PNAS

Supporting Information for

Structural Basis for Severe Pain Caused by Mutations in the S4-S5 Linkers of Voltage-Gated Sodium Channel $Na_V1.7$

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This PDF file includes:

Figure S1
Tables S1 to S3
Legends for Movies S1 to S3
SI References

Other supporting materials for this manuscript include the following:

Movies S1 to S3

Supporting Figure

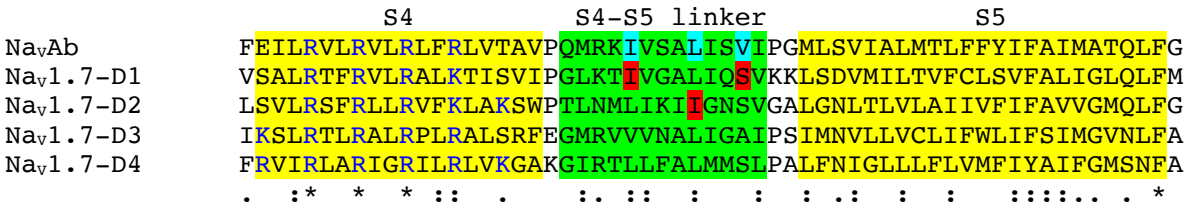

Figure S1. Multiple Amino Acid Sequence Alignments of Na_VAb and human $Na_V1.7$. Amino acid sequences in the region from S4 to S5 segments are shown.

Supporting Tables

Table S1. IEM Mutations

Domain	Location	Mutation ($Na_V1.7$)	Construct (Na_VAb)	Origin	Phenotype[a]	References
I	S4-S5	I234T	I199T		IEM	Ahn *et al.*, 2010
I	S4-S5	S241T	V126T	Flemish	Familial IEM[b]	Michiels et al., 2005
II	S4-S5	I848T	L123T	Chinese English	Familial IEM[b]	Drenth et al., 2008 Wu *et al.*, 2013 Yang et al., 2004

Reference List: (1); (2); (3); (4); (5).
[a]Familial IEM indicates at least two affected family members are known.
[b]Interestingly, the same mutation has been found in $Na_V1.4$ and $Na_V1.5$ (corresponding to residue I141V). These mutations lead to myotonia and exercise-induced polymorphic ventricular arrhythmia, respectively, caused by a negative shift in $V_{1/2}$ (6-9).

Table S2. Activation properties of Na_VAb and $Na_V1.7$ with IEM mutations

Mutation	Na_VAb Δ28			$Na_V1.7$			Reference
	$V_{1/2}$ (mV)	Δ $V_{1/2}$ (mV)	Slope (k) (mV)	$V_{1/2}$ (mV)	Δ $V_{1/2}$ (mV)	Slope (k) (mV)	
WT	-90 ± 1.5	-	9	-	-	-	Gamal El-Din et al., 2019[b]
I119T (I234T)[a]	-127.6 ± 1.5	-37.5	5.6	-43.1 ± 1.0	-17.9	7.5 ± 0.1	Ahn et al., 2010[c]
L123T (I848T)[a]	-105.5 ± 0.5	-15.5	6.6	-38.4 ± 1.0 -29.60 ± 0.50 -28.06 ± 5.42	-13.8 -7.5 -9.8	- 5.92 ± 0.43 8.99 ± 1.48	Cummins et al., 2004[c] Wu et al., 2013[c] Kerth et al., 2021[c]
V126T (S241T)[a]	-102 ± 0.4	-12	5.6	-34.0 ± 1.1	-8.4	6.52 ± 0.26	Lampert et al., 2006[c]

[a]Residue name and number for $Na_V1.7$
[b]Reference for $Na_VAb\Delta28$
[c]Reference for human $Na_V1.7$

Table S3. X-ray data collection and refinement statistics of $Na_VAb\Delta28$ structures with $Na_V1.7$ IEM mutations in the S4-S5 linker

$Na_VAb\Delta28$ with $Na_V1.7$ IEM mutations	**I119T (I234T)[a]** PDB: 8DIZ	**L123T (I848T)[a]** PDB: 8DJ0	**V126T (S241T)[a]** PDB: 8DJ1
Space group	I422	I422	I422
Cell dimensions (Å)	a = b = 124.8	a = b = 125.0	a = b = 123.4
	c = 192.4	c = 190.8	c = 191.1
	α =β=γ= 90°	α=β=γ= 90°	α=β=γ= 90°
Wavelength (Å)	1.00	1.00	1.00
Resolution (Å)[b]	50 – 2.75	50 – 2.70	50 – 3.10
	(2.80 – 2.75)	(2.75 – 2.70)	(3.15 – 3.10)
Number of reflections	20,054 (929)	20,400 (746)	13,526 (659)
Completeness (%)	99.4 (93.0)	96.5 (71.4)	98.6 (96.3)
Multiplicity	12.7 (4.6)	12.0 (4.1)	6.0 (5.4)
I/σI	16.5 (0.6)	14.0 (0.4)	12.8 (0.9)
CC½	0.997 (0.405)	1.0 (0.690)	0.994 (0.479)
R_{merge}	0.156 (1.318)[c]	0.148 (1.018)[c]	0.133 (1.917)[c]
R_{pim}	0.044 (0.592)	0.042 (0.429)	0.059 (0.891)
Refinement			
Resolution (Å)	50 – 2.75	50 – 2.70	50 – 3.10
No. reflections (work/free)	15,701/849	14,422/761	10,898/561
R_{work} / R_{free}	0.225/0.256	0.207/0.248	0.214/0.260
Number of atoms	2,013	2,207	2,158
B-factor	45.0	45.2	59.3
R.m.s. deviations			
Bond length (Å)	0.008	0.007	0.003
Bond angle (°)	1.055	0.925	0.554
MolProbity			
Overall score	2.4	2.3	1.5
Clashscore	7.5	7.7	5.4
Ramachandran favored (%)	92.8	96.6	96.6
Ramachandran outlier (%)	0.0	0.0	0.0
Rotamer outliers	5.5	8.3	0.0

[a]Residue name and number for $Na_V1.7$
[b]Values in parenthesis are for the highest resolution shell.
[c]Due to anisotropic diffraction

Supporting Movies

Movie S1. Transition of Na_VAb/I119T from the resting state to the activated state from the intracellular view. I119T and Val126 are shown as red and orange sticks, respectively. Ser132' and Asn211' from a neighboring subunit are shown as yellow and cyan sticks, respectively. S4 is colored in magenta, the S4-S5 linker in orange, and the pore module S5 to S6 in cyan. Dashes indicate distance in Å.

Movie S2. Transition of Na_VAb/L123T from the resting state to the activated state from the intracellular view. L123T, and Asn211' from a neighboring subunit are shown as red and cyan sticks, respectively. Dashes indicate distance in Å.

Movie S3. Transition of Na_VAb/V126T from the resting state to the activated state from the intracellular view. V126T, Ile216, and Asn211' from a neighboring subunit are shown as red, teal, and cyan sticks, respectively. Dashes indicate distance in Å.

References for Supporting Information

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