A Second-Generation Mouse Model of Cerebral Cavernous Malformation Pathogenesis

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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. CCM lesions are identified using magnetic resonance imaging (MRI). Histological analysis of these lesions shows grossly dilated blood vessels lacking intervening brain tissue. CCMs are dynamic in nature, growing over time from dilated blood vessels to multicavernous lesions with a decreased ability to form tight junctions, leading to bleeds and stroke. Epidemiologically, CCM occurs in either sporadic or familial forms, the latter showing an autosomal dominant pattern of inheritance. 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 CCMs.

First-Generation Mouse Model of CCM

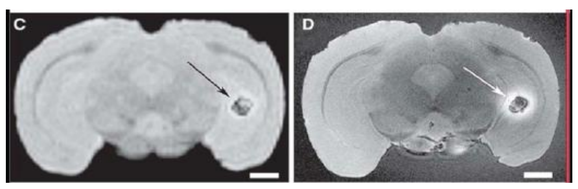


Figure 1: MRI of whole brain from *Ccm1*+/-, *Trp53*-/- mouse. Image adapted from Shenkar R, *et al*. Advanced magnetic resonance imaging of cerebral cavernous malformations: II imaging of murine models. Neurosurgery 2008; 63(4): 790-797.

Trp53 is a tumor suppressor gene and is part of a DNA repair pathway. Deletion of *Trp53* in mice leads to widespread DNA damage. We used homozygous *Trp53* deletion to sensitize our *Ccm1*+/- and *Ccm2*+/- mice to somatic mutations. Both *Ccm1*+/-,*Trp53*-/- and *Ccm2*+/-,*Trp53*-/- mouse models developed CCM lesions with high penetrance (Figure 1). Cloning and sequencing analysis, however, did not reveal any sequenceable second-site somatic mutations, as were seen in human CCM lesion tissues. We then examined the frequencies of the wild-type and mutant alleles at SNPs in and around *Ccm2* in a Ccm2+/-,Msh2-/- mouse and found evidence for a somatic loss of heterozygosity event that could be the second hit for lesion genesis (Figure 2). From these data, we hypothesized that deletion of *Trp53* could be causing large deletions and chromosomal abnormalities. In order to detect sequenceable mutations, a different sensitizing mutation was necessary. *Msh2* is part of the DNA mismatch repair system and inactivation of this gene cause point mutations and small insertions/deletions. Using *Msh2* knockout as a sensitizer mutation, then, would yield a mouse model of CCM bearing second-site somatic mutations in the CCM genes that are easily sequenceable.

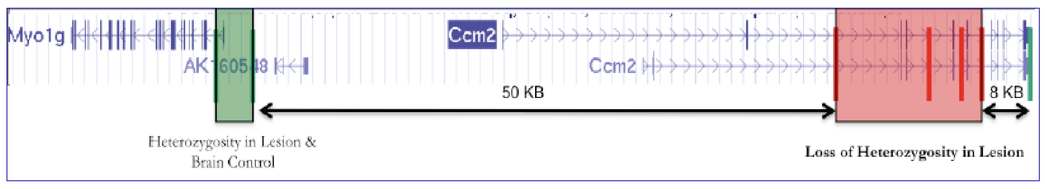


Figure 2: Summary of SNaPshot mapping showing a potential loss of heterozygosity event of the wild-type allele affecting somatic cells within the CCM lesion of a *Ccm2*+/-,*Msh2*-/- mouse.

Materials & Methods

- An *Msh2* null allele was developed by crossing mice with the CRE recombinase transgene to mice with an allele of *Msh2* flanked by loxP sites (provided by R Kucherlapati & M Kucherlapati)
- Mice with the genotype *Ccm1*+/-,*Msh2*-/- were bred using the scheme shown in Figure 3
- Between 120 and 140 days of age, 5 mice with the above genotype as well as some littermate controls were sacrificed and whole brains were removed
- Brains were fixed in formalin, then imaged by T2*-weighted gradient recall MRI
- After imaging, the brains were embedded in parafin, cut into 1mm coronal slices and stained with hematoxylin and eosin
- Imaging and H&E staining analyses were all performed blind to genotypes

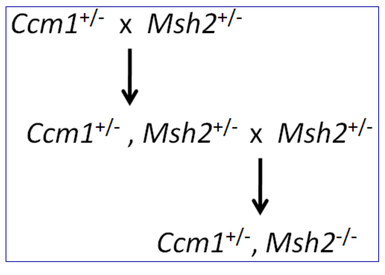
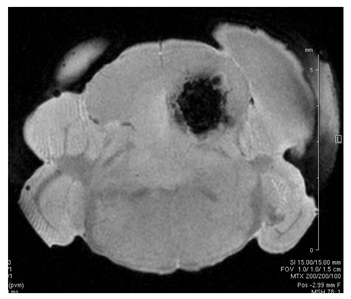
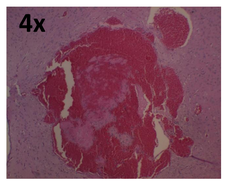


Figure 3: Breeding scheme to generate *Ccm1*+/-,*Msh2*-/- mice.

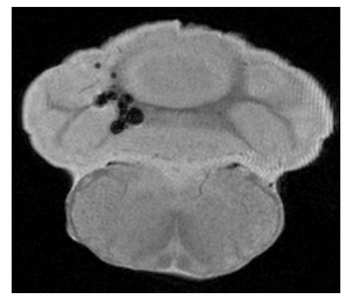
Results

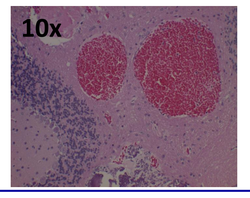
Mouse 78: Ccm1+/-, Msh2-/-



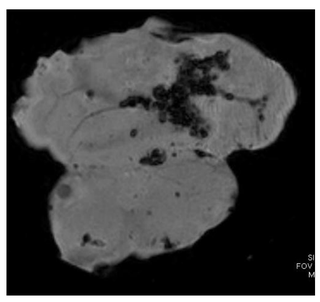


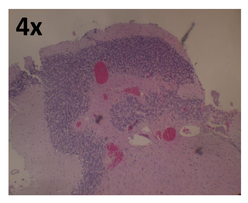
Mouse 79: Ccm1+/-, Msh2-/-



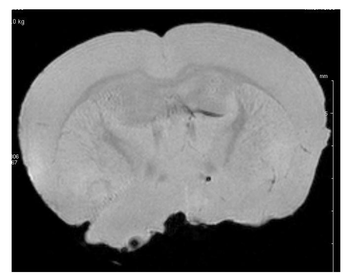


Mouse 81: Ccm1+/-, Msh2-/-





Mouse 83: Ccm1+/+, Msh2-/-



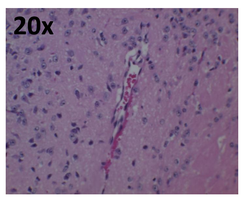


Figure 4: MRI of whole mouse brains and H&E staining of brains cut into 1mm coronal cross-sections (magnifications indicated on each picture). Lesions of various stages were seen in 3/5 mice with genotype Ccm1+/-,Msh2-/- (all shown above) and none of the controls (one shown above).

Table 1: MRI and histology results for 5 *Ccm1*+/-,*Msh2*-/- mice and 2 *Ccm1*+/+,*Msh2*-/- controls

Mouse #	MRI	Histology
Ccm1+/-,Msh2-/-		
69	1 punctate area (<0.1mm)	No vascular lesions
71	1 punctate area (<0.2mm)	3 dilated capillaries
78	1 multicavernous lesion	Large CCM lesion (Stage 2)
79	1 multicavernous lesion	Large CCM lesion, >10 caverns
81	2 multicavernous lesions	Many dispersed CCM lesions (Stage 1), dilated capillaries
Ccm1+/+,Msh2-/-		
73	1 punctate area (<0.2mm)	Prominent vessel or artifact
83	4 punctate areas (<0.1mm)	Prominent vessel or artifact

Conclusions

- *Msh2* knockout was successfully used as a sensitizer mutation to generate mice that develop CCM lesions
- 3/5 *Ccm1*+/-,*Msh2*-/- mice and 0/2 *Ccm1*+/+,*Msh2*-/- controls showed CCM lesions, shown by MRI and histology

Future Directions

- Replicate methods on a larger number of experimental and control mice
- Use *Msh2* as a sensitizer for *Ccm2*+/- mice
- Sequence lesion DNA to verify that CCM in these animals follows a similar genetic mechanism to that seen in humans
- Utilize this second-generation model to elucidate additional details of the mechanism behind CCM lesion genesis and growth