Epilepsy-causing mutations in Kv7.2 C-terminus affect binding and functional modulation by calmodulin

L. Manocchio[1], P. Ambrosino[1], A. Alaimo[2], S. Bartollino[1], M. De Maria[1], I. Mosca[1], C. Gomis-Perez[2], A. Alberdi[2], G. Scambia[3], G. Lesca[4], A. Villarroel[2], M. Taglialatela[1,2,5], M.V. Soldovieri[1]

[1]Dept. of Medicine and Health Science, Univ. of Molise, Campobasso (Italy)
[2]Biophysics Unit, CSIC, UPV/EHU, Univ. del País Vasco, Leioa (Spain)
[3]Dept. of Obstetrics and Gynecology, Catholic Univ. of Sacred Heart, Rome, Italy
[4]Dept. of Medical Genetics, Hospices Civils de Lyon, Lyon (France)
[5]Dept. of Neuroscience, Univ. 'Federico II', Naples (Italy)

Mutations in the *kcnq2* gene, encoding for Kv7.2 voltage-dependent K^+ channel, cause epilepsies with wide phenotypic heterogeneity, ranging from Benign Familial Neonatal Seizures (BFNS; *1*) to severe encephalopathies (*2*). Epilepsy-causing mutations in Kv7.2 often affect the cytoplasmic C-terminus, where the binding sites for several modulators have been identified, such as calmodulin (CaM; A and B helices; *3)*, sintaxin-1A *(4),* PIP_2 (*5*) and others.
In the present study, we have characterized the biochemical and functional consequences of BFNS-causing mutations (W344R, L351F, L351V, Y362C and R553Q; *6),* affecting the A (W344R, L351F, L351V) or the B (R553Q) helices or the inter-helical region (Y362C) of the Kv7.2 subunit. To this aim, the possible mutation-induced alterations in CaM binding on Kv7.2 subunits were investigated by using wild-type or mutant Kv7.2 C-terminal fragments encompassing the CaM binding domain and the entire CaM in semi-quantitative (Far-Western blotting) and quantitative biochemical (Surface Plasmon Resonance, SPR and dansylated CaM fluorescence) assays. In parallel, mutation-induced changes in CaM-dependent Kv7.2 current regulation were investigated by patch-clamp recordings in Chinese Haster Ovary (CHO) cells co-expressing Kv7.2 channels and CaM or CaM_{1234} (a CaM isoform unable to bind Ca^{2+}) (7).
The results obtained suggest that some of the mutations studied (L351F, Y362C, R553Q) caused a significant decrease in Kv7.2 CaM affinity. Functional experiments also revealed that CaM overexpression significantly increased the maximal current density elicited by Kv7.2 channels, with an even stronger potentiation when CaM_{1234} was co-expressed. Biotinylation experiments revealed that CaM- or CaM_{1234}-induced current potentation was not related to an increased plasma membrane expression of Kv7.2 subunits. Interestingly, Kv7.2 channels carrying the mutations altering CaM affinity (L351F, Y362C or R553Q) also induced specific alterations in the CaM-dependent Kv7.2 functional modulation. Notably, L351F- and R553Q-Kv7.2 channels showed a significant decrease in the maximal current density when expressed alone, whereas CaM overexpression partially (R553Q) or fully (L351F) restored this functional deficit. By contrast, W344R-Kv7.2 mutant channels showed a complete functional loss, despite any change in CaM affinity. Similar results were also obtained when mutant Kv7.2 subunits were co-expressed with wild-type Kv7.2/3 subunits to reproduce the genetic balance of affected individuals.
Taken together, the results obtained suggest that each BFNS-associated mutation produces specific biochemical and/or functional consequences, ranging from subtle alterations in CaM affinity which did not prompt functional changes (as observed for L351V mutation), to significant alterations both in CaM affinity and CaM-dependent functional regulation (as observed for L351F, Y362C and R553Q). Overall, the present study suggests that alterations in CaM-dependent Kv7.2 binding and/or modulation may be a pathogenetic mechanism for BFNS-causing mutations, and highlights CaM-dependent Kv7.2 current potentation as a possible target for novel pharmacological therapy against hyperexcitability diseases.

References

1. *Singh et al; Nat Genet, 1998.*
2. *Weckhuysen et al; Ann Neurol, 2012.*
3. *Yus-Näjera et al., J Biol Chem, 2002.*
4. *Regev et al., PLoS One, 2009.*
5. *Hernandez et al; J Gen Physiol, 2009.*
6. *Soldovieri et al; Hum Mutat, 2014.*
7. *Geiser et al; Cell, 1991*