FUNCTIONAL COMPARISON AMONG THREE VARIANTS AFFECTING THE SAME CRITICAL RESIDUE IN THE Kv7.2 POTASSIUM CHANNEL GENE IDENTIFIED IN PATIENTS WITH EPILEPSY AND/OR SEVERE DEVELOPMENTAL IMPAIRMENT

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Kv7.2 (KCNQ2) and Kv7.3 (KCNQ3) are voltage-gated potassium channels mainly expressed in the central nervous system where they form homo- or hetero-terameric channels underlying the so-called M-current, a slowly, non-inactivating potassium current that regulates neuronal firing. Structurally, Kv7.2 and Kv7.3 subunits are composed of six transmembrane segments (S1–S6) and a long C-terminal region; the S5 and S6 segments and the intervening linker form the pore and the inner pore gate, whereas the S1-S4 region forms the voltage sensor domain. Mutations in the gene encoding for Kv7.2, and more rarely Kv7.3, channels have been identified in patiens with a wide range of early-onset epilepsies ranging from benign familial neonatal seizures (BFNS) to early-onset epileptic encephalopathy (KCNQ2 encephalopathy) (Miceli et al., 2016). In addition, several mutations in both Kv7.2 and Kv7.3 genes have been more recently found sporadically in large cohorts of patients affected by severe developmental disorders and autism (DDD Study, 2017; Gilling et al., 2013). The molecular pathogenesis of such divergent clinical presentation of specific Kv7.2 or Kv7.3 variants is still unknown.

In the present work we compared the functional consequences prompted by two newly-identified de novo Kv7.2 missense mutations affecting a critical residue localized at the bottom part of S2 (R144W and R144G) found in patients with severe developmental impairment (DDD Study, 2017) with those of the R144Q variant, previously described in a patient with epileptic encephalopathy (Allen et al., 2013; Miceli et al., 2015).

To this aim, we introduced the specific mutations in the human Kv7.2 cDNA and expressed these plasmids in Chinese Hamster Ovay (CHO) cells by transient transfection; a plasmid encoding for a fluorescence protein was used as transfection marker. Electrophysiological experiments performed twenty-four hours after transfection, revealed that homomeric Kv7.2 R144Q, Kv7.2 R144W and Kv7.2 R144G channels are functional, exhibiting a leftward shift in activation voltage-dependence of about 19, 5 and 24 mV, respectively.

Furtermore, in order to reproduce the genetic balance of the patients and considering that the M-current is mainly formed by the assembly of Kv7.2 and Kv7.3 subunits, the functional consequences of the mutation were also evaluated in heteromeric channels formed upon co-expression of Kv7.2 and Kv7.3 subunits. We found that Kv7.2wt+Kv7.2 R144Q+Kv7.3, Kv7.2wt+Kv7.2 R144W+Kv7.3, and Kv7.2wt+Kv7.2 R144G+Kv7.3 heteromeric channels showed a current density equal to Kv7.2+Kv7.3 channels; in addition, in cells expressing Kv7.2wt+Kv7.2 R144G+Kv7.3 channels, a statistically significant leftward shift in activation voltage-dependence of about 7 mV was observed. No significant gating change was instead observed when

Kv7.2wt+Kv7.2 R144Q+Kv7.3 or Kv7.2wt+Kv7.2 R144W+Kv7.3 were investigated. The results obtained suggest that R144Q, R144W and R144G mutations induced a gain-of-function effect on Kv7.2 channels, with a more dramatic effect when R144 was replaced with an uncharged and small residue such as glycine. Although further studies are needed to clarify the unique genotype/phenotype correlation observed in the described mutations, the present results indicate the potential use of selective Kv7.2 blockers (Cheung et al., 2012; Miceli et al. 2017) as a personalized therapeutic approach in neurodevelopmental disorders caused by the described variants.

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