Unraveling the pathogenesis of novel ANKRD1 missense mutation causing familial dilated cardiomyopathy using human-induced pluripotent stem cells derived cardiomyocytes

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Introduction: Familial dilated cardiomyopathy (f-DCM) can be caused by mutations in 30 different genes encoding sarcomeric, cytoskeletal, mechanotransducers and transcription factors. One of the genes ANKRD1 encode Cardiac ankyrin repeat protein (CARP) which is involved in the mechanosensory unit located on the I-band of the sarcomere. Here we demonstrate a novel mutation in ANKRD1 gene causing f-DCM, and the usage of human-induced pluripotent (hiPSC) technology unraveling the pathogenesis of the disease.

Purpose: of the study:
1) Establishing patient-specific hiPSC-derived cardiomyocytes from a family harboring a mutation in ANKRD1 gene causing f-DCM; 2) Phenotypical assessment of the morphological and ultrastructural properties of the diseased cells upon CARP activation; 3) Functional assessment of the 3 component of excitation-contraction coupling at the single-cell and tissue level.

Methods and Results: Patient-specific hiPSC were generated from a family harboring missense mutation in the ANKRD1 gene, with a substitution of the amino acid arginine to cysteine at position 182 of the Cardiac ankyrin repeat protein (CARP). ANKRD1-mut hiPSC were differentiated into cardiomyocytes and later matured using hormonal treatment. Morphological assessment using immunofluorescence (IF) techniques revealed that ANKRD1-mut hiPSC-CMs demonstrate an attenuated response to hypertrophy (upon Angiotensin-2 and Phenylephrine treatment). In addition ANKRD1-mut hiPSC-CMs displayed a higher cellular death rate upon increasing substrate stiffness and starvation. Electrophysiological assessment of the ANKRD1-mut cardiac tissues exhibit no alternations in action potential durations and conduction velocities while assessment of calcium transients demonstrated slower kinetics. In addition forces were quantified at the single-cell and tissue level, and demonstrated reduced active tension values and abnormal post-extrasystolic potentiation, while only single-cell experiments have demonstrated decreased passive tensions. These phenotypical alternations might be attributed to decreased nuclear translocation of CARP upon phenylephrine treatment as detected in IF experiments.

Conclusion: Novel mutation in ANKRD1 were found and validated to be causing f-DCM, by revealing phenotypical alternation using hiPSC-CM technology. Increased cellular death rate and impaired nuclear translocation of CARP might be the underlying causes of the morphological and contractile abnormalities detected in vitro, leading to systolic dysfunction in the affected family.