Results: We identified aberrant RYR2 splicing in the RBM20 mutant myocardium characterised by an increased inclusion of an additional 24bp-exon. Furthermore we found a decrease of the major cardiac splice variant N2BA by real-time PCR and Western Blot. Titin Western Blots revealed an increase of the splice variant N2BA in the RBM20 mutants. Of note, an influence on splicing could be detected not only for the RBM20-p.P638L-mutant but also for the mutation p.PV914A located outside of the conserved RS-domain. The variant p.D888N showed no influence on the splicing of the gene analysed so far. In contrast to the rat hearts, in the human myocardium of RBM20-mutation carriers we did not find splicing defects in TRDN and NEKX.

Conclusion: We present here first data on a molecular defect of a novel RBM20-mutation located outside the conserved RS-region. Furthermore we show the influence of mutant RBM20 on splicing of RYR2 and TTN in the human explanted myocardium of three mutation carriers. The identification of the molecular pathomechanisms of the RBM20-mutations in the affected patients allows a differentiated insight into the disease mechanisms and might offer potential targets for curative treatment in the future. Furthermore the analysis of the splicing pattern helps to distinguish pathogenic variants like RBM20-p.PV914A from rare polymorphisms.

472 The impact of missense versus nonsense mutations in arrhythmogenic cardiomyopathy phenotype

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Background: Arrhythmogenic Cardiomyopathy (AC) is an inherited heart muscle disease associated with mutations in five genes mainly encoding desmosomal components. Missense mutations leading to a single nucleotide substitution have disputed pathogenicity in inherited cardiomyopathies whereas truncating and splice site mutations are considered disease-causing due to their impact on form and function of the resulting protein.

Purpose: Herein we describe the clinical features of two probands and their family members in whom a missense and a nonsense mutation in two desmosomal-encoding genes co-segregate.

Methods: A Next Generation Sequencing-based strategy comprising 150 inherited cardiomyopathy related genes was applied on the two index female cases (63y, 70y). Libraries were prepared according to the manufacturer’s instructions and sequenced on a MiSeq platform (Illumina). The Illumina Variant Studio software was used to filter and prioritize variants on the basis of genotype quality, allele frequencies, predicted pathogenicity, literature information. Subsequent conventional sequencing was used for cascade mutation screening. HL-1 cells have been transfected to ascertain localization of the mutated proteins.

Results: Two unrelated female probands (63 y-old, 70 y-old) carried a missense mutation in DSG2 (p.Asn266Ser). The 63 y-old proband carried an additional nonsense mutation in DSP (c.88G>T:p.Gln113X), as well her 64 y-old sister. Eight of the fifteen relatives from both families carried the DSG2 mutation: seven of them exhibited typical clinical AC features, whereas two young family members were unaffected at the ages of 6 and 12, respectively. The two DSP mutation carriers of family B were unaffected at the age of 44 and 66. No sudden cardiac death events occurred in both families. Haplotype analysis indicated that all the DSG2 mutation carriers shared the same haplotype suggesting a common founder allele. In vitro experiments on the cardiomyocyte cell line HL-1 showed correct localization of the mutated DSG2, whereas the mutated DSP was absent at the membrane level.

Conclusions: This study is calling into question whether nonsense mutations are of high relevance with respect to the missense ones in the pathogenesis of AC. Our results highlight the dominant negative effect of a known missense DSG2 mutation with a presumed common founder effect, whereas the pathogenic role of the DSP nonsense mutation needs further studies to elucidate its contribution to the disease phenotype.