**BACKGROUND**

Dilated Cardiomyopathy (DCM) is a common form of cardiomyopathies and is characterized by an enlarged left ventricle and reduced cardiac output. Left ventricular non-compaction cardiomyopathy (LVNC) is a rare genetic heart condition. LVNC is characterized by trabeculated myocardium, which develops during embryogenesis as the myocardial tissue does not compact completely. RBM20 is a splicing factor with specific expression in the heart and is preserved from fish to human. Furthermore, RBM20 is a well-known DCM causing gene.

**PURPOSE and AIM**

The aim of the study was to establish an in vitro induced pluripotent cell (iPSC) system from LVNC and DCM patients to gain insights into the pathophysiology caused by a genetic predisposition in the splicing factor RBM20.

**METHODS**

Donated somatic material from one LVNC patient, two DCM patients, and healthy controls were reprogrammed into an iPSC and subsequently differentiated into functional beating 2-month-old cardiomyocytes (CM). iPSC-CM were used in analyses to study LVNC and DCM by investigating splicing isoforms of RBM20 targets by RT-PCR, sarcomeric disarray with immunostaining, calcium handling parameters with Fluo-4 and FURA-2 probes and protein analysis with Western blots. The b-blocker Metoprolol and the calcium-channel blocker Verapamil were evaluated for their therapeutic potential in RBM20-mutant-based LVNC and DCM in addition. Isogenic RBM20 rescue (res) lines were generated from LVNC- and DCM-iPSC to analyse the direct contribution of the respective RBM20 mutations.

**RESULTS**

1: iPSC generation of RBM20-based LVNC and DCM

- IPSC generation with TRA-1-60
- Patient-specific IPSC
- Cardiac differentiation efficiency for all iPSC-CM is 89%. Analysis of CM purity at day 60-90 post IPSC-CM after cTNT staining and quantification with FLOW. Unsorted IPSC serve as negative control.

2: Molecular basis: shared and differential missplicing

- Shared and differential missplicing in LVNC and DCM-CM. Every dot represents one differentiation. Shared missplicing in TTN and RYR2. LVNC-specific missplicing in CAMKK2 and TRDN. DCM-specific missplicing in LDB3. p-value by Mann-Whitney test.

3: Sarcomeric disarray in LVNC and DCM

- Sarcomeric regularity is disturbed in LVNC- and DCM-CMs compared to control-CMs. The phenotype is reversed in the rescue lines. No of differentiations/measured pictures are control (6/112), LVNC (7/128), real/VNC (4/73), DCM (1/479). DCM (3/55) and real/DCM (4/71). p-value by Kruskal-Wallis against control. p-value by Mann-Whitney test.

4: Calcium handling: differential phenotypes for LVNC and DCM


5: Verapamil as therapeutic option

- Left: Metoprolol and Verapamil significantly reduce calcium leak in DCM-CM, end numbers DCM: real vs Verapamil (3/44 vs 3/44) and DCM basal vs Metoprolol (3/44 vs 3/44). Right: Verapamil slows fasted calcium kinetics in LVNC-CM and restores reaction to iso. No of differentiations/measured cells are basal/Verapamil treatment (3/38/3/40/3/41) and basal/Metoprolol treatment (2/22/2/22/22).

**CONCLUSION**

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The authors declare no conflict of interest.