# Skeletal muscle sodium channelopathies

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**Skeletal Muscle Sodium Channelopathies**

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**Purpose of review**

This is an update on skeletal muscle sodium channelopathies since knowledge in the field have dramatically increased the last years.

**Recent finding**

The relationship between two phenotypes and *SCN4A* have been confirmed with additional cases that remain extremely rare: 1) severe neonatal episodic laryngospasm mimicking encephalopathy, which should be actively searched for since patients respond well to sodium channel blockers; 2) congenital myasthenic syndromes, which have the particularity to be the first recessive Nav1.4 channelopathy. Deep DNA sequencing suggests the contribution of other ion channels in the clinical expressivity of sodium channelopathies, which may be one of the factors modulating the latter. The increased knowledge of channel molecular structure, the quantity of sodium channel blockers, and the availability of preclinical models would permit a most personalized choice of medication for patients suffering from these debilitating neuromuscular diseases.

**Summary**

Advances in the understanding of the molecular structure of voltage-gated sodium channels as well as availability of preclinical models would lead to improve medical care of patients suffering from skeletal muscle as well as other sodium channelopathies.

**Key Words**

- Skeletal muscle voltage-gated sodium channel Nav1.4
- Molecular structure
- New sodium channelopathies
- Hypokalaemic periodic paralysis
- Personalized medicine

## Introduction

Excitable cells are the hallmark of animals, found both in Protozoa and Metazoa, which give them the capacity of sensing and of reacting to an environmental modification. In Metazoa, excitable cells communicate with other cells by a unique mechanism common to perception, brain information processing, and motor action (1). This latter relies on the functioning of transmembrane voltage-gated channels that regulate rapid changes in ion fluxes, resulting in transient and drastic changes in cell membrane potential, the so-called action potential (AP). APs lead to liberation of a chemical signal that binds to specific receptors in neighboring cells, or of calcium within the cell to promote cell response such as secretion (neuron) or contraction (muscle). Due to their unique role in the depolarizing phase of AP, voltage-gated sodium channels (VGSC) are the direct targets for natural toxins and synthetic drugs that modulate cell excitability, or are the site for mutations causing diseases. In humans, distinct diseases are associated with mutations of nearly all isoforms of VGSC depending upon their cellular expression: epilepsies or hemiplegic migraines for neuronal isoforms (Nav1.1, Nav1.2, Nav1.6), cardiac arrhythmias for the heart isoform (Nav1.5), and myotonia or paralyses for the skeletal muscle isoform (Nav1.4) (2). However, the picture is far more complex than initially expected with distinct disease expressivity for each isoform. In this review, we will focus on recent advances in the comprehension of skeletal muscle sodium channelopathies. They were the first examples of defective VGSC as the direct cause of human diseases despite the late general belief that defective function of such an important channel would be lethal (3-5), and are still good drivers for a better understanding of VGSCs.

## Advances in basic understanding of Nav1.4 functioning

$Na_V1.4$ is the main VGSC isoform of mature and innervated skeletal muscle fibers. This heterodimer is composed of two not covalently bound transmembrane proteins, the α pore-forming subunit encoded by *SCN4A* and a single auxiliary β subunit encoded by *SCN1B*. To summarize a complex picture, the α subunit forms the pore, and is the site for the mutations causing skeletal muscle sodium channelopathies. As all α subunits of VGSCs, it is made of four domains (DI to DIV), each constituted of six transmembrane segments (S1 to S6) with 3 possible conformations: closed, activated and inactivated (Figure 1). The channel opening

(activation) in response to cell membrane depolarization is initiated by a motion into the extracellular space of positively charged amino acid residues (mainly arginine) at each third position on the transmembrane α-helix within each S4 segment. Inactivation designs the mechanism by which the VGSC is closed after its opening despite the maintenance of cell membrane depolarization. There are two types of inactivation: rapid (few milliseconds range) and slow (hundreds of milliseconds to seconds range). Grossly, rapid and slow inactivation terminates action potential and regulates the number of channels that may open during repetitive firing, respectively. Recent reviews focusing on the molecular architecture and structure-function relationship of VGSC are available, and the readers are referred to them for a more complete overview (6, 7). The β1 auxiliary subunit modulates the membrane expression level by interacting with extracellular matrix and cytoskeleton, influences the voltage-dependence and kinetics of channel gating, and might exert independent function in cell signaling (8). We will not further discuss on β1 despite its importance, since its dysfunction has surprisingly no effect on skeletal muscle in contrast to brain, peripheral nerve, and heart (9-12).

During the last few years, crystallization studies have dramatically increased knowledge of VGSCs. Crystals were obtained from the bacterial sodium channels *NaChBac* considered as the ancestors of VGSCs (13). In contrast to VGSCs, *NaChBac* are homotetramers, and only activation and fast inactivation can be studied, slow inactivation requiring physical linkage between the four domains. The first crystal structure of *NaChBac* was captured in the activated state (14). The rotation of the S4-S5 linkers responsible for the channel opening could be visualized as well as the mechanism of ion selectivity. The latter results from an anionic high field with ion dehydration by direct interaction with glutamate side chains. Crystallization of *NaChBac* in inactivated state was most recently obtained (15, 16). A profound conformation change reshaping the ion selectivity filter was observed confirming

the theorized mechanical coupling of activation and inactivation. Negatively charged amino acids were shown to obtrude the outer mouth of the channel as expected from biophysical models of VGSC inactivation. Crystallization studies of *NaChBac* have not only increased our basic understanding of VGSCs but also opened new avenues to design specific drugs modulating transmembrane $Na^+$ fluxes.

**New *SCN4A*-associated diseases and modulators**

Skeletal muscle sodium channelopathies are known since several decades to manifest as autosomal dominant syndromes with different degrees of recurrent attacks of muscle weakness (periodic paralyses) or muscle stiffness (myotonia) depending on the mutation. All described mutations are missense and affect the gating behavior or ion current passing through Nav1.4 (17, 18). Their prevalence has been recently estimated to be equal to 0.43/100.000 (19) and 1.19/100.000 (unpublished data), in the UK and France, respectively. They display differential electroneuromyographic (ENMG) patterns at needle and compound muscle action potentials (CMAP) recording after short and long exercise (20, 21). According to variations of blood $K^+$ levels during attacks of muscle weakness, patients are classified into hypo- (HOKPP, OMIM#170400), normo- (NormoPP, OMIM#170600) or hyperkalemic (HYPP, OMIM#170500) periodic paralysis (PP). Several forms of sodium channel myotonia (SCM, OMIM#608390) are distinguished according to their intensity, fluctuating evolution, or response to acetazolamide, and may be difficult to clinically distinguish each other without ENMG and molecular characterization. In Paramyotonia Congenita (PMC, OMIM#168300), symptoms and signs are aggravated by exercise and cold, and include some degree of muscle weakness reminiscent of HYPP.

A new and severe form of SCM have emerged these last years. Severe Neonatal Episodic Laryngospasm (SNEL, OMIM#608390) is caused by *de novo SCN4A* mutations (22, 23). In

the neonatal phase, the disease is characterized by attacks of muscle stiffness affecting the upper airways. This causes cyanosis and hypoxemia and may be mistaken for an epileptic encephalopathy. Five additional cases have been described, leading to a total of 8 described patients with 2 distinct mutations, the p.Gly1306Glu accounting for all but one cases (Figure 1) (24, 25). The authors always emphasized the diagnostic difficulty because ENMG is not informative in babies, delaying efficient therapeutic intervention resulting to permanent deficiencies caused by hypoxia or even death of the patient. This rare condition should be systematically discussed as a cause of neonatal epileptic encephalopathy since sodium channel blockers (mexiletine and carbamazepine) dramatically ameliorate its course. When patients survive, they later develop a severe SCM affecting the limb muscles and easily diagnosed by ENMG. Why the upper airways are the first muscle groups to be affected in SNEL, distinguishing it from the severe myotonia permanens condition — caused by *de novo* *SCN4A* mutations including the p.Gly1306Glu one — is currently unknown.

According to a key role of Nav1.4 for the genesis of the muscle AP at the neuromuscular junction (NMJ) (26), $Na_v1.4$ dysfunction was recently confirmed in congenital myasthenic syndrome (CMS, OMIM#614198) — a heterogeneous group of inherited rare diseases resulting from functional or structural changes at the NMJ (27, 28) — twelve years after the first report (29, 30). In the latter, only one (p. Val1442Glu) of the two compound *SCN4A* missense substitutions severely impaired $Na_v1.4$ gating, suggesting that the fatigable muscle weakness characteristic of CMS was dominantly inherited in the family (29). In the second patient, the phenotype resulting from one *SCN4A* missense mutation was for the first time recessively inherited (30). The inherited mutation was homozygous in the patient and substituted a charged amino acid residue of DIVS4 (p.Arg1457His). The two CMS-causing mutations dramatically favored the inactivation state, resulting in reduced availability of the mutant channels for opening and so a loss-of-function (Figure 2). This suggests that

p.Val1442Glu would exert its dominant effect through haploinsufficiency, the amount of wild-type channels being not able to compensate for the inactivation of mutant channels. Decremental muscle responses to repetitive nerve stimulation were recorded at ENMG investigations as expected for CMS but at nerve stimulation frequencies higher than usual in both patients (>10 Hz instead of 3 Hz). Why these two mutations cause a phenotype almost similar to CMS (i.e. blockade of neuromuscular transmission) rather than periodic paralysis (i.e. failure to generate/propagate muscle AP along sarcolemma), remains to be determined.

Patients suffering from PP may develop permanent muscle weakness related to late-onset permanent myopathy. In a recent survey, the percent of patients over 41 years with permanent muscle weakness was estimated to be equal to 68%, and with muscle fatigue to 89% (31). In addition, 89% of the surveyed patients reported muscle pain. The mechanisms at the origin of these symptoms are still unknown. Muscle weakness is probably the result of muscle changes including vacuolar myopathy and T-tubular aggregates. These myopathic features are direct consequences of the causing mutation since similar histopathological changes have been observed in all knock-in mouse models of PP developed so far (32-35).

The phenotype of each skeletal muscle sodium channelopathy is modulated by factors that may be genetics, epigenetics, and environmental, independently from their individual fluctuation with time. As physiologically expected, one genetic factor is the second *SCN4A* allele, the homozygosity of a dominant *SCN4A* mutation leading to a more severe phenotype (36). Another expected and recently documented genetics factor is the *ClCN1* gene, which encodes the skeletal muscle chloride channel ClC-1 and is mutated in Myotonia Congenita, the most frequent form of non-dystrophic myotonia (NDM) (37). *SCN4A* mutations may also modulate other neuromuscular phenotypes. Myotonic Dystrophy type 2 (DM2, OMIM#602668) is an adult onset muscle disease caused by a tetranucleotidic (CCTG) repeat expansion in the *CNBP* gene, combining muscle dystrophy and a mild myotonia often

noticeable only by ENMG. DM2 are not classified as a primary channelopathy since the multisystemic phenotype results from defective posttranslational processing of many transcripts including *ClCN1* one (38). Nevertheless, a combination of DM2 repeat expansion and a *SCN4A* missense mutation (p.Pro72Leu) located within the N-terminus, results in earlier and most severe myotonia in DM2 (39). This missense variation modestly shifted the voltage-dependence of activation toward hyperpolarization (-5 mV), which may favor membrane hyperexcitability.

**Advances into pathophysiology understanding of skeletal muscle sodium channelopathies**

The major advances made the last years in the field of skeletal muscle channelopathies concern HOKPP. HOKPP are typically associated with mutations of charged amino acid residues of the voltage sensor domain (VSD) — formed by the S4 segments — in the calcium Cav1.1 (60% of the cases) and Nav1.4 (20%) channels (Figure 1). The remaining 20% of cases, mostly isolated patients, remain not explained at the molecular level. The pathophysiology of HOKPP, especially the paradoxical sarcolemma depolarization in low extracellular $K^+$ concentration, had been remained mysterious until the demonstration of a potentiated gating pore current, also known as omega pore, at hyperpolarized potentials in all Nav1.4, then in Cav1.1, HOKPP mutant channels (34, 40-44). To note that aberrant gating pore currents resulting from VSD mutations in Nav1.5 (DIS4) have recently been demonstrated in patients with mixed arrhythmia and dilated cardiomyopathy, indicating that this defective molecular mechanism is not restricted to skeletal muscle sodium channelopathies (45).

Substitution of individual VSD gating charge allows inward cation current to flow through the gating pore at membrane potentials depending upon the position of the substituted amino acid.

In *NaChBac*, mutating the first or second charged residue resulted in gating pore current at hyperpolarized membrane potentials, where the homotetrameric channels are in resting states, but not at depolarized potentials (46). Conversely, mutating the third charged residue resulted in closed gating pore at hyperpolarized membrane potentials and opened gating pore with the α□pore activation. All S4 segments are also not equal regarding their potential to modulate gating pore current. Whereas substituting the charged residues of DI-DIIIS4 improved the gating pore currents at hyperpolarization, this had no effect when DIVS4 residues were replaced (47). A critical component of the proposed pathophysiological mechanism to account for paradoxical depolarization is the other ion conductances active at the muscle resting potential, especially $K^+$ and $Cl^-$ (18, 43). Accordingly, inhibition of the Na-K-2Cl (NKCC) cotransporter was shown to prevent muscle weakness in the two available mouse models of HOKPP (48, 49).

Additional mechanisms have been proposed as contributing to the reduced fiber excitability in HOKPP. A shift of voltage-dependence of inactivation toward hyperpolarization and prolonged recovery were previously reported (50, 51). The slower rate of recovery was recently confirmed (52), whereas decoupling between gating charge displacement and peak sodium current resulting in reduced $Na^+$ current density was reported (53). These mechanisms exert a loss-of-function effect contrasting with the gain-of-function effect of the gating pore current, and may contribute to the muscle hypoexcitability in HOKPP (Figure 2).

Finally, muscle hypoexcitability in PP may lead to secondary alterations that may themselves participate to the phenotype: careful investigation of a new mouse model with a single missense *SCN4A* mutation (p.Ile588Val) leading to myotonia and HYPP in humans and mice revealed general metabolic alterations that could result from abnormal AMPK activation (35).

**Pharmacology and treatment of skeletal muscle sodium channelopathies**

Binding of toxins or blockade of VGSC parts have historically played an important role in deciphering structure-function relationships, until more direct observation became possible through crystallization. It is noteworthy that most chemical compounds or toxins interact directly with the α pore. Mexiletine, the only treatment validated by a phase II randomized controlled study for NDM (54), globally acts by blocking Nav1.4 in its open state. Tarentula and sea anemone toxins modify the gating pore current by complex interactions with the VSD (55). Such fine knowledge of molecular structure is of importance since allosteric modifiers of Nav1.4 might be of safer use for therapy. The likelihood of repositioning known VGSC antagonists, the number of patented VGSC blockers and the possibility to chemically optimize them are promising approaches to improve drug-based treatments for NDM (56-59). Developing new panels of drugs toward personalized medicine will be facilitated by the existence of preclinical models of NDM that could be used to test any new candidate drug (60, 61). The sole limitation is the limited number of patients, which explains why funding of therapeutic trials have mainly relied on academia or charities even if promising molecules made their way from proofs of concept to preclinical models.

Clinical reasoning with some parts of chance led to the prophylactic use of carbonic anhydrase inhibitors (acetazolamide, dichlorphenamide) in PP since forty years without any pathophysiological evidence. This treatment has been shown only recently to be efficient in reducing the number of muscle weakness attacks in HOKPP with a much higher efficiency for Cav1.1 (≈56% of responders) than for Nav1.4 (<16%) related cases (62, 63). There were remaining questions on the safety of carbonic anhydrase inhibitors use in children, acidosis potentially interfering with bone development. A recent long-term follow-up of 3 children treated by acetazolamide during their first decade was recently reported with no side effect (64). Nevertheless, the reduced number of patients precludes from using this treatment without any precaution in children. Two drug-based therapeutic strategies for HOKPP now

emerge with pathophysiological knowledge: i) the use of NKCC transporter inhibitor bumetanide —a drug approved for treatment of several conditions in humans found to be efficient on muscle weakness in the two mouse models of HOKPP — to prevent the paradoxical depolarization resulting from the aberrant gating pore current, and ii) to directly block this aberrant current using guanidinium analogues (48, 49, 65). For all, it remains to determine whether they prevent the permanent muscle deficit, a therapeutic need also expressed for HYPP with no solution currently proposed.

## Conclusion

Skeletal muscle sodium channelopathies are model diseases that enabled to understand how mutations of the key-player in cell excitability modulate its functioning. Their essential features are the symptom fluctuation and their sensitivity to triggers such as exercise and hormones. These characteristics are still not well understood and deserve further studies. The more skeletal muscle sodium channelopathies — which affect a function easily investigated in animals and humans — are carefully studied, the more it may benefit to other, often more frequent, sodium channelopathies. Finally, skeletal muscle channelopathies should move to personalized medicine now that molecular characterization and preclinical models of individual mutation are available.

## Key points

- Crystallography of bacterial voltage-gated sodium channel has increased the knowledge of mammalian sodium channel functioning.
- New phenotypes related to muscle sodium channelopathies have done recognized such as Severe Neonatal Episodic Laryngospasm (SNEL) and recessive forms of congenital myasthenia.

- New therapeutic targets have emerged from the studies of mouse models of periodic paralysis.
- Personalized treatments for muscle sodium channelopathies could emerge from screening of pharmacological compound libraries.

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**Conflicts of Interest**

None.

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**Legends of the Figures**

**Figure 1**

Schematic representation of the transmembrane α pore-forming subunit of Nav1.4 with its four domains (DI-DIV), each composed of 6 transmembrane segments (numbered S1 to S6 from the amino to carboxy terminal ends). The disease-causing mutations listed in the text and those located within the voltage-sensor domain are indicated with their related diseases. The positively charged amino acid residues (from 4 in DIS4 to 7 in DIVS4) in each S4 voltage-sensor segment, and the related skeletal muscle sodium channelopathies when they are mutated, are indicated in distinct colors and forms as indicated. NDM: non dystrophic myotonia; HOKPP: hypokalemic periodic paralysis; PMC: paramyotonia congenita, HYPP: hyperkalemic periodic paralysis, normoPP: normokalemic periodic paralysis; CMS: congenital myasthenic syndromes.

**Figure 2**

Spectrum of skeletal muscle sodium channelopathies ranging from muscle stiffness (hyperexcitability of myofiber, SCM and PMC) to muscle weakness (hypoexcitability of myofiber, HYPP, HOKPP and CMS) and proposed molecular mechanisms accounting for their clinical diversity. Some form of weakness similar to HYPP may be observed in PMC, represented by dashed lines. All but CMS are caused by dominant missense mutations in the SCN4A gene, encoding the α pore subunit of Nav1.4. Gain-of-function mutations enhancing activation with (PMC) or without (SCM) persistent current would be the cause of non-dystrophic myotonia. Periodic paralyses would also result from gain-of-function mutations (impaired inactivation for HYPP and gating pore leak for HOKPP) that eventually mimic dominant-negative mutations since they would modify the function of the wild-type allele product. Loss-of-function (enhanced inactivation) mutations with haploinsufficiency would

occur in HOKPP as in the dominant form of CMS linked to Nav1.4, whereas recessive loss-of-function mutations would result in recessive CMS (star). SCM: sodium channel myotonia, PMC: paramyotonia congenita, HYPP: hyperkalemic periodic paralysis, HOKPP: hypokalemic periodic paralysis, CMS: congenital myasthenic syndromes.

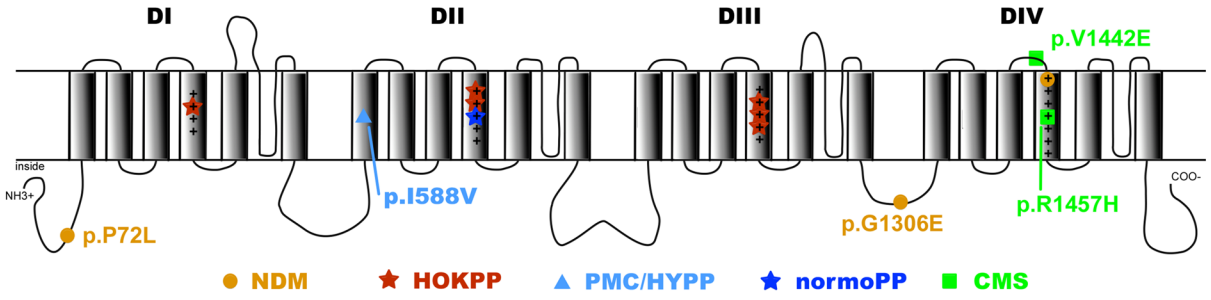
DI
DII
DIII
DIV
p.V1442E
inside
NH3+
COO-
p.I588V
p.P72L
p.G1306E
p.R1457H
NDM
HOKPP
PMC/HYPP
normoPP
CMS

Figure 1

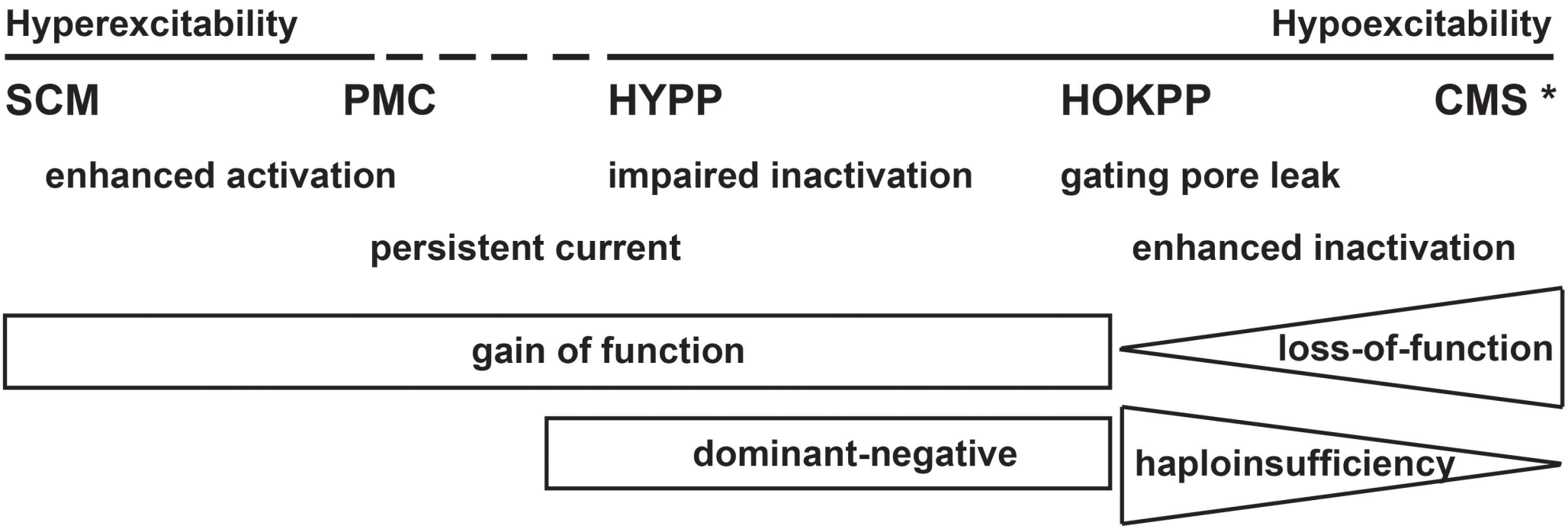
Hyperexcitability
Hypoexcitability
SCM
PMC
HYPP
HOKPP
CMS *
enhanced activation
impaired inactivation
gating pore leak
persistent current
enhanced inactivation
gain of function
loss-of-function
dominant-negative
haploinsufficiency

Figure 2