# Efficient Hemangioma Formation in Virally-Transduced *VHL* Floxed Mice is Serotype-Dependent

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## PURPOSE

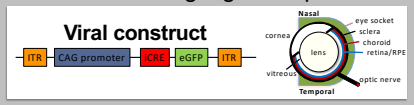
This study seeks to establish a rigorous mouse model of retinal hemangiomas using viral transduction of Cre recombinase in *VHL* floxed mice.

## BACKGROUND

- Targeted deletion of *VHL* in mouse melanocytes, rod photoreceptors, retinal neuronal progenitors, astrocytes and hematopoietic cells has not produced a robust mouse model with phenotypes closely mimicking hemangiomas in patients.
- Major characteristics are highly vascular regions associated with giant foamy cells at late stages, dilated "feeder" blood vessel and exudation
- Cre expression driven by the *SCL* promoter exhibit prominent vasculature, hemorrhage, exudates, anomalous capillary networks and localized fibrosis. Wang et al., 2018. Cancer Res 78(12):1266
  - Occasional "tumorlet" (foamy stromal cells) and "tumorlet" cell clusters were observed

## METHODS

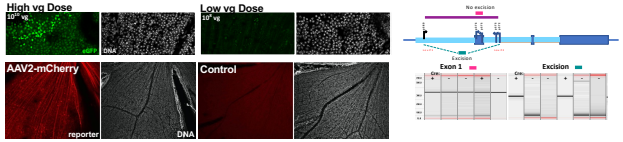
- **Virus:** "Agnostic" expression or Cre recombinase and eGFP or eGFP only (control) was directed using a general promoter. AAV serotypes 2, 6 or 9 were injected into the vitreous.




- ***in vivo* imaging:** Fluorescence Angiography (FA, Micron III), indirect ophthalmoscopy (IO)
- **Immunofluorescence (IF)**, blood vessels with stained with isolection IB4, microglia with anti-Iba1 and viral expression by intrinsic fluorescence or anti-GFP
- **Microscopy**, retinal sections or whole mounts with hematoxylin/eosin or IF were imaged on a light or Fluoview confocal microscope, respectively

## RETINAL SPECIFIC EXPRESSION OF CRE RECOMBINASE

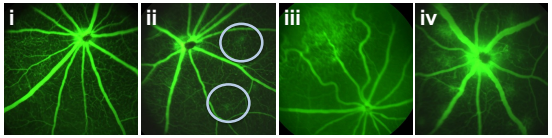
**B. Viral gene expression**




**Viral expression was observed at the injection site 3 days post-injection and broadly at 4 weeks in retinal whole mount IF for the reporter or PCR for loss of the floxed genomic DNA B).**

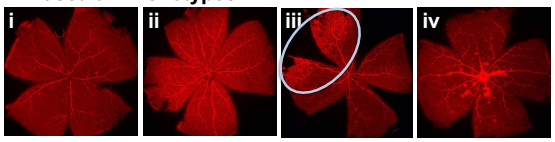
## AAV SEROTYPES INFLUENCE VASCULAR PATHOLOGIES

**A. Fundus Angiography**



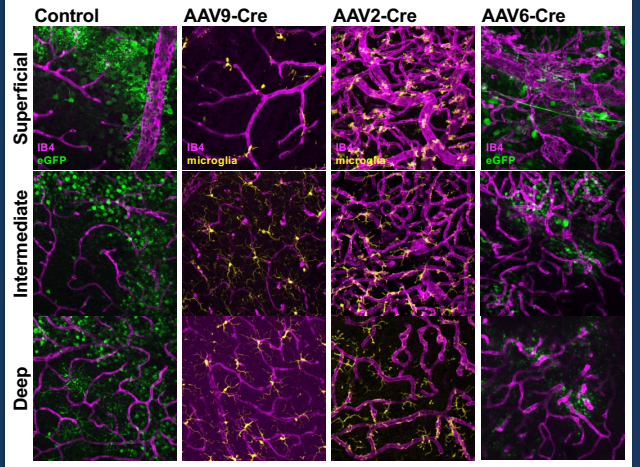

**A) Examples of control with no pathology (i), control with small exudates (ii, circles), large exudates in mice injected with AAV2-Cre (iii, also in controls, see below), and vessel dilation and multiple exudation sites vessels only in mice injected with AAV6-Cre (iv).**

**B. Vascular Phenotypes**




**B) No pathology from control (i) or AAV9-Cre (ii), or with hypervascularization over a large peripheral region with AAV2-Cre (iii, circle) or multiple sites of vascular proliferation in AAV6-Cre (iv) injected retinas. Endothelial cells are labelled with isolectin IB4 (red) in retinal whole mounts.**

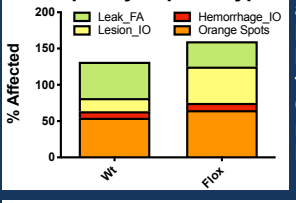
**C. Retinal vascular pathology**




**C) Control and AAV-9 injected retinas exhibit no pathology. AAV2- and AAV6-Cre exhibit distinct vascular pathology as a single broad region or multiple discrete loci, respectively. Microglia are associated with vascular pathology. GFP (green), microglia (Iba1, yellow) and endothelial cells (IB4, magenta).**

## INJECTION AND VIRAL TRANSDUCTION PHENOTYPES

**A. Frequency of phenotypes**




**Exudates, hemorrhage, and visually identified lesions are apparent using IO and FA. While frequent, they are not specific to Cre expression and are not effective predictors for hemangiomas.**

**Serotype Influences Hemangioma Formation**

| | Control* | AAV9 | AAV2 | AAV6 |
|---|---|---|---|---|
| Eyes injected | 30 | 4 | 30 | 20 |
| Eyes with hemangiomas | 0 | 0 | 9 | 17 |
| Serotype Efficiency | 0% | 0% | 30% | 85% |

***Control: combined AAV-reporter only injected into *VHL* floxed mice and AAV-Cre injected into wild type mice.**

## CONCLUSIONS

- **Single, large hemangiomas are characteristic of AAV2-Cre transduction and multiple discrete hemangiomas are characteristic of AAV6-Cre.**
- **The frequency of hemangiomas is much greater with AAV6-Cre, suggesting preferential targeting of critical tumorigenic cells.**
- ***In vivo* FA effectively and unambiguously identifies hemangiomas only with AAV6 transduction.**
- **These results suggest using AAV6-Cre in *VHL* floxed mice will be a useful model to study retinal hemangiomas in VHL with a simple, *in vivo* diagnostic to select mice for downstream studies.**

VHL Alliance

Research to Prevent Blindness

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