Neuro-glial-vascular alterations in a novel mouse model of small vessel disease

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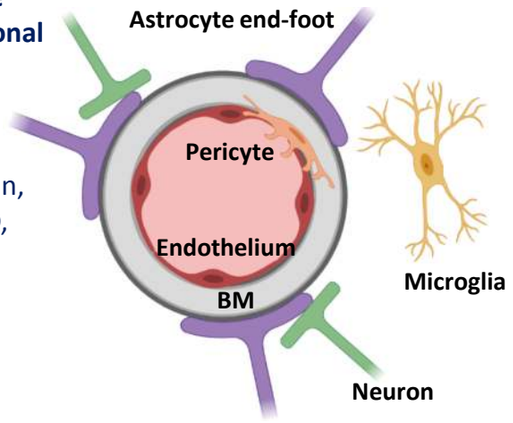
Introduction

- Cerebral small vessel disease (SVD) is a leading cause of vascular cognitive impairment (VCI) and dementia, albeit **the underlying molecular basis of SVD disease processes remains poorly understood**.
- Central to SVD pathophysiology is the disruption of the finely tuned interplay between the cells of the neuro-glial-vascular unit (NGVU)[1]
- Vital to NGVU integrity and function, is a complex meshwork of extracellular matrix (ECM) and basement membrane (BM) proteins.

Disruption of which could have profound effects on the functional integrity of the NGVU.



- Mutations in the genes ***COL4A1/A2*** (collagen IV alpha chains 1 and 2), a key BM protein, are known to cause familial SVD, and polymorphisms in these genes are also associated with sporadic SVD risk[2].
- This study aims to address how mutations in the *Col4a1 gene* impact upon brain health and function by investigating **cerebral perfusion, white matter integrity and neurovascular function in a Col4a1 mouse model.** Furthermore, it aims to characterise **SVD-like pathology in the model** and ultimately address the **effects of these changes on matrisome and NGVU integrity.**

Hypothesis & Aims

Overarching hypothesis:

Genetic risk factors (*COL4A1/A2*) → Matrisome defects → NGVU dysfunction → SVD → VCI

Aims:

- Characterise with imaging and histology SVD-like features in a *Col4a1* model
- Characterise the impact of a *Col4a1* mutation on brain perfusion and white matter integrity
- Characterise the impact of a *Col4a1* mutation on neurovascular function
- Characterise how a *Col4a1* mutation impacts upon matrisome function and NGVU integrity
- Investigate if perfusion and white matter changes are mechanistically related to matrisome and NGVU changes

Methods

***Col4a1* model:**

Col4a1$^{+/Svc}$ heterozygous mice carry a glycine to aspartic acid mutation [G1064D] in the *COL4A1* gene, leading to BM changes and haemorrhages[3].

Magnetic Resonance Imaging (MRI):

3-month male and female *Col4a1 (*n=14) and wild type litter mates (n=12) underwent MRI to assess structural (T2), white matter (diffusion tensor imaging [DTI]) and perfusion (arterial spin labelling [ASL]) changes.

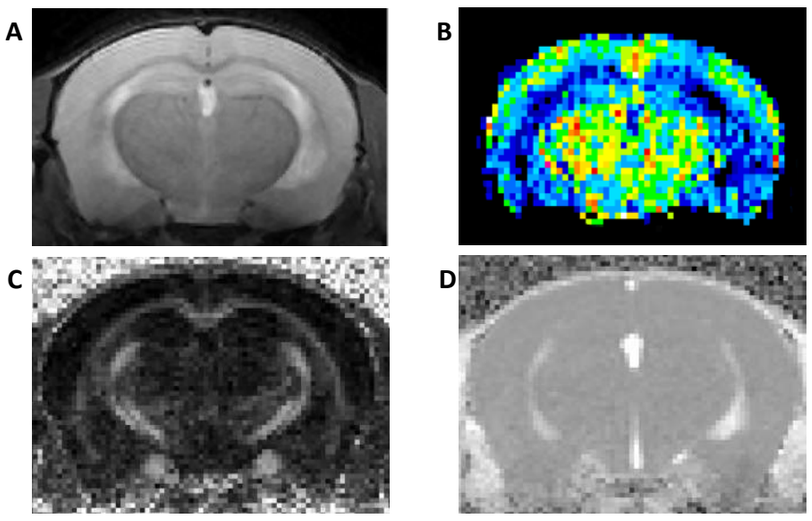


Figure 1. Representative MRI images of a 3 month old WT animal. (**A**) T2 weighted, (**B**) ASL and DTI; (**C**) Fractional Anisotropy (FA) and (**D**) Mean Diffusivity (MD) at the hippocampal level (Bregma level -2.06mm).

Neurovascular function:

Following MRI, neurovascular function was assessed by measuring blood flow responses to whisker stimulation by laser speckle contrast imaging.

Histology and Immunohistochemistry:

H&E and Perls' staining was used to assess vascular lesions, structural changes (H&E) and haemorrhages (Perls'). Furthermore white matter integrity was assessed with **Luxol Fast Blue** (LFB) and fibrinoid necrosis and vascular collagen deposition was assessed by **Martius Scarlet Blue** (MSB). Immunostaining for **myelin-associated glycoprotein** (MAG) was used to detect myelin debris.

Results

3 month *Col4a1* mice exhibit vascular changes, cerebral haemorrhages and fibrinoid necrosis

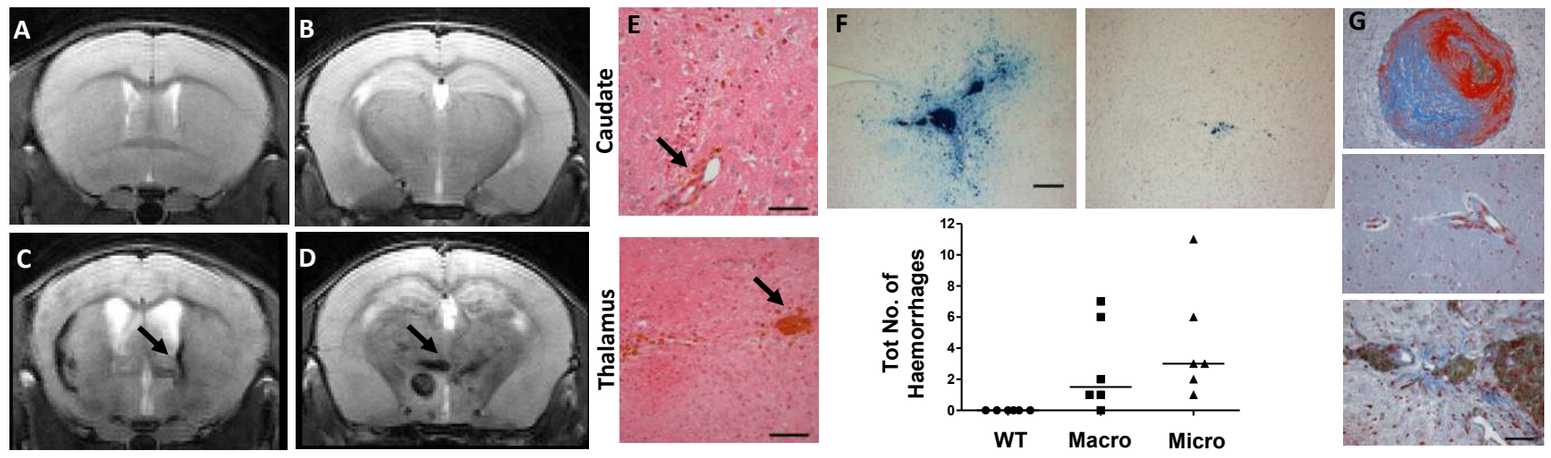


Figure 2. (**A-B**) WT T2-weighted MRI at striatal (left) and hippocampal (right) levels showing no radiological features. (**C-D**) Radiological features observed on T2-weighted MRI images of *Col4a1* mice, showing hypointense signals in caudate putamen and thalamus (black arrows). (**E**) Parallel pathological assessment (H&E) identified haemorrhagic lesions associated with hypointense signals. *Scale bar = 100µm.* No evidence of ischemic type lesions were found. (**F**) Further pathological assessment (Perls') identified two distinct types of cerebral haemorrhages, identifiable as macro (left) and micro (right) haemorrhages. *Scale bar = 200µm.* Vascular disruptions were associated with iron-positive hemosiderin-laden macrophages, consistent with old bleeds[4]. Number of macro and micro haemorrhages were assessed for each *Col4a1* mouse (*n*=6) based on Shih et al (2018). No haemorrhages were found in WT cases. (**G**) Fibrinoid necrosis (red in MSB stain) is observed in 4 out of 6 *Col4a1* mice, predominately in the thalamus (images), caudate putamen and hypothalamus. Fibrin accumulation, alongside vessel thickening and vessel wall enlargement are features which suggest blood brain barrier breakdown. *Scale bar = 50µm.* Collagen has also been observed to infiltrate the brain parenchyma (blue in MSB stain). *Scale bar = 100µm.*

3 month *Col4a1* mice show white matter microstructural alterations

A

Region of interest	*Biomarker*	*WT*	*Col4a1*	*Statistics*	*% Change*
Striatum Corpus Callosum	FA MD	0.49±0.03 9.18±0.88	0.45±0.04 10.59±1.5	*F*(1,24)=8.83, *p*=.007 *F*(1,24)=7.9, *p*=.010	-8.16 +15.36
Hippocampus Corpus Callosum	FA MD	0.36±0.04 8.1±0.35	0.34±0.06 9.5±2.27	*F*(1,24)=0.75, *p*=.395 (**ns**) *F*(1,24)=4.44, *p*=.046	-5.55 +17.28
Internal Capsule	FA MD	0.62±0.04 8.24±0.27	0.51±0.05 8.36±0.56	*F*(1,23)=37.7, *p*<.001 *F*(1,23)=0.42, *p*=.525 (**ns**)	-17.74 1.45

Abbreviations: FA (Fractional anisotropy), MD (Mean diffusivity)

Striatum corpus callosum (Bregma level -0.14mm)
Hippocampus corpus callosum (Bregma level -2.06mm)

B

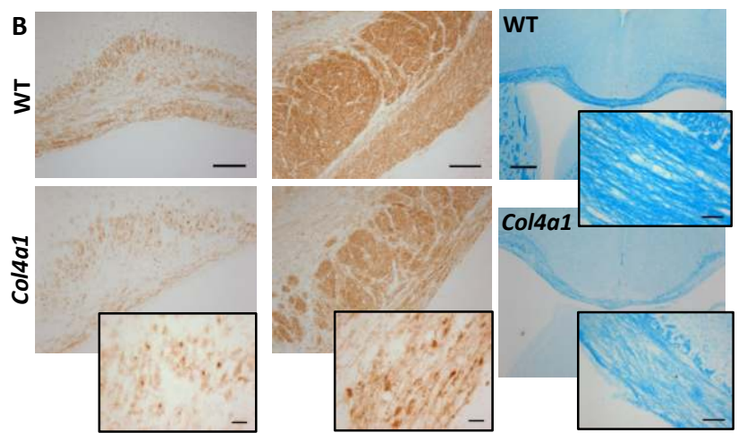


Figure 3. (**A**) Table displaying FA and MD biomarkers changes for *Col4a1* animals compared to WT. *Col4a1* mice exhibit significant reductions in FA in striatum corpus callosum and internal capsule, suggesting underlying microstructural alterations. MD (x$^{10\text{-}4}$mm^2/s) is increased in striatum and hippocampus corpus callosum, but not in the internal capsule. (**B**) *Col4a1* mice also exhibit high levels of MAG debris and vacuolation in striatum and hippocampus corpus callosum, internal capsule and optic tract (images) compared to WT. Scale bar = 100µm, 15µm insert. Pathology severity was graded on a scale from 0-3 (0 representing no debris and 3 extensive myelin debris) (**C**) Myelin pallor and disorganisation of fibres were also features observed in *Col4a1* corpus callosum (images, assessed with LFB stain). *Scale bar = 400µm, 25µm insert.*

3 month *Col4a1* mice show increases in cerebral blood flow in grey and white matter regions albeit neurovascular function (coupling) is preserved

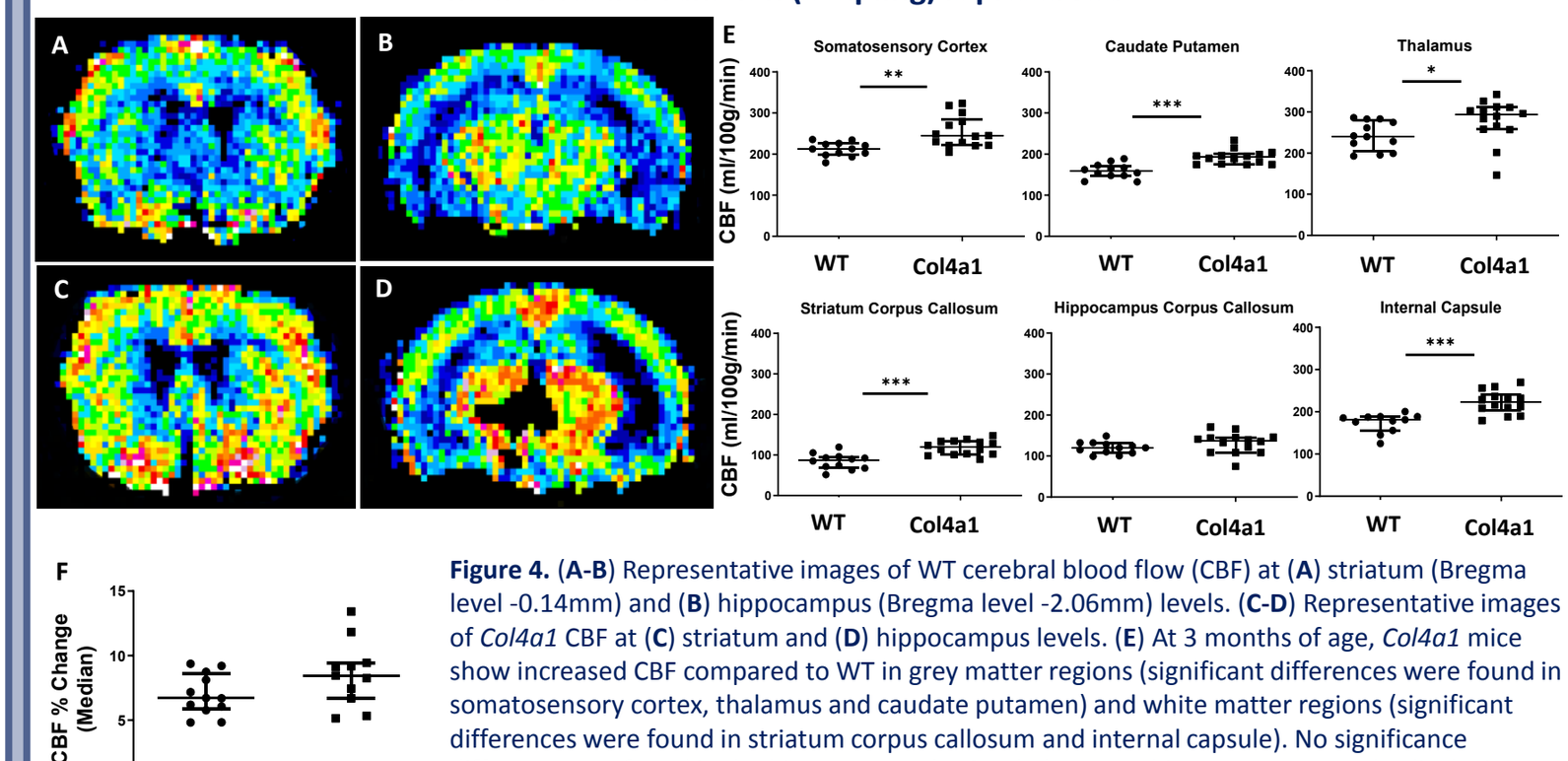


Figure 4. (**A-B**) Representative images of WT cerebral blood flow (CBF) at (**A**) striatum (Bregma level -0.14mm) and (**B**) hippocampus (Bregma level -2.06mm) levels. (**C-D**) Representative images of *Col4a1* CBF at (**C**) striatum and (**D**) hippocampus levels. (**E**) At 3 months of age, *Col4a1* mice show increased CBF compared to WT in grey matter regions (significant differences were found in somatosensory cortex, thalamus and caudate putamen) and white matter regions (significant differences were found in striatum corpus callosum and internal capsule). No significance difference was found in the hippocampus (*p*=0.55). *(Mann-Whitney U tests; graphs displaying median and interquartile range).* (**F**) Representative image of laser speckle contrast imaging recording (left) with ROI overlaying somatosensory/barrel cortex. At 3 months of age, neurovascular function/coupling in *Col4a1* mice is preserved *(Mann-Whitney U test, p*=.13*).*

Conclusions

- *Col4a1* mice exhibit **key radiological and pathological features of SVD**, including **vessel thickening, vessel wall enlargement, cerebral haemorrhage, fibrinoid necrosis and white matter alterations**.
- **White matter changes** may reflect **direct changes in oligodendrocytes/OPCs, myelination or indirect changes via endothelial cells (loss of signalling or trophic support)**
- **Increased CBF in grey and white matter regions** suggests **impaired auto-regulatory functions and/or vascular remodelling**, albeit cortical neurovascular coupling remains unaffected.

References

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Acknowledgements

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