HSC Public Health Agency
Research and Development

# Evidence Brief

## Targeting the compromised brain endothelial barrier function during cerebral malaria with AT2 receptor agonists

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### Why did we start? (The need for the research and/or why the work was commissioned)

A loss of endothelial integrity is central to the pathology of many prevalent conditions, including haemorrhagic viral infections, CNS ischemia, stroke, retinal haemorrhage, and cerebral malaria (CM). Notwithstanding therapeutic potential of interventions that could strengthen the inter-cellular junctions between endothelial cells, such efforts have been frustrated by lack of understanding of mechanisms regulating endothelial integrity and thus the identification of therapeutic targets. We have chosen CM to model compromised endothelial barrier function because we recently identified signalling pathways, which are crucial in the pathogenesis of this syndrome. As the deadliest manifestation of malaria, CM kills 20% of affected patients. While anti-malarial drugs are effective clearing parasites, they do not have specific effects against CM, therefore the need for specific adjunct therapy. To date, none of the clinical trials for CM have demonstrated sufficient efficacy. CM is caused by the interaction between *P. falciparum* infected erythrocytes and host brain endothelial cells, leading to the loss of endothelial cell junctions and ultimately the disruption of the blood-brain barrier.

Comparable vascular pathology and compromise of the blood-retinal barrier occurs in malaria retinopathy, which has led others to propose the retina as a surrogate/prognostic tissue for CM. We reported that endothelial integrity is protected from *P. falciparum*-induced disruption by activation of the angiotensin (Ang) II receptor type 2 (AT2). Although more than 120,000 publications introduce Ang II as *the* agonist, it is only a biased agonist of the G protein-coupled receptor AT2, since it is not able to activate G-proteins. However, we have recently identified the natural endogenous agonist (EA) for AT2, which activates Gαs. Our work in this field has also led to the identification of another compound that can be used as a tool to dissect AT2 signalling in endothelial cells, the first specific non-peptidic AT2 agonist (SNPA), which unlike other AT2 agonists, does not present cross reactivity with other Ang receptors.

Our US-Ireland collaboration brought together a team with complementary expertise in malaria, retinopathy and the angiotensin system. Together we hypothesized that the activation of specific intracellular pathways mediated by AT2 protects human brain and retinal endothelial cells (HBMECs/HRMECs) from *P. falciparum*-induced disruption of inter-endothelial junctions. Thus, our goal is to identify a lead compound that prevents CM by protecting endothelial integrity through stimulation of distinct intracellular signalling downstream of the AT2 receptor. Infection with *Plasmodium falciparum* carries a significant risk of cerebral malaria (CM). Children are particularly susceptible to human CM (HCM) which manifests as an acute neurovascular encephalopathy leading to high levels of mortality. Occurring in parallel with CM, malarial retinopathy (MR) is readily detected on ophthalmoscopy as one or more of: white-centered retinal haemorrhage, retinal whitening, and vessel discoloration. It leads to several distinct types of blood retinal barrier (BRB) breakdown. The precise molecular mechanisms underpinning CM and MR remain ill-defined, but parasitaemia is known to drive progressive neurovascular obstruction and inflammation leading to cerebral and retinal oedema and ischemia. Extensive clinical studies in patients with CM have shown that retinal examination is a useful approach for understanding pathology and an indicator for risk of mortality and morbidity. Fully understanding the cellular and molecular mechanisms that underpin CM and MR is important for developing new therapeutic approaches and in this regard the murine model of experimental CM (ECM) has proved to offer considerable value as has parallel evaluation of the retina during CM.

### What did we do? (Methods)

The Northern Ireland component of this project has focused attention on the linkage between retina and brain in CM. Given the retina's accessibility, we sought to gain unique insights into CM pathology could be gained by examining the neurovascular unit of the retina. Using the *P. berghei* animal model which we have set-up in Queen's, we investigated disease pathology without the need for human participants.
Specifically, we evaluated and characterised the retinal pathology of the retina during CM and explored the transcriptomic relationship between the retina and the brain using single-cell and spatial transcriptomic approaches. displayed reduced susceptibility to the neurological manifestations of CM.

| What answer did we get? (Findings) |
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| By generating the first single-cell RNA-sequencing dataset of endothelial cells in this model, our work provided a unique foundation for future studies. The findings not only address our initial research questions but also provide a valuable framework for ongoing exploration of CM pathogenesis and therapy development. For example, we have identified a novel marker, Apold1, uniquely expressed in our CM cohort that can be used as a potential target for therapeutic applications or a novel biomarker. We have reported leukocytes found in the retina which have not previously been investigated as well as vascular We further characterised the inflammatory response in CM, with a focus on microglia and other immune cells. Our investigation also evaluated EPCR (encoded by the gene procr). The impact of *procr* gene knockout in a heterozygous model, revealing that *Procr*$^{+/-}$ animals displayed reduced susceptibility to the neurological manifestations of CM. |
| What should be done now? (Practice/Policy Implications and/or Recommendations) |
| We are intending to build on unique datasets to fully understand vascular dysfunction in CM and, in particular, the retina. Apold1's presence in CM is of particular interest due to its potential as a novel therapeutic target. At present, however, its precise role in endothelial cells remains unclear and we are conducting additional experiments to understand how Apold1 actively drives infection associated pathology and its role in a protective response aimed at preserving endothelial function.<br>While challenges remain in translating these findings to the clinic, this work provides a foundation upon which future studies can build, whether through mechanistic dissection of Apold1, therapeutic testing of vascular modulators, or deeper spatial characterisation of the retina and brain in CM. By integrating molecular insights with therapeutic exploration, this thesis advances our understanding of CM and MR pathogenesis and provides a platform for more targeted and effective interventions in this devastating disease. |

## Final Report

### Background

A loss of endothelial integrity is central to the pathology of many prevalent conditions, including hemorrhagic viral infections, CNS ischemia, stroke, retinal hemorrhage, and cerebral malaria (CM). Notwithstanding therapeutic potential of interventions that could strengthen the inter-cellular junctions between endothelial cells, such efforts have been frustrated by lack of understanding of mechanisms regulating endothelial integrity and thus the identification of therapeutic targets. We have chosen CM to model compromised endothelial barrier function because we recently identified signaling pathways, which are crucial in the pathogenesis of this syndrome. As the deadliest manifestation of malaria, CM kills 20% of affected patients. While anti-malarial drugs are effective clearing parasites, they do not have specific effects against CM, therefore the need for specific adjunct therapy. To date, none of the clinical trials for CM have demonstrated sufficient efficacy. CM is caused by the interaction between *P. falciparum* infected erythrocytes and host brain endothelial cells, leading to the loss of endothelial cell junctions and ultimately the disruption of the blood-brain barrier.

Cerebral Malaria (CM) is a life-threatening encephalopathy caused by malaria infection, whereby infected RBCs accumulate in the brain vasculature, leading to severe neurological symptoms. With a high mortality rate, primarily among children, CM remains a major health concern. Malaria retinopathy, often present in CM patients, has become a key diagnostic feature, with ophthalmologists using fundus imaging to identify affected individuals with peripheral parasitaemia.

### Aims and Objectives

The specific aims of the QUB element of the US-Ireland project were to:

1. To conduct a comprehensive characterisation of the mouse model, employing *in vivo* retinal imaging modalities. To assess *ex vivo* brain and retina tissues to ascertain the effects of ECM on these tissues and cells of the NVU.
2. To determine whether the brain and the retina are transcriptomically analogous, based on the fact that little is known about the genes which were driving CM and underpinning the vascular pathology thus identifying new potential targets for CM therapies.
3. To investigate whether *Procr* knockdown modulates susceptibility to CM based on EPCR being reported to be implicated in CM by activation of protein C leading to a pro-inflammatory and pro-coagulation state. The relationship between this gene and *P. berghei* has not been investigated and we sought to use of heterozygous *procr*$^{+/-}$ animals which may be protective against the disease.

### Methods

We used the murine model of CM induced by *P.berghei* ANKA, which replicates the neurological signs observed in human CM cases. In mice, we utilised fundus imaging, OCT and fluorescein angiography to characterise the retinal manifestations of the disease. Based on this model we conducted a detailed transcriptomics assessment at single cell resolution is missing and this chapter utilised both single cell sequencing and spatial transcriptomics in murine CM tissues to elucidate the genes contributing to brain and retinal neurovascular breakdown in this disease.
Also investigated the role of EPCR in malaria pathogenesis using both *in vitro* and *in vivo* models. *In vitro*, we examined human retinal (HRMECs) and brain microvascular endothelial cells (HBMECs), while *in vivo* studies employed *P.berghei ANKA* infection in wild-type and *Procr* heterozygous (*Procr* $^{+/-}$) knockout mice.

### Personal and Public Involvement (PPI)

This was a discovery science project based on a tropical disease. We did not conduct PPIE as such although there was consistent attendance and presentation of our work at the NI Science Festival to promote public engagement.

### Findings

Our findings have shown that infected mice exhibit paler retinal blood vessels, indicative of red blood cell sequestration, alongside immune cells adhering to the vascular endothelium and circulating throughout the eye. Notably, OCT imaging revealed no significant retinal thinning. Post-mortem analysis confirmed the

presence of red blood cell aggregation in the vasculature, contributing to blockage. These results underscore the value of this murine model in studying the pathophysiology of CM and provide a non-invasive approach to understanding retinal changes without the need for clinical studies in comatose patients.

We have also identified the transcript, *Apold1,* which exhibited transcript upregulation only in the CM brains and may be contributing to BBB breakdown and endothelial cell damage in CM

*In vitro*, suppression of *Procr* expression impaired endothelial cell survival but partially restored tubulogenic capacity in HBMECs. *In vivo*, *Procr*$^{+/-}$ mice displayed reduced susceptibility to CM yet retained hallmark features of disease including paler vessels and leukostasis. Together, these findings suggest that while EPCR contributes to CM and MR pathogenesis by modulating mechanisms relating to parasite adhesion to the vasculature and enabling the maintenance of infection virulence

**Conclusion**
This project has provided novel insights into the pathogenesis of CM and MR through the combined use of sc-RNA seq, ST and *in vivo* murine models. By focusing on the NVU in both the retina and the brain, it was demonstrated that the retina may serve as a valid transcriptomic window into cerebral pathology, confirming that vascular leakage, barrier disruption and cytokine-driven inflammation are central hallmarks of CM.

From these analyses, we identified Apold1 as a novel gene of interest in CM, with potential roles in endothelial angiogenesis and barrier stability. Our data suggest that Apold1 may act as an adaptive response rather than a baseline driver of pathology. Future studies employing overexpression or knockout models will be required to determine its precise role and therapeutic potential. In parallel, this study also highlighted the importance of the Angpt2/Tie2 pathway, already targeted clinically in other vascular diseases, opening new avenues for translational research in CM.

Therapeutic exploration using *procr* $^{+/-}$ mice revealed that loss of EPCR does not protect against parasite infection. However, the observation that *procr* $^{+/-}$ animals appeared less susceptible to neurological manifestations raises mechanistic questions, already reported protective vascular adaptations seen in other conditions such as sickle cell disease. While complete EPCR loss may not offer a straightforward therapeutic strategy, targeted modulation or antibody blockade remains promising, particularly given supportive findings in human systems.

Collectively, our work contributes to both the mechanistic and translational understanding of CM. It has demonstrated the value of combining single-cell and spatial approaches with *in vivo* models to uncover new molecular targets, has identified Apold1 as a novel candidate gene, and has evaluated EPCR and Angpt2/Tie2 as potential therapeutic targets. Beyond CM, these findings reinforce the broader principle that transcriptomic approaches can uncover unexpected drivers of vascular pathology and highlight targets with cross-disease relevance.

While challenges remain in translating these findings to the clinic, this project provides a foundation upon which future studies can build, whether through mechanistic dissection of Apold1, therapeutic testing of vascular modulators, or deeper spatial characterisation of the retina and brain in CM. By integrating molecular insights with therapeutic exploration, this thesis advances our understanding of CM and MR pathogenesis and provides a platform for more targeted and effective interventions in this devastating disease

**Practice and Policy Implications/Recommendations**
N/A

**Pathway to Impact**
While challenges remain in translating these findings to the clinic, this project provides a foundation upon which future studies can build, whether through mechanistic dissection of Apold1, therapeutic testing of vascular modulators, or deeper spatial characterisation of the retina and brain in CM. By integrating molecular insights with therapeutic exploration, this thesis advances our understanding of CM and MR

pathogenesis and provides a platform for more targeted and effective interventions in this devastating disease

Publications

- McDonnell S, MacCormick IJ, Harkin K, Medina RJ, Rodriguez A, Stitt AW. From Bench to Bedside: Unraveling Cerebral Malaria and Malarial Retinopathy by Combining Clinical and Pre-Clinical Perspectives. Curr Eye Res. 2025 Feb 20:1-15. doi: 10.1080/02713683.2025.2463142 PMID: 39976257.
- Guduric-Fuchs J, Pedrini E, Bertelli PM, McDonnell S, Pathak V, McLoughlin K, O'Neill CL, Stitt AW, Medina RJ. A new gene signature for endothelial senescence identifies self-RNA sensing by retinoic acid-inducible gene I as a molecular facilitator of vascular aging. Aging Cell. 2024 Sep;23(9):e14240. doi: 10.1111/acel.14240. Epub 2024 Jun 21. PMID: 39422883; PMCID: PMC11488300.
- Bertelli PM, Pedrini E, Hughes D, McDonnell S, Pathak V, Peixoto E, Guduric-Fuchs J, Stitt AW, Medina RJ. Long term high glucose exposure induces premature senescence in retinal endothelial cells. Front Physiol. 2022 Aug 26;13:929118. doi: 10.3389/fphys.2022.929118. PMID: 36091370; PMCID: PMC9459081.

Abstracts/Conferences

- Poster Presentation: Deciphering the mechanisms underpinning retinal vascular cell pathophysiology in a murine model of cerebral malaria- Neuroplasticity in Brain Health and Disease: Advances in Techniques, Translation and Education (Newcastle University, April 2024)
- Oral Presentation: Deciphering mechanisms underlying vascular cell pathophysiology in malaria retinopathy (ISER, Buenos Aires, October 2024)
- Poster Presentation: Transcriptomic analysis of retinal and brain neurovascular pathology in a murine model of cerebral malaria (ARVO, Salt Lake City, May 2025)