Mutations in the potassium channel gene KCNQ4 underlie DFNA2, a subtype of autosomal dominant progressive, high-frequency hearing loss. In a previous study we identified a novel mutation, p.G296S, in the pore region of KCNQ4 channel that impaired its activity in two manners: it greatly reduced surface expression and, secondarily, abolished channel function. Moreover, G296S mutant exerted a strong dominant-negative effect on potassium currents by reducing the wild type KCNQ4 channel expression at the cell surface. This was the first study to identify a trafficking-dependent dominant mechanism for the loss of KCNQ4 channel function in DFNA2. In the present study we have investigated the pathogenic mechanism associated with all the described mutations identified up to date. To this end, we have generated a series of KCNQ4 mutants in order to identify specific motifs involved in channel retention/exportation. Altogether, our results provide insights into the pathogenesis of DFNA2 indicating that a defect in channel trafficking is the common mechanism for the KCNQ4 mutations identified up to date.

Abstract

Mutations in the potassium channel gene KCNQ4 underlie DFNA2, a subtype of autosomal dominant progressive, high-frequency hearing loss. In a previous study we identified a novel mutation, p.G296S, in the pore region of KCNQ4 channel that impaired its activity in two manners: it greatly reduced surface expression and, secondarily, abolished channel function. Moreover, G296S mutant exerted a strong dominant-negative effect on potassium currents by reducing the wild type KCNQ4 channel expression at the cell surface. This was the first study to identify a trafficking-dependent dominant mechanism for the loss of KCNQ4 channel function in DFNA2. In the present study we have investigated the pathogenic mechanism associated with all the described KCNQ4 mutations (F182L, W242X, E260K, D262V, L274H, W276S, L281S, G285C, G285S and G321S) that are located in different domains of the channel protein. F182L mutant showed a wild type-like cell-surface distribution in transiently transfected nonpermeabilised NIH3T3 fibroblasts and the recorded currents obtained in Xenopus oocytes resembled those of the wild-type, indicating this variant cannot be considered as pathogenic. All the remaining KCNQ4 mutants displayed distinct levels of defective cell-surface expression in NIH3T3 fibroblasts. Co-localization studies revealed these mutants were retained in the ER, unless W242X, which showed a clear co-localization with Golgi apparatus. Interestingly, this mutation results in a truncated KCNQ4 protein at the S5 transmembrane domain, before the pore region, that escapes the protein quality control in the ER but does not reach the cell surface at normal levels. On this base we are currently investigating the trafficking behaviour and electrophysiological properties of several KCNQ4 truncated proteins artificially generated in order to identify specific motifs involved in channel retention/exportation. Altogether, our results provide insights into the pathogenesis of DFNA2 indicating that a defect in channel trafficking is the common mechanism for the KCNQ4 mutations identified up to date.