Developmental Basis of Cardiac Inherited Diseases

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470 Extracardiac endothelium patterns embryonic coronary artery-venous connections

P. Perez-Pomares1, E. Cano1, R. Carmona3, A. Ruiz-Villalba1, A. Roja1, Y.-Y. Chau1, K.D. Wagner6, N. Wagner6, N.D. Hazely1, R. Muirza-Chapua1

1University of Malaga, Malaga, Spain; 2Max Delbruck Center for Molecular Medicine, Berlin, Germany; 3University of Navarra, Pamplona, Spain; 4Center Andalucia of Biologia Molecular y Medica Regenerativa (CABIMER), Stem Cells, Seville, Spain; 5Western General Hospital, Edinburgh, United Kingdom; 6Institut de Biologia Valrose, Nice, France

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/proepicardial (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 or the endothelium (Tie2Cre) reveals a critical role for these ST/PE-derived endothelial cells in the establishment of transmural coronary arterial-venous connections. Taken together, our results confirm that embryonic CoE is a developmental 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 ontogenetic factor for adult coronary disease.

471 DCM-associated RBM20-mutations lead to aberrant splicing of titin and ryanodin receptor 2 in the human myocardium

A. Gaertner-Rommel1, B. Klauke1, W.A. Linke2, U. Schulz3, K.T. Laser3, J. Gummert3, H. Milting1

1Heart and Diabetes Center NRW, Erich und Hanna Klessmann-Institut für Kardiovaskuläre Forschung und Medicine; 2Max Delbruck Center for Molecular Medicine, Berlin, Germany; 3Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

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.

We have identified the previously published RBM20 mutation, p.P638L, 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.V914A localised outside the RS-region.

Purpose: Though it is investigated that RBM20 is involved in alternative splicing in the rat heart little is known on aberrant myocardial splicing in human RBM20 carriers. Goal of this study was to gain insight into the mechanisms leading to cardiomyopathy by analysing expanded human myocardial myocardium of RBM20-mutation carriers.

Material and Methods: Cardiac tissue from two RBM20-p.P638L- and one RBM20-p.V914A-DCM-patients and a patient with the RBM20-variant p.D88BN was obtained during heart transplantation or the implantation of a ventricular assist device, respectively. Ventricular myocardium was used for RNA extraction, cDNA synthesis and splicing analysis of TTN, RYR2, LDB3, CAMK2D, NEXN and TRDN by Real-Time-PCR. The alternative splicing of Titin was additionally examined by Western Blot using cardiac tissue lysates.

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 splicing variant N2B 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 localised outside of the conserved RS-domain. The variant p.D88BN showed no influence on the splicing of the genes 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 NEXN.

Conclusion: We present here first data on a molecular defect of a novel RBM20-mutation localized outside the conserved RS-region. Furthermore we show the influence of mutant RBM20 on splicing of RYR2 and TTN in the human expanded myocardium of three mutation carriers. The identification of the molecular pathways/mechanisms 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

E. Lazzarini, B. Baue, M. Casoni, R. Calegini, I. Rigato, E. Carturan, S. Rizzo, G. Thiene, C. Bassi, K. Pilichou

University of Padova, Dept. of Cardiac, Thoracic and Vascular Sciences, Padova, Italy

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: 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 underwent cardiac transplantation (family A). The 70 y-old proband carried an additional nonsense mutation in DSP (c.845G>A, p.Gln115X), as well as 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. Haplotyping 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 proteins.

Conclusions: This study is calling into question whether missense 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.

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