The Fibrillin-1 Mutated Mouse Model Presents an Accelerated Cerebrovascular Aging Phenotype and Vulnerability to Mild Traumatic Brain Injury

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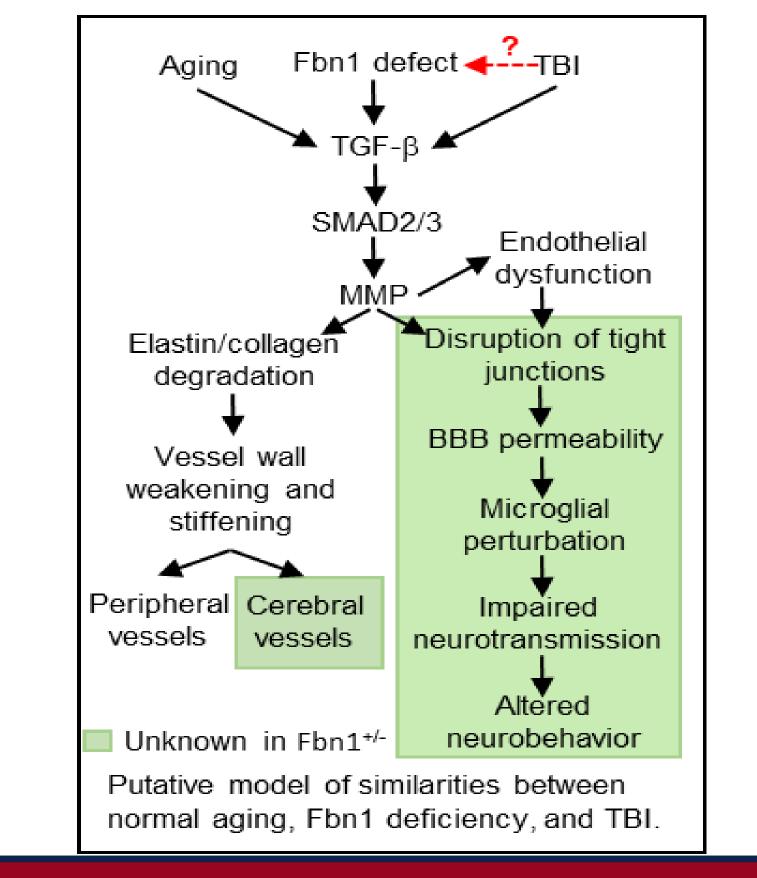


Introduction

- Age presents a significant risk for prolonged morbidity and mortality after traumatic brain injury (TBI), yet mechanisms associated with age-related cerebrovascular vulnerability following TBI remain unclear.
- Age-induced transforming growth factor-β (TGF-β) upregulation is implicated in cerebrovascular dysfunction, loss of bloodbrain barrier (BBB) integrity, and increased risk of neuroinflammation and cognitive impairment.
- Fibrillin-1 (*Fbn1*) mutation increases TGF-β availability and signaling in *Fbn1*+/- mice, inducing peripheral vascular dysfunction by 6-months of age.
- This study investigated Fbn1 deficiency on cerebrovascular integrity and increased vulnerability to TBI using a transgenic mouse model with constitutively upregulated TGF-β signaling to isolate the role of TGF- β in vascular aging and vulnerability to TBI.

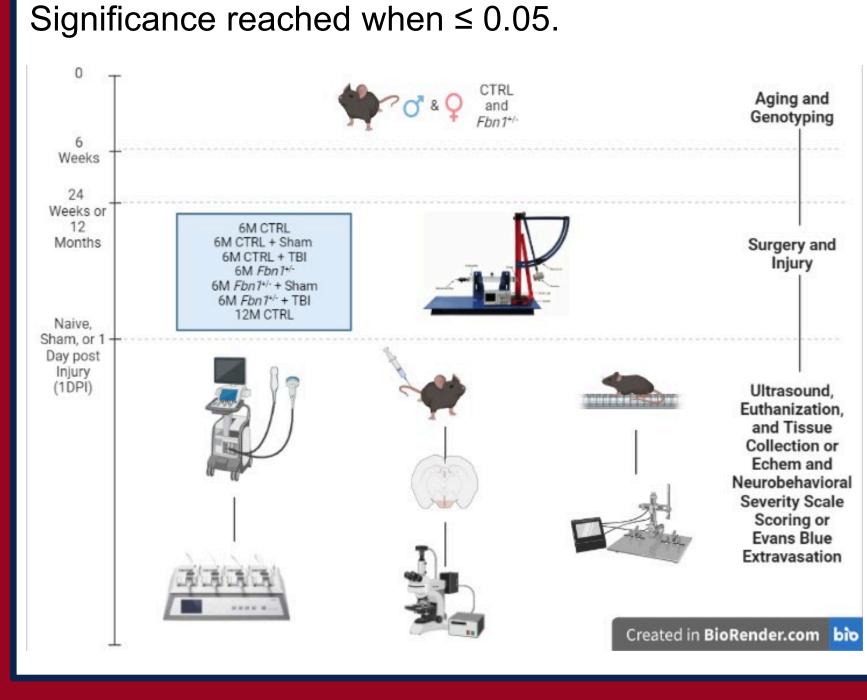
Hypothesis

Fbn1 mutation accelerates cerebrovascular rigidity, BBB permeability, and neurological alterations associated with aging, leaving the brain more vulnerable to diffuse TBI.

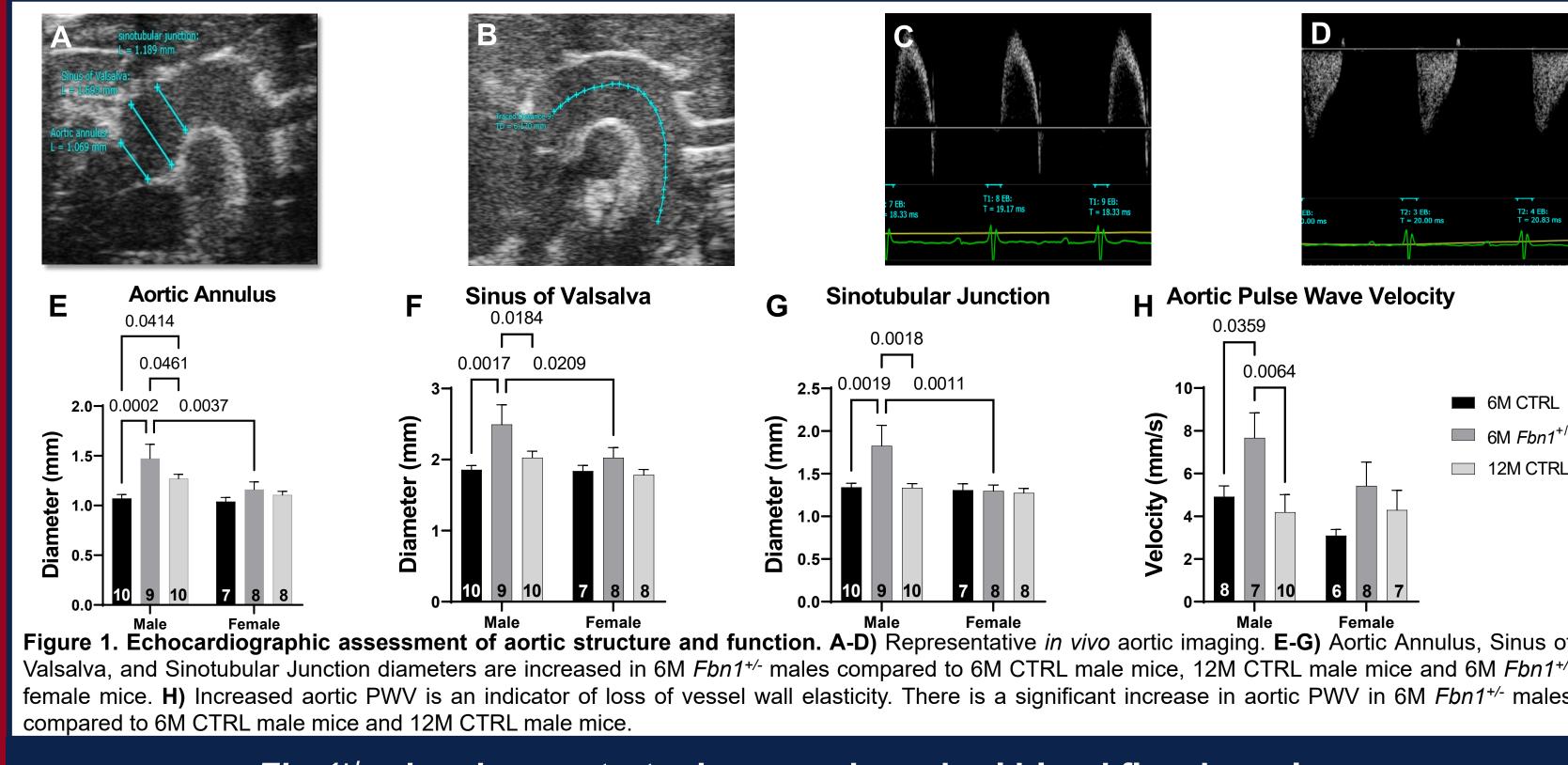


Experimental Design

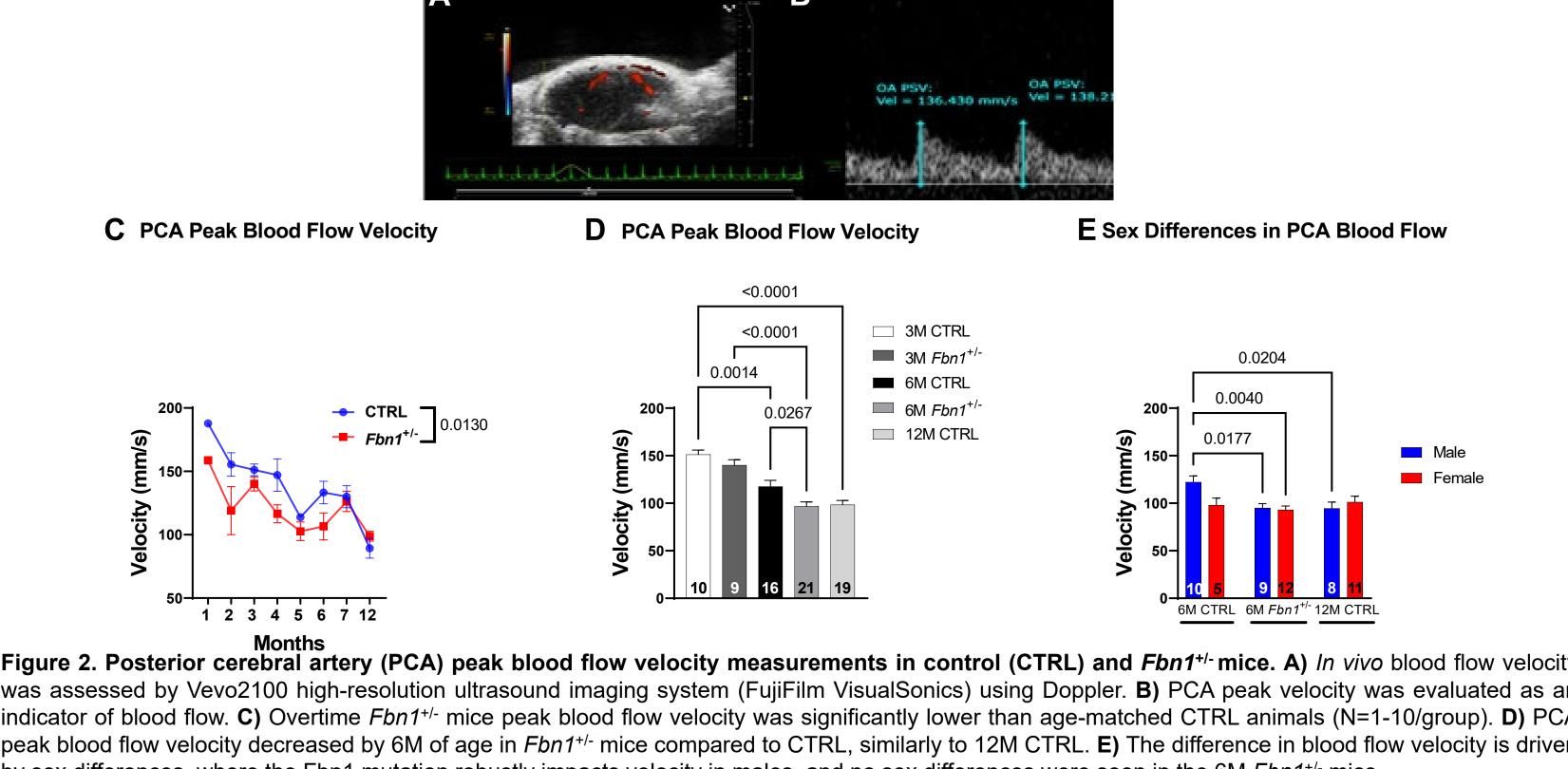
The following outcomes were compared between male and female 6-month-old (6M) Fbn1+/- and C57BL/6 wildtype (CTRL) mice and 12-month-old (12M) CTRL mice: PCA blood flow, PCA rupture point, BBB permeability, NSS outcomes, injury viability, microglial perturbation, and real-time glutamate neurotransmission using in vivo amperometry with glutamate selective microelectrode arrays. All graphs, curves, and statistical significances were determined and created with GraphPad Prism software. One-way analysis of variance (ANOVA), Two-way ANOVA, and Three-way ANOVA tests were performed on data sets with one, two, or three independent variables, respectively. Data reported as mean + SEM.



Fbn1^{+/-} mice demonstrate peripheral vascular dysfunction in males, but not females



Fbn1^{+/-} mice demonstrate decreased cerebral blood flow in males



by sex differences, where the Fbn1 mutation robustly impacts velocity in males, and no sex differences were seen in the 6M *Fbn1*+/- mice

Fbn1+/- mice demonstrate decreased vessel wall strength

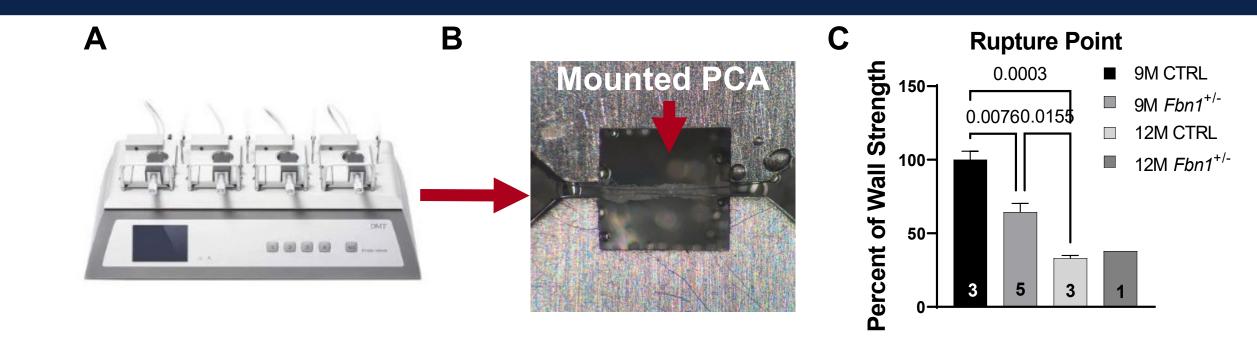


Figure 3. Maximum wall strength in 9M and 12M CTRL and Fbn1+/- mice. PCA segments (2mm) were isolated. A) Ex vivo assessment of vessel wall strength was performed using small vessel wire myography. B) Mounted PCA segments were stretched to the point of rupture and the maximum force recorded. C) Rupture point, a measure of wall strength, was decreased in 9M Fbn1+/- mice compared to age-matched CTRL but increased compared to 12M

Fbn1^{+/-} mice show increased neurobehavioral alterations

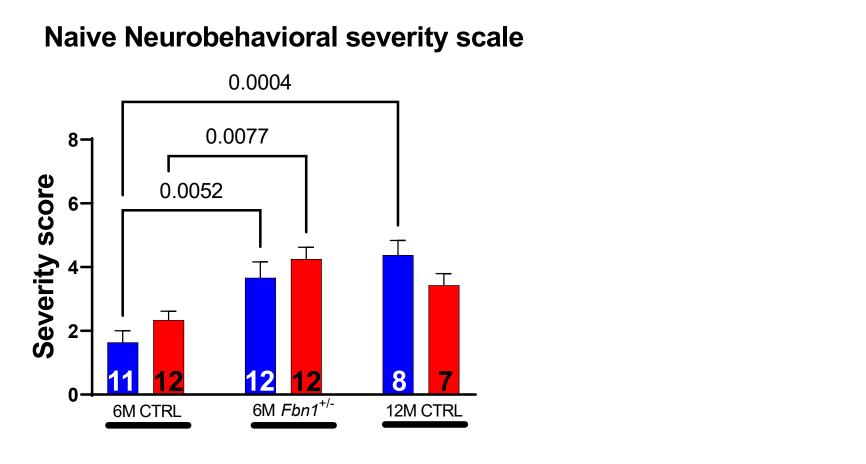


Figure 5. Neurobehavioral severity scale (NSS) scores in 6M and 12M CTRL and Fbn1+/- mice with and without injury. Mice were assessed using the vised NSS (Yarnell et al. 2016. Curr. Protoc. Neurosci.). A) There were significant increases in severity scale scores in 6M Fbn1+/-, 12M CTRL mice, and 3) Fbn1+/-+ 1 day-post-injury (1DPI) compared to 6M CTRL sex-matched mice.

Fbn1^{+/-} and female mice require lower FPI force to achieve inclusion criterion for mTBI

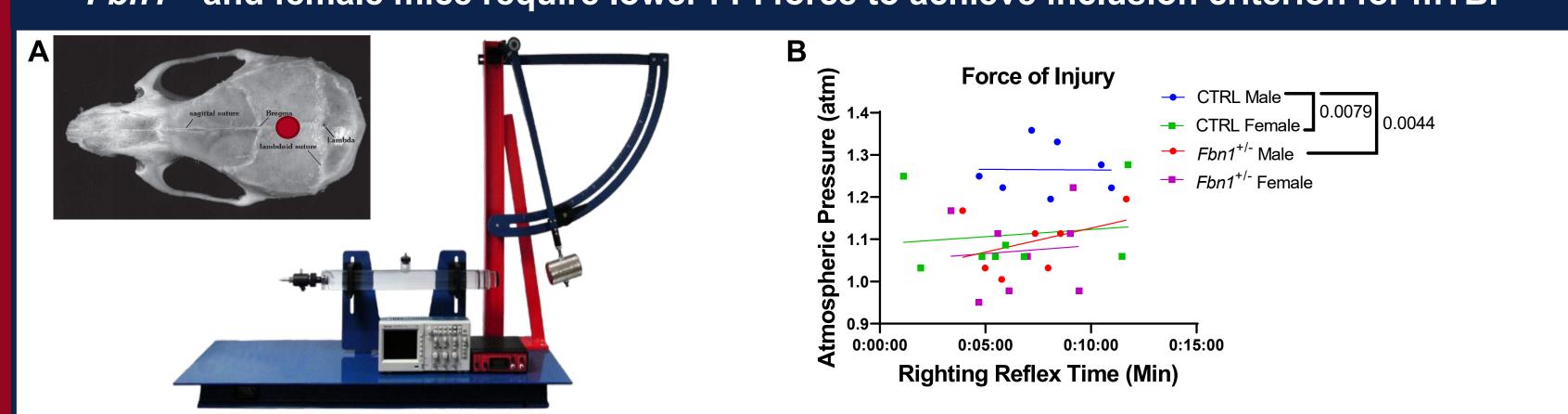
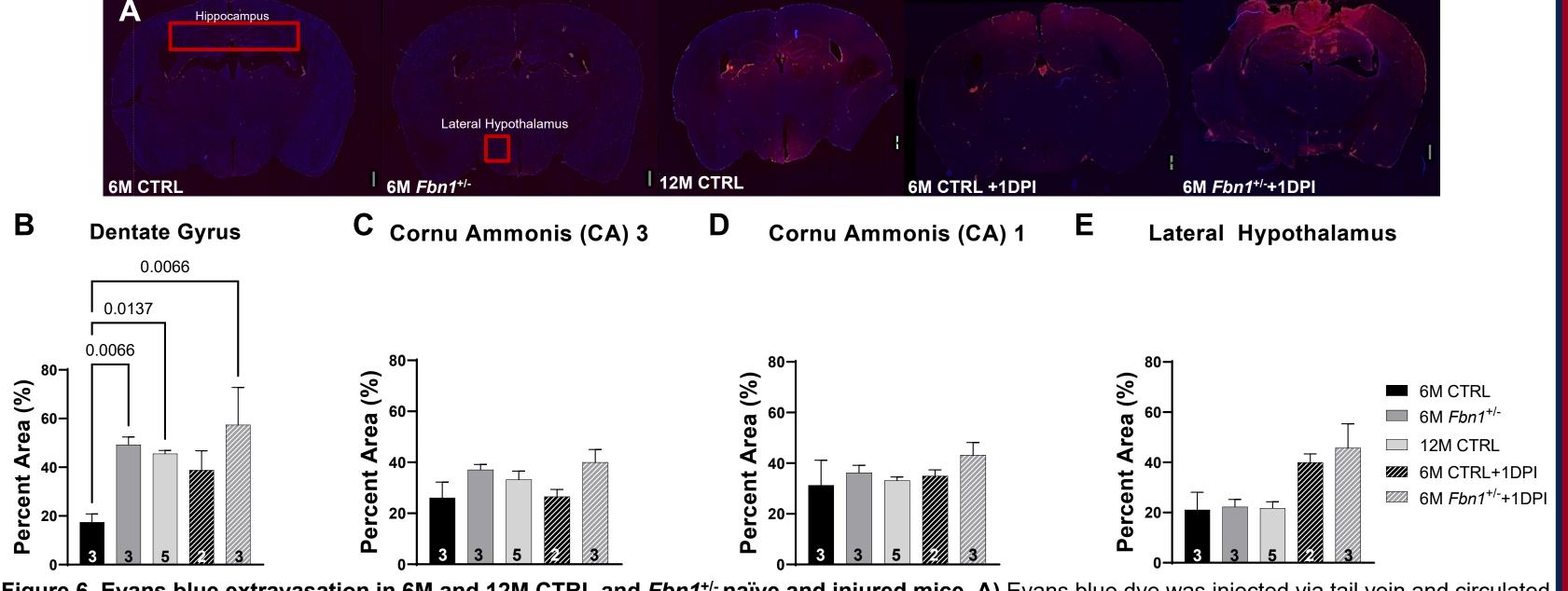


Figure 4. Force of injury in 6M CTRL and Fbn1+/ mice. A) Midline fluid percussion injury (FPI) was utilized. A cranial hub, secured during midlin craniectomy (red dot), was attached to the injury device where an adjustable pendulum falls and strikes the plunger, generating a fluid pulse that impacts the intact dural surface, resulting in diffuse axonal injury. B) A significantly lower injury force (atm) induced mild to moderate TBI in CTRL female and Fbn1 male mice compared to age-matched CTRL male mice, an indication of vulnerability to TBI in male Fbn1+/- mice (inclusion criterion = 5-10min righting reflex

Methods and Results

Fbn1^{+/-} mice BBB permeability is increased in the hippocampus and vulnerable to TBI



for 18hrs (Mendes, et al. Frontiers in neurology.). B) An increase in BBB permeability in the dentate gyrus of the hippocampus of 6M Fbn1+/- and 12M CTRL mice compared to 6M CTRL mice is approaching significance. C,D) The CA1 and CA3 of the hippocampus were not different. E) The lateral hypothalamus was used as a control region due to its fenestrated BBB, where no differences were seen. Qualitatively, extravasation was substantially increased in brains of 6M Fbn1+/-+1DPI compared to 6M CTRL+1DPI

Fbn1^{+/-} mice BBB permeability is more similar to older CTRL and Injured mice

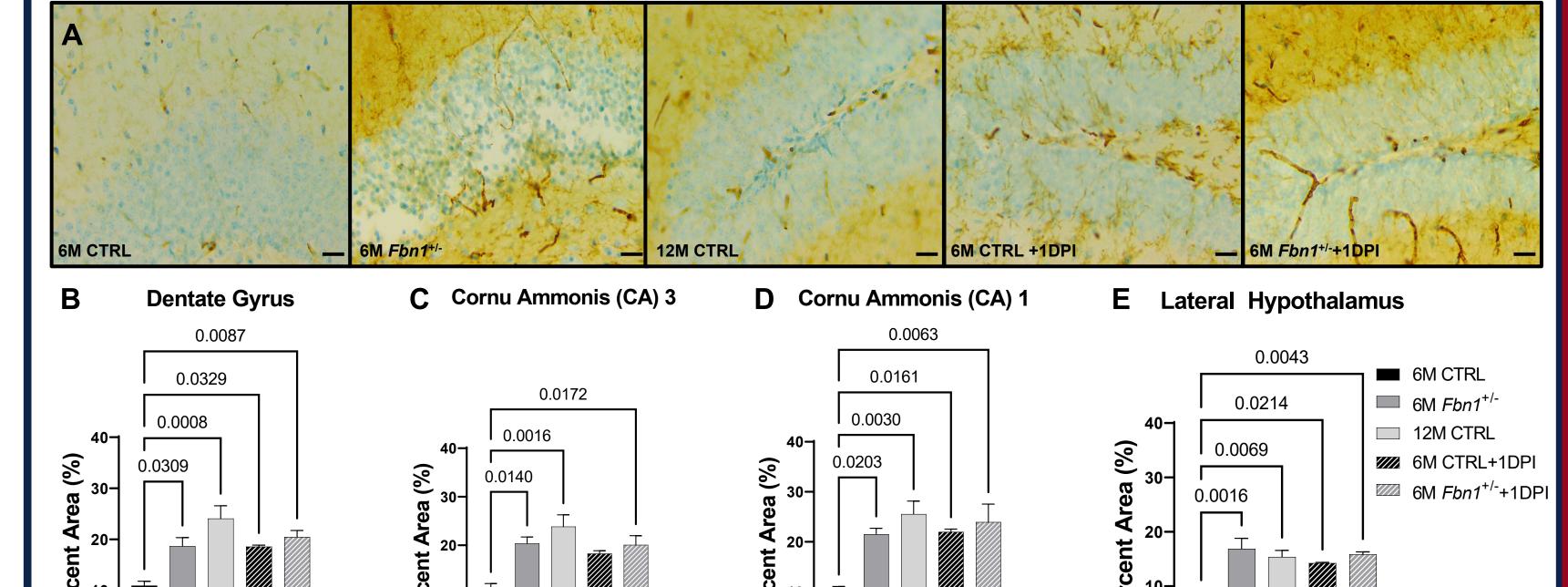
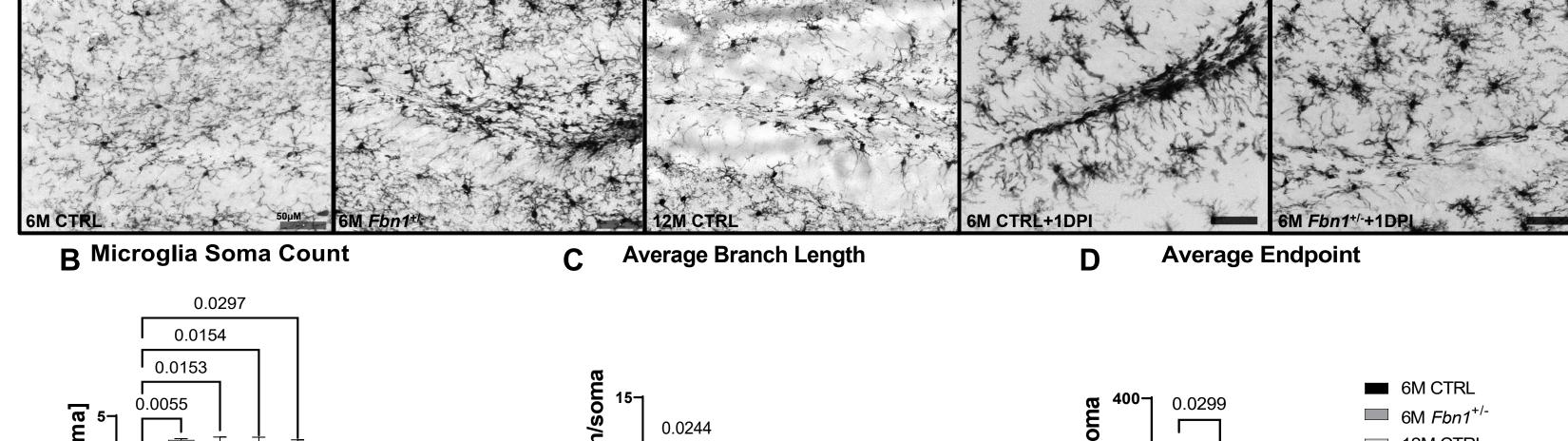
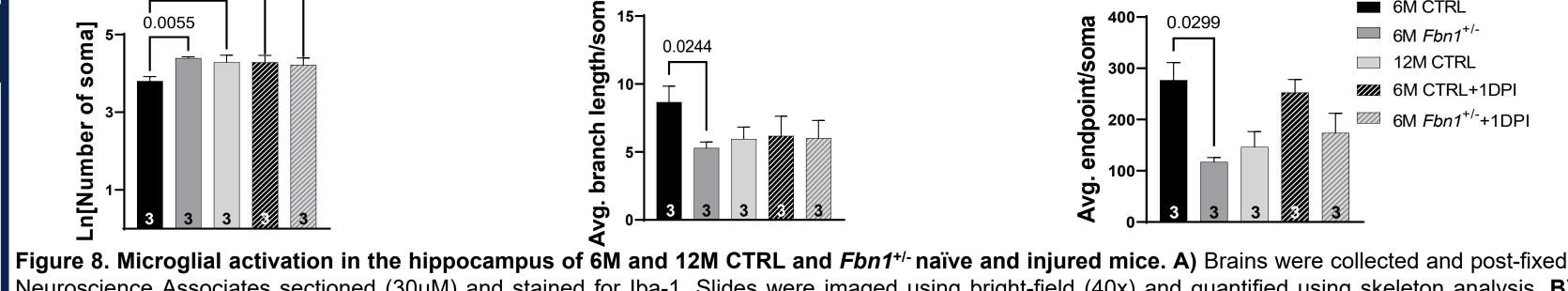


Figure 7. Immunoglobulin G (IgG) Staining in 6M and 12M CTRL and Fbn1+/- naïve and injured mice. A) Brains were collected and post-fixed. Neuroscience Associates sectioned (30µM) and stained for IgG. Slides were imaged using bright-field (40x) and quantified using intensity analysis (ImageJ). peak blood flow velocity decreased by 6M of age in Fbn1+/- mice compared to CTRL, similarly to 12M CTRL. E) The difference in blood flow velocity is driven BBB permeability was increased in the (B) dentate gyrus, (C) CA3, and (D) CA1 of the hippocampus and (E) lateral hypothalamus of injured mice, 6M Fbn1+/-, and 12M CTRL mice compared to 6M CTRL mice.

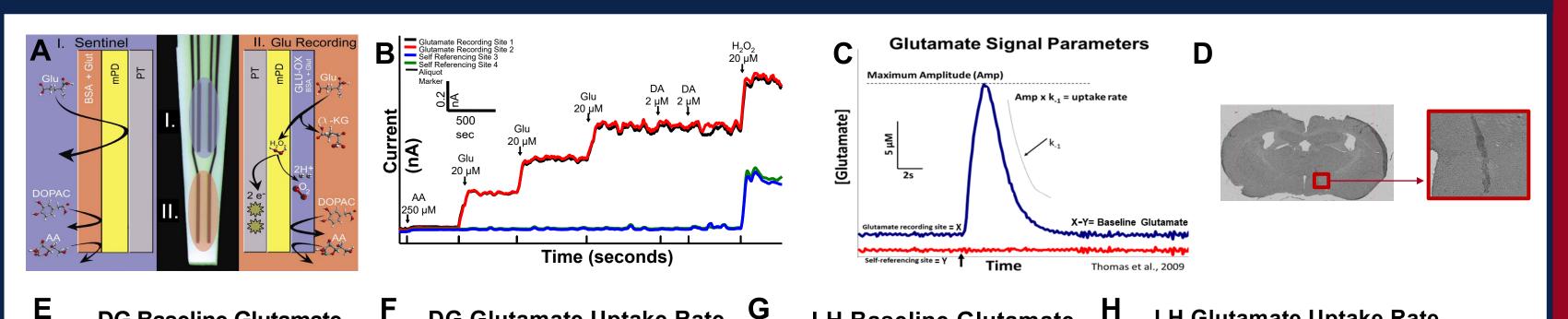
Neuroinflammation is increased in *Fbn1*+/- mice seen through microglia activation





Neuroscience Associates sectioned (30µM) and stained for Iba-1. Slides were imaged using bright-field (40x) and quantified using skeleton analysis. **B)** Increased soma count suggests increased recruitment of microglia in 6M Fbn1+/-, 12M CTRL, and 6MCTRL+1DPI mice compared to 6M CTRL mice. C,D) Decreased average branch length and endpoints per microglia are indicative of neuroinflammation in the 6M Fbn1+/- mice compared to 6M CTRL mice.

Altered glutamate neurotransmission in *Fbn1*+/- mice and 12M CTRL mice



DG Glutamate Uptake Rate G

Figure 9. In vivo amperometry using glutamate selective microelectrode arrays (MEAs) in the DG of the hippocampus and Lateral Hypothalamus (LH) of 6M and 12M CTRL and Fbn1+/- naïve mice. A) Representative MEA coating to achieve glutamate selectivity. B) MEA calibration performed to confirm glutamate selectivity. C) Representative glutamate peak and measures assessed. D) Representative image of MEA placement verification. I Baseline glutamate concentration levels are increased in 12M CTRL males compared to 6M CTRL male mice. F) In males, slower glutamate uptake rate approached significance between 6M CTRL and 12M CTRL mice indicative of age-related changes in glutamate clearance kinetics. **G)** Baseline glutamate concentration levels are increased in 12M CTRL females compared to 6M CTRL female mice. H) LH glutamate uptake rate is similar between groups.

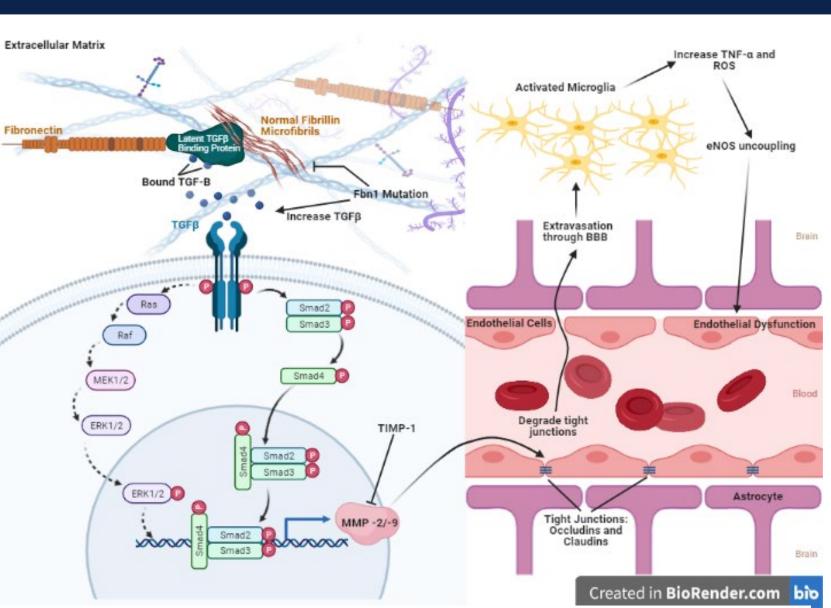
Conclusions

- These are the first in vivo and ex vivo experiments in the cerebrovasculature and the first known behavioral experiment in *Fbn1*^{+/-} mice.
- Fbn1+/- mice have phenotypes more like 12M CTRLs, supporting the hypothesis that constitutively expressed TGF-β can accelerate cerebrovascular aging, which was more robust in males. TBI data indicated changes in TGF-β may also disrupt the grey matter extracellular

Significance/Impact

- *Fbn1*+/- mice may be useful in teasing out specific mechanisms responsible for aging, cerebrovascular compromise, and increased vulnerability of the geriatric patient population to TBI.
- These effects are sex-dependent, where the role of sex hormones in neuroprotection requires preclinical and clinical investigation.
- These data indicate that response to TBI may also be affected by predisposed disorders and sex differences may be altered within those disorders.
- These results implicate TGF-β signaling and its downstream products as potential mechanisms and therapeutic targets for future directions addressing vulnerability to mTBI.

Potential Mechanism



Fbn1 mutation results in increased TGF-β signaling. This pathway leads to increased matrix metalloproteinases (MMPs) -2/-9, which are implicated in promoting degradation of tight junctions, BBB permeability, microglial perturbation, corresponding neurological alterations, and eNOS uncoupling leading to endothelial dysfunction; all mechanisms associated with age-related pathophysiology that can be targeted for therapeutic intervention in CTD, TBI, and abnormal aging.

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