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5188 Functional effects of p.D1690N and p.G1748D Nav1.5 mutations detected in compound heterozygosity in a patient with Brugada syndrome

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Purpose: In the present study we identified two compound heterozygous mutations (p.D1690N and p.G1748D) in the gene (SCN5A) encoding the alpha subunit of the cardiac Na^+ channels (Nav1.5) in a proband diagnosed of type 1 Brugada Syndrome. Furthermore, in the allele encoding p.D1690N mutation, the p.H558R polymorphism was also detected. Therefore, we analyzed the functional properties of the mutated channels as well as the putative modulator effects produced by the presence of the polymorphism.
Methods: Native (WT) and mutated human Nav1.5 channels were expressed in Chinese hamster ovary cells (CHO) and studied using the whole-cell patch-clamp.
Results: Separately, both p.D1690N and p.G1748D mutations produced a marked reduction in peak Na^+ current density (by 80% and 92% compared to WT, respectively), which was mainly attributed to their limited trafficking into the membrane. Furthermore, p.G1748D mutation shifted 14 mV rightward both activation and inactivation curves. p.G1748D also accelerated the time course of recovery from fast inactivation thus, p.G1748D profoundly affected the channel gating. Both p.D1690N and p.G1748D produced a marked dominant negative effect when cotransfected (0.5:0.5 ratio) with either WT or p.H558R channels. Indeed, p.D1690N+WT and p.G1748D+WT reduced peak Na^+ current density by 68% and 85%, respectively. Conversely, p.H558R was able to rescue defective trafficking of p.D1690N channels into the membrane when both, polymorphism and mutation, were in the same construct as demonstrated by using confocal microscopy of CHO cells transfected with GFP-tagged-Nav1.5 channels, generating currents that were undistinguishable from those generated by WT. Surprisingly, cotransfection with p.D1690N, either alone or together with the polymorphism (p.H558R-p.D1690N) completely restored the profound gating defects exhibited by p.G1748D channels while only slightly rescued their trafficking.
Conclusion: Our results add further support to the hypothesis that Nav1.5 subunits interact among them before trafficking into the membrane and shows that a missense mutation can "rescue" the defective gating produced by another missense mutation when present in different alleles.

5189 Genome wide association study identifies the kallikrein-kinin system to regulate plasma levels of mid-regional-proadrenomedullin and C-Terminal-proendothelin-1

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Purpose: Endothelin-1 (ET-1) and Adrenomedullin (ADM) are two potent vasoactive peptides circulating in plasma. Elevated levels of Plasma ET-1 and ADM, and their biologically stable surrogates, CT-proET-1 and MR-proADM, are predictors of cardiac death and development of (chronic) heart failure. The mechanisms by which ET-1 and ADM are regulated are largely unknown.
Methods: To gain further insights in ET-1 and ADM regulation we performed a genome wide association study on levels of MR-ProADM and CT-proET1 in 3,444 subjects of European descent with follow-up genotyping in an additional 3,230 subjects.
Results: A variant in KLKB1 and F12, both part of the kallikrein-kinin system, were associated with higher MR-proADM (4.46E-52, 5.90E-24) and higher CT-proET-1 levels (P = 1.23E-122, 1.26E-67). Further epistatic analyses between KLKB1 and F12 showed a significant interaction. In addition, a variant near the ADM gene was associated with MR-proADM (P = 1.05E-12) and a variant in EDN-1 was associated with CT-proET-1 (P = 1.49E-27). The total phenotypic variation explained by the genetic variants was 7.16% for MR-proADM and 14.61% for CT-proET-1. KLKB1 encodes for plasma kallikrein, a proteolytic enzyme known to release bradykinin and renin. The recognition sites of plasma kallikrein sites are also present in the precursors of ADM and ET-1. We cloned the precursor of ADM by in-vitro transcription and demonstrated the ability of plasma kallikrein to cleave the recombinant protein into multiple smaller peptides in-vitro.
Conclusion: We have identified a novel role for the kallikrein-kinin system in the regulation of MR-ProADM and CT-proET1.

NOVEL MECHANISMS AND IMAGING TOOLS IN ATHEROSCLEROSIS

5194 Impaired neointima formation in mice lacking the coronary artery disease risk gene ADAMTS-7 after cessation of blood flow

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Purpose: ADAMTS-7 has been identified as a coronary artery disease (CAD) risk gene in genome-wide association studies (GWAS). Interestingly, ADAMTS-7 plays rather a role in plaque formation than rupture, as hypothesized by a GWAS subgroup analysis. ADAMTS-7 is involved in rheumatoid arthritis pathogenesis, where it has been shown to degrade cartilage oligomeric matrix protein (COMP). Thus far, the role of ADAMTS-7 in atherosclerosis remains elusive. In this study, we investigated Adamts-7 in a murine knockout (KO) model regarding remodeling of injured arteries and metabolic parameters.
Methods: Adamts-7-KO-mice were generated by interrupting the Adamts-7 gene with an internal ribosome entry site followed by the beta-Gal sequence and a neomycin cassette. The transgene was confirmed using PCR, RT-PCR and LacZ-staining of various tissues. Phenotyping was carried out using common carotid artery ligation (CCAL). Ten days after ligation occluded and sham-treated vessels were harvested and morphometrically analyzed. COMP was detected by immunofluorescence in injured and sham-treated arteries. To investigate metabolic effects, KO- and WT-mice were fed a Western diet for 15 weeks. Weight was assessed weekly, and blood lipid levels before and after the diet. Atherosclerotic lesions at the sinus aortae were quantified after Oil-Red-O-staining.
Results: Adamts-7-KO-mice appeared normal in growth and behavior. After CCAL, arteries of WT-mice displayed significant increases of media surface area (MA), intima surface area (IA) and intima-media-ratio (IMR) compared to sham-treated vessels. By contrast, CCAL failed to induce neointima formation in Adamts-7-KO-mice. IA, MA, external elastic lamina circumference and IMR were all significantly lower in KO-mice. Immunofluorescence directed against COMP only revealed weak fluorescence in injured vessels of WT-mice, whereas no difference between the vessels was visible in KO-mice. After Western diet, blood lipid levels increased in both WT-and KO-mice. Thus, significant differences in weight gain, blood lipid levels or lipid deposition measured as lesion area were not observed between WT- and KO-mice.
Conclusions: We conclude that ADAMTS-7 plays an important role in the pathophysiology of CAD as it seems to be pivotal for the remodeling of arteries following vascular injury. The degradation of COMP may be part of the downstream signalling pathway. Since lack of Adamts-7 did not alter metabolic parameters or lipid deposition, we suggest that the association of ADAMTS-7 and CAD involves a remodelling mechanism in the vessel wall itself.

5195 Adventitial inflammation is associated with thin-cap atheromas and expression of matrix-degrading enzymes and occurs in coronary regions exposed to low endothelial shear stress

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Purpose: Vascular inflammation, a critical component of atherosclerosis, is thought to originate from the lumen and infiltrate the intima-media in advanced lesions. Low endothelial shear stress (ESS) is known to induce intima-media inflammation and plaque growth. In this study we investigated in vivo the role of adventitial inflammation in atherosclerosis and its relation to local ESS.

Abstract 5189 – Table 1. Summary associated loci

Locus	Trait	CHR	SNP	A1/A2	Discovery, N = 3,230			Replication, N = 3,444		Combined,N=6,674	
					Beta (se)	P	FRQ	Beta (se)	P	P-value	Nearest gene
1	ADM	4	rs4253238	C/T	0.027 (0.003)	6.93E-24	0.49	0.034 (0.003)	2.76E-34	4.46E-52	KLKB1*
	ET	4	rs4253238	C/T	4.811 (0.305)	4.07E-54	0.49	5.476 (0.312)	5.49E-66	1.23E-122	KLKB1*
	ADM	4	rs3733402	G/A	NA	NA	NA	0.035 (0.003)	1.88E-35	2.71E-36	KLKB1*
	ET	4	rs3733402	G/A	NA	NA	NA	5.501 (0.314)	1.37E-65	1.41E-68	KLKB1*
2	ADM	5	rs2731672	T/C	0.024 (0.003)	7.07E-14	0.24	0.020 (0.003)	6.19E-10	5.90E-24	F12*
	ET	5	rs2731672	T/C	5.041 (0.375)	3.85E-40	0.24	4.178 (0.375)	2.92E-28	1.26E-67	F12*
3	ET	6	rs5370	T/G	2.928 (0.379)	1.38E-14	0.22	2.983 (0.390)	2.49E-14	1.49E-27	EDN1*
4	ADM	11	rs2957692	G/A	0.017 (0.003)	2.46E-08	0.41	0.013 (0.003)	9.87E-06	1.05E-12	ADM*