A Second-Generation Mouse Model to Study Cerebral Cavernous Malformations Lesion Development

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Introduction

Cerebral cavernous malformations (CCM) are vascular abnormalities of the central nervous system that manifest as recurrent headaches, seizures and stroke. Histological analysis of these lesions shows grossly dilated, multicavernous blood vessels lacking intervening brain tissue. CCMs are dynamic in nature, growing over time from dilated blood vessels to multicavernous lesions that lose vascular integrity, leading to bleeds and stroke. Epidemiologically, CCM occurs in either sporadic or familial, autosomal dominant forms. The inherited forms of CCM are due to heterozygous germline mutations in one of three genes: *CCM1*/*KRIT1*, *CCM2*/malcavernin and *CCM3*/*PDCD10*. Previous work with human lesion tissue from inherited cases has shown that CCM follows a two-hit mechanism where the wild type allele must be somatically mutated for pathogenesis to occur. Our lab generated knockout alleles in mice for *Ccm1* and *Ccm2*. Homozygous null mice die mid-gestation and heterozygous animals do not develop CCM lesions.

Materials & Methods

- In keeping with the two-hit hypothesis, *Ccm1*+/- and *Ccm2*+/- mice were crossed into a sensitizer background promoting somatic mutation (*Msh2*-/-) to produce *Ccm1*+/-*Msh2*-/- and *Ccm2*+/-*Msh2*-/- mice and littermate controls (shown in Figure 1)
- Mice were sacrificed at 5 months of age and brains were removed
- Brains were fixed in formalin, then imaged by T2*-weighted gradient recall MRI
- After imaging, the brains were embedded in paraffin, cut into 1mm coronal slices and examined for Rho kinase activity, expression of KRIT1 and CCM2 proteins, and abnormal ultrastructure of the CCM lesions

Results

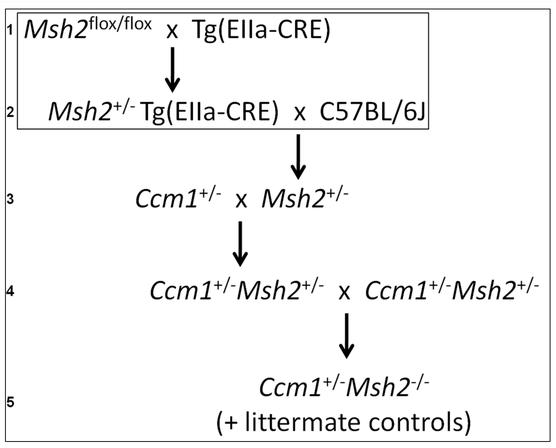

Figure 1: Example breeding scheme to generate Ccm1+/-Msh2-/- mice. An *Msh2* null allele was generated by crossing mice with the CRE recombinase transgene to mice with an allele of *Msh2* flanked by loxP sites (provided by R Kucherlapati). After back-crossing to a wild-type C57BL/6J mouse to eliminate CRE, *Msh2*+/- mice were mated with *Ccm1*+/- to produce double heterozygotes, which were then mated with each other to produce Ccm1+/-Msh2-/- mice as well as littermate controls.

Genotype	Total # of Mice Examined	Fraction with CCM Lesions
Ccm1+/-	3*	0/3
Ccm2+/-	3*	0/3
Msh2-/-	6	0/6
Ccm1+/-*Msh2*-/-	19	9/19
Ccm2+/-*Msh2*-/-	11	0/11

Table 1: CCM lesion penetrance was increased in *Ccm1+/-Msh2-/-* mice. While no CCM lesions were seen in the control genotypes, *Ccm1+/-Msh2-/-* mice showed lesions with a penetrance of 47%. *Ccm2+/-Msh2-/-* mice, however, did not display CCM lesions. * - only 3 mice were examined for these genotypes because these mice have been previously examined for CCM lesions and those results are published.

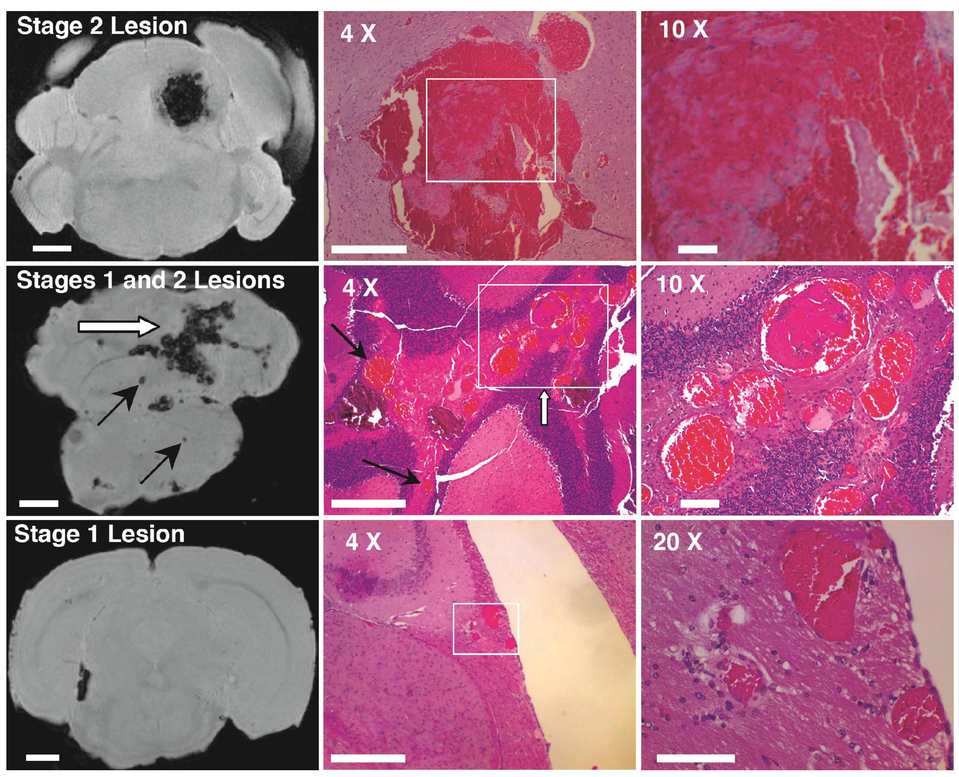

Figure 2: *Ccm1+/-Msh2-/-* mice show both solitary single caverns and multicavernous lesions. Brains were examined by *ex vivo* MRI (left panels) and hematoxylin & eosin staining (middle panels with white squares shown by higher resolution in the right panels). Solitary (Stage 1) caverns are indicated by black arrows and multicavernous (Stage 2) lesions are indicated by white arrows.

Results

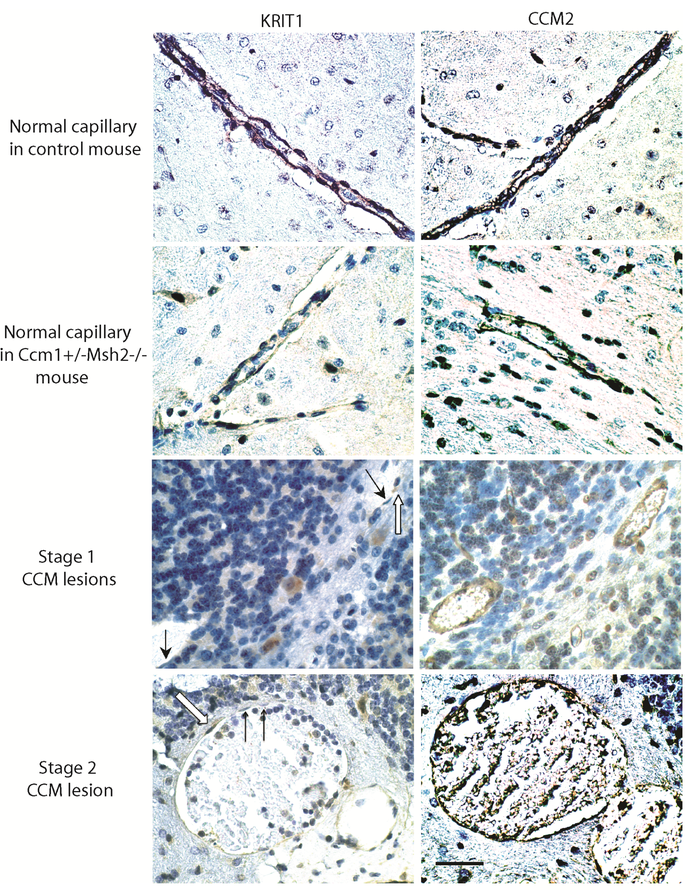

Figure 3: Reduced KRIT1 expression in the endothelium of lesions from *Ccm1+/-Msh2-/-* mice. Capillaries in *Msh2*-/- control brains (first row) showed prominent brown staining for both KRIT1 (left) and CCM2 (right). Consistent with the genotype of the mice, KRIT1 staining was reduced, while CCM2 staining remained at control levels in normal capillaries (second row) of the *Ccm1*+/-*Msh2*-/- mouse brain. Endothelial cells lining two Stage 1 CCM lesions (third row) and two caverns of a Stage 2 CCM lesion (fourth row) showed normal staining for CCM2 (right), but either reduced (white arrow) or no staining (black arrow) for KRIT1 (left).

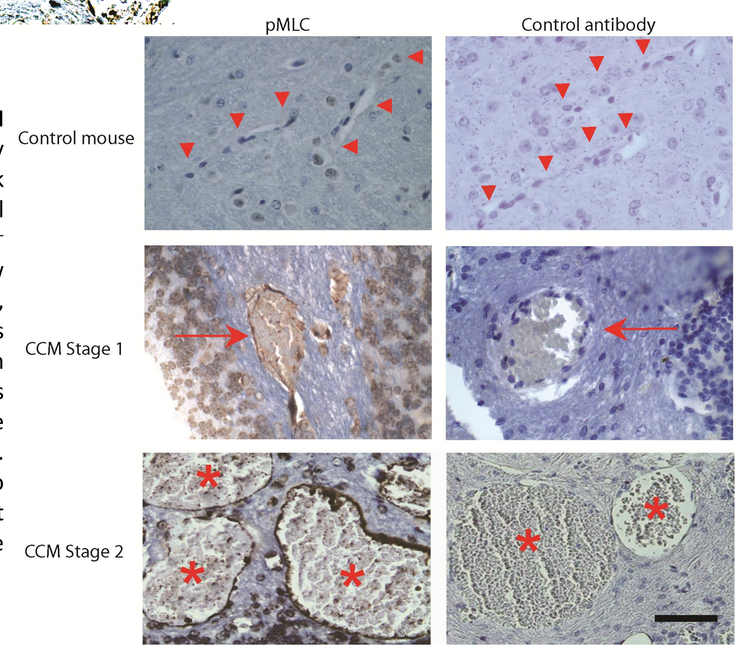

Figure 4: ROCK activation in mouse CCM lesions. ROCK activation was assessed by pMLC immunohistochemistry (dark brown staining, left panels). Normal capillaries (arrowheads) from an *Msh2*-/- control mouse (top row) do not show evidence of ROCK activation. By contrast, in *Ccm1*+/-*Msh2*-/- mice, endothelial cells (ECs) lining Stage 1 lesions (arrows) stain weakly for pMLC (middle row) and ECs lining three caverns (asterisks) of a Stage 2 lesion show stronger pMLC staining. Corresponding serial sections show no staining with an isotype control (right panels). All tissue sections were counterstained blue with hematoxylin.

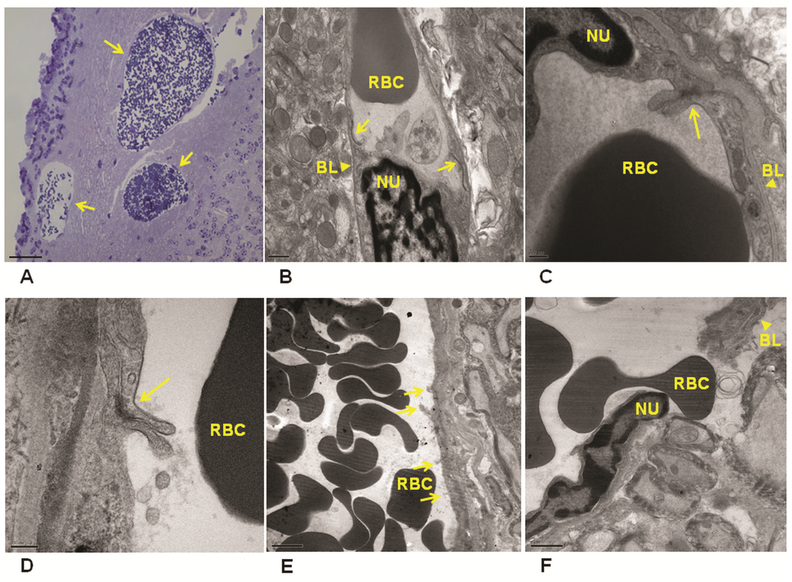

Figure 5: Electron microscopy reveals abnormal ultrastructure of CCM lesion endothelium. (A) Three CCM lesions (arrows) in a *Ccm1*+/-*Msh2*-/- mouse brain shown by Toluidine Blue staining. (B) Intact tight junctions (arrows) and basal lamina (arrowheads) are present within a normal capillary in a control C57BL/6 mouse. Intact tight junctions (arrows) and basal lamina (arrowheads) are also present within a normal capillary (C) and a CCM lesion (D) in a *Ccm1*+/-*Msh2*-/- mouse brain. (E) In the same lesion filopodia (arrows) are present. (F) A tight junction and the basal lamina (arrowhead) are both broken in this lesion and erythrocyte extravasation is visible. RBC = red blood cell; NU = endothelial nucleus; BL = basal lamina.

Conclusions

- *Msh2* knockout was successfully used as a sensitizer mutation in *Ccm1*+ heterozygotes to generate mice that develop CCM lesions
- CCM lesions that develop show the hallmarks of the disease by MRI, IHC and EM

Future Directions

- Utilize this second-generation model to elucidate additional details of the mechanism behind CCM lesion genesis and growth
- Test the efficacy of therapeutic strategies to halt lesion genesis and progression