

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

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## 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 study<sup>1</sup> 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

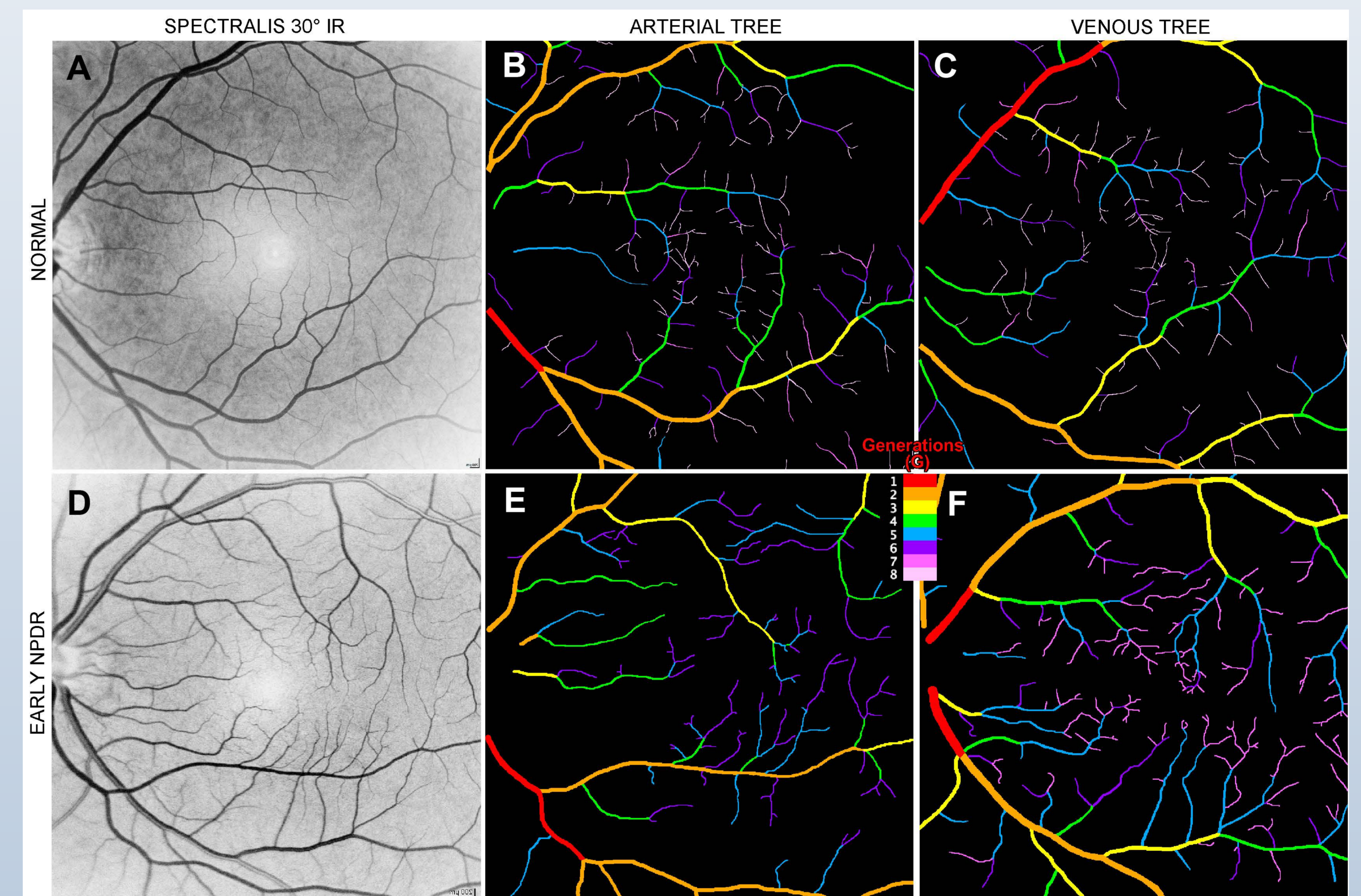
Arterial and venous patterns were extracted from images of 6 normal control subjects and 3 early NPDR subjects (mild and moderate) obtained by Heidelberg Spectralis® 30 degree imaging following fluorescein angiography (FA). The binary vascular patterns were mapped by VESGEN to yield branching generations ( $G_v$ ) and quantified by parameters that include densities of vessel length ( $L_v$ ), area ( $A_v$ ) and number ( $N_v$ ). Peripheral blood of diabetics and controls was collected for CD34<sup>+</sup> CAC isolation. RAS gene expressions in CACs were measured by qPCR for Mas receptor for Ang-(1-7). Vasoreparative function of CACs was assessed by migration ability toward CXCL12 (SDF-1) using QCM 5 $\mu$ M 96-well chemotaxis cell migration assay.

## RESULTS

By VESGEN analysis, vessel density measured by  $L_v$ ,  $A_v$ , and  $N_v$  in early NPDR was greater than in normal retina (Figure 1). For example,  $L_v$  was  $2.00 \pm 0.06E-2$  px/px<sup>2</sup> in NPDR veins for all branching generations compared to  $9.85 \pm 0.68E-3$  px/px<sup>2</sup> in control, and  $1.64 \pm 0.13E-2$  px/px<sup>2</sup> compared to  $9.18 \pm 0.99E-3$  px/px<sup>2</sup> in arteries. Results, which are slightly updated from our abstract submission, were confirmed by other parameters such as  $A_v$  and  $N_v$ . 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<sup>+</sup> 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 study<sup>1</sup> 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 suggest the protective RAS axis within diabetic CACs is lost early in DR and is associated with increased vascular remodeling evidenced by VESGEN analysis.



**Figure 1 Increased Vascular Density in Early-Stage NPDR**

Vessel density increased in both arteries and veins during early NPDR (a-c) compared to normal patients (d-f) in vascular trees analyzed by NASA's VESGEN software extracted from 30 degree Spectralis® fluorescein images. Branching generations in the arterial and venous trees (pseudo-colored per legend for Branching Generations,  $G_1$ - $G_7$ ) were automatically analyzed by VESGEN according to physiological vascular rules. All mapped vessels were enlarged slightly to visualize the small vessels. Vessel density was quantified by VESGEN parameters such as densities of vessel length ( $L_v$ ), area ( $A_v$ ) and number ( $N_v$ ). For example,  $L_v$  was  $2.00 \pm 0.06E-2$  px/px<sup>2</sup> in NPDR veins for all branching generations compared to  $9.85 \pm 0.68E-3$  px/px<sup>2</sup> in controls, and  $1.64 \pm 0.13E-2$  px/px<sup>2</sup> compared to  $9.18 \pm 0.99E-3$  px/px<sup>2</sup> in arteries. Results were confirmed by other parameters that include  $A_v$  and  $N_v$ .

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