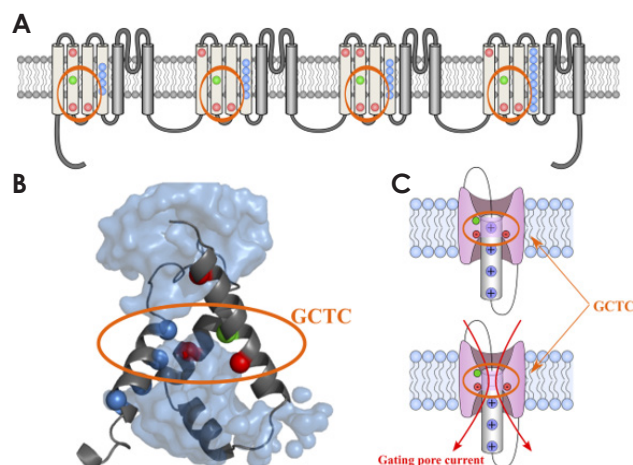


Figure 1 Schematic showing modular domain organisation of voltage-gated ion channels and structural basis for gating pore currents The S1-S4 helices form the voltage sensor domain (VSD), with conserved residues in S1-S3 forming the gating charge transfer centre (GCTC) that prevents leak current flowing when positively charged residues (blue) in the S4 helix move across the membrane. Mutations that neutralise S4 charged residues can induce gating pore currents. Image taken from¹

I would hope that all readers are familiar with ionic pore (α) current measurements, but many may not know much about gating charge recordings or the mechanism by which omega or gating pore currents arise. The schematic in Figure 1 nicely illustrates the structural basis of this blog, using the Na_v1.4 channel as an exemplar voltage-gated

ion channel with its modular arrangement of VSD and pore domains. Charged (arginine or lysine) residues in the S4 helix move in response to membrane voltage (creating gating charge Q currents) and couple to ionic pore activation and inactivation, where ion permeation and selectivity are controlled by residues in the central pore domain (PD). Negatively charged, aromatic and hydrophobic residues in the S1-S3 helices in the gating charge transfer centre (GCTC) or hydrophobic constriction site (HCS) form strong ionic pairs with the S4 helix and prevents ion leak and water wire formation when the S4 helix charged residues move helically within the VSD and become exposed to the extracellular or intracellular milieu. Crucially, mutations in charged S4 residues found in patients with neurological and muscular diseases can disrupt this tight molecular seal and result in ectopic flow of cationic gating pore (omega) currents into or out of the cell across a range of membrane voltages (see reviews^{1,2,3}). Gating pore currents are carried by H⁺ or monovalent cations but are very small (0.2-4% of central ionic pore current) and so can be hard to measure. These S4 mutations also affect voltage-dependent gating of ion channels, and both processes can contribute to complex disease patho-physiology, complicating the classification of this type of patient-derived channelopathy variant.

Gating pore currents were first discovered during structure-function mutagenesis studies of brain Na_v1.2 and Shaker and prokaryotic K_v channels in the 1990s and 2000s, when charged residues in the S4 helix were changed to better understand gating mechanisms and distinguish between the two theories of VSD function, 'S3-S4 paddle' and 'sliding helix'.^{2,3} As expected neutralisation of S4 charges altered the voltage-dependence of channel activation, but also produced ectopic 'omega' gating pore currents carried by monovalent cations or protons (after histidine substitution); these early experiments relied on heterologous expression in *Xenopus* oocytes where the small currents were more easily detectable.^{4,5} Mutation of extracellular R1 or R2 residues on the upper end of the S4 helix can lead to inward omega currents at rest, which close when the S4 helix moves upward during depolarisation and channel activation and the remaining intact HCS can prevent water wire formation; conversely, mutation of central and cytoplasmic R2, R3 or R4 S4 arginines in Na_v1.2 and Shaker and K_v7.x channels can lead to outward currents during membrane depolarisation (Figures 2, 3). Thus, the size and voltage-dependence of gating pore currents depends on the location of the altered charged residue on the S4 helix and the ionic conditions, so they can exhibit state-dependence just like central pore ionic currents. Ion channel gating pore currents and 'water wires' were initially revealed by structure-function mutagenesis experiments and voltage clamp recordings over the last 20 years, and have more recently been resolved by patch clamp recordings in mammalian cells, and even visualised in high resolution cryo-EM structures of ion channel protein subunits,⁶ making for a fascinating biophysical story by itself. Below I will outline the latest

chapter, which involves growing awareness that similar VSD S4 helix arginine mutations occur in a growing number of human pathologies affecting muscle cells and neurons, and that the gating pore currents produced can contribute to this novel class of channelopathy.

Gating pore channelopathies

I'm sure many people who have read dense and dry journal articles on ion channel gating biophysics and VSD mutagenesis failed to make the connection between this work and human patients. Indeed, it took me several decades from my PhD in Australia in the early 90s (reading Bertil Hille's textbook and learning about gating charge movement in skeletal muscle from Angela Dulhunty) to working up targets for a virtual rare channelopathy disease biotech here in Cambridge (UK) in the mid-2010s for these worlds to overlap. The field has thus evolved from mutating charged residues in the S4 helix of various exemplar voltage-gated ion channels to understand their structure-function, to studying human patient and disease-linked S4 mutations in more detail to try and link altered biophysics (pore gating, omega currents) with disease phenotypes, and this work is still ongoing thanks to greater awareness of gating pore currents. I will start this section with perhaps the best known examples of pathological gating pore currents (Na_v1.4 and Ca_v1.1 in skeletal muscle myotonias), before moving onto work involving Na_v1.5, Ca_v1.3 and Ca_v1.4 channels and TRPM7 receptors linked to peripheral pathologies, and then concluding with recent CNS examples of gating pore currents in K_v7.2/3 and Na_v1.2 channels linked to epilepsy and autism.

It is also important for this discussion to discriminate between disease-related mutations in the VSD S4 helix that produce changes in voltage-dependent gating of the central ionic channel pore, and specific variants in positively charged residues that can promote occurrence of omega gating pore currents. Table (Figure 2) from the review by Jurkat-Rott et al. (2012)⁷ is a good place to start, as it shows that not all VSD S4 R variants linked to human pathologies produce omega currents.

Skeletal muscle myotonias

I first came across gating pore channelopathies in a drug discovery setting when selecting targets for a rare disease virtual biotech company I co-founded with colleagues in 2014 (Inovion Pharma). We identified gain-of-function (GoF) mutations in Na_v1.4 channels linked to several types of skeletal muscle myotonia (e.g. paramyotonia congenita) that increased current by decreasing inactivation and thus might be treatable with classical Na_v1.x pore blockers, but other forms of muscle pathology are linked to gating pore currents in Na_v1.4 and Ca_v1.1 channels.^{7,8,9} In a sign of how science has rapidly progressed since I first became aware of omega currents, Bill Catterall's group managed to visualise the VSD gating pore using cryo-EM of a homology model of

motif	Shaker	Kv7.1	Kv7.2	Kv11.1	Nav1.1				Nav1.4				Nav1.5				Cav1.1				Cav2.1				omega current
					I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	
	359	228	198	525	213	856	1313	1636	216	666	1126	1448	216	805	1300	1623	162	525	894	1233	192	580	1343	1658	
R0	A	R	R	K	S	S	K	R	S	S	K	R	S	S	K	R	K	S	K	A	R	S	K	S	
X	I	G	S	T	A	V	S	V	A	V	S	V	A	V	S	V	A	V	I	F	T	V	S	F	
X	L	I	L	A	L	L	L	I	L	L	L	I	L	L	L	I	L	L	L	F	L	L	L	L	
R1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
X	V	F	F	L	T	S	T	L	T	S	T	L	T	S	T	L	A	C	V	L	A	A	V	L	
X	I	L	L	L	F	F	L	A	F	F	L	A	F	F	L	A	F	I	L	F	V	L	L	F	
R2	R	Q	Q	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
X	L	I	I	L	V	L	A	I	V	L	A	I	V	L	A	I	V	L	V	V	V	L	V	A	
X	V	L	L	V	L	L	L	G	L	L	L	G	L	L	L	G	L	L	L	M	L	L	L	A	
R3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
X	V	M	M	V	A	V	P	I	A	V	P	V	A	V	P	I	P	I	P	L	P	I	P	L	
X	F	L	I	A	L	F	L	L	L	F	L	L	L	F	L	L	L	F	L	I	L	F	L	I	
R4	R	H	R	R	K	K	R	R	K	K	R	R	K	K	R	R	R	K	R	K	K	K	K	K	
X	I	V	M	K	T	L	A	L	T	L	A	L	T	L	A	L	L	I	A	L	L	V	T	L	
X	F	D	D	L	I	A	L	I	I	A	L	I	I	A	L	I	V	T	I	L	V	T	I	L	
R5	K	R	R	D	S	K	S	K	T	K	S	R	S	K	S	R	S	K	N	S	S	K	K	R	
X	L	Q	R	R	V	S	R	G	V	S	R	G	V	S	R	G	G	Y	R	R	G	Y	R	Q	
X	S	G	G	Y	I	W	F	A	I	W	F	A	I	W	F	A	V	W	A	A	I	W	L	G	
R6	R	G	G	S	P	P	E	K	P	P	E	K	S	P	E	K	P	T	K	E	P	A	P	Y	

Figure 2 Arrangement of VSD S4 helix charged residues (R= arginine, K= lysine) in R0-R6 sequence across voltage-gated K_v , $Na_v1.x$ and Ca_v ion channels . Disease-related S4 residues in red. Inward or outward omega currents may be produced after membrane hyperpolarisation (R1-R2) or depolarisation (R3-R6) respectively, but evidence is lacking except for those discussed below. Taken reference⁷ under open access CC4.0 license.

a muscle disease $Na_v1.4$ channel.⁶

Potassium-sensitive Hypokalemic Periodic Paralysis (HypoPP) is caused by charge neutralising variants of R1 or R2 arginine residues in the upper part of VSD S4 helices of $Ca_v1.1$ (D II-IV) and $Na_v1.4$ (D I-III) channels that allow cationic 'leak' omega currents to flow into the muscle cells through resting channels at hyperpolarised membrane potentials, leading to Na^+ accumulation, chronic depolarisation and blunted action potential firing and ultimately muscle wasting and flaccid paralysis (opposite of muscle stiffness with pore-related GoF $Na_v1.4$ mutations). Interestingly, French researchers showed that another HypoPP mutation in S3 of D III of $Ca_v1.1$ (V876E) indirectly induced omega currents and Na^+ accumulation at rest in muscle fibres after ionic changes or pH challenge.¹¹ There is still some debate over the role of proton conduction through HypoPP channels,

although it is clear that disease-linked histidine substitutions allow for significant H^+ current and cellular acidification,¹² and this pH-sensitivity may help explain why diet and exercise can trigger attacks. In contrast, Normokalemic Periodic Paralysis (NormoPP) is produced by mutations in R3 arginine residues lower down the VSD S4 helix,¹³ which allow an ectopic outward current to flow at depolarised membrane potentials when $Na_v1.4$ channels are activated and/or inactivated (Figure 3). While changes to the VSD can alter gating and some $Na_v1.4$ and $Ca_v1.1$ VSD mutations reduce channel expression (but not in NormoPP), the ectopic omega currents are thought to be the main drivers of pathophysiology as they uniquely produce membrane depolarisation. The Cannon group, a major player in the field of myotonia, created mouse knock-in models of HypoPP expressing VSD S4 histidine variants in $Ca_v1.1$ (R1 in D II) and $Na_v1.4$ both of which recapitulated the low

"From early mutagenesis to patient-derived models, we're finally closing the loop between ion channel biophysics and disease, showing that even tiny gating pore currents can drive pathology. Integrating precise electrophysiology with translational models is now essential for next-generation drug discovery."

Marc Rogers, Founder and Director, Albion Drug Discovery Services Ltd

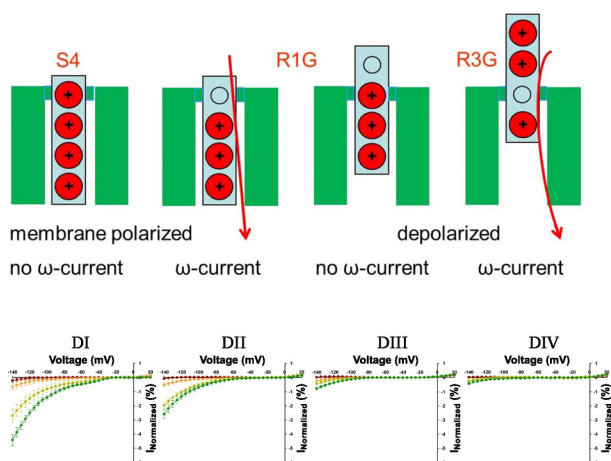


Figure 3 Gating pore mutations in the VSD of Cav1.1 and Nav1.4 channels induce resting or activated state pore currents in Hypokalemic and Normokalemic Periodic Paralysis (HypoPP and NormoPP). Upper, Taken from ⁷ under open license; Lower, taken from ¹⁰ open access article.

K⁺-induced depolarisation and loss of muscle excitability and muscle weakness, and an ectopic inward leak current in muscle fibres at rest indicative of omega currents.¹⁴ The Ca_v1.1 mutant mouse also exhibited a vacuolar myopathy affecting transverse tubules and triad junctions which impaired Ca²⁺ release, as these channels couple electro-mechanically to sarcoplasmic stores.

Na_v1.5 cardiac myopathy

Alongside numerous mutations in Na_v1.5 channels linked to arrhythmia (Brugada syndrome, LQT3), there are a subset that produce both arrhythmia and dilated cardiac myopathy

(DCM) and many of these variants alter charged arginine residues in the VSD S4 segment of domain I (R219H, R222Q, R225W) and domain II (R225P and R814W). Whilst several groups have shown these variants to alter the magnitude, voltage-dependence and kinetics of transient, persistent/late, ramp and window currents carried through the central pore, as might be expected for changes in the VSD, Mohamed Chahine's group in Canada also demonstrated that they produce gating pore currents.^{15,16,17} These omega currents are small (<100 pA) but can reach ~3% of the peak inward current density in heterologous expression systems, similar to ramp and window current through the main pore. Na_v1.5 DCM-associated omega currents typically flow at positive potentials (Figure 4) as the mutated arginine residues lie at the bottom of the VSD S4 segment and only enter the membrane electric field after large depolarisations, but they can also flow inwardly at negative potentials after a long depolarisation, both conditions occurring during and after a long cardiac action potential.

It still remains uncertain how each of the biophysical effects of these VSD S4 mutations in Na_v1.5 channels contribute to both arrhythmia and DCM in certain patients, but it is likely that a combination of altered current voltage-dependence and kinetics can lead to small but sustained Na⁺ influx and Ca²⁺ dysregulation to produce muscle remodelling and gene expression, which may be exacerbated by additional proton or cation 'leak current' through gating pores that further impacts intramuscular signalling (pH, Na⁺, Ca²⁺), contractile function and cellular homeostasis. Significantly, the DCM variant R219H produces little change in Na_v1.5 central pore current amplitude or biophysics but induces a large proton-specific gating pore leak current, and expressing this variant in human iPSC-derived ventricular and atrial-like cardiomyocytes recapitulates many of the electrophysiological, morphological and functional deficits

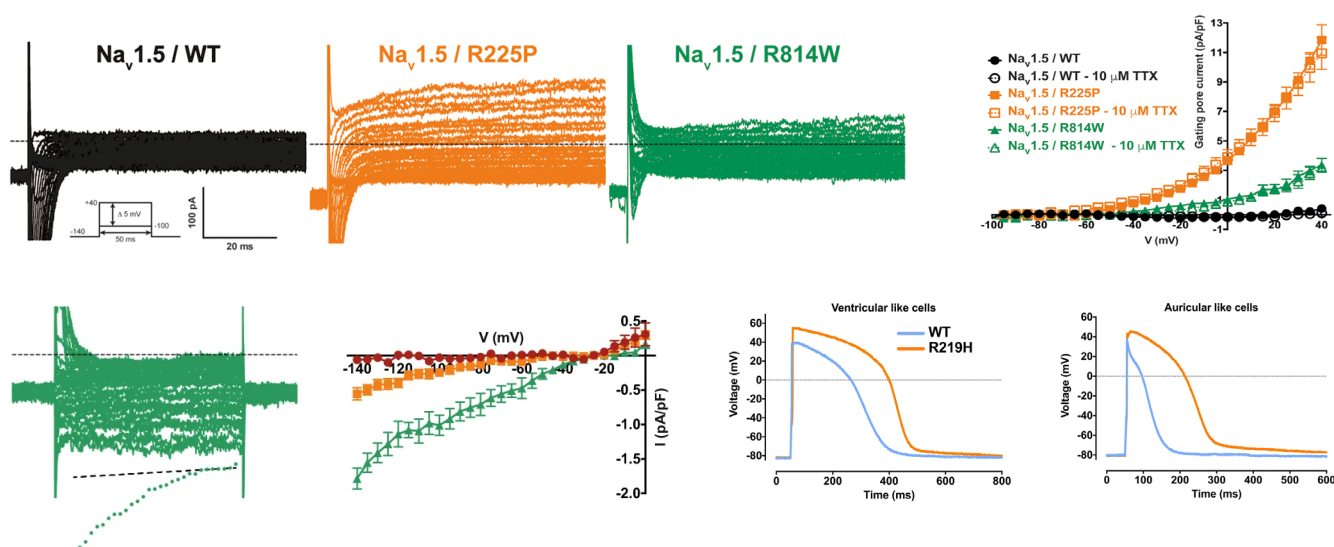


Figure 4 Na_v1.5 VSD S4 helix mutations in dilated cardiomyopathy patients can induce outward or inward gating pore currents and prolong iPSC cardiomyocyte action potentials. Upper taken from ¹⁶ and Lower taken from ¹⁷ under CC4.0 licenses.

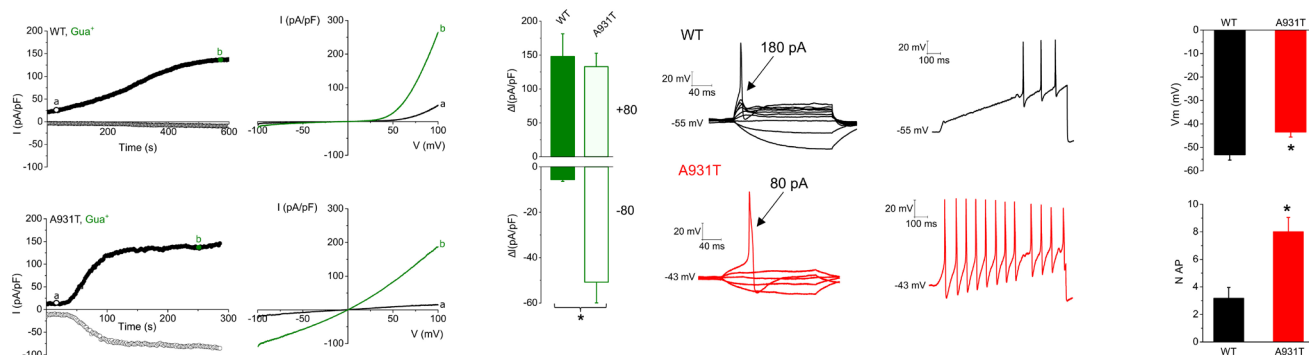


Figure 5 A novel VSD gating pore current in TRPM7 S3 helix mutant linked to trigeminal neuralgia induces an inward current that excites trigeminal neurons. Taken from ¹⁸ open access PNAS article.

of DCM,¹⁷ confirming the pathological significance of this family of cardiac gating pore channelopathies.

TRPM7 pain-related gating pore mutation

As the tight seal during VSD movement involves both positively charged residues on the S4 helix and negatively charged and hydrophobic residues in other parts of the VSD, it is possible that gating pore currents can be produced by mutations in the latter side-chains, and a recent example of this was published for a TRPM7 variant identified in a patient with trigeminal neuralgia.¹⁸ The A931T variant in the S3 domain alters TRPM7 rectification and divalent cation selectivity by inducing a large inward Na^+ current, as well as affecting activation voltage-dependence and current kinetics. The ectopic inward current is inhibited by the TRPM7 gating modulator NS8593 but insensitive to the pore blocker Gd^{3+} and activator Naltriben, suggesting it represents a novel permeation pathway linked to structural changes in the VSD; this was confirmed by its permeability to guanidinium ions but not NMDG, common features of gating pore currents in other ion channels (Figure 5). Site-directed mutagenesis based on a TRPM7 cryo-EM structure suggests that A931T disrupts an interaction with hydrophobic residues in the S3-S4 cleft, opening up a water wire conduction pathway. Significantly, the increased inward (depolarising) cation current in A931T channels increases membrane excitability and action potential firing when expressed in rodent trigeminal neurons, offering a translational link to the trigeminal pain of the patient.

$\text{Ca}_v1.x$ gating pore channelopathies linked to peripheral disease indications

Taking a lead from the extensive work on VSD S4 gating pore current mutations in $\text{Ca}_v1.1$ channels of HypoPP channels, an academic collaboration between Austrian and UK researchers showed that the R990H variant in VSD domain III of $\text{Ca}_v1.3$ channels found in human aldosterone-producing adenomas also produces omega currents.¹⁹ Functional profiling confirmed predictions from molecular

modelling that 'water wires' carried gating pore currents at rest (as R990H sits at the bottom of the S4 helix) but not after activation by membrane depolarisation (Figure 6). As expected, omega current biophysics and amplitude depended on the number and position of positively charged arginine residues on the S4 helix bundle and their interacting counter-charges in each VSD domain, and the nature of the variant amino acid residue. Other biophysical changes in R990H mutant $\text{Ca}_v1.3$ channels are limited to a small depolarising shift in activation and slightly reduced macroscopic and gating charge currents, suggesting little change in surface expression or central pore function. Thus, cancer pathology in patients with human adenomas may be causally linked to persistent inward currents in $\text{Ca}_v1.3$ channels at resting membrane potentials, in contrast to mutations in other regions of the $\text{Ca}_v1.3$ channel that induce gain-of-function changes in central pore function.

Similarly, a leading Ca_v channel academic group in Austria recently detailed two pathological mutations in the VSD S4 helices of $\text{Ca}_v1.4$ channels linked to congenital stationary night-blindness type 2.²⁰ R964G in VSD III and R1288L in VSD IV were found in male patients and produce marked loss-of-function in $\text{Ca}_v1.4$ currents with complex biophysical effects, affecting synaptic transmission from retinal photoreceptors. Charge neutralisation in the VSD S4 helix significantly decreased current density, but there was no change in protein expression or number or open probability of single channels on the cell surface, suggesting that reduced macroscopic current was due to reduced ion permeation through the central pore. There were also significant changes in $\text{Ca}_v1.4$ current biophysics and voltage-dependence, as expected from fundamental changes to VSD residues important for channel gating. While a reduction in gating charge current was shown for both variants, the authors did not publish any recordings of omega gating pore currents. Rather, $\text{Ca}_v1.1$ and NavAb channel homology structures were used for molecular dynamic modelling that showed greater propensity for formation of 'water wires' through the VSD of resting R964G and activated R1288L mutant $\text{Ca}_v1.4$ channels. It

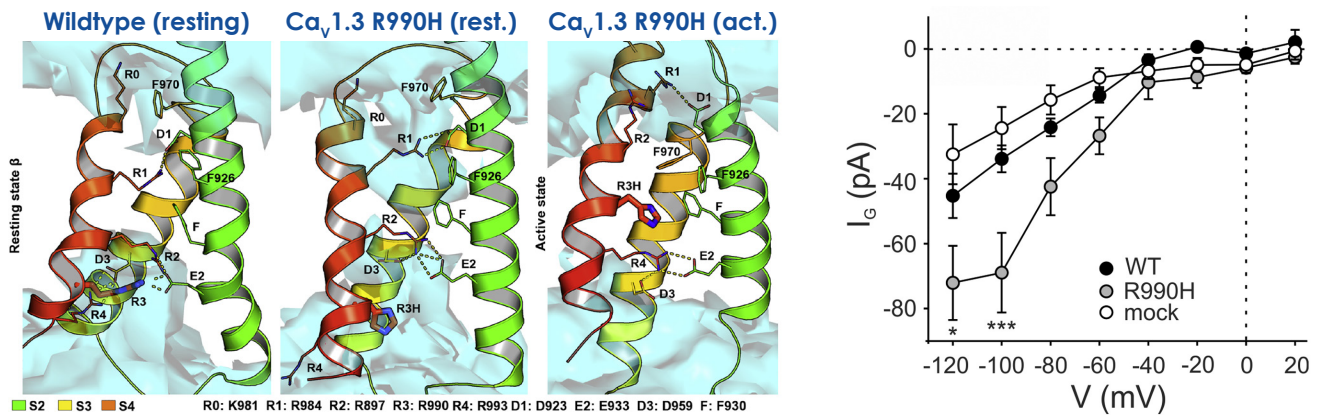


Figure 6 VSD water wires and inward gating pore current in R990H mutant $\text{Ca}_v1.3$ channels found patients with aldosterone-producing adenomas. Images from open access journal article ¹⁹.

will be important to see this theoretical work confirmed experimentally, and thus align the effect of $\text{Ca}_v1.4$ gating charge mutations with those seen in $\text{Ca}_v1.3$ and HypoPP $\text{Ca}_v1.1$ channels.

Examples of pathological gating pore current channelopathies have also been published for CNS diseases involving $\text{K}_v7.2$ and $\text{Na}_v1.2$ channel variants in epilepsy and autism patients (recent review ²¹).

$\text{K}_v7.2/7.3$ VSD S4 helix variants linked to epilepsy

As well as being expressed in peripheral sensory neurons where they constitute the M-current that controls membrane excitability, $\text{K}_v7.2$ and $\text{K}_v7.3$ channels are expressed in mammalian CNS neurons (e.g. hippocampus, neocortex and cerebellum) and regulate cell and network connectivity and activity, which makes them attractive targets for anti-epileptic drugs (Retigabine/Ezogabine, XEN1101/azetulkaner, BHV-7000/Opakalim). Mutations in $\text{K}_v7.2$ (and more rarely $\text{K}_v7.3$) are linked to a rare neonatal autosomal dominant form of epilepsy called benign familial neonatal seizures (BFNS), producing a haploinsufficiency and mild loss-of-function. The genotype-phenotype relationship is complex and includes mutations in the intracellular loops as well as the VSD which produce changes in channel protein trafficking or voltage-dependence. Of note for this blog are BFNS patients with mutations in positively charged S4 residues in the VSD, where charge neutralisation of proximal (R201 on the exterior end of the S4 helix) and distal arginines (R213 towards the cytoplasmic end) alter M-current voltage-dependence and gating as expected for VSD changes, but alteration of the central R207 residue also induces the appearance of omega gating pore currents (Figure 7). These are only seen at positive membrane potentials, suggesting that a novel ion conductance pathway appears when the S4 helix rotates completely and exposes the central R4 residue to the outer plasma membrane.²² An earlier study in a German family showed that the R207W mutation produced similar biophysical changes to the

ionic pore current of $\text{K}_v7.2$ channels and was associated with BFNS and late onset myokymia (peripheral nerve hyperexcitability, PNH) attributed to altered excitability of spinal cord motoneurons, whereas the less drastic R214W variant was found in another family with BFNS but no myokymia.²³ No gating pore current measurements were made in the earlier study, but the authors did confirm that heteromeric channels containing wt $\text{K}_v7.2$ and $\text{K}_v7.3$ and mutant $\text{K}_v7.2$ subunits were less affected than mutant homomers, better reflecting the pathophysiological situation in patient neurons. The significance of omega currents in some BFNS patients is not clear, with the more pronounced

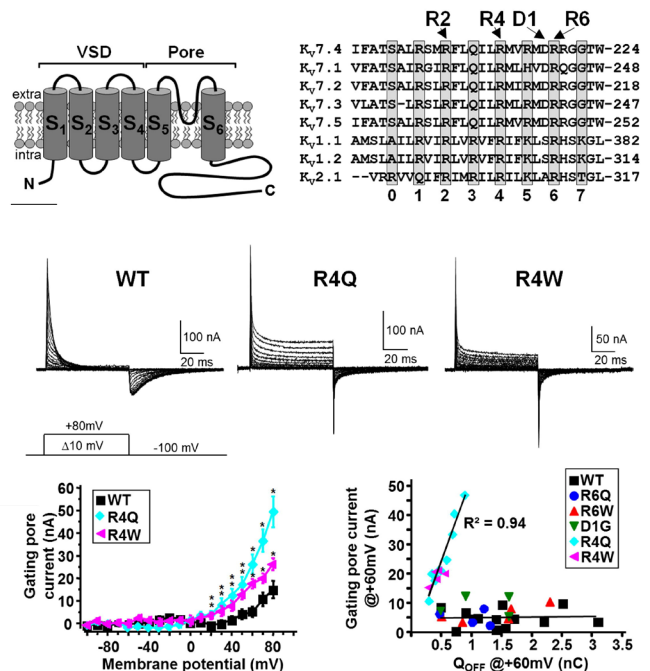


Figure 7 VSD R4 arginine mutation R207 in $\text{K}_v7.2$ channel linked to benign familial neonatal epilepsy produces outward gating pore currents. Images taken from open access journal article ²².

slowing of M-current activation and depolarising shift in activation most likely to contribute to reduced inhibition of neuronal firing.

K_v7.3 gating pore current variants in autism patients

Bill Catterall (RIP) and his colleagues at University of Washington in Seattle followed up their ground-breaking biophysical work on ion channel VSD structure-function in the 2000s by digging into extensive new genetic databases of patients with neurodevelopmental disorders, noting an unexpectedly high number of *de novo* missense mutations in charged arginine residues of the VSD across a number of voltage-gated ion channels (Ca_v1.2, Ca_v1.4; Ca_v2.1, Ca_v2.2, Ca_v2.3; Ca_v3.1, Ca_v3.2, Ca_v3.3; K_v2.1, K_v3.1, K_v3.3, K_v7.3, K_v10.1, K_v10.2; Na_v1.1, Na_v1.2, Na_v1.3, Na_v1.6, Na_v1.7) were present in autism patients.²⁴ While these S4 helix variants would be expected to affect voltage-dependent biophysics of these ion channels, work remains to see if they also produce omega currents (see below for Na_v1.2). The authors started to test this with K_v7.3 channels as they found five autism patient families with R227Q, R230C/H or R236C/L mutations across the length of the S4 helix (see Figure 8). They found that R1Q and R2C autism variants induced large (4% of central pore ionic current) inward gating pore currents at negative membrane potentials and outward omega currents at depolarised membrane potential, while the R4C mutation only generated outward gating pore currents positive to resting membrane potential (i.e. typical activation range for M-current).

The pathophysiological significance of these large inward and outward K_v7.3 gating pore currents was uncertain, especially as they are reduced 2-3 fold in physiological Na⁺ compared to artificial K⁺-rich solutions. However, the authors hypothesised that significant ectopic Na⁺ influx and efflux through K_v7.3 channels in CNS neurons could disrupt membrane potential and excitability (particularly given their likely location at the axon initial segment that integrates neurotransmission), and showed that knock-in of the K_v7.3 R2C variant into mice significantly decreased spontaneous and evoked action potential firing and increased spike frequency accommodation in dopaminergic neurons in brain areas linked to autism and social behaviour. The R2C variant increased firing threshold and prolonged single AP duration and reduced the size of the afterhyperpolarisation, opposite to the effect of K_v7.3 knockdown, confirming a gain-of-function biophysical effect that leads to a pathophysiological inhibition of CNS neuron function and network signalling. Much like Na_v1.4 and Ca_v1.1 omega current mutations in HypoPP, these large non-selective gating pore currents in autism-variant K_v7.3 channels likely introduce a 'shunting' effect on membrane conductance which depolarises the resting potential and inactivates Na_v1.x channels, along with biophysical changes to K_v7.x channels themselves, both of which would affect excitation-inhibition (E/I) balance in the brain.

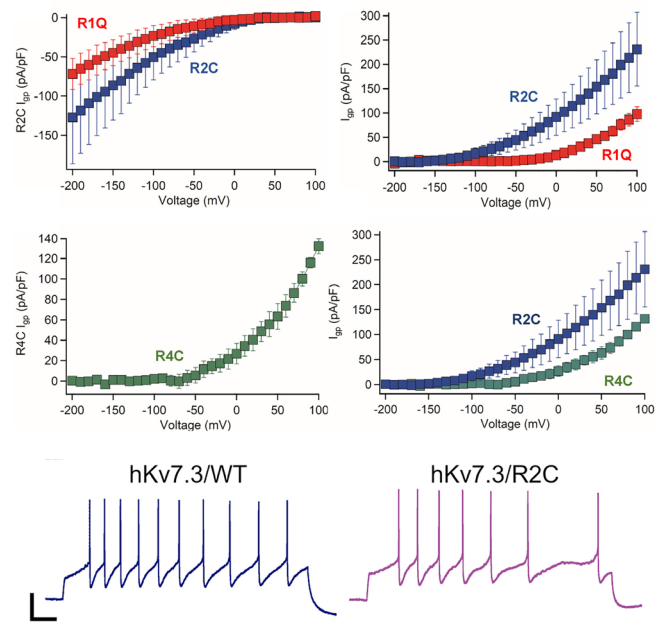


Figure 8 Large gating pore currents produced by VSD S4 helix arginine mutations in autism families affect neuronal excitability. Images taken from open access article²⁴.

Na_v1.2 gating pore GoF mutations in autism

The same group followed up this work by looking at autism-related variants in Na_v1.2 channels, several of which affect S4 arginine residues in human D II (R853Q) and D IV (R1626H and R1629H).²⁵ These R1 and R2 mutations produce the expected inward omega currents at hyperpolarised potentials when Na_v1.2 channels are at rest, including proton currents for the histidine substitutions, but are much smaller (0.1% central pore current) than those seen in K_v7.3 channels (Figure 9). There were also significant changes to voltage-dependent gating and inactivation, as expected for altered VSD structure-function. Introduction of these autism-related Na_v1.2 parameters and omega currents into an *in silico* model of mouse neocortical interneurons enhanced excitability by increasing single and repetitive action potential firing and thereby altering input/output modulation and E/I balance. This effect was opposite to the autism-related K_v7.3 gating pore current mutations, perhaps as they are selective for K⁺ whereas the Na_v1.2 variants flux protons and cations, and are much smaller and less likely to produce depolarising block seen with Na_v1.4 HypoPP gating pore mutations.

The same laboratory has just published on a R854Q mutant mouse which showed an autism-like phenotype including repetitive behaviour and impaired social interactions and risk avoidance.²⁶ However, further *in silico* modelling based on *in vitro* biophysical measurements showed that the ectopic omega pore current partly offsets the reduction in

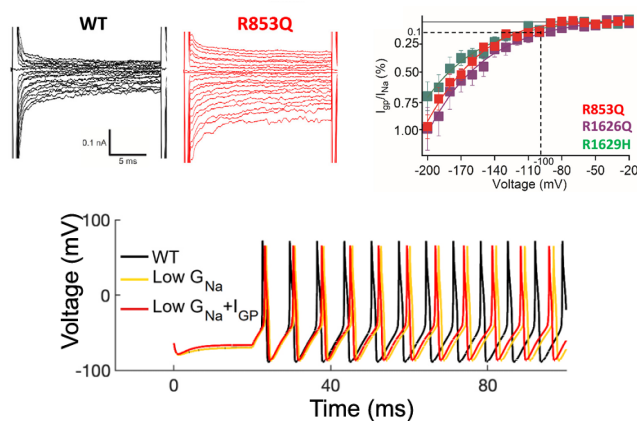


Figure 9 Large inward gating pore currents produced by Na_v1.2 VSD S4 helix arginine mutations in autism families affect neuronal excitability. Images taken from open access article ²⁵.

Na_v1.2 channel conductance (channel density), but overall this variant results in decreased cortical excitability (Figure 9). Notably, these electrophysiological and behavioural changes were rescued by the K_v7.x channel opener Retigabine, likely by reducing slow inactivation of mutant Na_v1.2 channels, although the characteristic autism trait of altered social interaction remained. In contrast, preliminary data the group revealed at the Tübingen channelopathy conference in 2025 showed that the Na_v1.2 R1630H variant (VSD IV) associated with seizures *in vivo* only shows a GoF effect of increased ectopic gating pore currents, consistent with increased neuronal excitability.

It is also worth mentioning that LoF mutations (e.g. haploinsufficiency) in Na_v1.2 are also found in autism and DEE patients, but it is thought that these may be more closely linked to intellectual disability.²⁷

Na_v1.2 gating pore variants linked to DEE epilepsy

Further evidence for the complex interplay between human genetics and Na_v1.x channel patho-physiology, which can upset simplistic theories of geno-phenotype relationships, comes from the fact that Steve Petrou's laboratory in Australia also recently made an R854Q knock-in mouse, to better understand the onset and overlap between different forms of DEE and epilepsy.²⁸ They classified this late onset DEE as a LoF based on reduced Na_v1.2 current density, and like Eltokhi and colleagues above found it reduced neuronal excitability and network synchronicity of mouse cortical neurons using an *ex vivo* multi-electrode array assay (similar to the more realistic *in silico* results that included both ectopic gating pore currents and reduced peak conductance). They also found that these deficits were rescued by Retigabine. In contrast, a GoF Na_v1.2 variant (R1882Q) increased cortical excitability and was linked to early onset DEE. An independent biophysical

study of both these Na_v1.2 variants in HEK cells showed that R1182Q was a GoF exhibiting altered inactivation and enhanced persistent currents, while R853Q was a LoF with decreased current amplitude and persistent currents and hyperpolarised inactivation.²⁹ It was only when R853Q channels were expressed in *Xenopus* oocytes that large gating pore currents could be detected, highlighting the need for comprehensive biophysical approaches to reliably characterise disease-related arginine residues and detect technically-challenging omega currents that may only conduct ~1% of peak pore current.

Also, other Na_v1.2 arginine variants across all four VSDs are found in patients with a wide range of neurological syndromes such as DEE, ataxia and epilepsy (e.g. Benign Familial Neonatal-infantile Seizures), but it is not yet determined if these effects are linked to biophysical or protein LoF or GoF, ectopic omega currents, or other biophysical changes affecting CNS development, excitability and plasticity. Preliminary data from the Eltokhi group shows that DEE (R220G) and BFNIS (R223Q) arginine substitutions in D I produce large inward cation omega currents at rest along with other biophysical changes, so this story continues to develop.

Drug Discovery opportunities and disease treatments for gating pore channelopathies

While this phenomena of 'ectopic' ion channel gating pore currents is an interesting biophysical mechanism important to understanding structure-function of voltage-gated transmembrane proteins, it is also relevant to a surprisingly wide range of human disease channelopathies. However, imagining treatments for these patients throw up a number of challenges for drug discovery efforts.

Pharmacological inhibition

The obvious idea is to try and pharmacologically block disease-related gating pore currents that flow across the VSD of mutant ion channel subunits – perhaps best exemplified by hyperpolarisation-activated inward currents seen in Na_v1.2 (autism), Na_v1.4 and Ca_v1.1 (HypoPP), Na_v1.5 (DCM), Ca_v1.3 (aldosteronism) and K_v7.x (autism) channels described above. For example, ectopic omega currents produced by mutated S4 arginine residues in Shaker K_v and Na_v1.x channels are reduced by μM concentrations of permeant trivalent (La³⁺, Gd³⁺) and mM levels of divalent cations (Ca²⁺, Mg²⁺, Zn²⁺),^{1,4} but these are non-selective and would affect many other cationic pores and channels. Other charged ions are less or non-permeant (Li⁺, TEA) so cannot be considered to be omega current blockers, while guanidinium ions are highly permeant, but these effects also depend on the nature and location of the S4 arginine variant and the voltage-dependence of their permeation.¹ Most significant is the fact that omega currents through mutated Na_v1.4 channels can be selectively inhibited by

some large hydrophobic xylyl guanidinium analogues, which apparently do not affect current through the central pore.³⁰

Achieving selectivity for block of narrow water-filled VSD omega pores is surprising but not impossible, especially as central ionic pores of most channels are larger and have a different profile of charged and hydrophobic residues, and some are not water filled. However, this work has not been followed up, and like many I imagine that the major challenge to pharmacologically targeting omega currents lies in the fact that transmembrane protein domains are built from highly conserved motifs shared across many families of voltage-gated ion channels. This sequence homology will likely make it very hard to design or discover ligands that are selective modulators/binders to ectopic gating current pores, as opposed to the potent and selective gating modifier small molecules and toxins that bind to other regions of VSDs of Na_v1.x and K_v7.x channels. It will also be desirable to avoid effects on wildtype VSDs and gating charge movement of non-diseased channels in the same sub/family, as well as avoiding perturbation of cationic permeation through a diverse array of many other voltage- and ligand-gated (e.g. TRP, P2X, GluR) channel proteins.

Given these caveats I would like to highlight more tractable alternative mechanisms to treat gating pore current channelopathies below.

Toxin peptides

The second option, alluded to above, is the fact that evolution and natural selection have produced a wide range of animal peptide toxins that potently bind with some specificity to extracellular loops of various ion channel VSDs to modify activation or inactivation gating, and thus hold potential as leads to discover and design gating pore current inhibitors:

- scorpion toxins bind to D IV S3-S4 (site 3) and modulate gating of Nav1.x channels; binding is highest at resting state so may be useful for R1 or R2 mutations?
- scorpion toxins bind to D II S3-S4 (site 4) with preference for resting or activated state of Na_v1.x channels, but only inhibitory peptides may be able to reduce gating pore currents
- Spider toxins (Protoxin-II, Huwentoxin-IV) bind to resting state of Na_v1.x D II S3-S4 linker and inhibit gating, and have proven amenable to protein engineering
- Protoxin-I also binds to D IV S3-S4 residues in Ca_v3.x T-type channels to inhibit their gating
- ω-Agatoxin binds to S3-S4 linker in D IV of Ca_v2.1 channels to inhibit gating
- Hanatoxin and other ICKs bind to S3-S4 linker of K_v channel subunits in resting state to inhibit gating

Notably, these classes of gating modifier toxin peptides

are different to those that block the external vestibule of the main ionic pore of voltage-gated ion channels (e.g. TTX, Charybdotoxin). The challenge here is that specific VSD-binding omega current blocking toxin peptides have yet to be identified, and their 'landing sites' on or close to the VSD S4 helix would be small and likely conserved across channel isoforms. Such gating pore toxin peptides may already be known, but I have yet to find any publications that has tested gating modifier toxins for actions against gating pore currents. However, the recent advent of rapid and accessible AI/ML software and computational methods for protein folding, binding and ligand design has enabled a rapid evolution in capabilities to design *de novo* proteins, including those targeting ion channels with potency and selectivity (Na_v1.7,³¹ ASIC1a³²). It thus remains possible that a similar approach could be used to design novel artificial peptide scaffolds from scratch, or 'evolve' known peptides and toxins, to specifically bind to the VSD and block disease-related gating pore currents.

VSD gating pore antibodies

A third approach similar to the concept of toxin peptides are antibodies, which typically bind to small extracellular epitopes on transmembrane proteins including the VSD and S5-S6 E3 pore turret regions (see my previous Nanion blog³³). While ion channel antibody 'binders' are relatively easy to identify, it has proven much harder to find antibodies that can functionally modulate ion channel activity, and I am not aware of many that alter voltage-dependent gating through binding to VSDs. There may be a greater chance of discovering and/or designing nanobodies that can selectively bind the VSD gating pores, as their smaller size enables access to cryptic and novel binding pockets, and some have recently been shown to alter HCN4 channel gating.³⁴

Gene therapies

Given the rare and specific amino acid changes that underlie gating pore channelopathies, the most specific and targeted approach for patients may be genetic therapies. Taking inspiration from ongoing clinical studies for rare Dravet epilepsy and Developmental and Epileptic Encephalopathies (DEE) affecting Na_v1.1, Na_v1.2 and Na_v1.6 channels (e.g. Stoke and Encoded Therapeutics, Praxis Precision Medicines) and preclinical work on Na_v1.7 channels for pain (Navega and Sangamo Therapeutics), one could imagine at least three types of genetic treatments that might be able to 'correct' or suppress VSD S4 arginine gating pore mutations:

- Gene editing (CRISPR, base or prime editing) to revert the amino acid sequence to wildtype
- RNA therapies (e.g. ASOs) to suppress mutant gene activity and RNA production (as most patients are heterozygous)

- Epigenetic therapies to suppress expression of the mutant allele gene/RNA/protein

There is growing industry awareness, patient and family campaigning and regulatory support for designing disease- and patient-specific genetic treatments, backed-up by validated reagents and assays and many strikingly successful preclinical and clinical ion channel programs. For example, such precision medicine approaches are rapidly advancing in the clinic to treat $\text{Na}_v1.x$ mutations linked to DEE, epilepsy and autism,³⁵ and preclinical work with human iPSC neurons and animal disease models includes using CRISPR activation to correct $\text{Na}_v1.2$ haploinsufficiency linked to autism.³⁶ Along similar lines, a recent preclinical paper on a $\text{Na}_v1.2$ S4 arginine gating pore mutation linked to DEE and autism showed that behavioural and electrophysiological symptoms could be partially rescued by the $\text{K}_v7.x$ opener Retigabine,²⁶ illustrating the potential for pharmacogenetic approaches to compensate for biophysical and phenotypical effects of gating pore channelopathies.

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