doi: 10.4149/gpb_2015014

Commentary

The first disease connection for $Ca_v2.2$ channels

Norbert Weiss

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

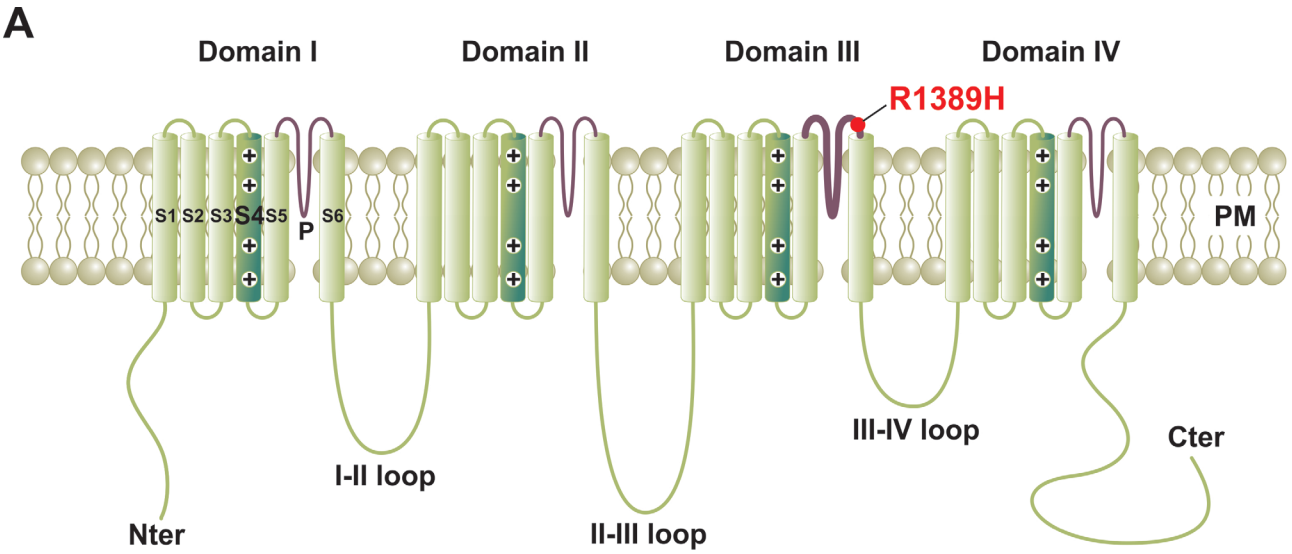
Commentary to: CACNA1B mutation is linked to unique myoclonus-dystonia syndrome. (Hum. Mol. Genet. 2015, pp. 987–993).

Key words: Calcium channel — $Ca_v2.2$ channel — Channelopathies — Myoclonus-dystonia syndrome

Fast neurotransmitter release in the central and peripheral nervous system relies on the tight control of Ca^{2+} influx into presynaptic nerve endings and the synchronized exocytosis of neurotransmitter vesicles. Neuronal voltage-gated Ca^{2+} channels (VGCCs), especially $Ca_v2.1$ and $Ca_v2.2$ channels and most likely Ca_v3 T-type channels (Weiss et al. 2012a, 2012b; Weiss and Zamponi 2013a), by virtue of their biochemical coupling with the vesicle-docking/release machinery, are perfectly suited to support depolarization-evoked neurotransmitter release, where they support a transient Ca^{2+} microdomain of relatively high concentration (10 to 50 μM) within the active zone of the synapse and essential to trigger the fusion of presynaptic vesicles with the plasma membrane (Weiss and Zamponi 2012, 2013b). In turn, numerous neurotransmitters released into the synaptic cleft activate G-protein coupled receptors to produce a potent negative feedback over the Ca^{2+} channel, essential to fine-tune synaptic activity and neuronal communication (Proft and Weiss 2015). Because of this essential contribution to synaptic activity, alteration in channel gating by modulatory proteins (Mallmann et al. 2013) or channel mutations is likely to alter neuronal function that eventually leads to a diseased state. Hence, a number of congenital mutations in the *CACNA1A* gene encoding the $Ca_v2.1$ channel are linked to several neurologic disorders including familial hemiplegic migraine type-1 (FHM-1), episodic ataxia type-2 (EA-2) and spinocerebellar ataxia type-6 (SCA-6) (Weiss et al. 2007; Pietrobon 2010). Those mutations usually affect structural determinants that are essential for proper channel gating including the voltage sensor, the inactivation gate and the pore selectivity filter. Surprisingly, mutations in the closely related $Ca_v2.2$ channel have never been linked to any human congenital disorders. In a recent issue of journal Human Molecular Genetics, Groen and colleagues (Groen et al. 2015) report for the first time a congenital mutation in the $Ca_v2.2$ channel in a family with a myoclonus-dystonia-like syndrome.

Myoclonus-dystonia (M-D) is a movement disorder that typically affects the upper half of the body. Individuals experience quick, involuntary muscle jerking or twitching (myoclonus) and more than a half of affected individuals also develop dystonia. In some cases, M-D is also accompanied by psychological symptoms such as obsessive-compulsive disorders, anxiety and depression. Although sporadic cases of M-D have been reported, many are inherited dominant and result from mutations in the *SGCE* gene encoding the epsilon-sarcoglycan. However, because sporadic cases of M-D have similar symptoms to familial cases, it is possible that even though the mutation is not present in *SGCE*, many of these will also have a genetic cause that has not yet been identified. Using exome sequencing and linkage analysis in a three-generation family with M-D-like syndrome, Groen and colleagues identified a missense mutation in the *CACNA1B* gene and resulting in the substitution of an arginine residue at position 1389 to a histidine (R1389H) in the $Ca_v2.2$ channel. This mutation is located in the pore-forming loop linking segments S5 and S6 of the third transmembrane domain of the channel, a region essential for calcium conductivity. In addition, sequence analysis of this region indicates that arginine 1389 in the $Ca_v2.2$ channel is highly conserved between the other six closely related high-voltage-gated Ca^{2+} channels suggesting an essential role of this residue in channel function (Figure 1). Using electrophysiological recording from tsA-201 cells

Correspondence to: Norbert Weiss, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo nám. 2., 166 10 Prague-Dejvice, Czech Republic
E-mail: weiss@uochb.cas.cz



B

```
Cav2.2   --KGKFFYCTDESKELERDCRGQYLDYEKEE---VEAQPRQWKKYDFHYDNVLWALLTLFTVSTGEGWPMVLKHSVDATYEEQGPSPGYRME
Cav2.1   LFKGKFFHCTDESKEFEKDCRGKYLLYEKNE---VKARDREWKKYEFHYDNVLWALLTLFTVSTGEGWPQVLKHSVDATFENQGPSPGYR--
Cav2.3   --KGKFFYCTDSSKDTEKECIGNYVDHEKNK---MEVKGREWKRHEFHYDNIIWALLTLFTVSTGEGWPQVLQHSVDVTEEDRGPSRSNRME

Cav1.1   --KGKFFRCTDLSKMTEEECRGYYYVYKDGDPMQIELRHREWVHSDFHFDNVLSAMMSLFTVSTFEGWPQLLYKAIDSNAEDVGPIYNNRVE
Cav1.2   --KGKLYTCSDSSKQTEAECKGNYITYKDGEVDHPIIQPRSWENSKFDFDNVLAAMMALFTVSTFEGWPELLYRSIDSHTEDKGPIYNYRVE
Cav1.3   --KGKFYRCTDEAKSNPEECRGLFILYKDGDVDSPVVRERIWQNSDFNFDNVLSAMMALFTVSTFEGWPALLYKAIDSNGENIGPIYNHRVE
Cav1.4   --KGKFYTCTDEAKHTPQECKGSFLVYPDGDVSRPLVRERLWVNSDFNFDNVLSAMMALFTVSTFEGWPALLYKAIDAYAEDHGPIYNYRVE
           ***:: *:* :*    :* * :  : . .      : * * . .*.:**:: *:::****** **** :* :::*   *: **  . *
```

Figure 1. A. Schematic membrane topology of the Ca_v-pore forming subunit of voltage-gated Ca^{2+} including $Ca_v2.2$ channel. It consists of four repeats (domain I to domain IV) each composed of six transmembrane segments (S1 to S6). The S4 segment of each of the four domains, rich in positively charged residues, presumably contribute to the voltage-sensitivity of the channel while the P loops between segments S5 and S6 form the pore selectivity filter. The location of the M-D mutation R1389H in the third P-loop is presented (red dot). Ancillary subunits are not represented (Geisler et al. 2014). **B.** Protein sequence alignment of the third P-loop of $Ca_v2.2$ channel with the corresponding sequence of the closely related high-voltage-gated Ca^{2+} channels including $Ca_v2.1$, $Ca_v2.3$ and $Ca_v1.x$ channels is presented. The arginine residue at position 1389 in the $Ca_v2.2$ channel is highly conserved between the channel isoforms, suggesting an essential role in channel function. PM, plasma membrane.

expressing the $Ca_v2.2$ channel carrying the M-D R1389H mutation ($Ca_v2.2^{R1389H}$), Groen and colleagues found a larger whole-cell voltage-activated Ca^{2+} current in cells expressing the $Ca_v2.2^{R1389H}$ mutated channel compared to cells expressing the wild-type channel. In contrast, the other whole-cell parameters including voltage-dependence of activation and inactivation and ion selectivity were found unaltered. At the single protein level, these authors also found a decreased single channel conductance of $Ca_v2.2^{R1389H}$ channels, likely due to the unique low conductance open state observed with mutated channels compared to wild-type channels which present an additional open state of higher conductance. Decreased single channel conductance in $Ca_v2.2^{R1389H}$ channels is apparently inconsistent with an increased whole-cell current. Although an increase in the surface expression of the mutated channel could contribute to the increased whole-cell current, this possibility remains unlikely considering the location of the mutation in a region that is not expected to affect the trafficking of the channel. In contrast, and as proposed by the authors, an increase of the opening duration of single channels is likely to cause the increased whole-cell current observed in cells expressing the M-D mutant channel.

The novel and important findings of Groen and Colleagues report a unique inherited mutation in the *CACNA1A* gene and providing the first disease connection for $Ca_v2.2$ channels. Alteration in channel gating produced by the M-D mutation is consistent with the location of this mutation in the pore-forming region of the channel, essential for ion conduction. Regardless the alteration on single channel gating, the observation that mutated channels produce an increased Ca^{2+} influx is likely to affect synaptic activity and

the release of neurotransmitter. In addition, considering that G-protein-dependent inhibition of VGCCs is affected by channels openings (Patil et al. 1996; Weiss et al. 2006), increased of the opening duration of channels carrying the M-D mutation could potentially reduce G-protein-mediated inhibitory pathway, thereby contributing to sustained presynaptic Ca^{2+} entry and neurotransmitter release. Finally, these findings also raise interesting questions about the possible contribution of $Ca_v2.2$ channels in diseased states in general. For instance, elevated level of $Ca_v2.2$ antibody has been reported in individuals with autoimmune autonomic ganglionopathy (Kimpinski et al. 2009), and more recently with autoimmune encephalopathis (Finkel and Koh 2013; Kamiya-Matsuoka et al. 2014). Although the causal link has not been yet established, it is likely that antibodies direct to $Ca_v2.2$ channels could also alter the Ca^{2+} permeability and neuronal function of the channel. There is no doubt that further studies will provide important insights into the contribution of $Ca_v2.2$ channels to diseased states and possible therapeutic avenues.

Overall, the findings of Groen and colleagues provide novel insights into the genetic of M-D and established $Ca_v2.2$ channels as an integral component of channelopathies.

Acknowledgment. Work in the Weiss laboratory is supported by the Czech Science Foundation (15-13556S), the Czech Ministry of Education Youth and Sports (7AMB15FR015), and the Institute of Organic Chemistry and Biochemistry.

References

Finkel L., Koh S. (2013): N-type calcium channel antibody-mediated autoimmune encephalitis: An unlikely cause of a common presentation. Epilepsy Behav. Case Rep. **1,** 92–96 http://dx.doi.org/10.1016/j.ebcr.2013.06.001

Geisler S., Clemens L., Schöpf C. L., Obermair G. J. (2015): Emerging evidence for specific neuronal functions of auxiliary calcium channel alpha2delta subunits. Gen. Physiol. Biophys. **34,** 105–118

Groen J. L., Andrade A., Ritz K., Jalalzadeh H., Haagmans M., Bradley T. E., Jongejan A., Verbeek D. S., Nurnberg P., Denome S. et al. (2015): CACNA1B mutation is linked to unique myoclonus-dystonia syndrome. Hum. Mol. Genet. **24,** 987–993 http://dx.doi.org/10.1093/hmg/ddu513

Kamiya-Matsuoka C., Blas-Boria D.,Williams M. D., Garciarena P., Tummala S., Tremont-Lukats I. W. (2014): N-type calcium channel antibody-mediated paraneoplastic limbic encephalitis: a diagnostic challenge. J. Neurol. Sci. **338,** 188–190 http://dx.doi.org/10.1016/j.jns.2014.01.004

Kimpinski K., Iodice V., Vernino S., Sandroni P., Low P. A. (2009): Association of N-type calcium channel autoimmunity in patients with autoimmune autonomic ganglionopathy. Auton. Neurosci. **150,** 136–139 http://dx.doi.org/10.1016/j.autneu.2009.06.002

Mallmann R. T., Wilmes T., Lichvarova L., Bührer A., Lohmüller B., Castonguay J., Lacinova L., Klugbauer N. (2013): Tetraspanin-13 modulates voltage-gated CaV2.2 Ca2+ channels. Sci. Rep. **3,** 1777 http://dx.doi.org/10.1038/srep01777

Patil P. G., de Leon M., Reed R. R., Dubel S., Snutch T. P., Yue D. T. (1996): Elementary events underlying voltage-dependent G-protein inhibition of N-type calcium channels. Biophys. J. **71,** 2509–2521 http://dx.doi.org/10.1016/S0006-3495(96)79444-4

Pietrobon D. (2010): CaV2.1 channelopathies. Pflügers Arch. **460,** 375–393 http://dx.doi.org/10.1007/s00424-010-0802-8

Proft J., Weiss N. (2015): G protein regulation of neuronal calcium channels: back to the future. Mol. Pharmacol. **87,** 890–906 http://dx.doi.org/10.1124/mol.114.096008

Weiss N., Arnoult C., Feltz A., De Waard M. (2006): Contribution of the kinetics of G protein dissociation to the characteristic modifications of N-type calcium channel activity. Neurosci. Res. **56,** 332–343 http://dx.doi.org/10.1016/j.neures.2006.08.002

Weiss, N., Tournier-Lasserve E., De Waard M. (2007): Role of P/Q calcium channel in familial hemiplegic migraine. Med. Sci. (Paris) **23,** 53–63 (in French) http://dx.doi.org/10.1051/medsci/200723153

Weiss N., Zamponi G. W. (2012): Regulation of voltage-gated calcium channels by synaptic proteins. Adv. Exp. Med. Biol. **740,** 759–775 http://dx.doi.org/10.1007/978-94-007-2888-2_33

Weiss N., Hameed S., Fernandez-Fernandez J. M., Fablet K., Karmazinova M., Poillot C., Proft J., Chen L., Bidaud I., Monteil A. et al. (2012a): A Ca(v)3.2/syntaxin-1A signaling complex controls T-type channel activity and low-threshold exocytosis. J. Biol. Chem. **287,** 2810–2818 http://dx.doi.org/10.1074/jbc.M111.290882

Weiss N., Zamponi G. W., De Waard M. (2012b): How do T-type calcium channels control low-threshold exocytosis? Commun. Integr. Biol. **5,** 377–380 http://dx.doi.org/10.4161/cib.19997

Weiss N., Zamponi G. W. (2013a): Control of low-threshold exocytosis by T-type calcium channels. Biochim. Biophys. Acta **1828,** 1579–1586 http://dx.doi.org/10.1016/j.bbamem.2012.07.031

Weiss N., Zamponi G. W. (2013b): Reciprocal regulation of neuronal calcium channels by synaptic proteins. In: Modulation of Presynaptic Calcium Channels (Eds. G. Stephensand S. Mochida), pp. 61–78, Springer