A COMMON MECHANISM OF DEFECTIVE CHANNEL TRAFFICKING UNDERLYING DFNA2 HEARING LOSS RESULT IN DIFFERENT CELL SURFACE EXPRESSION LEVELS OF *KCNQ4* MUTANTS

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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. 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.

Mutations Identified in the *KCNQ4* Gene

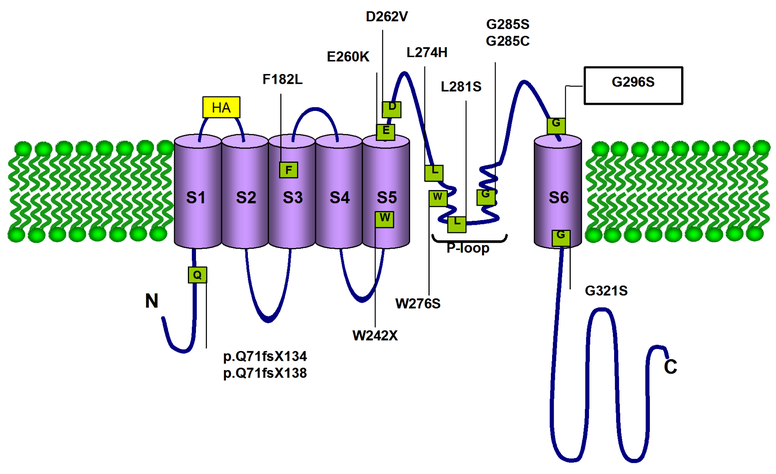

Figure 1. Graphical representation of the *KCNQ4* voltage-gated potasium channel. The described mutations are shown.

Table 1.Cell surface, reticulum and Golgi co-localization of the mutants.

	Cell surface expression	RE Co-localization	Golgi Co-localization
WT	+++++	++	+
G296S	+	++++	+
F182L	+++++	++++	+
L274H	++	++++	+
W276S	+++	++++	+
L281S	+	++++	+
G285S	+++	++++	+
G285C	+++	++++	+
G321S	+++	++++	+
W242X	++	-	++++
E260K	+	++++	+
D262V	+	+++	+

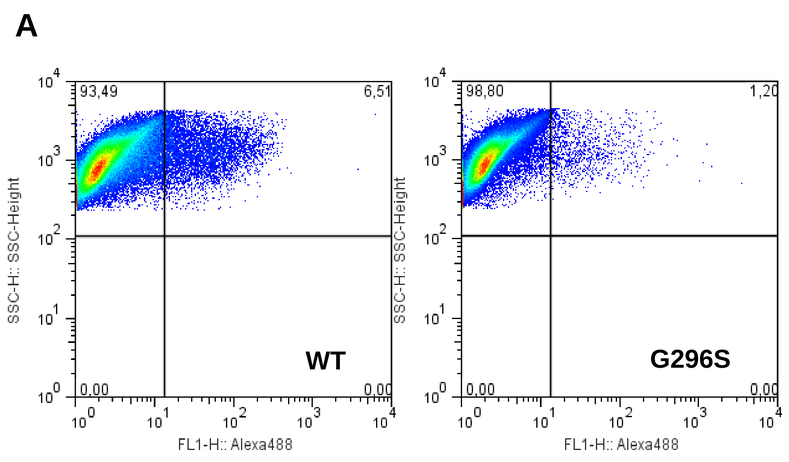

Figure 2. A. Representative flow cytometry graphic showing *KCNQ4* cell surface expression in NIH3T3 were permebilized and labeled with anti-HA alexa fluor 488 conjugated. B. Graphic representation of cell surface expression of KCNQ4 mutants. The values were normalized to those obtained for the wild type.

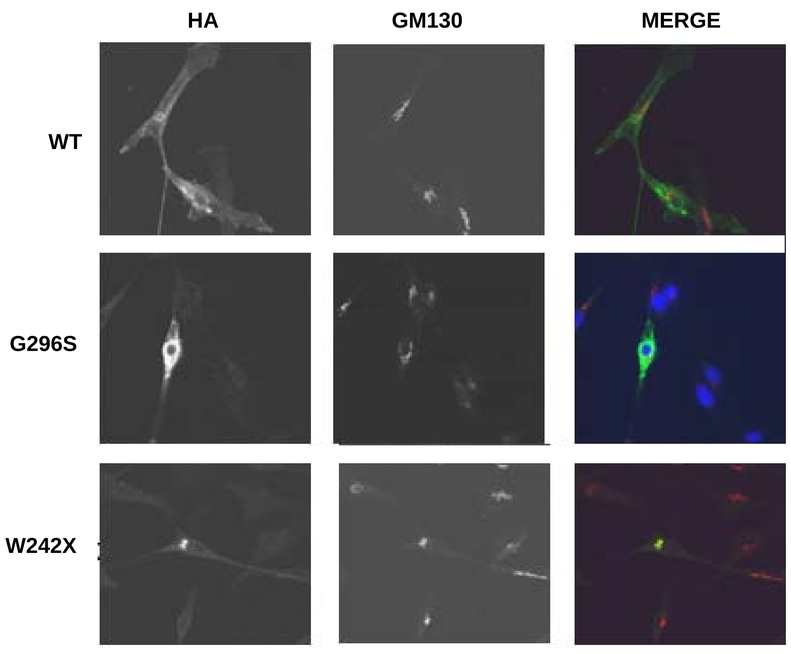

Figure 4. Detection of sub-cellular localization of mutants *KCNQ4* channels. HA-tagged mutants *KCNQ4* were double stained in permeabilized cells with anti-HA alexa fluor 488 conjugated and anti-GM130 alexa fluor 594 conjugated (Golgi marker) .Only the mutant W242X showed a clear co-localization Golgi.

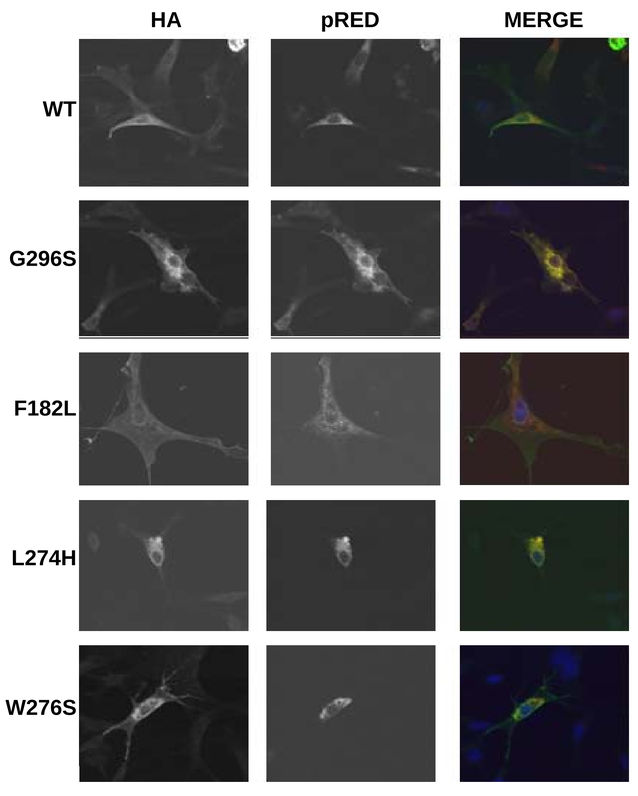

Figure 3. Detection of sub-cellular localization of mutants KCNQ4 channels. NIH3T3 cells were co-transfected with a plasmid containing the different mutants and pDSRed2-ER plasmid. Most of the KCNQ4 mutants are retained in the endoplasmid reticulum.

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Conclusions

1- F182L mutant showed a wild type-like cell surface distribution and localization, indicating this variant cannot be considerated as pathogenic. All the reamining mutants displayed a defective cell-surface expression.

2- Flow cytometry experiments show that the mutant have different cell surface expression.

3- Co-localization experiment showed that these mutants were retained in the ER unless W242X which showed a co-localization with Golgi apparatus.

4- We are investigating the expression and current recordings of the *KCNQ4* mutants in *Xenopus* oocytes.