Developmental Basis of Cardiac Inherited Diseases

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470 Extracardiac endothelium patterns embryonic coronary artery-venous connections
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Recent reports suggest that mammalian embryonic coronary endothelium (CoE) develops from the sinus venosus and ventricular endocardium. Although several studies in non-mammalian vertebrates have proven that extracardiac endothelial cells also participate in CoE development, the contribution of such cells to mammalian CoE is regarded to be minor and non-significant. Using classic (WT1Cre) and novel (G2-Gata4Cre) mouse transgenic models for the study of coronary vascular development, we show that extracardiac septum transversum/proepicardium (ST/PE)-derived endothelial cells are required for proper coronary development. Our results indicate that, at least, 20% of embryonic coronary arterial and capillary endothelial cells derive from the ST/PE. Conditional deletion of the epicardial lineage gene WT1 in the ST/PE (G2-Gata4Cre) or the endothelium (Tie2Cre) reveals a critical role for these ST/PE-derived endothelial cells in the establishment of transmural ventricular coronary artery-venous connections. Taken together, our results confirm that embryonic CoE is a development- mental mosaic forming from different endothelial sources, and that extracardiac cells are necessary for the completion of coronary morphogenesis. These data contribute to our understanding of some coronary congenital anomalies and suggest embryonic CoE heterogeneity as an ontogenic factor for adult coronary disease.

471 DCM-associated RBM20-mutations lead to aberrant splicing of titin and ryanodin receptor 2 in the human myocardium
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Background: Mutations in human RBM20 have previously been shown to cause dilated cardiomyopathy (DCM). RBM20 is predominantly expressed in the heart. Within the nucleus it partially colocalises with other splice factors and binds RNA. Deep sequencing of the cardiac transcriptome of a human RBM20 mutant and of a RBM20 deficient rat model revealed RBM20 dependent regulation of myocardial alternative splicing.

Results: We identified the previously published RBM20 mutation, p.(Phe638Leu), which is localised in the highly conserved RS-region, in a large German family. The mutation is highly penetrant leading to terminal heart failure and/or sudden cardiac death. Additionally we have discovered the new RBM20 mutation p.(Val914A) localised outside the RS-region.

Conclusions: Although RBM20 mutants and patients with a RBM20-deficient rat model revealed RBM20-dependent regulation of myocardial alternative splicing, we have identified the previously published RBM20 mutation, p.(Phe638Leu), which is localised in the highly conserved RS-region, in a large German family. The mutation is highly penetrant leading to terminal heart failure and/or sudden cardiac death. Additionally, we have discovered the new RBM20 mutation p.(Val914A) localised outside the RS-region.

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 desmosome-encoding genes co-segregate.

Methods: Sanger sequencing of the two AC-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 underwent cardiac transplantation (family A). The 70-y-old proband carried an additional nonsense mutation in DSP (c.88G>A, 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 56. 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.