Association between Increased Vascular Density and Loss of Protective RAS in Early-Stage NPDR

Krishnan Radhakrishnan1,2, Sneha Raghanundan3, Ruchi J. Vyas3, Amanda C. Vu4, Douglas Bryant5, Duan Yaqian5, Brenda E. Knecht5, Maria B. Grant1,6, KV Chalam2, Patricia Parsons-Wingerter3

1Clinical Epidemiology Research Center, CT Healthcare System, U.S. Department of Veterans Affairs, West Haven CT; 2Department of Internal Medicine, College of Medicine, University of Kentucky, Lexington, Kentucky; 3Space Life Sciences Research Branch, Ames Research Center, National Aeronautics and Space Administration (NASA), Moffett Field CA; 4NASA SLSTP Summer Internship Program & Department of Biomedical Engineering, University of California, Berkeley CA; 5Department of Ophthalmology, The Eugene and Marilyn Glick Eye Institute, Indiana University School of Medicine, Indianapolis, IN; 6Department of Integrative and Cellular Physiology, Indiana University School of Medicine, Indianapolis, IN; 7Department of Ophthalmology, University of Florida, Jacksonville, Florida

PURPOSE
Our hypothesis predicts that retinal blood vessels increase in density during early-stage progression to moderate nonproliferative diabetic retinopathy (NPDR). The prevailing paradigm of NPDR progression is that vessels drop out prior to abnormal, vision-impairing regrowth at late-stage proliferative diabetic retinopathy (DR). However, surprising results for our previous preliminary study1 with NASA's VESSEL GENERATION Analysis (VESGEN) software showed that vessels proliferated considerably during moderate NPDR compared to dropout at both mild and severe NPDR. Validation of our hypothesis will support development of successful early-stage regenerative therapies such as vascular repair by circulating angiogenic cells (CACs). The renin-angiotensin system (RAS) is implicated in the pathogenesis of DR and in the function of CACs, a critical bone marrow-derived population that is instrumental in vascular repair.

METHODS
Arterial and venous patterns were extracted from images of 6 normal control subjects and 3 early NPDR subjects (mild and moderate) obtained by Heidelberg Spectralis® 30 degree imaging following fluorescein angiography (FA). The binary vascular patterns were mapped by VESGEN to yield branching generations (G) and quantified that include densities of vessel length (L), area (A) and number (N). Peripheral blood of diabetics and controls was collected for CD34+ CAC isolation. RAS gene expressions in CACs were measured by qPCR for Mas receptor (MasR) and the protective RAS at this stage of DR. NPDR was associated with CD34+ CAC migratory dysfunction toward CXCL12, which was corrected with Ang-(1-7) pretreatment prior to CXCL12 exposure.

RESULTS
By VESGEN analysis, vessel density measured by L, A, and N in early NPDR was greater than in normal retina (Figure 1). For example, L was 2.00 ± 0.06E-2 px/px2 in NPDR veins for all branching generations compared to 9.85 ± 0.68E-3 px/px2 in control, and 1.64 ± 0.13E-2 px/px2 compared to 9.18 ± 0.99E-3 px/px2 in arteries. Results, which are slightly updated from our abstract submission, were confirmed by other parameters such as A and N. The expression of Mas in CACs was reduced in NPDR relative to control, indicating possible loss of compensation of the protective RAS at this stage of DR. NPDR was associated with CD34+ CAC migratory dysfunction toward CXCL12, which was corrected with Ang-(1-7) pretreatment prior to CXCL12 exposure.

CONCLUSIONS
For our ongoing longitudinal study, preliminary evidence by VESGEN indicates that vascular density increased in early NPDR compared to normal retinas. The results are the first independent confirmation of our previous study1. If validated by more complete analysis, the VESGEN discovery is potentially of value for determining optimal therapies at early stages of NPDR, when regenerative vascular treatments are more likely to be successful. These data further support the protective RAS axis within diabetic CACs is lost early in DR and is associated with increased vascular remodeling evidenced by VESGEN analysis.