# Early-onset epileptic encephalopathy caused by a reduced sensitivity of Kv7.2 potassium channel subunits to $PIP_2$

M. V. Soldovieri[1], P. Ambrosino[1], M. De Maria[1], I. Mosca[1], P. Striano[2], S. Weckhuysen[3], E. C. Cooper[4], and M. Taglialatela[1,5]

[1]Dept. of Medicine and Health Science, University of Molise, Campobasso (Italy)
[2]Lab. of Neurogenetics, Dept. of Neuroscience, Institute 'G. Gaslini', Genova, Italy
[3]Neurogenetics Group, Dept. of Molecular Genetics, VIB, Antwerp, Belgium; Institut du Cerveau et de la Moelle épinière, Hopital de la Pitié-Salpêtrière, Paris (France)
[4]Dept. of Neurology and Dept. of Neuroscience and Molecular and Human Genetics, Baylor College of Medicine, Houston, TX
[5]Dept. of Neuroscience, University of Naples 'Federico II', Naples (Italy)

Kv7.2 and Kv7.3 (KCNQ2, KCNQ3) channels underlie the M-current ($I_{KM}$), a potassium-selective neuronal current, limiting repetitive firing and causing spike frequency adaptation.
Kv7.2 and Kv7.3 subunits are characterized by the presence of six transmembrane segments and a long C-terminus containing domains required for homo- or heteromeric subunit assembly and for regulation by a complex network of mutually interacting molecules, such as $PIP_2$, calmodulin, syntaxyn, and others[1]. Mutations in the KCNQ2 gene are responsible for early-onset neonatal seizures with wide clinical outcome, ranging from Benign Familial Neonatal Seizures (BFNS)[2] to severe Early-Onset Epileptic Encephalopathy (EOEE)[3]. The molecular basis for such phenotypic heterogeneity are still debated, although distinct mutation-induced changes in $K^+$ channel function, including both loss-[4,5] and gain-of function,[6] appear to play a major role.
In the present study, we have investigated the biochemical/functional consequences prompted by a recurrent Kv7.2 C-terminal mutation (R325G)[7] found in three independent cases with severe epileptic encephalopathy and profound developmental delay[8]. The mutation was engineered in a KCNQ2-encoding plasmid and expressed by transient transfection in Chinese Hamster Ovary (CHO) cells. Biochemical and electrophysiological experiments were performed 1 day after transfection.
Western-blot experiments performed on CHO cells expressing wild-type or mutant Kv7.2 subunits revealed that the R325G mutation failed to interfere with total or plasma membrane levels of Kv7.2 subunits; however, patch-clamp recordings revealed that homomeric Kv7.2-R325G channels were non-functional. To mimic the genetic balance of EOEE-affected patients, carrying the mutation on a single allele, and since $I_{KM}$ is mainly formed by Kv7.2/3 heteromers, we also co-transfected Kv7.2-R325G together with wild-type Kv7.2 and Kv7.3 subunits. Under this experimental condition, mutant subunits significantly suppressed wild-type channel function, suggesting a dominant-negative effect.
Experiments in which Kv7.2-R325G subunits were rendered TEA-insensitive by an additional mutation in the pore region (Y284C)[9] and co-expressed with wild-type Kv7.2 or in which Kv7.2-R325G subunits were co-expressed with wild-type Kv7.3 subunits demonstrated the ability of mutant subunits to be incorporated into heteromeric channels with either Kv7.2 or Kv7.3 wild-type subunits.
In Kv7 channels, the short cytoplasmic region immediately past the $S_6$ segment, where the R325 residue resides, provides a critical attachment site for $PIP_2$ binding during channel gating[10]. Increasing cellular $PIP_2$ levels by co-expression of a PI(4)P5-kinase (PIP5K)[11] led to a partial recovery of homomeric Kv7.2-R325G channel function, a result consistent with this residue being critical for conferring $PIP_2$-dependent Kv7.2 current potentiation[12]. Furthermore, currents carried by heteromeric channels incorporating Kv7.2-R325G subunits were more readily inhibited than wild-type channels upon $PIP_2$ depletion by activation of a voltage-gated phosphatase (VSP) from zebrafish[13], and recovered more slowly during $PIP_2$ resynthesis upon VSP switch-off.
Altogether, a decreased sensitivity to $PIP_2$ appears as the primary molecular defect responsible for Kv7.2-EOEE in individuals carrying the R325G variant, further expanding the range of pathogenetic mechanisms exploitable for individually-tailored therapeutic targeting in Kv7.2-related epilepsies.