Disruption of the architecture of the junctional sarcoplasmic reticulum in recessive catecholaminergic polymorphic ventricular tachycardia is caused by the er-shaping proteins reep5 and climp63

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Background: The recessive variant of Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), a highly lethal inherited arrhythmic disease, is caused by loss of functions mutations in the genes encoding cardiac Calsequestrin (CASQ2) and Triadin (TRD). Disease-mediated decrease in expression of either CASQ2 and TRD in cardiomyocytes, beside affecting calcium handling, profoundly affects the architecture of the junctional SR (jSR) cisternae that appears enlarged and fragmented thus potentially modifying calcium-induced calcium-release.

Purpose: The present study explores the involvement of SR structural proteins in a recessive CPVT mouse model. We focused on the role of REEP5 and CLIMP63 in shaping the SR in cardiomyocytes in CASQ2-KO mice. The two proteins exert opposing actions: while REEP5 promotes the membrane curvature that helps forming tubules, CLIMP63 promotes the formation of flat cisternal "sheets".

Methods: In cardiac tissue of WT and CASQ2-KO mice we compared the transcriptional, translational and post-translational profile of genes encoding for proteins that regulate the ER-architecture. We studied protein interaction and localization by co-immunoprecipitation and immunofluorescence. We processed protein extracts to evaluate the extent of palmitoylation of CLIMP63.

Results: Our data demonstrate transcriptional repression of REEP5 ($p<0.05$), and upregulation of CLIMP63 at the transcriptional and translational level ($p<0.05$) in the heart of CASQ2-KO mice compared to controls. We also investigated the interplay between RyR2 and CLIMP63 by co-immunoprecipitation and documented that in the heart of CASQ2-KO mice this interaction is doubled as compared to WT. Interestingly, we observed that in cardiomyocytes of CASQ2-KO mice the co-localization between RyR2 and CLIMP63 is not affected by the absence of CASQ2. Since it has been shown that palmitoylation is a post-translational modification that regulated the turnover and the retention of CLIMP63 in the endoplasmic reticulum, we therefore hypothesized that the increased abundance of CLIMP63 and its association with RyR2 could be mediated by an increased level in palmitoylation. In agreement with our hypothesis we observed that the amount of protein that is affinity-purified through palmitoylated cysteines is increased in CASQ2-KO mice compared to WT suggesting that this modification, by slowing the turnover of the protein, mediates its accumulation and leads to expansion of SR cisternae.

Conclusion: Our data, represent the first evidence that post translational modifications of CLIMP63 contribute to the loss of SR homeostatic environment and SR integrity in CPVT mice by breaking the balance between REEP5 and CLIMP63 and therefore reducing the formation of curved tubular membranes in favour of the flat sheet morphology.