Quantifying expression defects of LQT2 associated hERG mutations

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Objectives: Congenital long QT syndrome (LQTS) is an autosomal dominant inherited disorder characterized by a prolongation of the QT interval on the body surface electrocardiogram, which is associated with a markedly increased risk of ventricular arrhythmias and sudden cardiac arrest. Long QT syndrome type 2 (LQTS2) is caused by mutations in human ether-a-go-go related gene (hERG) K^+ channels and accounts for ˜40 % of LQTS cases. There are over 500 LQTS2 mutations reported, with the majority of missense mutations resulting in protein expression defects: ie. fewer hERG channels at the cell membrane. Clinical presentation of patients with LQTS2 can range from death *in utero* to being asymptomatic into old age and we hypothesize that some of this clinical variability can be explained by the variable impact of different mutations on hERG channel expression. However, hERG channels are tetrameric proteins, so in LQTS2 patients will contain a mixture of wild-type (WT) and mutant subunits and this should be taken into account when estimating mutant expression levels. The situation is further complicated by the presence of alternatively spliced variants of hERG, such as hERG1b. Our aim was to quantify the expression phenotype of LQTS2 mutants in the background of WT hERG1a or WT hERG1b isoforms.

Methods: Using the Flp-In system, we generated inducible stable HEK293 cell lines harbouring WT or mutant hERG1a channels with a C-terminal flag tag. To assess the expression phenotype of mutant channels, we transiently transfected hERG1a mutants (C-terminal HA tag) into stable cell lines expressing WT or mutant hERG1a (C-terminal flag tag). Alternatively, to assess the dominant negative phenotype of each mutant, we transiently transfected WT-hERG1a (C-terminal HA tag) or WT-hERG1b (C-terminal flag tag) into WT or mutant hERG1a stable cell lines. 48 hours after transfection, cells were harvested and proteins separated using SDS-page gel electrophoresis and detected on western blot. Expression levels were quantified from the fully-glycosylated (FG) form of the transiently transfected protein using a Li-Cor Oddysey imaging system. Expression levels were normalised to the level of GFP expression to control for transfection efficiency. All data are presented as FG/GFP ratios normalised to WT controls.

Results: We studied four LQTS2 mutations located in different regions of the hERG channel: R56Q (N-terminus), R582C (extracellular S5-P linker), A614V (pore helix) and S818L (C-terminus). When HA-tagged mutants were transiently transfected into cells stably expressing hERG1a with the same mutation, all mutants showed reduced expression compared to WT (0.92 ± 0.05 for R56Q, 0.34 ± 0.03 for R582C, 0.16 ± 0.02 for A614V, and 0.11 ± 0.01 for S818L). When each mutant was transiently transfected into a WT hERG1a stable cell line, no increase in mutant protein levels was observed suggesting that WT co-expression does not rescue expression defective mutants. Also, in the reverse experiment, that is transiently transfected WT hERG1a in the mutant stable cell line, we did not observe any dominant-negative suppression of WT hERG1a (i.e. WT hERG1a FG protein levels were similar to those when WT hERG1a was transfected into the WT hERG1a stable cells). In contrast, we did observe dominant-negative suppression of WT hERG1b, with WT hERG 1b levels suppressed by $40 \pm 8\%$ for R56Q, $29 \pm 6\%$ for R582C, $64 \pm 5\%$ for A614V and $24 \pm 10\%$ for S818L.

Conclusions: All four LQTS2 mutants tested showed expression defects, ranging from an 8% reduction for R56Q to an 89% reduction for S818L. None of the mutants were rescued by co-expression with WT and none caused a dominant negative suppression of WT hERG1a channels. However, we did observe dominant negative suppression of WT hERG1b and the level of suppression was mutant specific. There is good evidence to suggest that in native tissues hERG channels assemble as hERG1a/1b and therefore the dominant negative suppression of hERG1b is physiologically relevant and should be considered when assessing LQTS2 mutant phenotypes.