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PS212.
Increased CircularRNA-16 in Acutely Symptomatic Carotid Plaques: A Novel Mediator of Carotid Plaque Rupture
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Objectives: Circular RNAs (circRNAs) are dynamically expressed during development and possess binding sites for microRNAs (miRs), small RNAs that negatively regulate gene expression. We recently demonstrated that miR-221, which is associated with vascular smooth muscle cell (VSMC) proliferation and inhibition of apoptosis, is decreased in acutely symptomatic carotid plaques. Because circRNA-16 possesses binding sites for miR-221 through seed sequences found within, we hypothesized that circRNA-16 is increased in acutely symptomatic carotid plaques.

Methods: Relative changes in gene expression levels of circRNA-16 were compared using a real-time polymerase chain reaction (PCR) assay and the ΔΔCt method. All samples were run in duplicate; mean and standard error were calculated. One-way analysis of variance with the Tukey test was used to determine significance between groups.

Results: Expression of circRNA-16 was confirmed in human VSMC using PCR and resistance to RNase H. To investigate its role in carotid plaque rupture, levels of circRNA-16 were quantified in patients undergoing urgent carotid endarterectomy for acute neurologic symptoms (n = 27), compared with asymptomatic carotid plaques (n = 19). In contrast to miR-221, circRNA-16 is increased in the urgent group compared with the asymptomatic carotid plaque group (1.51 ± 0.26 vs 1.00 ± 0.10, P = .03; Fig).

Conclusions: We demonstrate circRNA-16 levels are increased and miR-221 levels decreased in acutely ruptured carotid plaques. Furthermore, our data suggest that a circRNA-16/miR-221 axis may be important in fibrous cap degradation and rupture during the transition from a stable to an unstable carotid atherosclerotic plaque.

Author Disclosures: H. A. Bazan: Nothing to disclose; D. Lightell: Nothing to disclose; W. Sternbergh: Nothing to disclose; T. Woods: Nothing to disclose.

PS214.
Agreement of Repeatability Coefficients for Within-Subject Carotid Artery Velocities with Snoring Application
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Objectives: To determine (1) within-subject variance in carotid artery velocities as measured by ultrasound (US) and (2) if snoring significantly alters carotid velocities.

Methods: Eight individuals underwent 32 bilateral carotid artery US exams by two Registered Vascular Technologist (RVTs; four exams/subject, four subjects/RVT). Steps were taken to ensure measurements were made at identical sites under similar parameters for subsequent exams. Peak systolic velocity (PSV) and end-diastolic velocity (EDV) were measured at eight locations. For each location, limits of agreement was used to calculate PSV and EDV repeatability coefficients, which are the limits within which 95% of the differences will lie for two measurements made on the same subject. Repeatability coefficients were then used to determine significance for observed velocity changes in carotid arteries with and without stenosis during mock snoring. They were also used to access velocity variability in patients scheduled for carotid endarterectomies (CEAs). All US exams were performed in an Intersocietal Accreditation Commission vascular testing accredited laboratory by RVTs.

Results: Repeatability coefficients for RVT A ranged from 34 to 44 cm/s for PSV and 10 to 22 cm/s for EDV. For RVT B they ranged from 30 to 65 and 8 to 19 cm/s for PSV and EDV, respectively. Maximum values occurred on the ostia of the internal carotid artery (ICA) and in the proximal ICA for RVTs A and B, respectively. In nonstenosed arteries, snoring most frequently caused a significant change in the PSV of the proximal ICA and in the EDV of the mid-ICA where it occurred 15% of the time (three of 20 arteries). However, the effect of snoring was greatest in stenosed arteries. In 38% (three of eight) snoring caused a significant increase in the PSV and EDV of the proximal ICA and in the PSV of the mid-ICA.

Conclusions: Repeatability coefficients can be used to determine significant changes in carotid artery velocities within stenosis categories as measured by US. There is a possible connection between snoring, carotid velocities, and stenosis that warrants further investigation to determine if it is part of the mechanism by which snoring may increase stroke risk.
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PS216.
Zizimin1 Overexpression Impairs Vascular Morphogenesis
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Objectives: The Rho subfamily of small GTPases, including RhoA, Rac1, and Cdc42, regulates diverse cellular functions, including polarity, migration, and actin-based cytoskeleton dynamics. Our prior studies established an essential role for Cdc42 in vascular network assembly, demonstrating that the genetic inactivation of Cdc42 yields defective vascular morphogenesis due to impaired migration of endothelial precursor cells. We have further shown that protein kinase Giota and glycogen synthase kinase-3 have further shown that protein kinase Ciota and glycogen synthase kinase-3β are downstream effectors of Cdc42 and are involved in mediating vascular network assembly. However, the guanine nucleotide exchange factors (GEFs) that activate Cdc42, remain unknown.

Methods: We performed affinity pulldown assays using a nucleotide-free Cdc42 G15A mutant that specifically binds to Cdc42 GEFs. Mass spectrometric analysis identified Zizimin1, an upstream regulatory protein, as a candidate Cdc42 GEF.

Results: During vasculogenesis in embryoid bodies (EBs) differentiated from embryonic stem cells, Zizimin1 is highly expressed in aggregated endothelial cell precursors before vascular network formation. Surprisingly, stable overexpression of Zizimin1 in EBs resulted in the inhibition of blood vessel formation compared with control, evidenced by immunohistochemistry demonstrating loss of vascular network development. Affinity pulldown assay helped to elucidate that overexpression of Zizimin1 increases Cdc42 activity; however, the activation of Rac1 and RhoA is significantly inhibited.

Conclusions: Because Rac1 and RhoA signaling has been reported to play an essential role in embryonic blood vessel formation, our results suggest that the interplay between Rho GTPases guides vascular network assembly during development. Furthermore, these findings provide novel insights into the mechanisms of embryonic vasculogenesis and also important new information for the design of potential proangiogenic and/or antiangiogenic therapies.

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C8j: Poster Session—Research (2)
PS218.
Plasma and Patterning: The New Focus for the Development of Nanocomposite Vascular Grafts
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Objectives: Despite prophylactic antibiotics and aseptic technique, prosthetic graft infections continue to cause significant morbidity and mortality. In contemporary reports, in vivo models have tested a conduit’s infectability using concentrations from 104 to 109 colony-forming units (CFU) per mL. Using an in vitro model, we evaluated the impact of inoculation concentrations on prosthetic graft attachment.

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PS220.
Quantitative In Vitro Model for the Study of Bacterial Attachment on Vascular Conduits
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