Abstract: P137

Cardiomyopathy-associated arginine-mutations within the RS-domain of RBM20 lead to mislocalization of the protein

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Introduction
Mutations in human RBM20 have previously been shown to cause dilated cardiomyopathy (DCM). Within the nucleus RBM20 partially colocalizes with other splice factors, binds RNA and has a major role in myocardial alternative splicing.

We have identified in our patient cohort the previously published RBM20 mutation p.P638L and two novel mutations, p.S635C and p.S635F, which are localized in the highly conserved RS-domain. The mutations are highly penetrant leading to terminal heart failure and/or to sudden cardiac death. Previous studies from our laboratory showed subcellular mislocalisation of these mutant RBM20-proteins presumably due to aberrant phosphorylation. Though cardiomyopathy-associated mutations affecting arginines within the conserved RS-domain are known from the literature, little is known on the molecular pathomechanisms of these mutations.

Purpose
Goal of this study was to gain further insight into mutation-associated mislocalisation of RBM20 by analyzing the effects of arginine-mutations within the conserved RS-domain on the intracellular distribution of RBM20. Furthermore, we proved if the position of the affected serine within the RS-domain has an effect on nuclear localization.

Methods
To compare the localization of RBM20 wildtype and mutants we constructed RBM20-EYFP protein chimera and analyzed their subcellular localization in different cell lines. To estimate the influence of phosphorylation we analyzed the localization of phosphomimetic mutants of RBM20. We analyzed the cellular localization of protein mutants related to the arginines on positions 632, 634, 636 and 641 and the serines at positions 635, 637 and 640 of RBM20.

Results
Comparable to serine mutations, mutations concerning the highly conserved arginines within the RS-domain also lead to a subcellular mislocalization of RBM20. Interestingly, the kind and position of amino acid exchange seems to be important for the extent of nuclear misdistribution. Comparing the intracellular localization of serine non-phosphomimetic and phosphomimetic mutants we could show that in contrast to serines at positions 635 and 637 the exchange of the serine at position 640 does not lead to a complete loss of nuclear localization of RBM20.

Conclusions
We present here first data showing that mutations affecting the conserved arginines within the RS-domain of RBM20 lead to a subcellular mislocalisation of the protein likely leading to aberrant splicing. Furthermore, our results indicate that the highly conserved amino acids within the RS-domain play different roles in nuclear
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