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COMMENTARY

Map3k3^{I441M} knock-in mouse model of cerebral cavernous malformations

Cheng Lv†, Ruofei Li†, Liang Yu†, and Yibo Wang*

State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Abstract

The *Map3k3*^{I441M} knock-in mouse model reveals an age-dependent mechanism in cerebral cavernous malformation (CCM) pathogenesis, wherein PI3K pathway activation is required for lesion formation in adults but not juveniles. Notably, rapamycin treatment effectively inhibited lesions across age groups, underscoring mammalian target of rapamycin (mTOR) inhibition as a potential therapy. This commentary highlights mechanistic insights from the *Map3k3*^{I441M} knock-in mouse model, emphasizing the age-dependent role of PI3K signaling in CCM formation. It discusses the potential synergy between *MAP3K3* and *PIK3CA* mutations, explores the therapeutic potential of mTOR inhibition, and considers the potential influence of pre-conceptional environmental exposures on CCM susceptibility.

Keywords: Cerebral cavernous malformations; *Map3k3* mutation; Mouse model

†*These authors contributed equally to this work.*

***Corresponding author:**
Yibo Wang
(wangyibo@fuwaihospital.org)

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1. Introduction

Cerebral cavernous malformations (CCMs) (Mendelian Inheritance in Man [MIM]: 116860) are among the most common vascular anomalies affecting the central nervous system, with a prevalence of 0.16–0.5% in the general population, primarily estimated from studies in developed countries utilizing magnetic resonance imaging (MRI).[1,2] Data from developing regions remain limited, and prevalence may be underestimated due to restricted access to diagnostic neuroimaging and specialized care. These lesions are characterized by fragile, abnormally dilated capillaries prone to hemorrhage, often resulting in seizures, focal neurological deficits, or even life-threatening intracranial bleeding. Approximately 85% of cases are sporadic.[3] Previous studies have identified loss-of-function mutations in one of the three CCM genes—*CCM1/KRIT1* (MIM: 604214), *CCM2/MGC4607* (MIM: 607929), and *CCM3/PDCD10* (MIM: 609118)—as causative for CCM lesions.[4] However, in a subset of cases, the underlying pathogenic mechanisms remain unclear.

This commentary aims to highlight the significance of the novel *Map3k3*^{I441M} knock-in (KI) mouse model developed by Xu *et al.*[5] in elucidating CCM pathogenesis, particularly for CCM5 associated with *MAP3K3* mutations, and to discuss its implications and the remaining questions.

In addition to germline mutations, somatic mutations also play a fundamental role in the pathogenesis of human disease. Advances in sequencing technologies have greatly enhanced our ability to dissect disease etiology and uncover critical mechanistic insights.

In March 2021, Hong *et al.*[3] first identified somatic *PIK3CA* and *MAP3K3* mutations through sequencing of 84 CCM lesions, which were associated with different phenotypes defined as CCM4 (MIM: 619538) and CCM5 (MIM: 621032), respectively. The findings were independently validated by Weng *et al.*[6] in April 2021 in a separate cohort of 38 CCM lesions, reporting similar mutation frequencies. In both studies, *MAP3K3* somatic mutations were found to be mutually exclusive with *CCM1*, *CCM2*, and *CCM3* mutations, but often concurrent with *PIK3CA* somatic mutations.

Using single-cell transcriptomic analysis, Ren *et al.*[7] compared lesion features across different mutation types and found no distinct transcriptional differences among them. Lesions harboring different mutations consistently exhibited enhanced endothelial angiogenic activity, an immune-activated endothelial-to-mesenchymal transition state, and a heightened smooth muscle cell phenotypic transformation pattern. These findings highlight the importance of developing CCM mouse models harboring diverse somatic and/or germline mutations to better explore the underlying disease mechanisms.

2. Discussion

The study by Xu *et al.*[5] presents a significant advance by establishing a novel *Map3k3*^{I441M} KI mouse model. Mouse models serve as indispensable tools in modern biomedical research, with applications spanning basic research, disease mechanism investigation, drug discovery, and functional genomics. These animal models have revolutionized our understanding of human diseases by enabling precise genetic manipulations that recapitulate pathological processes in a controlled experimental system.

Previous studies have successfully modeled CCMs in mice by introducing the pathogenic *Map3k3*^{I441M} mutation into cerebral endothelial cells through adeno-associated virus (AAV)-mediated gene delivery, recapitulating key histopathological features of human CCMs.[8,9] The AAV model can mimic a state of localized, low-frequency somatic mutations, consistent with observations in real-world patients.[3,6] This approach has enabled researchers to investigate lesion initiation, growth dynamics, and potential therapeutic interventions in a living organism. However, two critical limitations of this model are the overexpression of the *Map3k3*^{I441M} mutation and its short-term effect. Moreover, a phenomenon—spontaneous regression of over 50% of induced lesions during adulthood—was observed in these AAV models, which is inconsistent with human CCM pathogenesis.[8]

Xu *et al.*[5] successfully established a novel *Map3k3*^{I441M} KI mouse model on the C57BL/6J genetic background using clustered regularly interspaced short palindromic repeats/Cas9-mediated precision genome editing technology. To achieve endothelial-specific mutation expression, they employed a sophisticated genetic strategy by crossing these mice with *Cdh5*-Cre^{ERT2} transgenic animals, enabling tamoxifen-inducible, restricted expression of the mutation in cerebrovascular endothelial cells. Comprehensive characterization revealed that this innovative model effectively recapitulates key pathological hallmarks of human CCMs. Lesion formation followed a distinct temporal pattern, with both lesion number and average volume showing significant increases during the first two post-natal months. From months 2–5, CCM lesions reached a plateau phase, maintaining remarkable stability in both quantity and mean volume.

Histopathological examination confirmed that the vascular lesions exhibited all characteristic features of human CCMs, including grossly dilated vascular channels lined by thin endothelial walls, ultrastructural defects in endothelial cell junctions, and prominent perivascular hemosiderin deposition indicative of chronic microhemorrhages. However, it is worth noting that the KI model may exaggerate the effects of mutations, as almost all target cells express the mutant allele, which may not accurately represent the pathogenic potential of low-frequency somatic mutations in patients. Due to the complexity of human physiology and the long-term effects of low-abundance mutant cells, this model cannot fully replicate the variant allele frequencies observed in human cases. Given that it is not feasible to replicate the full scale of human disease progression in mouse models, AAV and KI models are most appropriate for short-term studies within the mouse lifespan. Each model has distinct strengths and is valuable for elucidating disease mechanisms, advancing drug discovery, and supporting functional genomics research. Accordingly, the KI model serves as a valuable alternative, supplementary tool, and means of validation.

The study by Xu *et al.*[5] also provides mechanistic insights into the critical role of the PI3K signaling pathway in CCM pathogenesis through comprehensive investigations using the *Map3k3*^{I441M} KI mouse model. The results reveal an age-dependent dichotomy in CCM development: In juvenile mice, heterozygous expression of the *Map3k3*^{I441M} mutation alone was sufficient to induce CCM-like lesions. In striking contrast, adult mice (≥3 months old) exhibited a more complex pathogenesis, in which the *Map3k3*^{I441M} mutation alone was insufficient to induce lesions, requiring concurrent PI3K pathway activation for CCM formation.

Previous studies have reported cases with concomitant somatic mutations in both *MAP3K3* and *PIK3CA*;[3,6] however, these cases did not exhibit more severe phenotypes

compared to those harboring a single mutation.[3,7] In these sporadic CCMs with dual mutations, the variant allele frequencies were relatively similar.[3,7] Combined with the present study by Xu *et al*.,[5] these observations raise several intriguing questions: (i) Do the mutations co-occur within the same cell? (ii) do lesions in cases with dual mutations arise later than those with only *MAP3K3* mutations? and (iii) does the potential synergistic effect of *MAP3K3* and *PIK3CA* mutations within a single cell contribute to CCM formation? These questions highlight the need for further studies to elucidate the cellular and temporal dynamics underlying these mutations and their pathogenic interactions.

The present clinical guidelines recommend surgical resection for symptomatic CCMs following multidisciplinary evaluation, while conservative management with monitoring is standard for asymptomatic lesions or those located in high-risk regions, such as the brainstem.[4] Spinal cavernous malformations (SCMs) similarly require individualized management, with surgical resection offering favorable outcomes for symptomatic intramedullary lesions when performed before the onset of severe neurological deficits.[10] For high-risk, inoperable CCMs or SCMs, stereotactic radiosurgery has been explored as an alternative treatment; however, its efficacy remains controversial due to variable obliteration rates (34–54%) and the risk of hemorrhage during the latency period.[11] Nevertheless, the persistent hemorrhage risk in these cases underscores the urgent need for pharmacotherapies.

Recent advances have identified MEKK3–KLF2/4 signaling[12,13] and local iodothyronine deiodinase type 2 (DIO2) upregulation (converting thyroxine to active triiodothyronine [T3])[14] as key adaptive responses in CCM pathogenesis. Exogenous DIO2/T3 supplementation attenuated pathology in *Pdcd10* knockout models,[13] and ponatinib inhibited lesion formation in *KRIT1* knockouts.[15] The study by Xu *et al*.[5] now demonstrates rapamycin's efficacy against *Map3k3*^{I441M}-driven lesions across age groups, establishing mammalian target of rapamycin (mTOR) inhibition as a translatable strategy. Beyond its therapeutic applications, this model also enables exploration of pre-conceptional influences on CCM susceptibility—an unexplored frontier.

Environmental exposures in prospective parents (e.g., tobacco, alcohol, pro-inflammatory diets, chronic stress, and sleep disorders) may compromise gametic DNA integrity or epigenetic programming,[16] potentially increasing offspring vulnerability to *de novo* germline mutations (e.g., *KRIT1*, *CCM2*, and *PDCD10*), somatic mutations (e.g., *MAP3K3* and *PIK3CA*), or endothelial DNA repair defects. Conversely, protective factors, such as adherence to a Mediterranean diet or stress reduction strategies may enhance resilience pathways, potentially including DIO2-mediated adaptation.[13] Critically, no direct epidemiological or experimental evidence currently links parental exposures to CCM outcomes in offspring. Future research should integrate epidemiological studies correlating parental exposures with sporadic CCM incidence, alongside controlled animal models testing pre-conceptional stressors in *Map3k3*^{I441M} lineages to assess impacts on endothelial-specific pathways (e.g., KLF2/4, PI3K/mTOR, and DIO2).

3. Conclusion

CCM5 (MIM: 621032), associated with *MAP3K3* mutations, is characterized by a relatively low risk of hemorrhage[3] and displays Zabramski type 2 features on MRI.[6] The development of a genetically accurate mouse model for CCM5 offers a valuable platform for studying disease mechanisms. In juvenile mice, the *Map3k3*^{I441M} mutation alone leads to fully penetrant CCM formation. In contrast, adult mice require additional activation of the PI3K signaling pathway to develop significant lesions, suggesting age-dependent susceptibility and signaling pathway synergy. Notably, pharmacological treatment with rapamycin effectively suppressed lesion progression, highlighting a potential therapeutic avenue.

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Conflict of interest

Yibo Wang is an Editorial Board Member of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions

Conceptualization: Yibo Wang
Writing – original draft: Cheng Lv, Ruofei Li, Liang Yu
Writing – review & editing: Yibo Wang

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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