

## Mutations of KCNQ2 and KCNQ3 identified in benign neonatal convulsions cause channel dysfunction despite proper expression on the cell membrane

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**Objective:** To investigate the physiological and cell biological consequences of two abnormalities of the genes encoding two subunits of KCNQ channel, KCNQ2 and KCNQ3; a deletion mutation of KCNQ2 (c.910-2delTTC or TTT: F304del) and a missense mutation of KCNQ3 (c.925T>C: W309R). Both abnormalities were found in benign neonatal convulsion epilepsy phenotypes and deduced to cause single amino acid mutations. In general, mutations of KCNQ channels cause haplo-insufficiency in the channel function, and often interfere with proper expression of the channel on the cell membrane.

**Methods:** cDNAs of KCNQ2 and KCNQ3 were PCR cloned and the mutations were introduced. Either wild type or mutant cDNA was transfected in HEK cells. Electrophysiological properties of reconstituted KCNQ K<sup>+</sup> channels were then studied. Both molecules were detected by Western blotting and immunostaining of transfected cells with anti-KCNQ2 and anti-KCNQ3 antibodies.

**Results:** Homomeric KCNQ K<sup>+</sup> channels reconstituted with the mutant KCNQ molecules showed virtually no K<sup>+</sup> current whereas those with wild type molecules exhibited robust K currents in response to voltage stimulations. The expression levels and molecular sizes of both wild type and mutant KCNQ molecules detected by Western blotting were equal suggesting equal expression and similar post-translational modification in the cell. Immunostaining confirmed the expression of both molecules on the plasma membrane.

**Conclusion:** Our findings indicate that both mutants undermine KCNQ K<sup>+</sup> channel function per se without trafficking disturbance in the cell.