

PROJECT TITLE **Crosstalk of Aging and Metabolic Syndrome in Atherosclerosis**
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ABSTRACT OF PROJECT

Metabolic syndrome (MetS), a cluster of modifiable cardiovascular (CV) risk factors including hyperglycemia, visceral obesity, and dyslipidemia, significantly accelerates atherosclerosis, amplifying the risk of cardiovascular morbidity and mortality in older adults with MetS. Aging and MetS share overlapping pathogenic mechanisms that can synergistically accelerate atherosclerosis. Despite its clinical importance, the molecular mechanisms by which MetS drives age-related vasculopathy are incompletely understood. The proposed project is part of a larger research initiative to uncover novel molecular pathways and protein networks that drive accelerated atherosclerosis in MetS as a function of age. Using a mouse model of combined MetS and atherosclerosis developed in our lab, we will study the interaction of aging with MetS on atherosclerotic lesion formation. Specifically, MetS and non-MetS mice (both sexes) on standard laboratory diet will be aged to 6 months and 1 year, followed by animal harvests for collection of plasma, heart and aortic vessels. The summer student working on this project will perform histological staining of aortic root sections to assess the severity of atherosclerotic lesions in our mice genotypes, including data analysis and data interpretation. The student will also attain familiarity with the relevant scientific literature in the field.

BACKGROUND AND SIGNIFICANCE

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, accounting for 19.9 million global deaths in 2021. Mortality rates due to CVD increase exponentially with age, accounting for >40% deaths in individuals aged 65–74 and nearly 60% in those aged 85 and older. Metabolic syndrome (MetS), defined by a cluster of conditions that include central obesity, hyperglycemia and dyslipidemia, significantly increases the risk of atherosclerotic cardiovascular disease. MetS patients have 2-4-fold greater risks of developing atherosclerotic complications.

Aging and MetS share overlapping pathogenic mechanisms that synergistically accelerate atherosclerosis. Aging promotes vascular cell senescence and immune dysregulation, creating a pro-inflammatory milieu that destabilizes plaques. Hyperglycemia and insulin resistance, characteristic of MetS, exacerbate these processes, creating a highly pro-atherogenic environment. Both MetS and aging amplify oxidative stress, impair endothelial function and reduce nitric oxide bioavailability. This interplay leads to accelerated plaque growth, calcification, and plaque instability, significantly increasing the cardiovascular risk in older adults with MetS. Despite the clinical significance of this intersection, the molecular pathways linking aging and MetS in atherosclerosis remain incompletely understood, highlighting a critical gap for targeted interventions.

The proposed work is part of a larger research initiative to elucidate the molecular mechanisms by which aging accelerates atherosclerosis in MetS.

GOALS AND OBJECTIVES

Goal: To determine the impact of aging on atherosclerotic lesion formation in a mouse model of combined MetS and atherosclerosis (KKAy^{+/-}ApoE^{-/-}) vs. non-MetS atherosclerotic mice (KKAy^{-/-}ApoE^{-/-}).

Objectives:

1. To compare body weight, blood glucose, plasma cholesterol and triglyceride levels in MetS atherosclerotic vs non-MetS atherosclerotic mice at 6 months and 1 year of age.

2. To compare and quantify atherosclerotic lesions in MetS atherosclerotic vs non-MetS atherosclerotic mice at 6 months and 1 year of age.

Experimental design: We will use a mouse model of combined MetS and atherosclerosis developed in our lab by crossing male agouti KKAY^{+/+} (a murine model of MetS) with female ApoE^{-/-} mice (a murine model of atherosclerosis). Upon weaning (4-wks-age), mice will be randomly allocated to experimental groups: a) 6-month-old MetS KKAY^{+/+}ApoE^{-/-}, b) 6-month-old non-MetS KKAY^{-/-}ApoE^{-/-}, c) 1-year-old MetS KKAY^{+/+}ApoE^{-/-} and d) 1-year-old non-MetS KKAY^{-/-}ApoE^{-/-}. Mice will be maintained on a standard laboratory diet for the duration of the study. Body weight and non-fasted blood glucose levels will be monitored monthly. At study endpoints (6 months and 1 year of age), mice will be euthanized following overnight fasting; blood, heart and aortic vessels will be collected for molecular studies. Both male and female mice will be utilized in this study.

We have already completed the animal experiments described above and all tissue samples collected from our mouse genotypes are already in hand. Due to the limited time frame of this fellowship, the summer student will focus on the analysis of blood and tissue samples previously collected from our mouse genotypes.

INVESTIGATIVE METHODS TO BE USED

Analyses of Plasma samples: Plasma triglycerides and total cholesterol levels will be measured using standard enzymatic kits (purchased from Fisher, Biovision, Sigma).

Aortic root morphometry: Aortic root sections (8–10-micron thickness) of formalin-fixed, OCT-embedded frozen hearts will be cut at the point where valve leaflets are first evident. Tissue sections will be stained with Oil Red O, H&E and Masson Trichrome to measure lipid burden, plaque area and collagen content, respectively. Image capture will be accomplished using an automated Slide Scanner.

Immunofluorescent staining of aortic root sections: will be conducted to detect lesion SMC abundance, immune cell infiltration and cell proliferation, per established protocols. Briefly, sections will be co-stained with i) ACTA-2 and Galectin-3 to detect inflammatory VSMCs and ii) ACTA-2 and Ki67 to detect proliferative VSMCs. Images will be captured via confocal microscopy.

PROPOSED METHOD OF DATA ANALYSIS

Morphometric and immunofluorescent images will be analyzed using ImageJ software. We will obtain multiple images from tissues collected from 3-5 mice per genotype for each sex and age group, which should be sufficient to visualize changes between the groups and determine whether the expected differences are evident. Data from each group will be compiled, plotted and compared across all conditions. All data sets will be checked for normality and homogeneity of variance, followed by appropriate statistical analyses (parametric or non-parametric). For parametric data, statistical significance will be analyzed by one-way or two-way ANOVA followed by Tukey HSD pos-hoc or unpaired two-tailed Student's t-test, as appropriate. For non-parametric data, Kruskal-Wallis followed by Dunn's post-hoc or Mann Whitney U/Wilcoxon Sign Rank tests will be used, as appropriate; $p \leq 0.05$ is considered statistically significant.

SIGNIFICANCE OF ANTICIPATED FINDINGS

Expected Outcome. We predict that aging will significantly accelerate the development and progression of atherosclerotic lesions in MetS mice. If time and resources permit, aortic vessels derived from our mice genotypes will be used in proteomics profiling to determine the link between lesion formation and differentially expressed proteins in MetS vs non-MetS mice as a function of age.

Impact. The overarching goal of this study is to uncover novel molecular pathways and protein networks, including protein-protein interactions, that associate with accelerated atherosclerosis in MetS as a function of age. Findings from this study will inform our future research directions and lay a foundation for additional

mechanistic studies to delineate the molecular interplay between aging and MetS in atherosclerotic vascular disease.

SUMMER RESEARCH FELLOW TRAINING/MENTORING PLAN

The proposed study will offer research and training opportunities for two students, and we anticipate that these students will work concurrently on this project. Therefore, we request support for two summer students for this study.

Plan for Training/Mentoring: The summer research fellows will be supervised and mentored by Dr. Priya Raman. The students will receive hands-on training from Dr. Raman and her laboratory personnel during the first 2-3 weeks of the program. Upon demonstration of adequate independence, the summer fellows will be expected to run independent experiments under close supervision by Dr. Raman and her team. Dr. Raman will meet with the summer students weekly to discuss the proposed research question, relevant scientific literature, and progression of experiments and data and will also provide necessary guidance on the approaches proposed in the project. Dr. Raman will be responsible for student training in all aspects of this project, including data analysis, graphical presentations, interpretation of data and poster preparation and presentation. The summer fellows will also receive training in critically reading the scientific literature relevant to the project. Dr. Raman is a member of the HBVD and DOM RFAs and the summer students will be expected to participate in weekly group meetings organized by the HBVD and DOM RFAs. These meetings will develop the student's research horizons and enhance his/her scientific presentation and perception skills. At the end of the training period, the students will be expected to submit a brief report summarizing the project and results and present their data during NEOMED's Annual Poster Day.

Description of Resources available: The summer students will have access to Dr. Raman's laboratory, histology core, microscopes, necessary supplies and other department core facilities, as needed.

Site where the research will be conducted: This project will be conducted in Dr. Raman's laboratory at NEOMED.