cause of morbidity and mortality in DMD patients. We hypothesized that abnormal [Ca2+]i handling in DMD iPSC-CMs results in depressed positive inotropic response to β-adrenergic stimulation. DMD iPSC-CMs were generated from male and female patients.

Methods and results: To test the hypothesis, [Ca2+]i transients and contractions were recorded from stimulated iPSC-CMs clusters using fura-2 fluorescence and video edge detector, in the absence and presence of the β-adrenergic agonist isoproterenol, which increases SR Ca2+ release through PKA-regulated RyR2. The results demonstrated concentration-dependent positive inotropic and lusitropic effects in healthy iPSC-CMs, in both [Ca2+]i transient and contraction parameters. In contrast, compared to healthy iPSC-CMs, the [Ca2+]i transient and contraction]i of the female and male DMD iPSC-CMs displayed attenuated inotropic responses to isoproterenol. To decipher the underlying mechanism, we determined SR Ca2+ release in DMD iPSC-CMs by means of a brief application of caffeine. The caffeine-induced Ca2+ signal area of DMD iPSC-CMs (male and female) and amplitude (female) were smaller than control. In addition, Seahorse XF analyzer demonstrated decreased (compared to control) oxidative phosphorylation accompanied by a correlated increase in glycolysis in DMD iPSC-CMs. Accordingly, mass spectrometry analysis showed a dramatic fall in phosphocreatine levels in DMD iPSC-CMs.

Conclusion: In summary, DMD iPSC-CMs exhibit an attenuated β-adrenergic inotropic response, metabolic deficits and reduced energy stores.

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Oral session 17
Poster session 4
Name of presenter: Marie-Louise Bang

Abstract # 115

Myopalladin is upregulated in dilated cardiomyopathies patients and myopalladin knockout mice develop cardiac dilation and dysfunction following pressure overload

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Background: Myopalladin (MYPN) is a 145 kDa striated muscle-specific sarcomeric protein belonging to the palladin (PALLD)/MYPN/myotilin family of actin-associated immunoglobulin-containing proteins in the Z-line. MYPN gene mutations are causative for dilated (DCM), hypertrophic and restrictive cardiomyopathy.

Methods and results: To determine whether MYPN expression is altered during disease, we performed qRT-PCR on cardiac biopsies from DCM (n=8) and ischemic cardiomyopathy (ICM) (n=8) patients vs. healthy subjects (n=5). This revealed a 2.7 fold upregulation of MYPN mRNA in DCM patients as well as a similar 2.5 fold upregulation of PALLD mRNA, encoding the 200 kDa striated muscle-specific PALLD isoform, structurally homologous to MYPN. In contrast, levels of transcripts encoding the ubiquitously expressed smaller isoforms of PALLD were unaltered. As previously reported also MYPN's interaction partner ANKRD1/CARP was 4.5 fold upregulated in DCM patients.

To determine the functional role of MYPN, we generated MYPN knockout (MKO) mice, which had a normal life span but were significantly smaller compared to wildtype (WT) controls. Cardiac analyses of MKO mice showed the development of progressive cardiac dilation and decreased fractional shortening starting from 4 months of age, which was associated decreased tension generation and increased resting tension in ventricular myofibrils. Following transaortic constriction, MKO mice exhibited a normal hypertrophic response, but developed severe cardiac dilation and systolic dysfunction, associated with upregulation of CARP protein and reduced CSQ2 protein and Akt-473 phosphorylation levels.

Conclusion: Furthermore, analyses on isolated adult cardiomyocytes showed reduced sarcomere contractility, and calcium spark frequency and amplitude in MKO mice compared to WT, consistent with altered calcium signaling.

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Poster session 4
Name of presenter: Larissa M. Dorsch

Abstract # 116

Cardiomyopathy-related Tropomyosin mutations impair calcium handling: the influence of mutation location

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Background: Mutations within the α-Tropomyosin gene (TPM1) can cause dominantly inherited cardiomyopathies (CM), including dilated (DCM), hypertrophic (HCM), and restrictive CM (RCM). How mutations in the same gene lead to such variability in clinical manifestation is largely unknown. Our aim is to study the functional consequence of different TPM1 mutations in more detail.

Methods and results: Wildtype and mutant forms of TPM1 (E62Q (HCM, RCM), T201M (DCM), M281T (HCM), and double mutant E62Q-M281T (RCM)) were overexpressed in HL-1 cardiomyocytes. Calcium transients (CaT) were measured 48h and 72h after double transfection of TPM1 and red fluorescent protein (RFP). Compared to wildtype, overexpression of mutant TPM1 for 48h significantly reduced CaT amplitudes to different levels dependent on the mutation, with T201M and M281T having the strongest effect. The reduction was enhanced after 72h overexpression. No significant differences in relaxation parameters were observed between wildtype and mutant TPM1 forms. Using different ratios of RFP and V5-tagged T201M, we were able to express T201M in a dose-dependent manner, which enables future studies on the effect of mutant doses.

Conclusion: This study shows that location of mutations in TPM1 gene determines the degree of calcium handling dysfunction. Overexpression of mutant TPM1 leads to time-dependent progressive deterioration of calcium transients. In ongoing experiments, we will determine if calcium handling dysfunction is influenced by the dose of mutant protein. These results may explain the variable clinical outcomes in patients carrying different mutations within TPM1 gene.