P-47

Identification of intronic DNA sequences regulating titin expression in a dilated cardiomyopathy human induced pluripotent stem cell model

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Introduction: Mutations that truncate the sarcomere protein titin (*TTNtvs*) are known to be a major cause of dilated cardiomyopathy (*DCM*). The aim of this study was to detect regulatory DNA regions that influence *TTN* gene expression. Manipulating those regions by microRNAs or small molecules could increase intact titin in cardiomyocytes and could provide new treatment options for *DCM*.
Methods: Deletions were implemented in human induced pluripotent stem cells (hiPSC) by transfecting with CRISPR/Cas9 guide RNA to target two putative regulatory regions, previously identified on bioinformatics analysis. Cloned cells were expanded and differentiated to iPS cardiomyocytes. After verification of *TTN* deletions by next generation sequencing, real-time quantitative PCR (qPCR) was applied to assess *TTN* RNA expression in heterozygous and homozygous mutated in comparison to WT clones.
Results: One deletion spanned 13,000 basepairs (13kb) upstream of *TTN* exon 1 excluding the TATA box and included a previously known SNP as well as a superenhancer region. Another separate deletion targeted a highly conserved region within Intron 1 of *TTN*. qPCR performed for two separate DNA sequences in exon 133 and exon 254 showed no significant difference in relative *TTN* expression compared to WT clones (see grey bars in Figure) in iPS derived cardiomyocyte clones carrying the 13kb deletion upstream of *TTN* (see striped bars in Figure). In contrast, homozygous and heterozygous deletions in Intron 1 significantly decreased *TTN* gene expression (see dotted bars in Figure; p-values: Intron 1 del (-/-) ex 133=0,02, Intron 1 del (-/-) ex 254=0,05, Intron 1 del (+/-) ex 133=0,04, Intron 1 del (+/-) ex 254=0,02) in iPS derived cardiomyocytes.
Conclusions: *TTN* Intron 1 plays an important role in *TTN* gene expression regulation and might be a relevant treatment target in *DCM* caused by titin-truncating mutations (*TTNtvs*).