Compound heterozygosis in the kcnq3 gene in a patient with early-onset Epileptic Encephalopathy (EOEE)

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Early-onset Epileptic Encephalopathy (EOEE) is a severe form of epilepsy in which epileptiform activity contributes to a progressive cerebral dysfunction. Seizures are often pharmacoresistant and early death can occur1. In a group of patients, a genetic origin of the disease has been identified, and knowledge of the underlying genetic cause is likely to improve treatment opportunities and clinical outcome. Recently, de novo mutations in the kcnq3 gene have been identified heterozygosisly in patients affected by EOEE2,3. The kcnq3 gene encodes for neuronal Kv7.3 subunits assembling with Kv7.2 subunits to form voltage-dependent K+ channels playing a crucial role in the regulation of neuronal excitability4, and characterized by an absolute functional requirement for phosphatidylinositol 4,5-bisphosphate (PIP2)5. In the present study, we have identified a patient affected by EOEE carrying two newly-identified missense mutations in the kcnq3 gene in compound heterozygosis, each inherited by a clinically asymptomatic parent, thus leading to an apparent autosomal recessive inheritance pattern of the phenotype. In particular, the V359L mutation was of maternal origin, whereas the D542N mutation was of paternal origin. Both mutations affect residues located in the long C-terminal tail of the Kv7.3 subunit, a critical region for Kv7 channel regulation and gating6. To investigate their functional consequences, each variant was engineered in a human kcnq3 plasmid and expressed in CHO cells by transient transfection. Patch-clamp recordings revealed that, opposite to wild-type Kv7.3 channels, Kv7.3 V359L or Kv7.3 D542N channels failed to carry K+ currents above background levels upon membrane depolarization. To mimic the genetic balance of the individuals carrying a single Kv7.3 mutation, as well as that of the EOEE-affected patient carrying both mutations, one in each Kv7.3 allele, we performed additional patch-clamp recordings in CHO cells co-expressing Kv7.2+Kv7.3 (control; transfection cDNAs ratio 1:1), Kv7.2+Kv7.3+Kv7.3 V359L or Kv7.2+Kv7.3+Kv7.3 D542N (healthy Kv7.3 mutation carriers; transfection cDNAs ratio 1:0.5:0.5), or Kv7.2+Kv7.3 V359L+Kv7.3 D542N (proband; transfection cDNAs ratio 1:0.5:0.5). The results obtained show that CHO cells co-expressing either mutant Kv7.3 subunits with wild-type Kv7.2+Kv7.3 subunits elicit currents identical to those measured in cells expressing only wild-type Kv7.2+Kv7.3 channels; by contrast, when both mutant Kv7.3 subunits were simultaneously co-expressed with wild-type Kv7.2 subunits, a significant reduction in maximal currents was measured. These results suggest a loss-of-function effect prompted by mutant Kv7.3 subunits on heteromeric channels with Kv7.2 subunits, only when simultaneously co-expressed. Initial experiments using a voltage-sensitive phosphatase (VSP) from zebrafish to deplete membrane PIP2 levels, revealed that each Kv7.3 variant reduced channel sensitivity to PIP2, with additional effects when subunits carrying each variant are co-expressed. Based on these results and given the poor effectiveness of traditional antiepileptic drugs in the treatment of EOEEs, we tested the ability of retigabine (10 μM), a known Kv7 opener, to counteract mutation-induced functional alterations. Notably, retigabine restored

the currents measured in cells co-expressing Kv7.2+Kv7.3 V359L+Kv7.3 D542N to the same levels than those in cells expressing Kv7.2+Kv7.3 subunits. These results may provide a plausible molecular explanation for the severe phenotype observed in the proband, and for the lack of phenotype in the other family members carrying a single kcnq3 variant; moreover, they provide a rationale for the use of Kv7 channels activators for the pharmacological treatment of patients affected by EOEE carrying Kv7 loss-of-function mutations.

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