Exon 11 skipping of SCN10A coding for voltage-gated sodium channels in dorsal root ganglia

Jana Schirmeyer¹, Karol Szafinski², Enrico Leipold¹, Christian Mawrin³, Matthias Platzer², and Stefan H Heinemann¹,*

¹Department of Biophysics; Center for Molecular Biomedicine; Friedrich Schiller University Jena and Jena University Hospital; Jena, Germany; ²Genome Analysis; Leibniz Institute for Age Research; Fritz Lipmann Institute; Jena, Germany; ³Department of Neuropathology; University of Magdeburg; Magdeburg, Germany

Keywords: sodium channel, patch clamp, exon skipping, protein kinase A, pain

The voltage-gated sodium channel NaV1.8 (encoded by SCN10A) is predominantly expressed in dorsal root ganglia (DRG) and plays a critical role in pain perception. We analyzed SCN10A transcripts isolated from human DRGs using deep sequencing and found a novel splice variant lacking exon 11, which codes for 98 amino acids of the domain I/II linker. Quantitative PCR analysis revealed an abundance of this variant of up to 5–10% in human, while no such variants were detected in mouse or rat. Since no obvious functional differences between channels with and without the exon-11 sequence were detected, it is suggested that SCN10A exon 11 skipping in humans is a tolerated event.

Introduction

Voltage-gated sodium (Na⁺) channels are important constituents in the membranes of excitable cells because they mediate the fast rising phase of action potentials and thus enable cellular excitation. The family of mammalian Na⁺ channels comprises nine large α-subunit isoforms (NaV1.1–1.9) with different functional characteristics and four β-subunits (β1–4), which associate with an α-subunit and modulate its functions (for review see ref. 1). The α-subunit of NaV1.8 channels has a pseudotetrameric structure; a central pore is surrounded by four homologous domains that are linked by large cytoplasmic loops (Fig. 1A). Together with the N- and C-termini, these loops are involved in the regulation of channel functions by means of posttranslational modification and interaction with various intracellular signaling molecules.²

NaV1.8 is primarily expressed in afferent neurons of dorsal root ganglia (DRG);³,⁴ it is involved in pain signaling⁵,⁶ and its activity is highly regulated. For example, NaV1.8 interacts with the rat NaV1.8 channel and masks a putative ER retention motif (sequence RRR) in the domain I/II linker, thus leading to enhanced surface expression of NaV1.8.⁷ Furthermore, phosphorylation of rat NaV1.8 by p38 mitogen-activated protein kinase (MAPK) at two serine residues located in the exon 11-encoded fragment of the domain I/II linker (Fig. 1B, S551 and S556) leads to enhanced current in DRG neurons.⁸ Moreover, rat NaV1.8 has been shown to be regulated by protein kinase A (PKA) phosphorylation at five consensus sites located in the domain I/II linker.⁹ Stimulation of PKA by the adenylyl cyclase activator forskolin (FSK) resulted in an increase in NaV1.8 peak current.⁹ However, these functional regulations were investigated using rat NaV1.8; a sequence alignment with the human protein reveals fewer putative phosphorylation sites and the sequence KRR instead of the putative ER retention signal sequence RRR (Fig. 1B).

Alternative mRNA splicing is a cellular mechanism utilized to adjust channel functions as was abundantly shown for NaV1.3,¹⁰ NaV1.5¹¹,¹² and NaV1.7.¹³ Much less is known about the alternative splicing of SCN10A, the gene encoding NaV1.8. The only reported regulation of SCN10A on the level of mRNA is the alternative usage of NAGNAG tandem acceptors at the end of intron 16 which results in the inclusion or exclusion of glutamine at position 1030 in the domain II/III linker.¹⁴,¹⁵

In this study we describe a splice variant unique to human SCN10A mRNA resulting from skipping of exon 11.

Results and Discussion

Roche 454 sequencing of cDNA obtained from human DRG neurons revealed a novel splice variant of SCN10A, explained by skipping of exon 11, which has a length of 294 nt. As a result, there exist two isoforms of the human NaV1.8 protein: the shortened NaV1.8Δe11 and the canonical NaV1.8. The abundance of the Δe11 mRNA isoform was 31% (206 of 669 reads and 233 of 741 reads, for two sequencing directions) in the 454 sequence data. The occurrence of the Δe11 isoform was independently analyzed in DRGs of three additional human individuals (Fig. 1C), and the fraction of Δe11 mRNA was approximately 5 to 10% in these samples. High-precision quantitative measurements using PCR-based techniques were precluded by the relatively large length difference of the isoforms, which predict an isoform-specific bias in PCR efficiency.¹⁶ For the same reason, our PCR-based
The exon 11-encoded sequence includes three putative PKα consensus sites (rXXS) for human naV1.8 and four in rat and mouse (gray shaded serines). Furthermore, there is a putative p38 site (broken boxes, consensus SP) and an ER retention motif (box, RRR) in the rat and mouse sequence that is not conserved in human.

Figure 1. Skipping of exon 11 in SCN10A. (A) Topological model of human NaV1.8 highlighting the position of amino acid residues encoded by exon 11 (e11, gray) in the cytosolic linker connecting domains I and II. (B) Multiple sequence alignment of the region coding for exon 11 of SCN10A for human, rat, and mouse. The exon 11-encoded sequence includes three putative PKα consensus sites (RXXS) for human NaV1.8 and four in rat and mouse (gray shaded serines). Furthermore, there is a putative p38 site (broken boxes, consensus SP) and an ER retention motif (box, RRR) in the rat and mouse sequence that is not conserved in human. (C) Evidence of the existence of the Δe11 isoform in three human individuals (lane 2–4), but not in rat (lane 5) or mouse (lane 6). Positive controls were performed on the expression constructs used in this study and show hNaV1.8 (lane 7), hNaV1.8-Δe11 (lane 8), a 9:1 mixture of hNaV1.8 and hNaV1.8-Δe11 (lane 9), mNaV1.8 (lane 10), and rNaV1.8 (lane 10). Reference bands of a size marker are indicated in lanes 1 and 12. +e11: full-length isoform (note, the species-specific PCR amplicons are very similar in size: 493, 501 and 493 bp for the three species, respectively); Δe11: short isoform with skipped exon 11; asterisk: unspecific PCR product (lane 3), as revealed by cloning and Sanger sequencing.

Figure 2. Parameters characterizing the voltage dependence of channel activation (Fig. 2B) were almost identical for the two channel types with $V_{1/2}$ values of $-16.2 \pm 1.4$ mV and $-15.3 \pm 1.3$ mV and $k$ values of $18.4 \pm 0.5$ mV and $19.0 \pm 0.3$ mV for hNaV1.8 (n = 30) and hNaV1.8-Δe11 (n = 23), respectively.

Steady-state inactivation (Fig. 2C) for both channel isoforms showed that $V_{1/2}$ was $-76.1 \pm 1.3$ mV and $-77.0 \pm 1.8$ mV and $k_1$ was $8.0 \pm 0.3$ mV and $7.7 \pm 0.3$ mV for hNaV1.8 (n = 27) and hNaV1.8-Δe11 (n = 16), respectively.

Recovery from fast inactivation (Fig. 2D) occurred with time constants of $\tau_{fast} = 3.5 \pm 0.5$ ms and $4.2 \pm 0.5$ ms, $\tau_{slow} = 23.8 \pm 3.0$ ms and $25.9 \pm 3.6$ ms for hNaV1.8 (n = 15) and hNaV1.8-Δe11 (n = 15), respectively. The fraction of the fast recovering current amplitude was $0.49 \pm 0.05$ for hNaV1.8 and $0.50 \pm 0.05$ for hNaV1.8-Δe11 (all $P > 0.2$). Thus, voltage dependence of fast inactivation and recovery from fast inactivation for hNaV1.8 did not differ from hNaV1.8-Δe11 (Fig. 2).

Time courses of channel activation and inactivation were determined applying a Hodgkin-Huxley fit function with $m = 3$ and $b = 1$ gates to current responses resulting from 40 ms test pulses to $-30$, $-10$, and $30$ mV from a holding potential of $-120$ mV. No differences between hNaV1.8 and hNaV1.8-Δe11 were observed (Fig. 2E). Ramp currents evoked by slow (200 mV/s) voltage-ramp protocols revealed no difference between NaV1.8 and NaV1.8-Δe11 in ramp current amplitude either (all $P > 0.05$, Figure 2F).

As reported previously, heterologous expression of rat NaV1.8 in non-neuronal cells is restricted because of an ER-retention...
motif (sequence RRR) in a region of the domain-I/II linker that is part of exon 11. This motif is not conserved between rodents and human since human NaV1.8 harbors a KRR motif. To check for an enhanced membrane trafficking of hNaV1.8Δe11, we transfected HEK 293 cells, which normally allow only very poor expression of NaV1.8. For both channel types only very small current densities (2.8 ± 2.3 pA/pF, n = 31 for hNaV1.8 and 4.2 ± 2.0 pA/pF, n = 37 for hNaV1.8Δe11 at 10 mV, P > 0.6) were observed; the current density values were in the range of non-transfected HEK 293 cells (5.1 ± 0.7 pA/pF, n = 5).

ND7/23 cells – a hybridoma cell line of mouse neuroblastoma and rat DRG neurons –, transfected with either NaV1.8 or NaV1.8Δe11, were assayed in the whole-cell patch-clamp mode using physiological solutions to retain activity of intracellular enzymes. Under control conditions, the two channel types did not show differences regarding parameters of voltage-dependence of channel activation. Vm was –15.9 ± 1.2 mV and km was 16.7 ± 1.0 mV (n = 7) for hNaV1.8 and –15.9 ± 1.5 mV and 16.3 ± 0.8 mV (n = 8) for hNaV1.8Δe11, respectively. As a positive control, forskolin stimulation of rat NaV1.8 was monitored according to Fitzgerald et al.9 rNaV1.8 showed a strong response to forskolin stimulation regarding an increase in the peak current amplitude by a factor of 1.48 ± 0.07 (P < 0.002) with a single-exponential time constant of 36.3 ± 4.9 s at 0 mV (n = 11). Voltage of half-maximal activation per gate was shifted to the left by about 6.8 ± 0.7 mV (n = 9; P < 0.001) compared with control. However, hNaV1.8 did not respond to forskolin application. Peak current amplitude was only insignificantly altered by a factor of 1.03 ± 0.08 (n = 7; P = 0.76). The current-voltage relationship was also not affected (n = 6; P = 0.98). hNaV1.8Δe11 behaved like the long channel isoform (Ipeak/Ictrl = 1.02 ± 0.10, n = 8, P = 0.59; IV: n = 5, P = 0.42; Figure 3).

A substantial deletion of the domain I/II linker apparently has almost no immediate functional consequences for the channel protein. This finding is compatible with an observation by Faber et al.17 who described the mutation L554P in human NaV1.8, resulting in the formation of a PPP motif within the sequence encoded by exon 11. Upon expression in mouse DRG neurons, NaV1.8-L554P channels revealed no differences compared with wild-type channels regarding their voltage dependence of activation and steady-state inactivation.17 Only recovery from inactivation seemed to be slightly faster for the mutant, and increased ramp current amplitudes were reported.17 No such effect was observed for hNaV1.8 and hNaV1.8Δe11, but the inactivation properties of hNaV1.8 measured in Neuro-2A cells are strongly different from those described for transfected DRG neurons.17
The domain I/II linker contains several putative protein interaction sites presumably contributing to the physiological regulation of NaV channels. It was shown that rat NaV1.8 possesses an ER retention/retrieval motif in the region encoded by exon 11; this motif can be masked by NaV\(\beta\)_3 to allow for membrane trafficking. Furthermore, it is commonly observed that NaV1.8 channels can be expressed in neuronal cells, while heterologous expression in HEK 293 cells is very poor. A likely explanation could be the endogenous expression of NaV\(\beta\)_1 and NaV\(\beta\)_3 in neuronal cells, such as in ND7/23, affecting the retention mechanism. However, here we did not observe any difference in functional channel expression of both isoforms, neither in neuronal nor in HEK 293 cells. Since human Na\(_{\text{a},1.8}\) channels contain a KRR motif, this might be a weaker ER retention signal or a motif that does not interact with the NaV\(\beta\)_3 subunit. Thus, the KRR retention motif can be excluded as a molecular reason for the weak expression of Na\(_{\text{a},1.8}\) channels in HEK 293 cells.

Figure 3. Stimulation of protein kinase A phosphorylation by the adenyl cyclase activator forskolin. (A) Current traces evoked by depolarization from -80 mV to 0 mV before and after extracellular application of 10 µM forskolin (FSK) for Na\(_{\text{a},1.8}\) from rat, hNa\(_{\text{a},1.8}\), and hNa\(_{\text{a},1.8}\)–Δe11 in ND7/23 cells. (B) Current-voltage relationships for the channels in (A) before and after FSK application normalized to the peak inward current and averaged for 6–8 cells ± s.e.m. (C) Time course of peak current changes upon FSK stimulation normalized to control level for Na\(_{\text{a},1.8}\) from rat, hNa\(_{\text{a},1.8}\), and hNa\(_{\text{a},1.8}\)–Δe11. Application of FSK starts at \(t = 0\) s. Data points are mean values for 7–11 cells each ± s.e.m.

Tissues, RNA extraction, and reverse transcription
Human DRG tissue was sampled during routine autopsy for neuropathological examinations in accordance with ethical regulations of the state of Saxony-Anhalt and the European Communities Council Directives, and approved by the Institutional Review Board of the Otto-von-Guericke University, Magdeburg, Germany. Samples were frozen on dry ice immediately after sampling, anonymized, and stored at –80 °C. Neuropathological examinations revealed no pathological alterations in the human DRGs analyzed. Rats and mice were fed standard chow and water ad libitum, and animal care procedures were performed according to local guidelines of animal protection, approved by the Thuringian Animal Care Council (registration number: 02–101/13). Tissues from adult C57/B6-J mice and Wistar rats, killed by cervical dislocation, were frozen on dry ice and stored at –80 °C. DRGs were pooled from one specimen. Total RNA from DRG was purified using an RNeasy Mini Kit (Qiagen) and subsequently reversed transcribed using the SuperScript™III First-Strand Synthesis System (Invitrogen).

Isform identification
An RT-PCR amplicon spanning exon 11 was included in an amplicon tiling path across human SCN10A mRNA. RT-PCR was performed using Taq polymerase (BioLine BioMix Red,
214 Channels Volume 8 issue 3

Briefly, borosilicate glass patch pipettes (Deutsche Dynal GmbH, Hamburg, Germany) were used for visual identification of individual transfected cells. Electrophysiological experiments were performed at constant temperature (19–21 °C). Data analysis was performed using FitMaster (HEKA Elektronik) and IgorPro (WaveMetrics, Lake Oswego, OR, USA) software. Data are presented as mean ± standard error of the mean (n = number of independent experiments). Data sets were tested for statistical significance using a two-sided Student’s t test with unequal variances when appropriate.

**Current-voltage relationships**

From a holding potential of −120 mV, test depolarizations in the range from −70 to 50 mV in steps of 10 mV were applied at an interval of 5 s. The peak currents were fit with a Hodgkin-Huxley activation formalism involving m = 3 activation gates and a linear single-channel characteristic:

\[
I(V) = G_{\text{max}}(V - E_{\text{rev}}) \frac{1}{\left(1 + e^{-(V-V_c)/k_w}\right)^3}
\]

where \( I \) is the peak current and \( V \) is the test pulse voltage. \( V_c \) is the voltage of half-maximal gate activation, \( k_w \) is the corresponding slope factor, \( G_{\text{max}} \) is the maximal conductance of all channels and \( E_{\text{rev}} \) the reversal potential.

**Voltage ramp recordings**

Voltage ramps from −80 to 20 mV in 500 ms were applied. Ramp currents were normalized to the maximal transient inward currents recorded in the IV and plotted against the ramp voltage.

**Inactivation kinetics**

The kinetics of activation and fast channel inactivation were analyzed using a Hodgkin-Huxley function to fit the current decay during depolarizing pulses to −30, −10, 10, and 30 mV from a holding potential of −120 mV:

\[
I(t) = I(0) h(t)
\]

\[
h(t) = h_{\inf} + (1 - h_{\inf}) \left( r_{12} e^{-t/\tau_{h1}} + (1 - r_{12}) e^{-t/\tau_{h2}} \right)
\]

where \( I \) is the current, \( \tau_{h1} \) and \( \tau_{h2} \) are the fast and slow time constants describing the current decay, \( r_{12} \) is the amplitude ratio of the fast and the slow component, and \( h_{\inf} \) is the steady-state inactivation, \( \tau_m \) is the time constant describing activation kinetics.

**Steady-state inactivation**

From a holding potential of −120 mV cells were conditioned for 500 ms at voltages ranging from −120 to −20 mV in steps of 10 mV. Subsequently, peak current was determined at 0 mV. The repetition interval was 20 s. The peak current plotted vs. the conditioning voltage was described with a Boltzmann function:
with the half-maximal inactivation voltage $V_h$ and the corresponding slope factor $k_h$.  

**Recovery from fast inactivation**

From holding a potential of -120 mV channels were inactivated with a 10-ms pulse to 0 mV. A second pulse to 0 mV, applied after a variable time interval at -120 mV, was used to assay the recovery of the channels from fast inactivation:

$$\frac{I(t)}{I_1} = a_t + a_0 e^{-\frac{V_h}{k_h}} 2 e^{-\frac{t}{\tau_{fast}}}$$  

with time $t$, the current amplitudes $I_1$ (first pulse) and $I_2$ (second pulse) and the time constants for the fast and slow components of the recovery from inactivation $\tau_{fast}$ and $\tau_{slow}$, respectively.

**Solutions and chemicals**

In most experiments the bath solution contained (in mM): 150 NaCl, 2 KCl, 1.5 CaCl$_2$, 1 MgCl$_2$, 10 HEPES (pH 7.4 with NaOH) and the patch pipettes contained (in mM): 35 NaCl, 105 CsF, 10 EGTA, 10 HEPES (pH 7.4 with CsOH). Forskolin (Sigma-Aldrich, Steinheim, Germany) experiments were performed in bath solution containing (in mM): 100 NaCl, 5 KCl, 2.5 MgCl$_2$, 40 TEA, 0.01 CdCl$_2$, 10 glucose, 2 Na$_2$HPO$_4$, 5 HEPES (pH 7.4 with NaOH); the corresponding pipette solution contained (in mM): 120 CsCl, 10 NaCl, 2.5 KCl, 10 MgCl$_2$, 0.5 Na ATP, 5 EGTA, 5 HEPES (pH 7.4 with CsOH). Forskolin was dissolved in bath solution and applied focally to the cells at a concentration of 10 µM. To completely block Na+ channels endogenous to Neuro-2A and ND7/23 cells, external solutions were supplemented with 300 nM tetrodotoxin. In addition, the holding potential was set to -80 mV in all forskolin experiments to optimize cell viability.

**References**

1. Goldin AL. Evolution of voltage-gated Na(+) channels. J Exp Biol 2002; 205:575-84; PMID:11907047
2. Malik-Hall M, Poon WY, Baker MD, Wood JN, Okuse K. Sensory neuron proteins interact with the intracellular domains of sodium channel Nav1.8. Brain Res Mol Brain Res 2003; 110:298-304; PMID:12591166; http://dx.doi.org/10.1016/S0169-328X(02)00661-5
3. Renganathan M, Cummins TR, Hormuzdiar WN, Waxman SG. TTX-resistant voltage-dependent sodium channel Nav1.8 splice variants and their regulation by protein kinase A. J Neurophysiol 2008; 99:2241-50; PMID:18039289
4. Renganathan M, Cummins TR, Waxman SG. Contribution of Nav1.8 sodium channels to post-operative, but not post-operative, pain states. Pain 2010; 150:8-16;  PMID:20028484 ; http://dx.doi.org/10.1016/j.pain.2009.09.012
5. Joshi SK, Mikusa JP, Hernandez G, Han C, Ahs HS, Person AK, Hoejmovers JG, Gerrits MM, Pierre T, et al. Gain-of-function Nav1.8 mutations in painful neuropathy. Proc Natl Acad Sci U S A 2012; 109:19444-49; PMID:23153351; http://dx.doi.org/10.1073/pnas.1216080109
6. Zimmermann K, Leffler A, Babes A, Cendan CM, Plattner M, Heinemann SH. A subtle alternative protein kinase increases current density in dorsal root ganglion neurons. J Neurosci 2008; 28:3190-201; PMID:18350422; http://dx.doi.org/10.1523/JNEUROSCI.4403-07.2008
7. Zhang ZN, Li Q, Liu C, Wang HB, Wang Q, Bao L. Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures. Nature 2007; 447:855-8; PMID:17568746; http://dx.doi.org/10.1038/nature05880
8. Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. Nature 1996; 379:257-62; PMID:8538791; http://dx.doi.org/10.1038/379257a0
9. Fitzgerald EM, Okuse K, Wood JN, Dolphin AC, Moss SJ. cAMP-dependent phosphorylation of the tetrodotoxin-resistant voltage-dependent sodium channel SNS. J Physiol 1999; 516:433-46; PMID:10087343; http://dx.doi.org/10.1111/j.1104-3859.1999.7733x
10. Thimmappaya R, Neelands T, Niforatos W, Davis-Tabor RA, Choi W, Putnam CR, Knoyer PE, Packer J, Gopalakrishnan M, Fahynek CR, et al. Distribution and functional characterization of human Nav.3 splice variants. Eur J Neurosci 2005; 22:329-41; PMID:16062990; http://dx.doi.org/10.1111/j.1460-9568.2005.04155.x
11. Makielki JC, Ye B, Valdivia CR, Pagel MD, et al. Distribution and functional characterization of human Nav1.8 splice variants. Eur J Neurosci 2005; 22:1-9; PMID:16029190; http://dx.doi.org/10.1111/j.1460-9568.2005.04155.x
12. Chatterjee A, Dahlhund L, Eriksson A, Knupp J, Chahine M. Biophysical properties of human Nav1.7 splice variants and their regulation by protein kinase A. J Neurophysiol 2008; 99:2241-50; PMID:18337362; http://dx.doi.org/10.1152/jn.01350.2007
13. Kerr NC, Holmes FE, Wyrnick D. Novel isoforms of the sodium channel Nav1.8 and Nav1.9 are produced by a conserved mechanism in mouse and rat. J Biol Chem 2004; 279:24826-33; PMID:15047701; http://dx.doi.org/10.1074/jbc.M412812200
14. Schirmeyer J, Szafranski K, Leipold E, Mawrin C, Thimmapaya R, Neelands T, Niforatos W, Kage K, Han P, et al. Distribution and functional characterization of human Nav.3 splice variants. Eur J Neurosci 2005; 22:1-9; PMID:16029190; http://dx.doi.org/10.1111/j.1460-9568.2005.04155.x
15. Makielski JC, Ye B, Valdivia CR, Pagel MD, et al. Distribution and functional characterization of human Nav1.8 splice variants. Eur J Neurosci 2005; 22:1-9; PMID:16029190; http://dx.doi.org/10.1111/j.1460-9568.2005.04155.x
16. Schindler S, Heimer M, Platzer M, Szafrański K. Comparison of methods for quantification of subtle splice variants. Electrophoresis 2009; 30:3674-81; PMID:19862747; http://dx.doi.org/10.1002/elps.200900292
17. Faber CG, Lauria G, Merkies IS, Cheng X, Han C, Ahs HS, Person AK, Hoejmovers JG, Gerrits MM, Pierre T, et al. Gain-of-function Nav1.8 mutations in painful neuropathy. Proc Natl Acad Sci U S A 2012; 109:19444-49; PMID:23153351; http://dx.doi.org/10.1073/pnas.1216080109
18. John VH, Main MJ, Powell AJ, Gladwell ZM, Hick C, Sidhu HS, Clare JJ, Tate S, Tresize DJ. Heterologous expression and functional analysis of rat Nav1.8 (SNS) voltage-gated sodium channels in the dorsal root ganglion neuroblastoma cell line ND7-23. Neuropharmacology 2004; 46:425-38; PMID:14975698; http://dx.doi.org/10.1016/j.neuropharm.2003.09.018
19. Liu C, Li Q, Su Y, Bao L. Prostaglandin E2 promotes Nav1.8 trafficking via its intracellular RRR motif through the protein kinase A pathway. Traffic 2010; 11:405-17; PMID:20028484; http://dx.doi.org/10.1111/j.1600-0854.2009.01027.x
20. Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. Nature 1996; 379:257-62; PMID:8538791; http://dx.doi.org/10.1038/379257a0
21. Chen H, Gordon D, Heimann SH. Modulation of cloned skeletal muscle sodium channels by the scorpion toxins Lqh II, Lqh III, and Lqh alpha/T. Pfuhls Arch 2000; 439:423-32; PMID:10678738; http://dx.doi.org/10.1002/0000240505995
22. Wetzl M, Platte M, Heinemann SH. Channelopathies of cloned skeletal muscle sodium channels by the scorpion toxins Lqh II, Lqh III, and Lqh alpha/T. Pfuhls Arch 2000; 439:423-32; PMID:10678738; http://dx.doi.org/10.1002/0000240505995