
**22-VIRC-470-AHA-VD**

**Genetic Ablation of CCDC92 Protects against Vascular Inflammation and Atherosclerosis**

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**Background:** Genome-wide association studies have uncovered specific genetic variants of the coiled-coil domain containing 92 (CCDC92) gene that are associated with coronary artery disease and type 2 diabetes. However, the biological function of CCDC92 in cardiovascular disease remains unclear. This study aims to investigate the impact of CCDC92 on atherosclerosis, a leading cause of cardiovascular disease, and dissect underlying mechanisms.

**Methods:** To investigate the effects of CCDC92 on atherosclerosis development in vivo, we used Ccdc92 knockout (KO) mice and littermate wild-type mice (in an Apoe KO background) fed a high-cholesterol diet for 18 weeks. To provide a comprehensive characterization of cellular identity within the atherosclerotic lesion, we applied single-cell sequencing to the whole atherosclerotic aorta from Ccdc92 KO/ApoE KO and littermate control mice on high-cholesterol diet for 12 weeks.

**Results:** Ccdc92 KO significantly reduced atherosclerotic plaque by sixty percent compared with littermate control mice measured by en face analysis of atherosclerotic lesions in the aortic tree after Oil Red O staining (n = 11-12/group; P < 0.01). The single-cell sequencing analysis identified 12 cell populations in the atherosclerotic aorta. Cell-specific gene set enrichment analysis further indicated that Ccdc92 deficiency had protective effects on multiple vascular cell populations. Specifically, in populations of endothelial cells (ECs), CCDC92 regulates cell inflammation and fatty acid metabolism. Using human coronary artery ECs and mouse aortic EC from Ccdc92 KO and wild-type mice, we demonstrated that deletion of CCDC92 significantly inhibited proinflammatory adhesion molecules and cytokines induced by tumor necrosis factor-α. Interestingly, adenosine-mediated CCDC92 overexpression increased lipid droplet accumulation (to an average of four-fold increase) in human coronary artery ECs exposed to oleic acid overload.

**Conclusions:** Our findings revealed the proatherogenic effects of CCDC92 in vivo and demonstrated a critical role of CCDC92 in EC dysfunction during atherosclerosis development. These data add an important new mechanism associated with atherosclerosis and provide a potential new target for atherosclerotic disease.

**Author Disclosures: W. Du: Nothing to disclose. D. Song: Nothing to disclose. L. Ren: Nothing to disclose. R.C. Becker: Other, Basking Biosciences, Ionis, Novartis, Bayer. Y. Fan: Nothing to disclose.**

**22-VIRC-508-AHA-VD**

**Mangiferin Conjugated Gold Nanoparticles Protect against the Development of Abdominal Aortic Aneurysm in an Apoe−/− Mouse Model**

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**Background:** There are no drugs to prevent the growth of abdominal aortic aneurysm (AAA), responsible for approximately 200,000 deaths in the world each year. There is a growing interest in natural anti-inflammatory compounds using nanotechnology-based drug delivery. Mangiferin (MCF) is one such phytochemical isolated from Mangifera indica. Conjugation of MCF on gold nanoparticles (MCF-AuNPs) enhances bioavailability, through cellular penetration, presenting new opportunities toward the design of innovative nanomedicine agents. Recently, we have demonstrated the unique applications of MGF-AuNPs as an immunomodulatory therapeutic agent in the treatment of metastatic breast and prostate cancers. Here we investigated whether MCF-AuNPs prevent the development of AAA.

**Methods:** Apo−/− mice were subjected to angiotensin (AngI, 1 µg/mg) (kg)-induced AAA. MCF-AuNPs (approximately 7 mg/mg) mouse) or starch (5)-AuNPs were administered daily, a week before AngI and continued for 28 days (n = 8-12 per group).

**Results:** The incidences of AAA were significantly attenuated with MCF-AuNPs than AngI group (P < 0.01), associated with a decrease in maximal inner-luminal diameter (P < 0.001), pulse wave velocity (P < 0.001), distensibility (P < 0.05), and radial strain (P < 0.05). Degradation of elastin (P < 0.001) and proinflammatory cytokines (P < 0.01) and apoptotic cell death (P < 0.01) were significantly reduced in the aorta of MCF-AuNPs-treated Apo−/− mice than the AngI group.
group. Mechanistically, Notch1, its ligands Jag1, Dll4 and downstream targets (HeyL, Nfibs, pStats3), were significantly reduced by MCF-AuNPs in the aorta (P < 0.01) than the Angi1 group. In the macrophages overexpressed with activated Notch1 (NICO), MCF-AuNPs significantly diminished the expression of Jag1 (P < 0.01), HeyL (P < 0.01), I6P (P < 0.01), and Nfib (P < 0.001). MCF-AuNPs also prevented the nuclear translation of NICD and its downstream effector pStats3 in the macrophages transfected with NICD plasmid.

Conclusions: Our studies provide compelling preclinical evidence of the protective effects of MCF-AuNPs on AAA development through inactivation of Notch1 signaling, thus present realistic potential toward clinical translation of this nanocarrier for use as a noninvasive effective treatment for AAA.


22-VIRC-501-AHA-VD

Interleukin-6 Levels and Cardiovascular Events in the Cardiovascular Inflammation Reduction Trial: Consistent Associations for Incident Coronary, Cerebrovascular, and Peripheral Artery Disease

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Background: Inflammation is causally related to atherothrombosis. Interleukin-1β (IL-1β) and IL-18 require NLRP3 inflammasome for activation and have downstream effects on IL-6, a marker previously associated with high risk of coronary artery and cerebrovascular disease (CCVD). However, data pertaining to peripheral artery disease (PAD) are sparse and could offer druggable targets in this disease.

Methods: We conducted a prospective cohort study of 4248 patients with type 2 diabetes or metabolic syndrome and prior coronary artery disease who participated in the National Institutes of Health-funded Cardiovascular Inflammation Reduction Trial. Participants were followed for up to 5 years for incident CCVD and symptomatic PAD events. Randomized treatment with low-dose methotrexate vs placebo had no effect on event rates or plasma levels of inflammatory biomarkers. Baseline levels of IL-1β, IL-18, and IL-6 were tested for association with incident vascular events. Kaplan-Meier curves and Cox proportional hazards models (adjusted for traditional risk factors) were estimated.

Results: In multivariable adjusted analyses, hazard ratios for the lowest (referent) to highest baseline quartiles of IL-6 were 1.0, 1.5, 1.8, and 2.0 (P-trend < 0.001) and 1.0, 1.2, 2.5, and 2.0 (P-trend = 0.04) for incident CCVD and PAD (n = 349) and PAD (n = 87), respectively. Baseline IL-6 levels above versus below the median (2.50 pg/mL) were associated with a 56% increased risk of CCVD (hazard ratio, 1.56; 95% confidence interval, 1.26-1.93) and a 113% increased risk of PAD (hazard ratio, 2.13; 95% confidence interval, 1.36-3.32) (Figure). Baseline levels of IL-1β and IL-18 did not associate with incident CCVD or PAD.

Conclusions: In this contemporary cohort of secondary prevention patients, elevated IL-6 was associated with both incident CCVD and PAD. These data support exploration of direct IL-6 inhibition for PAD prevention, a strategy currently being pursued to reduce risk of coronary artery and cerebrovascular disease.


22-VIRC-621-AHA-VD

Trpm2 Deficiency in Macrophages Protects Mice against Atherosclerosis

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Background: Atherosclerosis and its major complications, ischemic heart diseases, and stroke are the leading causes of mortality worldwide. The prominent feature of atherosclerosis is the formation of foam cells, the infiltrated macrophages overloaded with lipids. Lipid uptake by macrophages is mainly mediated by CD36. Previously we reported that (1) Global Trpm2 deletion attenuates atherosclerosis and (2) TRPM2 and CD36 enhance the activation of each other in macrophages, thereby promoting foam cell formation. Thus, we hypothesized that macrophage specific Trpm2 deletion protects mice against atherosclerosis.

Methods: Macrophage-specific Trpm2 deletion in was achieved by crossing Apoe^{-/-}Trpm2^{floxed} mice with Cdl1b-cre mice. A high-fat diet was used to induce atherosclerosis, and total serum cholesterol level was measured. Oil Red O (ORO) staining was used to evaluate atherosclerotic lesion size. Immunofluorescence staining of Mac-1, F4/80, and CD80 was used to examine the macrophage content in the plaques, which was further confirmed by flow cytometry analysis of the immune cell populations in digested aorta. Inflammation of the aorta was examined by WB analysis of expression of NLRP3, ASC, caspase-1, IL-1β, macrophage chemokine protein-1, and migration-inhibitory factor.

Results: Macrophage-specific Trpm2 deletion (1) did not influence the serum total cholesterol level, (2) decreased plaque lesion ratio as shown by en face aorta ORO staining and reduced plaque size as shown by aortic root ORO staining, (3) reduced macrophage content and immune cell infiltration in the plaques, and (4) inhibited NLRP3 in mice.

Conclusion: Macrophage-specific Trpm2 deletion attenuates High-fat diet-induced atherosclerosis in Apoe^{-/-} mice.