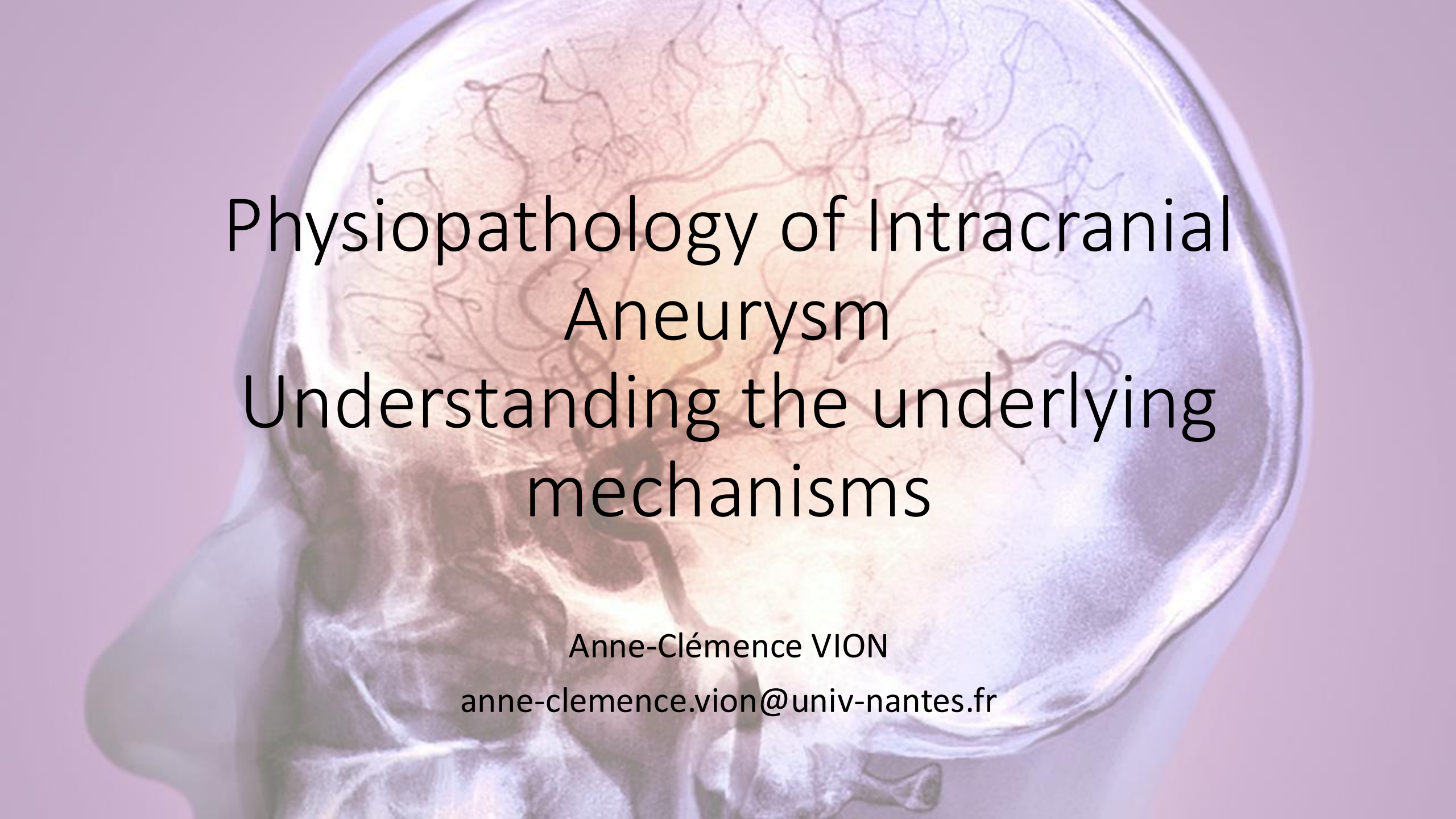
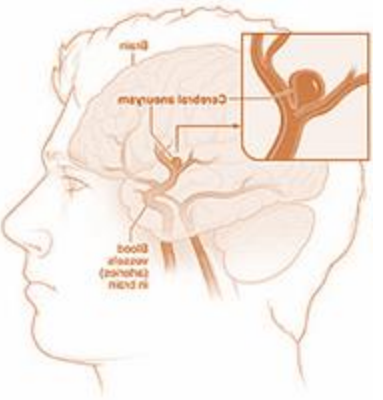


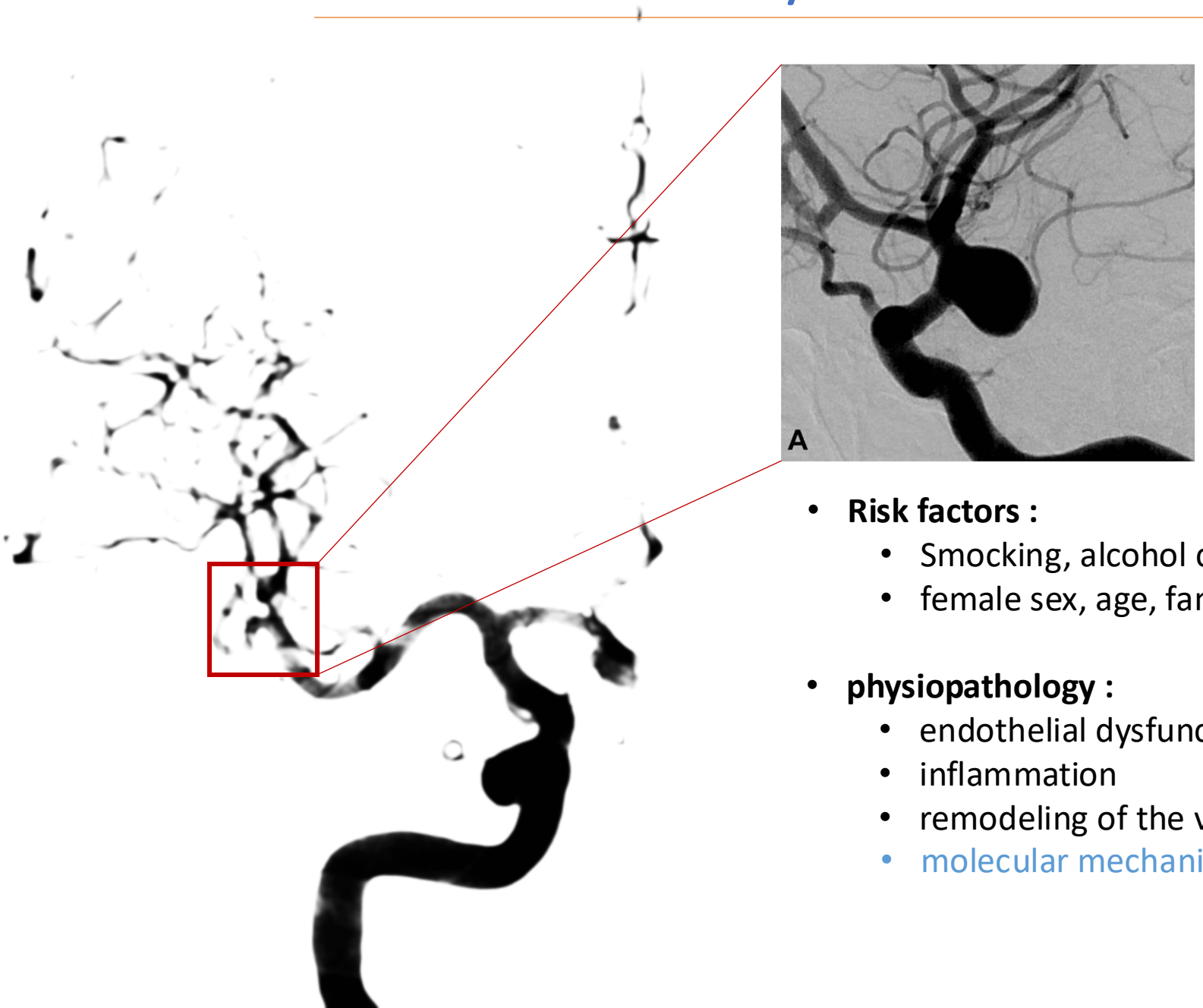
Physiopathology of Intracranial Aneurysm
Understanding the underlying mechanisms

Anne-Clémence VION

anne-clemence.vion@univ-nantes.fr

Intracranial Aneurysm : causes and consequences





- 3% of the general population
- unpredictable rupture
- 50% of death in case of rupture

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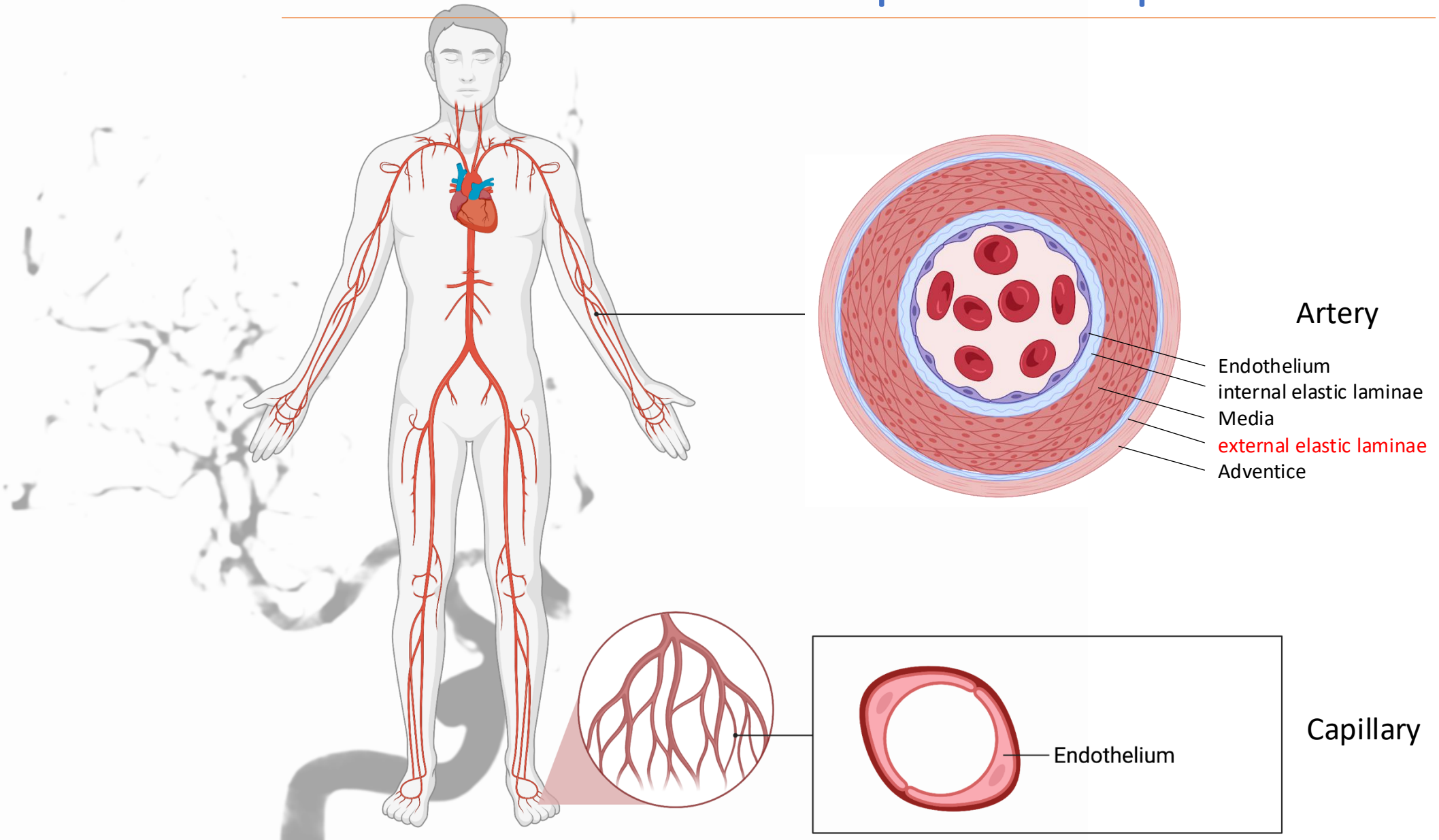
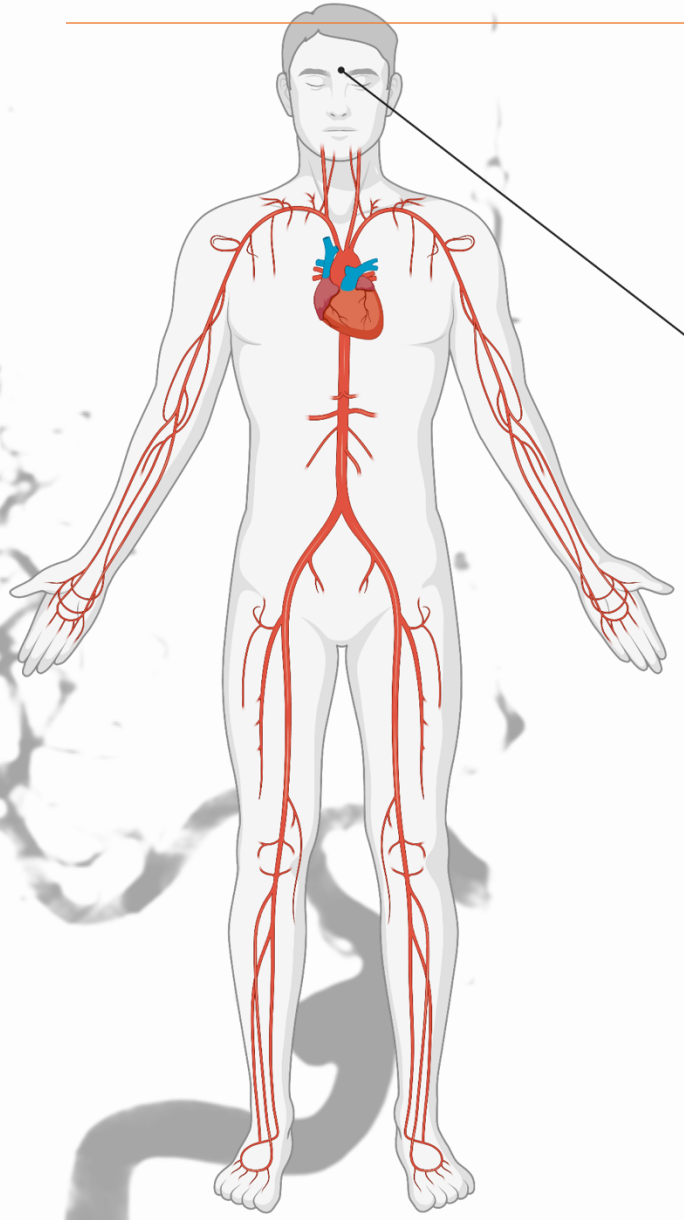
  - endothelial dysfunction
  - inflammation
  - remodeling of the vascular wall
  - molecular mechanisms at play are mostly unknown

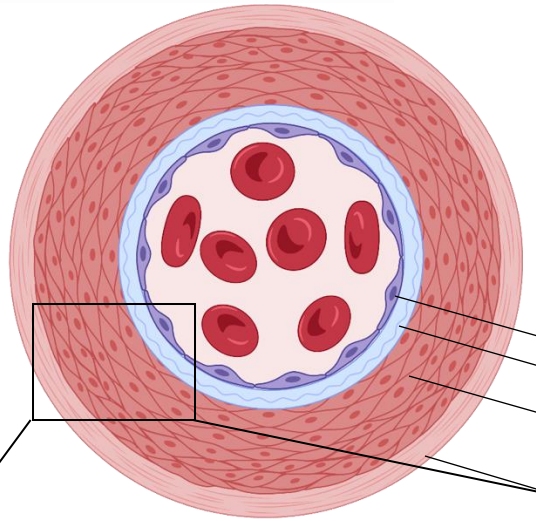
Cerebral arteries : a specific composition



Created with BioRender.com

Cerebral arteries : a specific composition



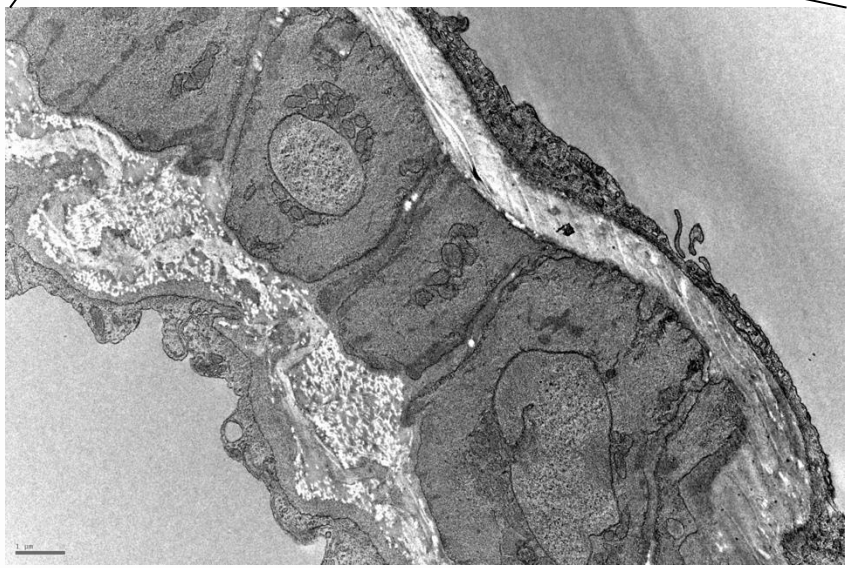


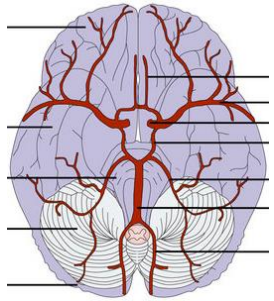
NO external elastic laminae

Cerebral artery

Endothelium
internal elastic laminae
Media

Adventicia

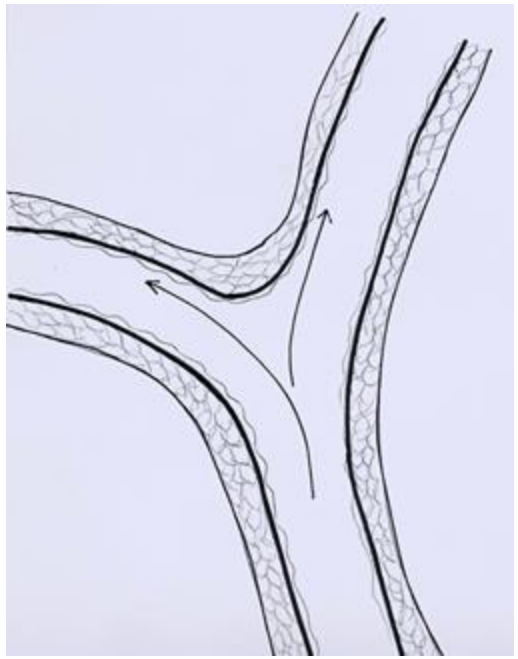


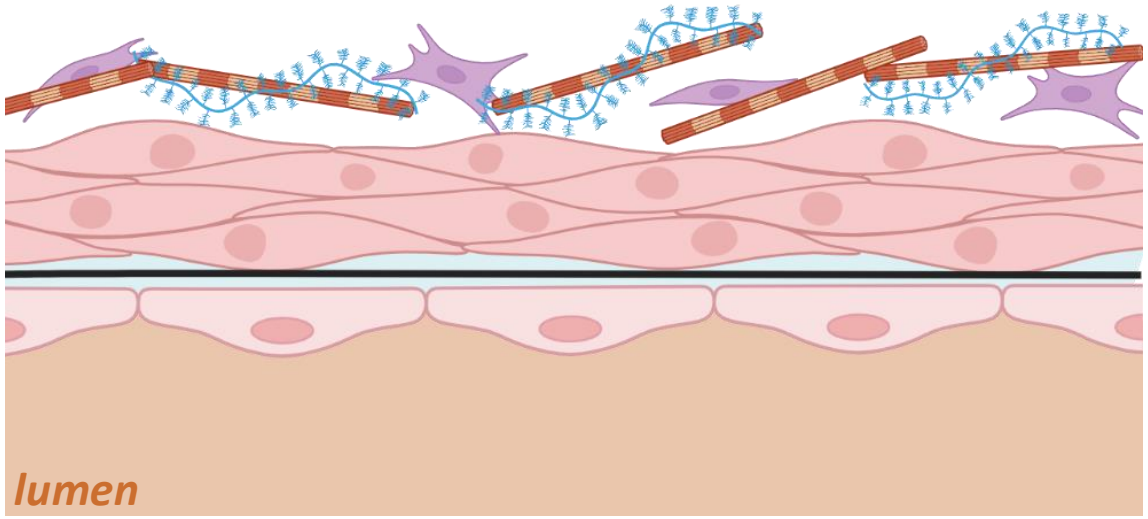


open angle at bifurcation

Created with BioRender.com

Natural history of Intracranial Aneurysm





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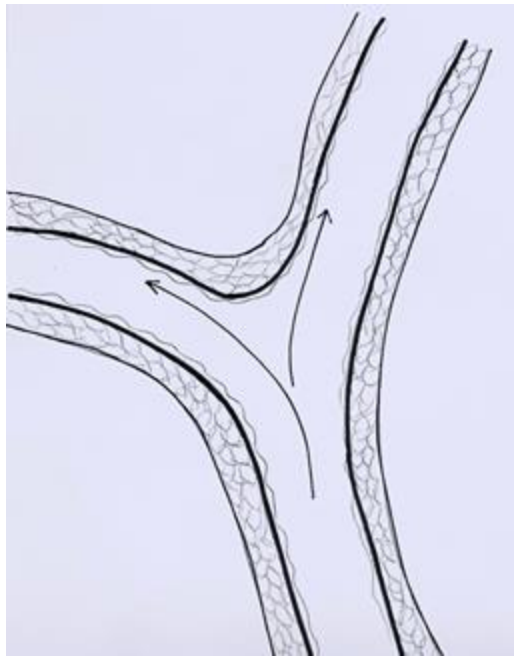
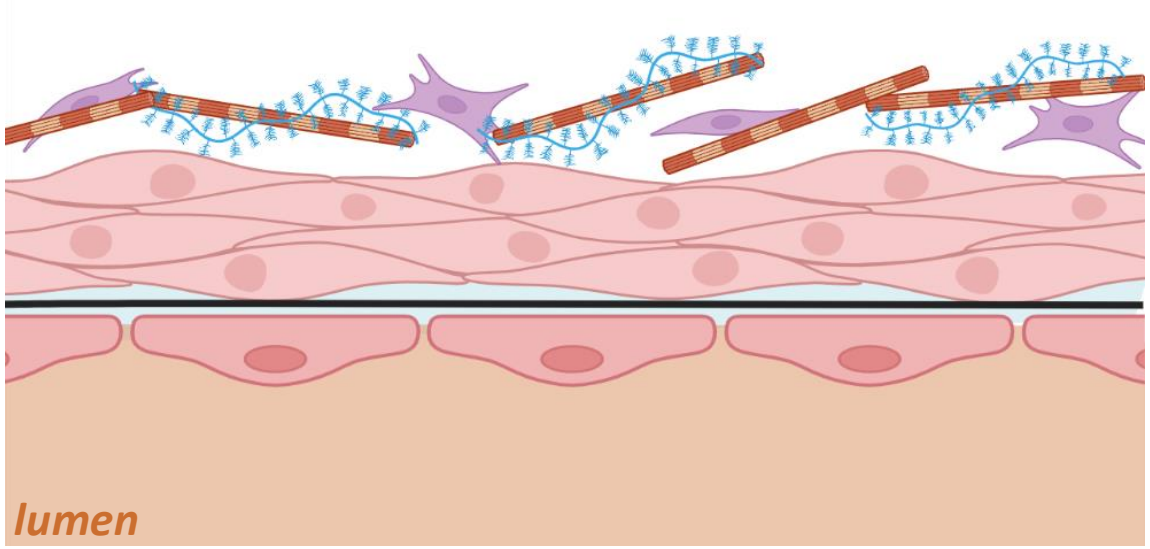




endothelial dysfunction

endothelial cell

SMC

internal elastic laminae

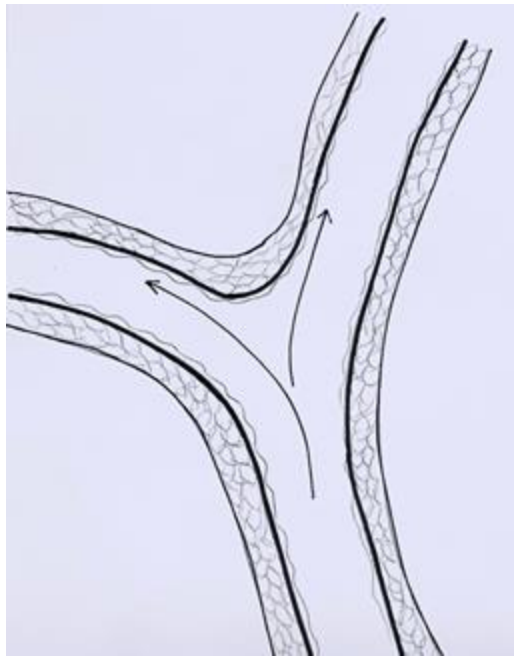
Fibroblastes

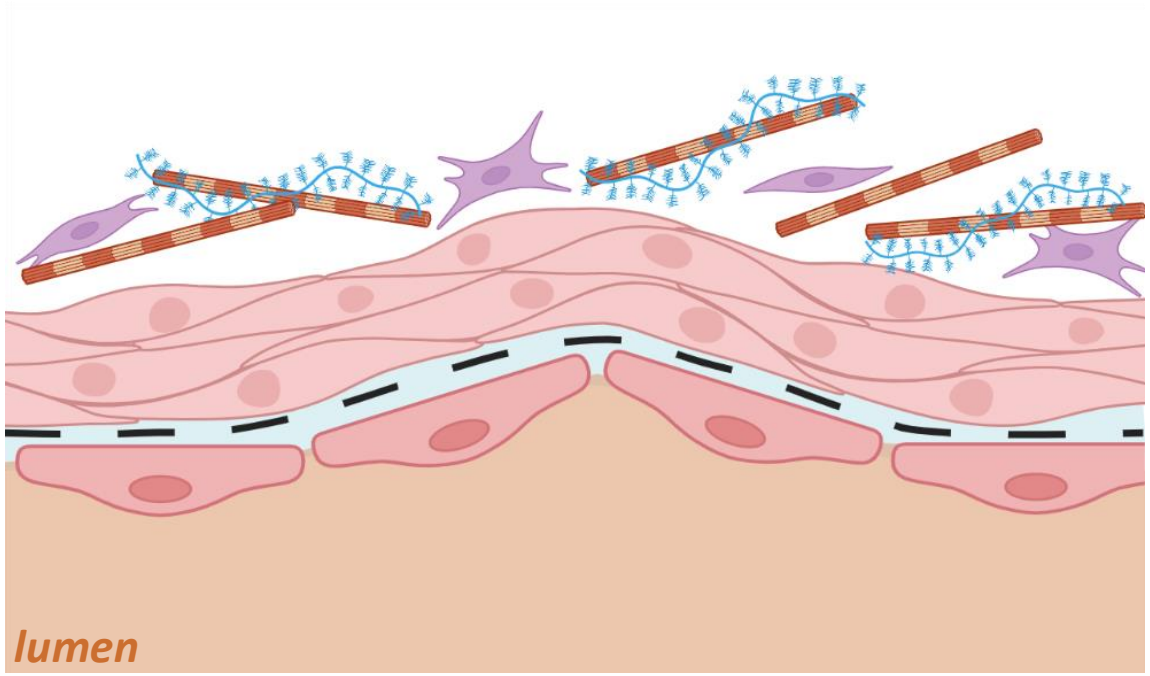
Proteoglycan

collagen fibers

Sheinberg *et al.*, Neurosurg, 2019

Natural history of Intracranial Aneurysm





endothelial cell

SMC

internal elastic laminae

Fibroblastes

Proteoglycan

collagen fibers

internal elastic laminae fragmentation

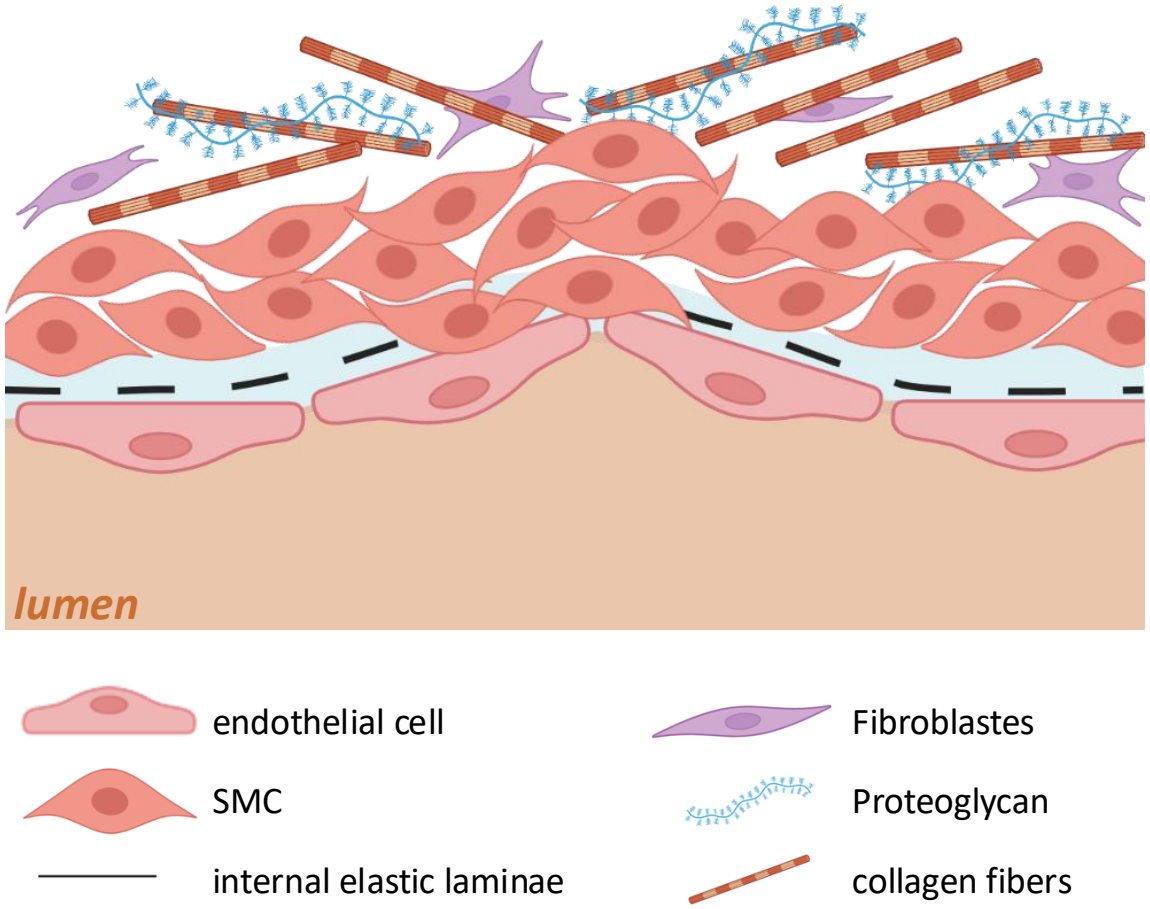
Surgery on rat
Hashimoto model

Futami *et al.*, Stroke, 1995

Natural history of Intracranial Aneurysm

secretory and proliferative phenotype

Animal model
Rat with elastase injection

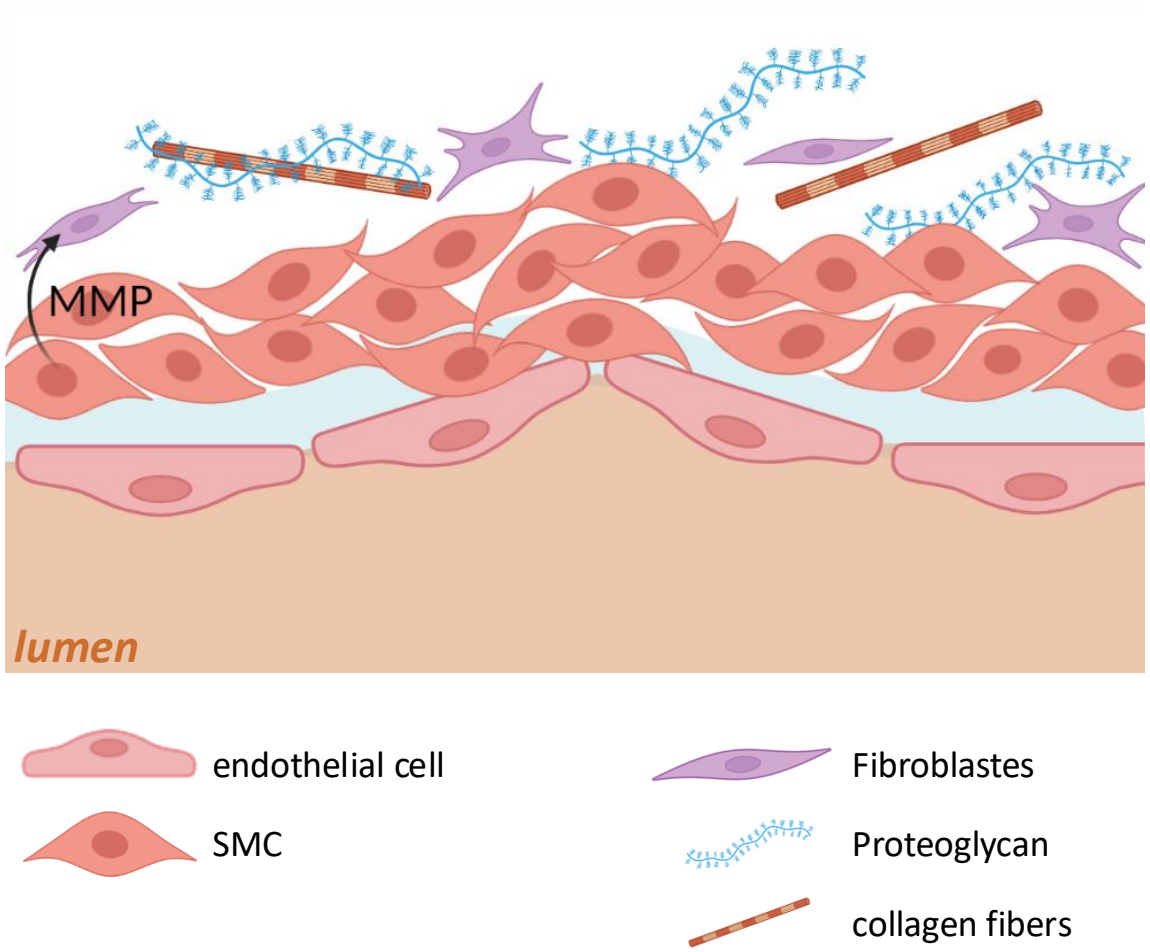


Li *et al.*, J Neuroinflammation, 2020

Natural history of Intracranial Aneurysm

matrix metaloproteinases secretion

Animal model
Rabbit with CCA ligation

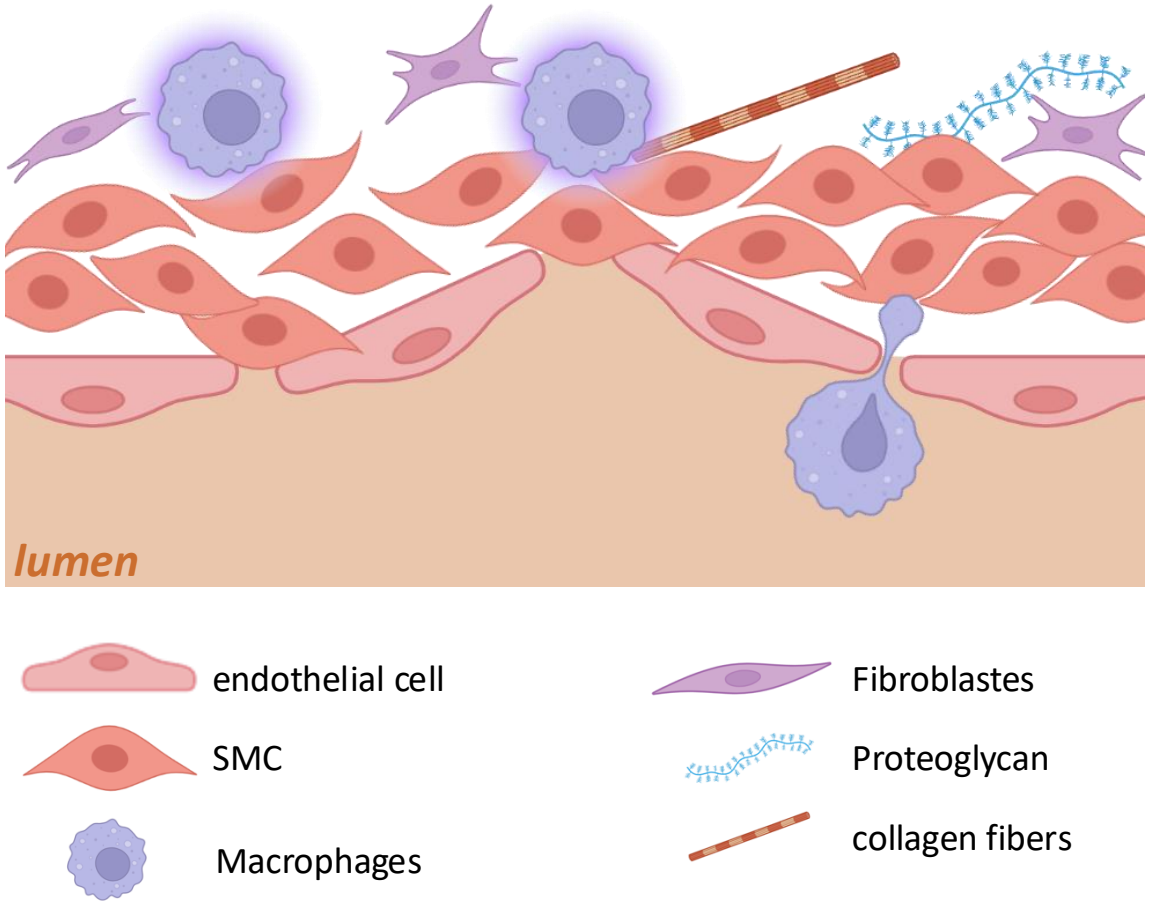


Mandelbaum *et al.*, PLoS ONE, 2013

Natural history of Intracranial Aneurysm

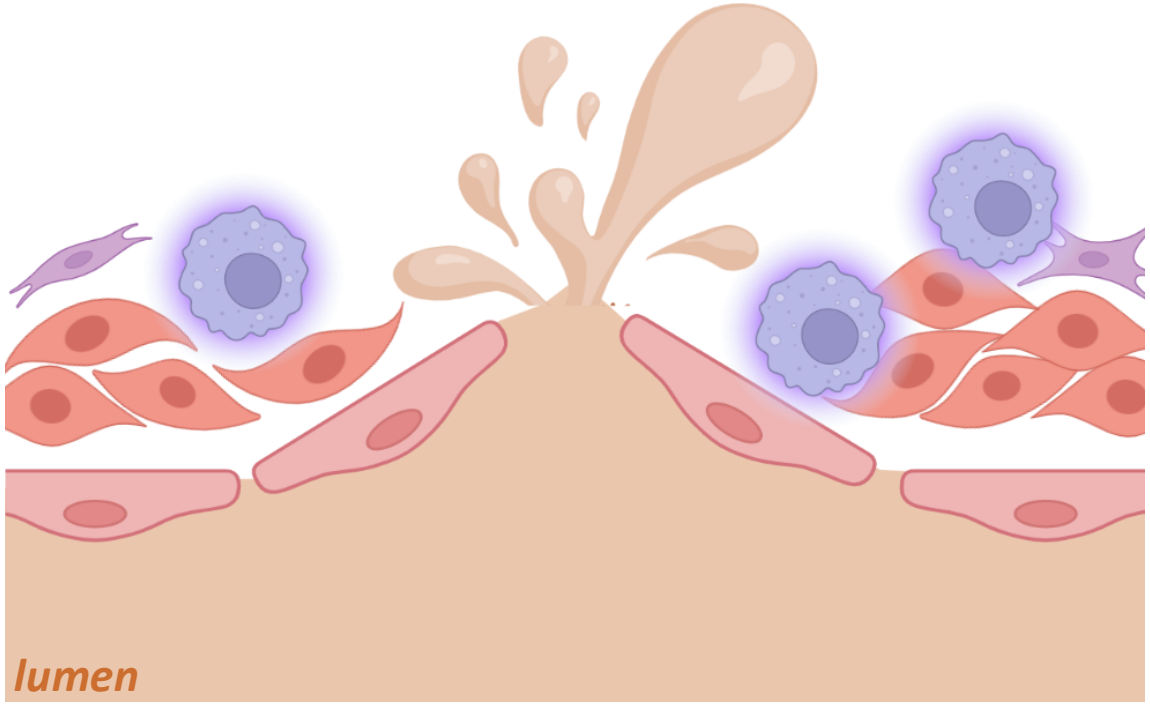
Inflammatory cells recruitement

SMC apoptosis



Aoki *et al.*, Circulation, 2007

Natural history of Intracranial Aneurysm



endothelial cell

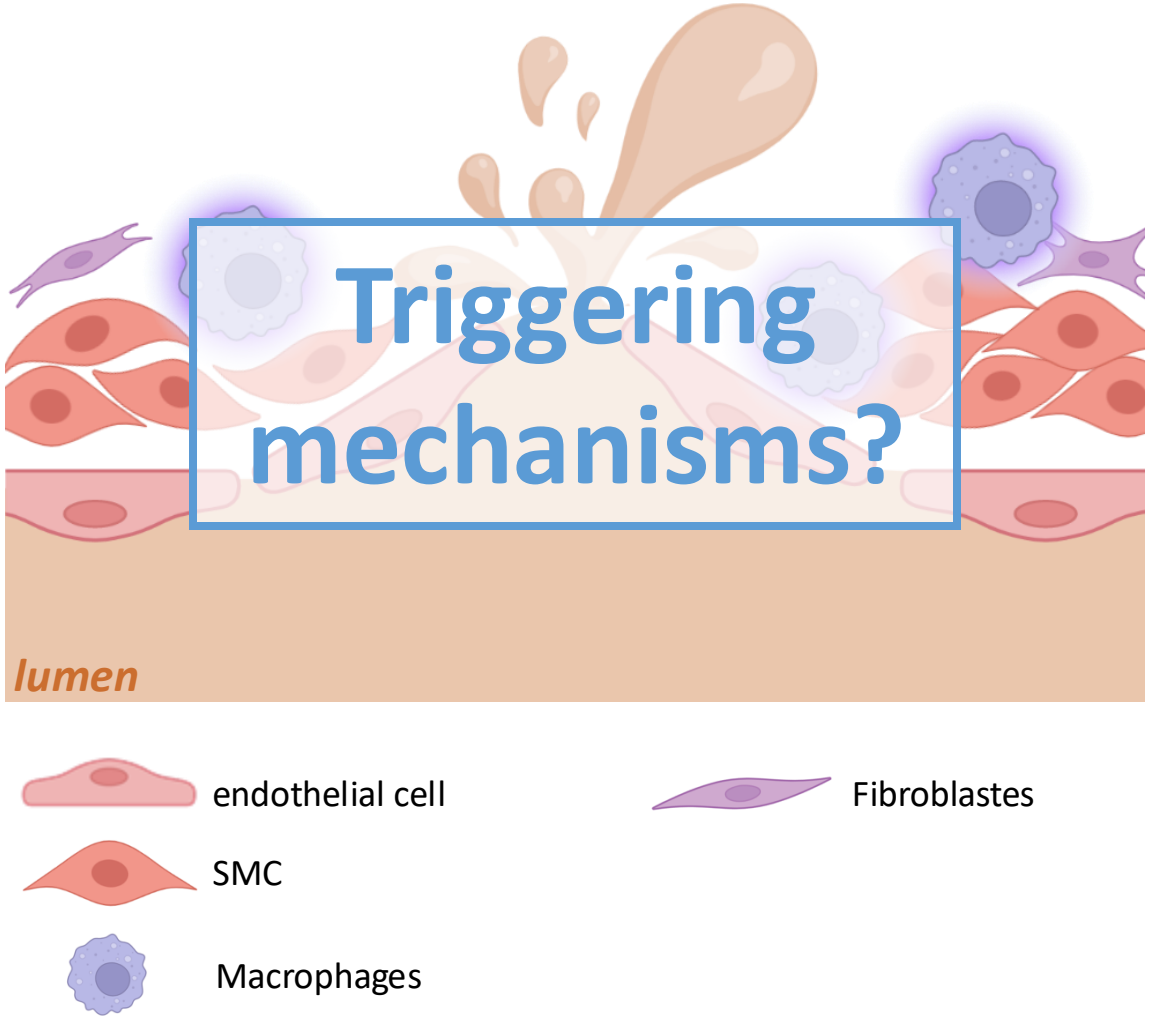
Fibroblastes

SMC

Macrophages

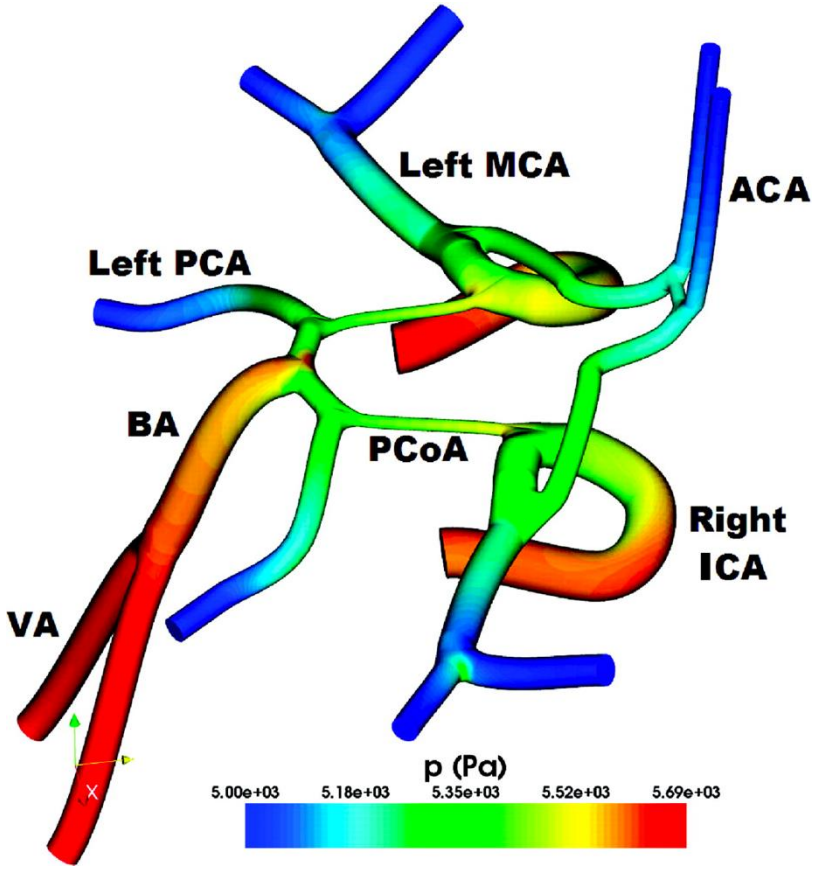
Kondo *et al.*, Stroke, 1998

Natural history of Intracranial Aneurysm



What happens within the cells ?

Intracranial Aneurysm : causes and consequences



Sandve Alnæs. *Stroke*. 2007

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- **Risk factors :**
  - Smocking, alcohol consumption, hypertension
  - female sex, age, familial history

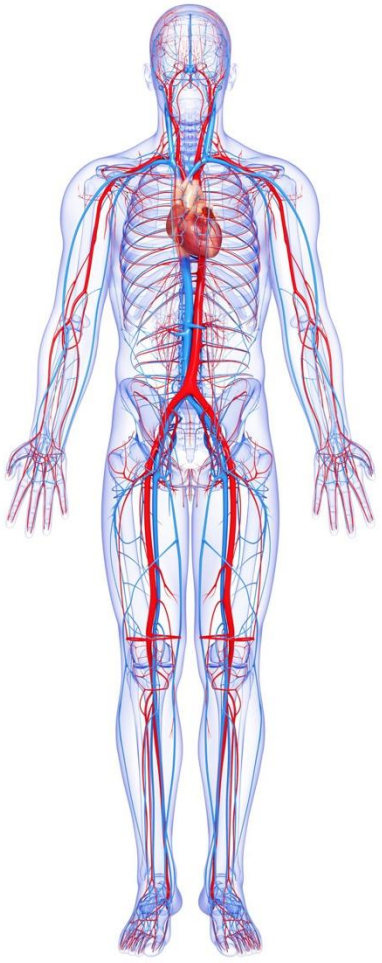
- **physiopathology :**
  - endothelial dysfunction
  - inflammation
  - remodeling of the vascular wall
  - molecular mechanisms at play are mostly unknown
  - associated with altered **hemodynamics**

Just 2 slides of physics :

What are hemodynamics ?

It is everything that concern the dynamics of blood flow.
It gathers in one term the physical laws that govern the flow of blood in the blood vessels, ie, in an hydraulic circuit.

As any system it is highly controlled

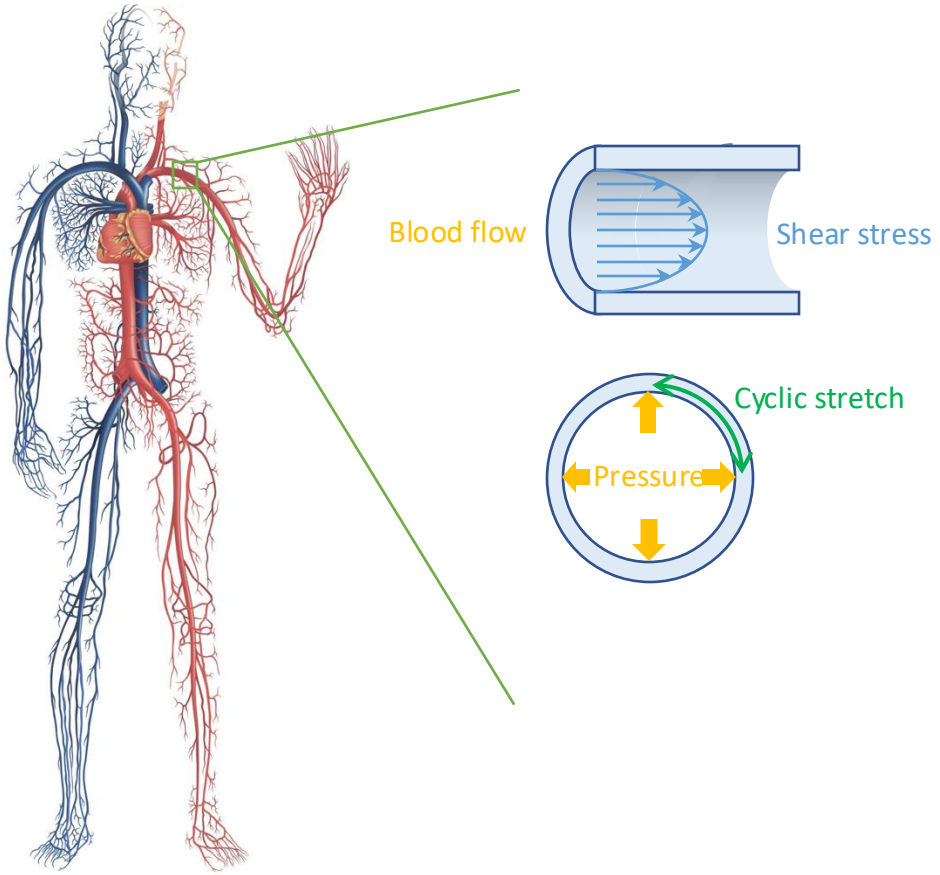


What blood flow is made to ?

Blood flow ensures the transportation of :
nutrients, hormones, metabolic wastes, O_2 and CO_2 throughout the body

to maintain cell-level metabolism, the regulation of the pH, osmotic pressure and temperature of the whole body, and the protection from microbial and mechanical harms.

Just 2 slides of physics :

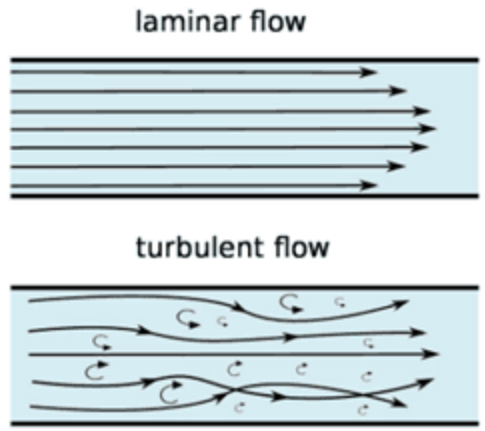


Shear stress
Hagen-Poiseuille Law

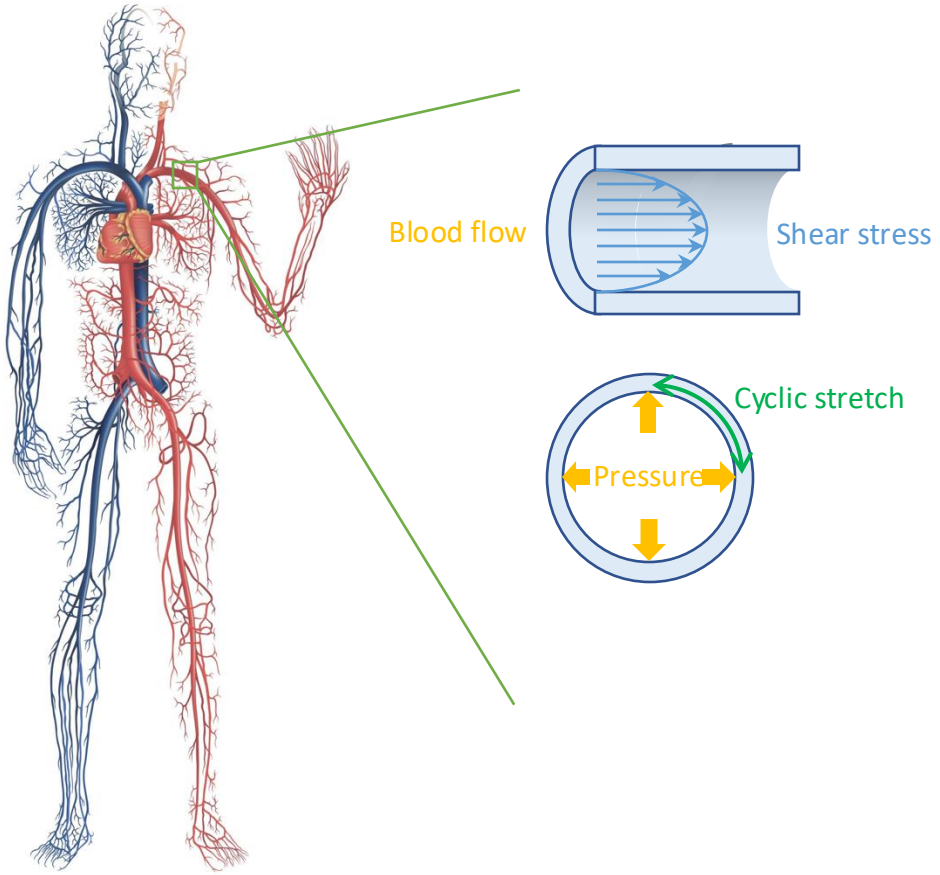
$$\tau = \frac{4\,\mu\,.\,Q}{\pi\,.\,r^3}$$

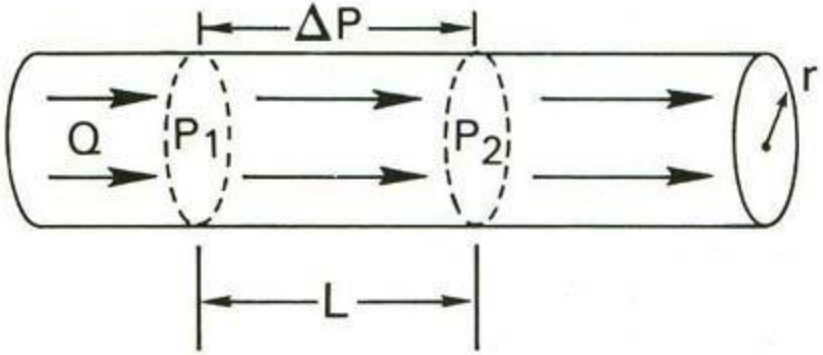
Perturbation of the flow

$$Re = \frac{\text{inertia forces}}{\text{viscous forces}} = \frac{\rho\,.\,V\,.\,D}{\mu}$$



Just 2 slides of physics :





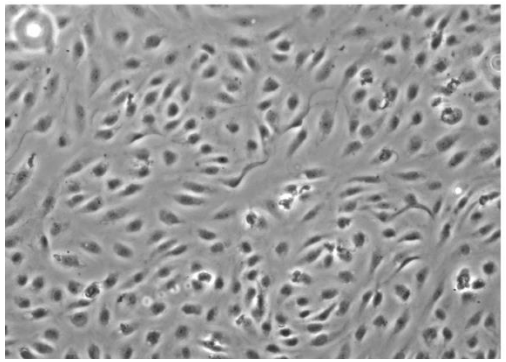
POISEUILLE'S LAW

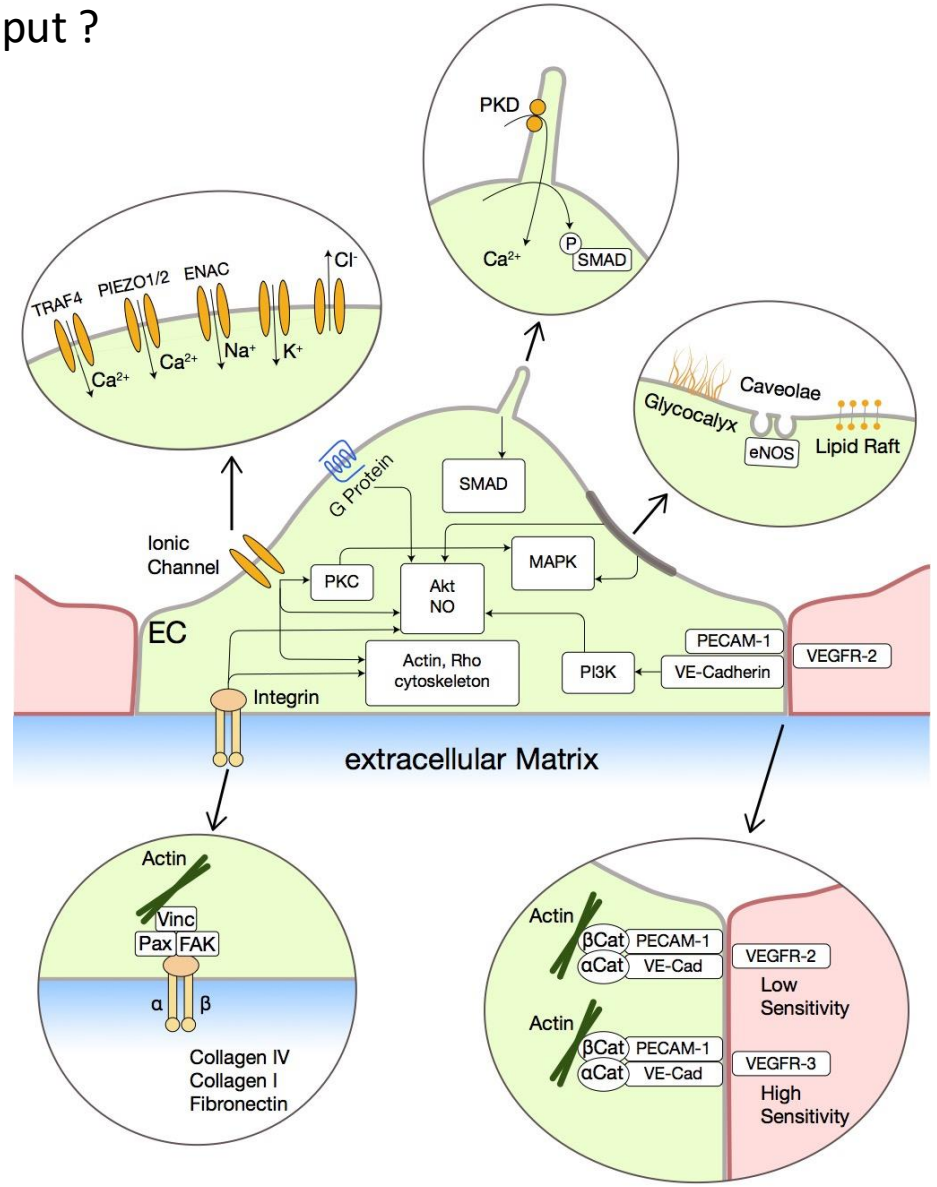
$$Q = \frac{\Delta P \; r^4 \; \pi}{\eta L \; 8}$$

The endothelial cell is dedicated to sense blood flow

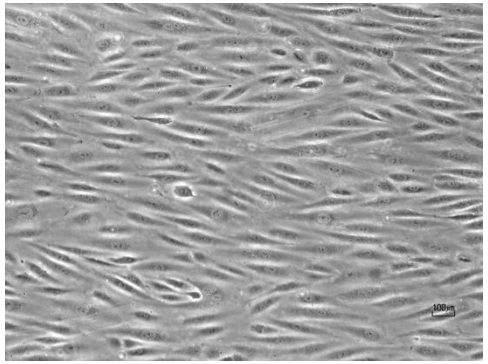
How a cell can “feel” a mechanical input ?

No shear stress



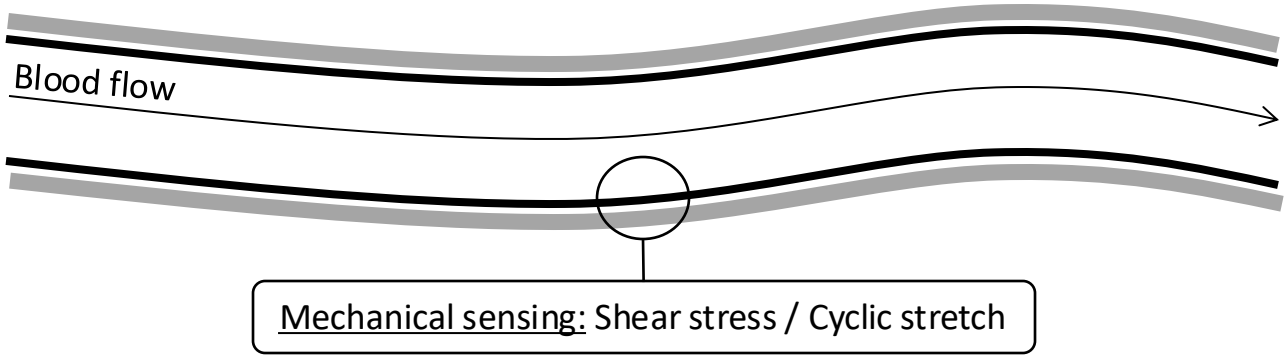


Physiological shear stress



Journal of Biomechanics 38 (2005) 1949–1971
Antioxid Redox Signal. 2016 Sep 1;25(7):373-88.
J Clin Invest. 2016 Mar 1;126(3):821-8.

physiological and pathological response to flow



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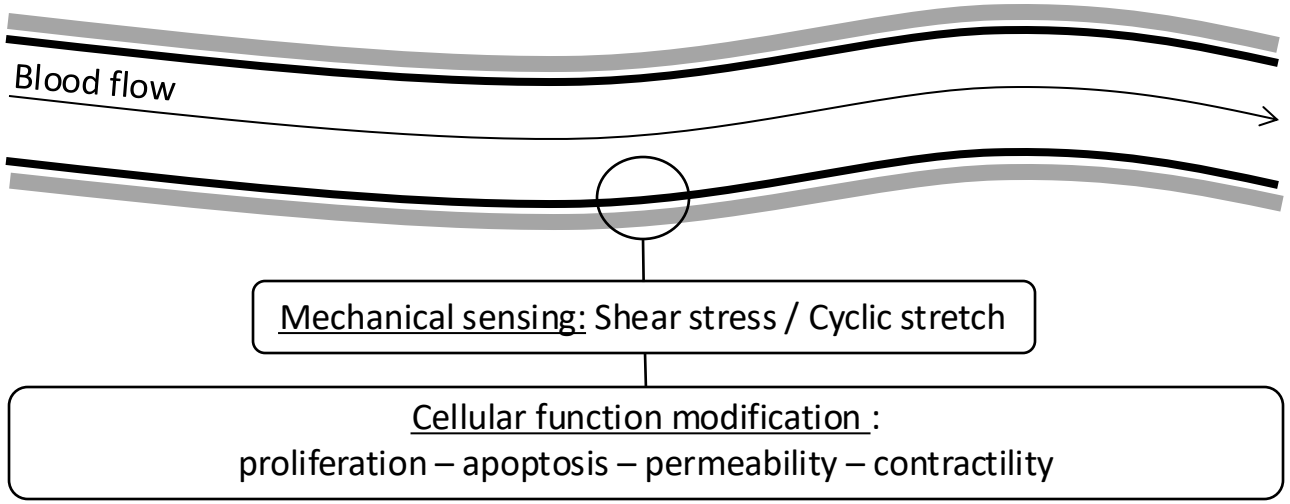

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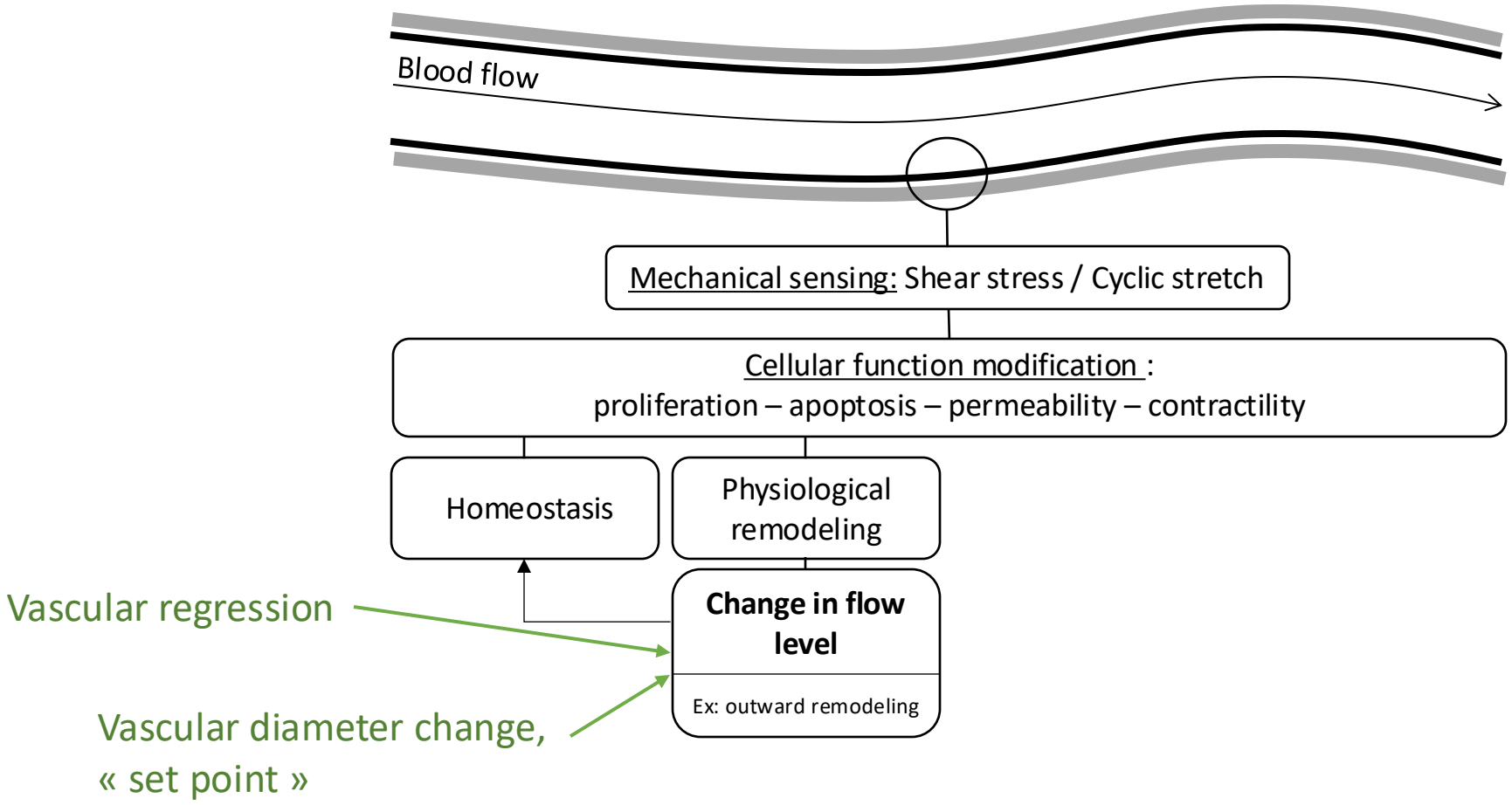

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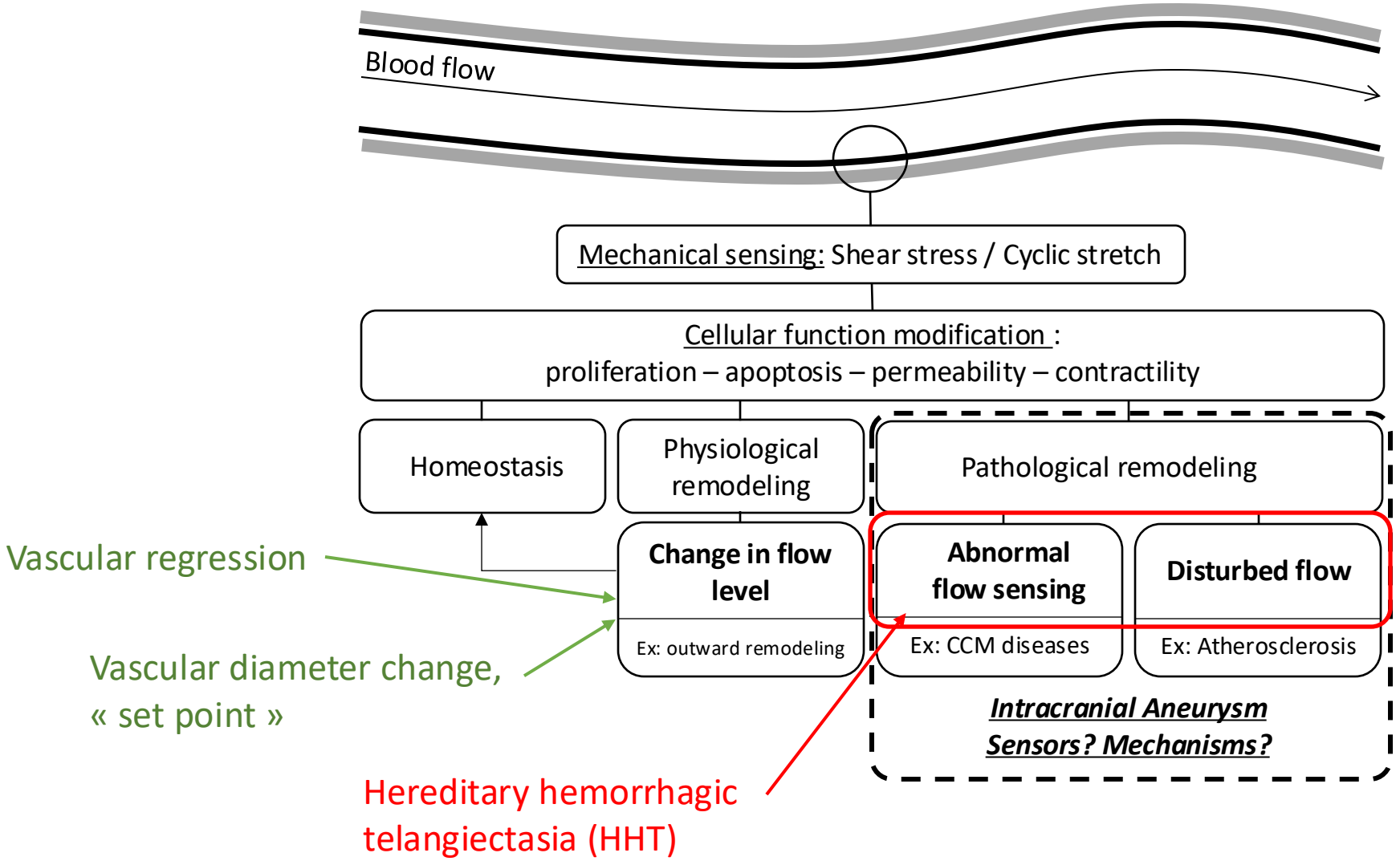

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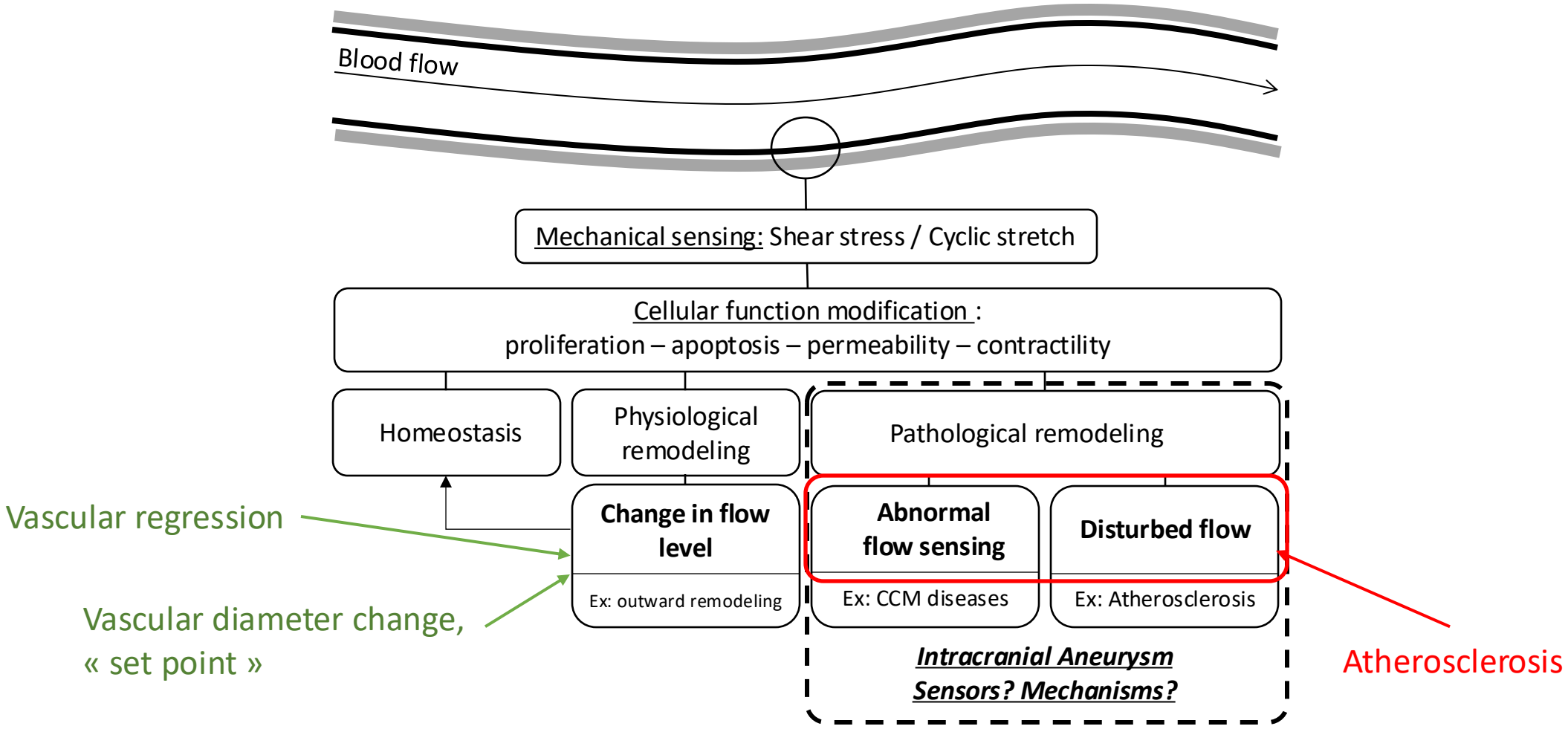
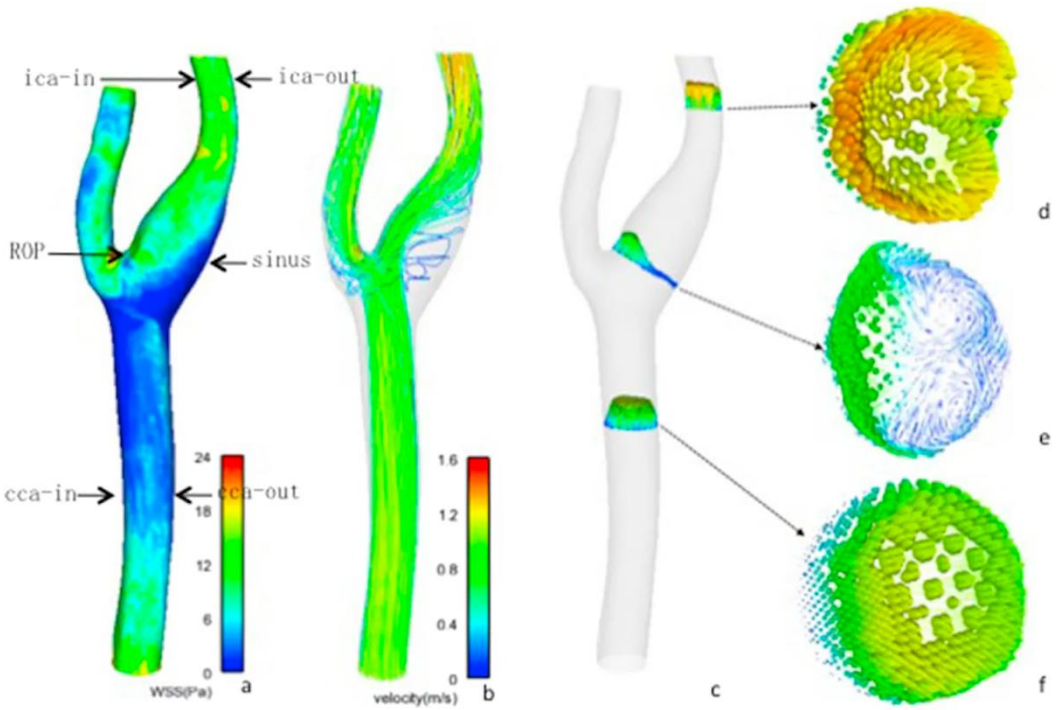
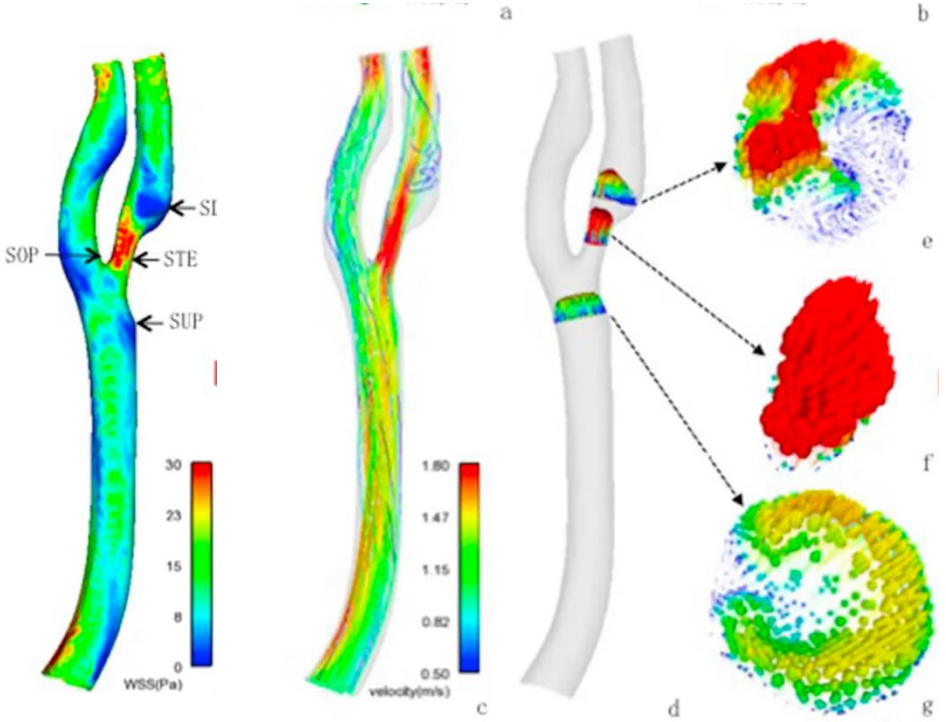


Hemodynamics at bifurcation sites : Carotides and atherosclerosis

Normal carotid bifurcation

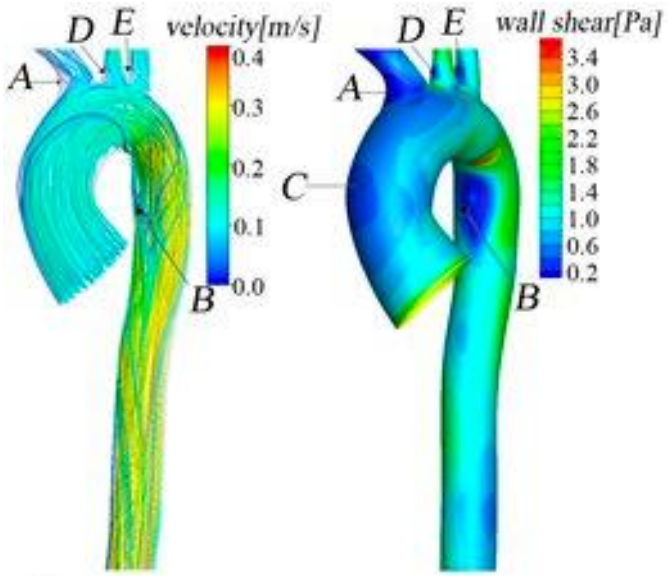


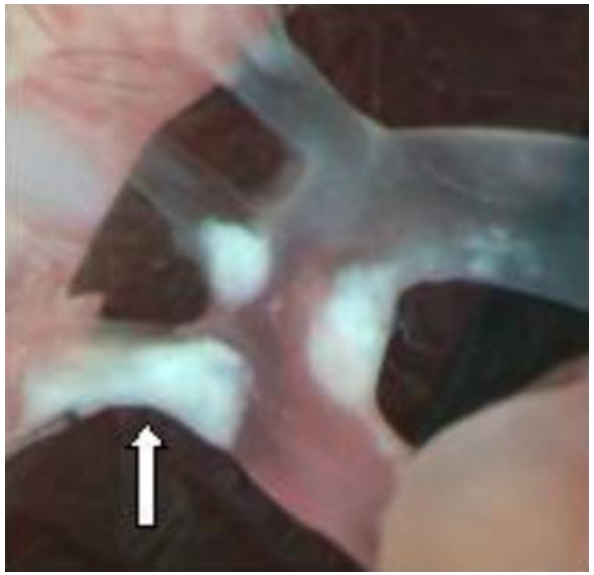
carotid bifurcation with atherosclerosis



Li, X. Scientific Reports volume 8, Article number: 5493 (2018)

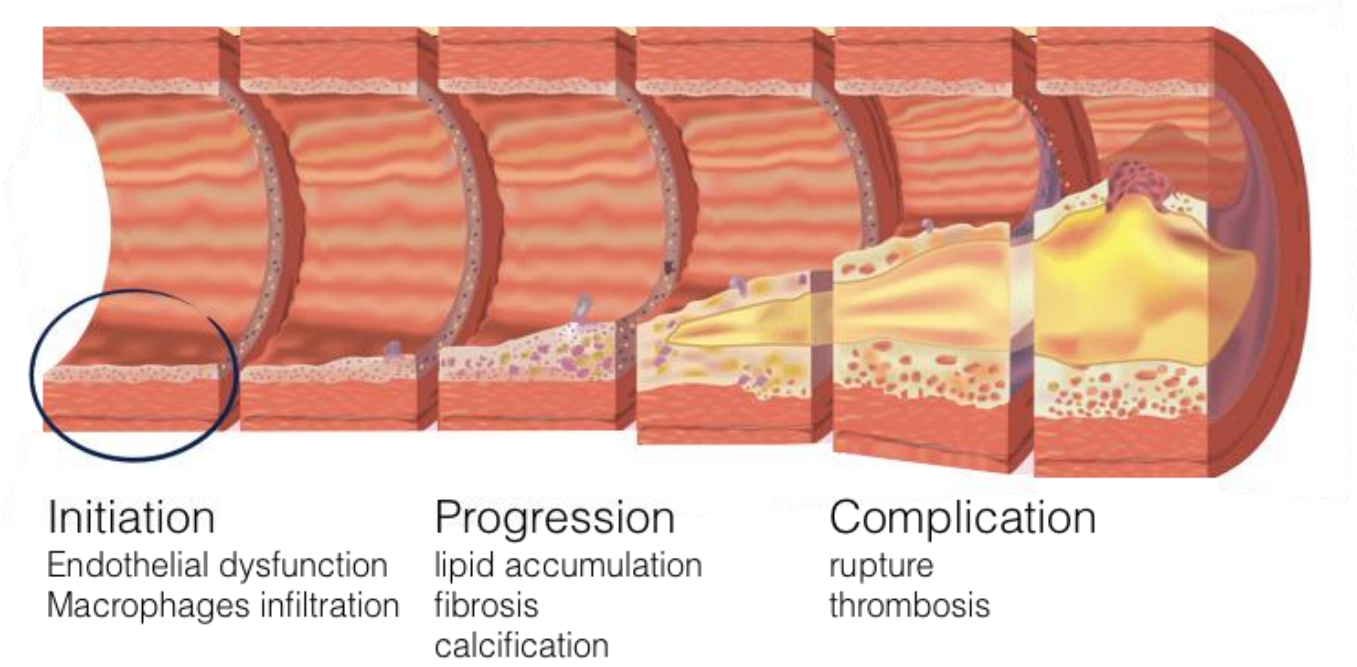
Hemodynamics in curvatures: Aortic arch and atherosclerosis





Liu, X. PLoS One. 2014 Nov 18;9(11):e112395.

Disturbed flow and atherosclerosis

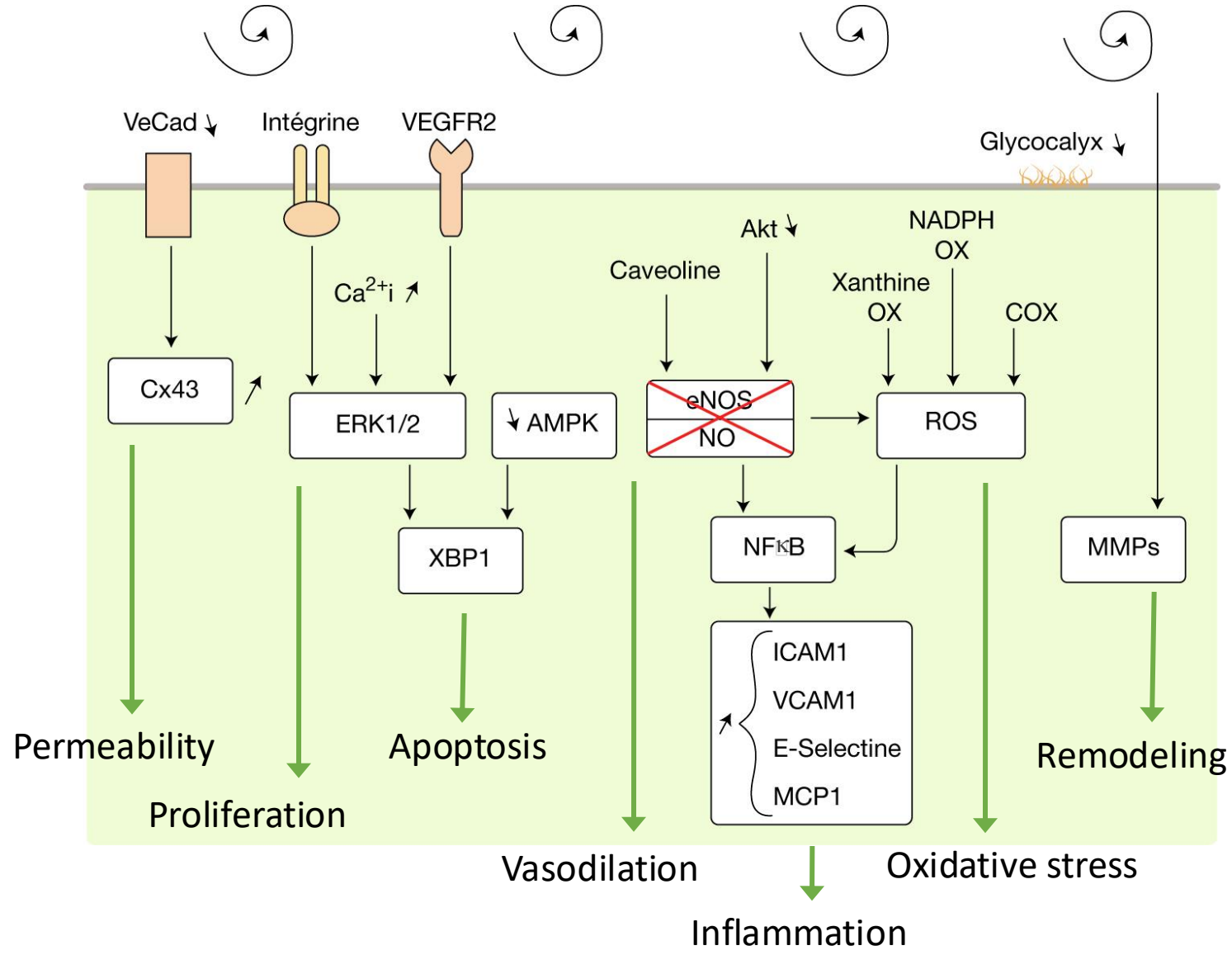


Branched points / Disturbed Flow / Low shear stress

Endothelial Dysfunction

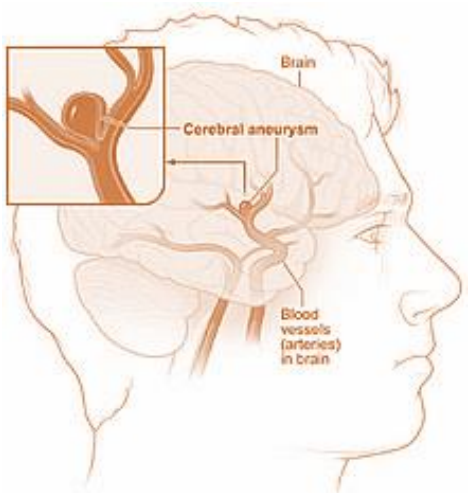
Vascular tone	Cell shape	Apoptosis	Proliferation	Inflammation	Thrombosis

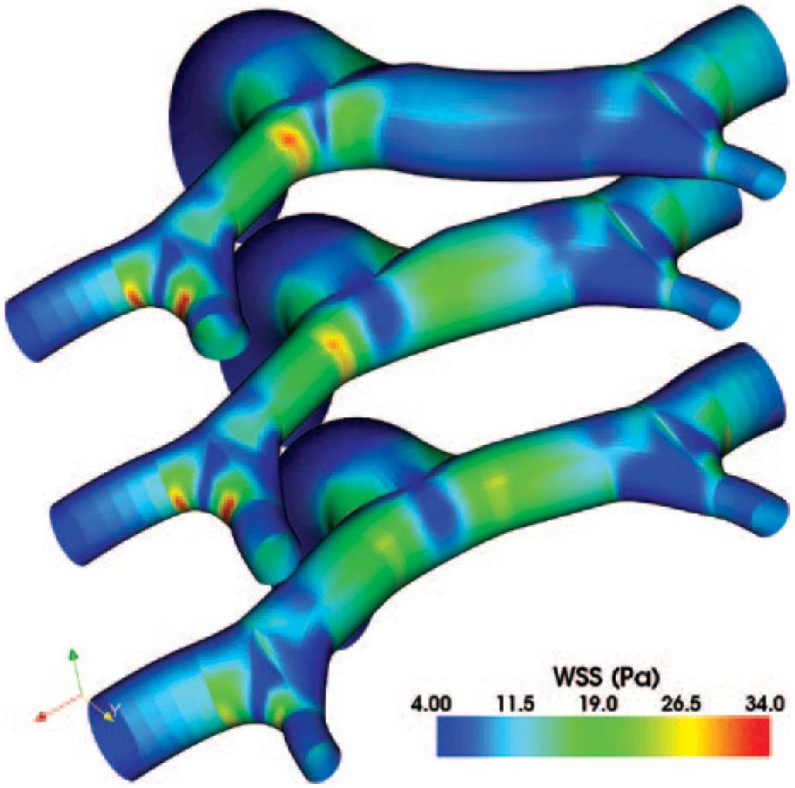
Disturbed flow and atherosclerosis



Chiu et al, Physiol Rev 2011; 391: 327-87

Hemodynamics at bifurcation sites : Circle of willis and IA





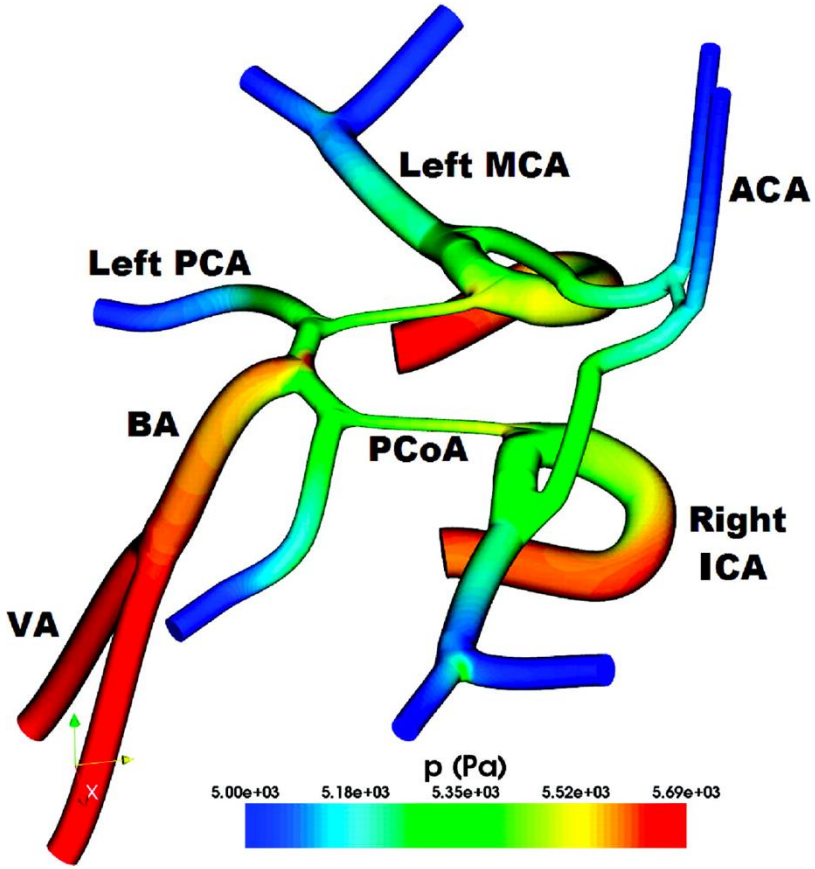
Alnaes, MS. Stroke. 2007;38:2500-2505.

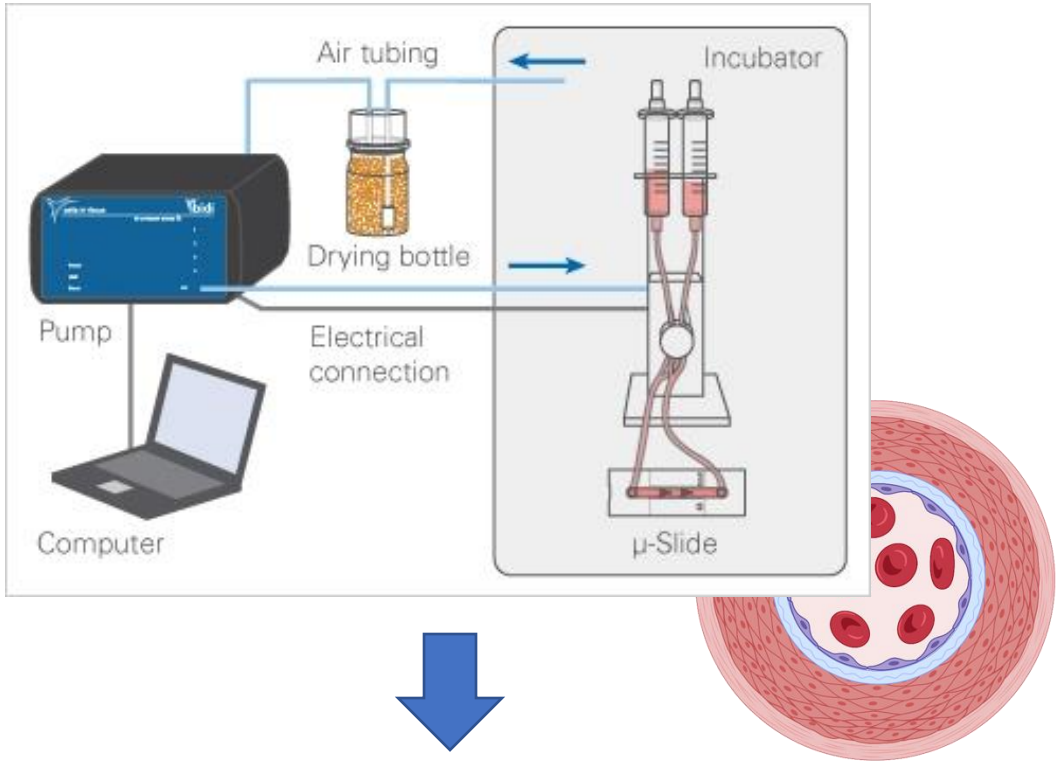
- 3% of the general population
- unpredictable rupture
- 50% of death in case of rupture

- **No diagnostic tool**
- **No pharmacological treatment**

- **Risk factors :**
  - Smocking, alcohol consumption, hypertension
  - female sex, age, familial history

Starting from the hemodynamic peculiarity?





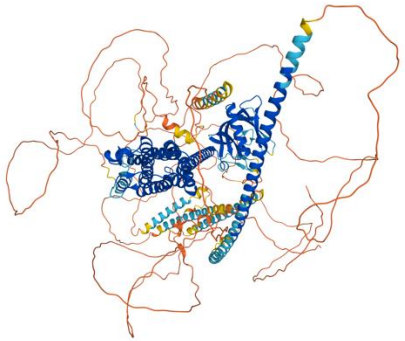
Cells analysis

- genes
- proteins
- shapes
- activation

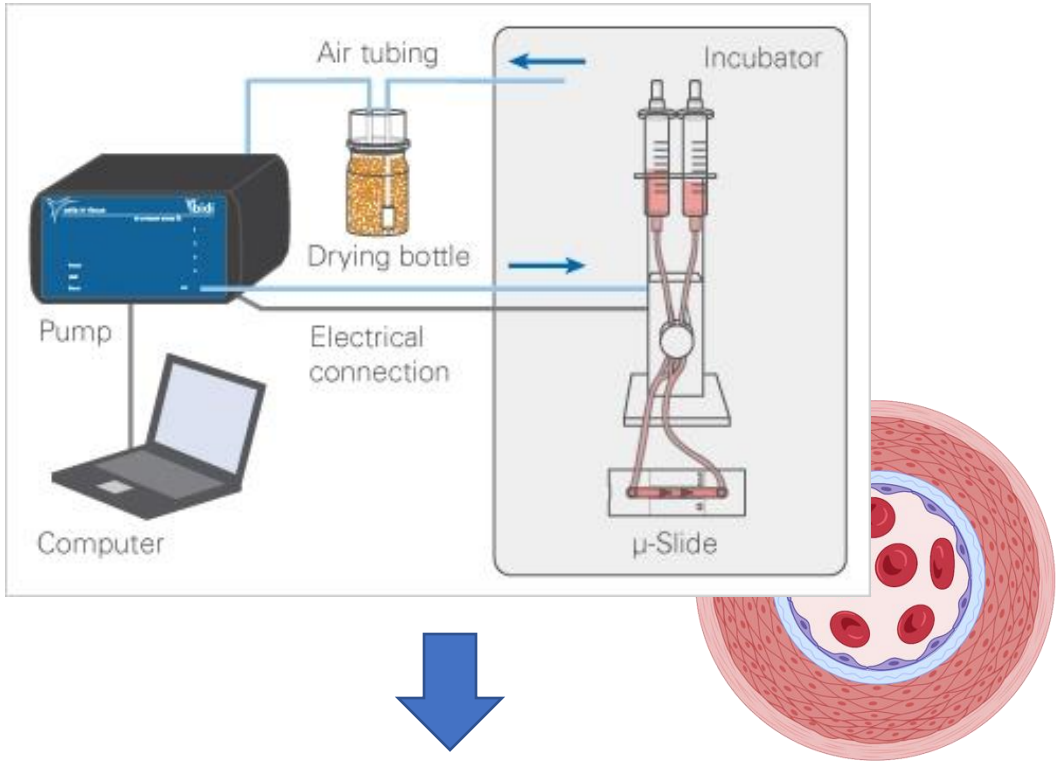
Identification of new mecanosensitive proteins

Identification of a protein sensitive to blood flow velocity variation

ARHGEF18



More active when blood flow is physiological
less active when blood flow is abnormal

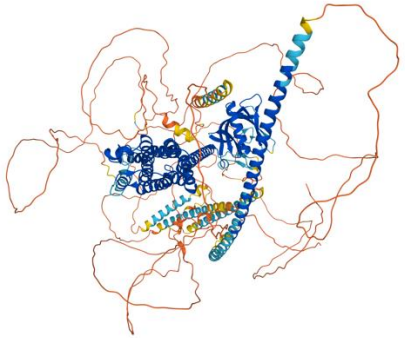


Cells analysis

genes → proteins → shapes → activation

ARHGEF18 identification in endothelial cells

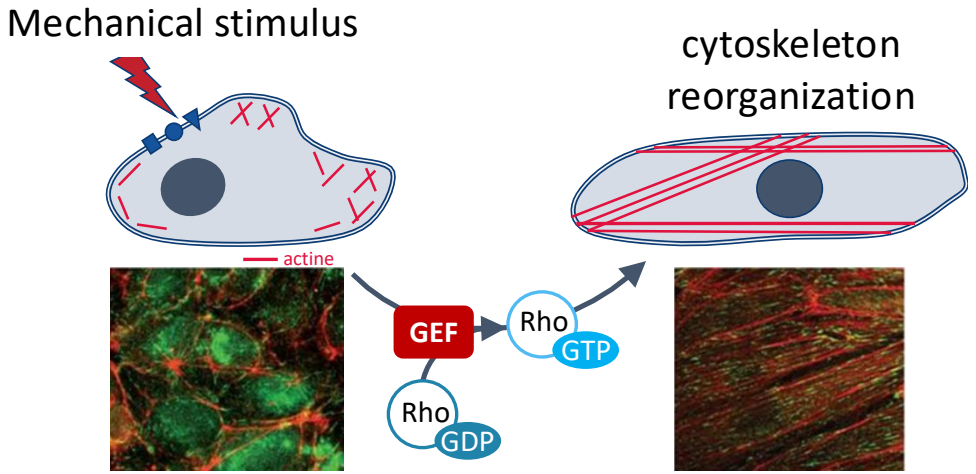
ARHGEF18



Well described in epithelial cells

Guanin echange factor -> RhoA activation

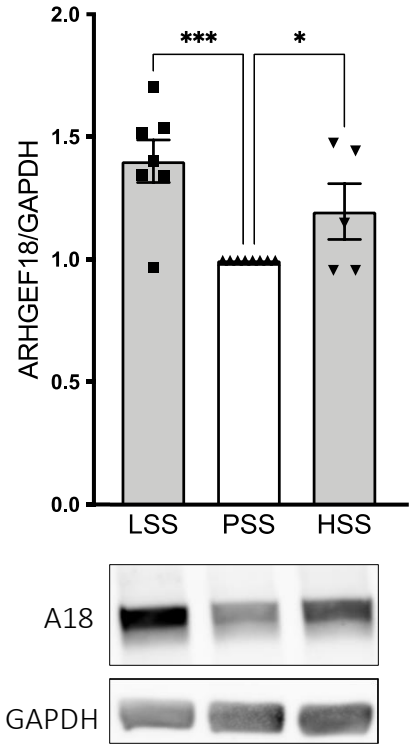
Cell-cell adhesion
Matrix adhesion and cell migration
ROS formation



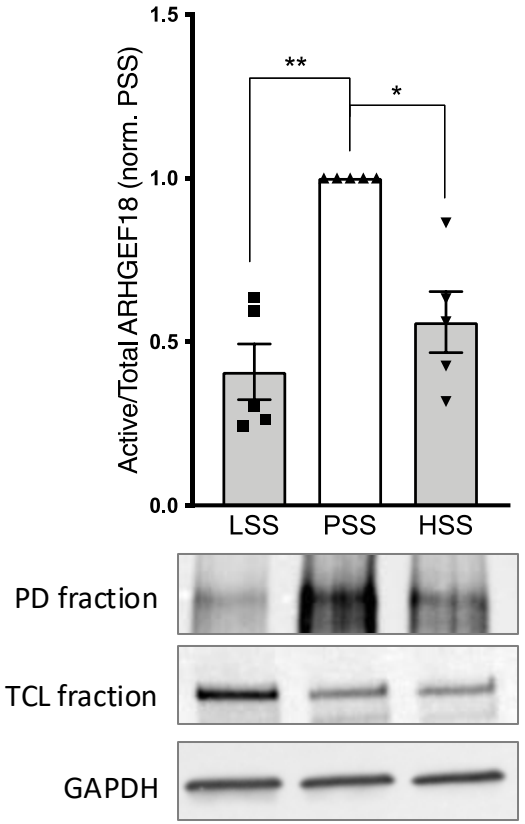
https://www.biorxiv.org/content/10.1101/2022.09.10.507283v1

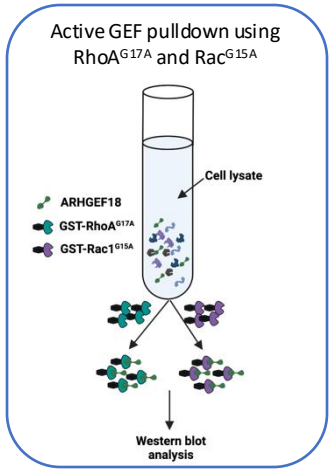
ARHGEF18 expression and activity is flow sensitive

Protein level



Guanin exchange activity



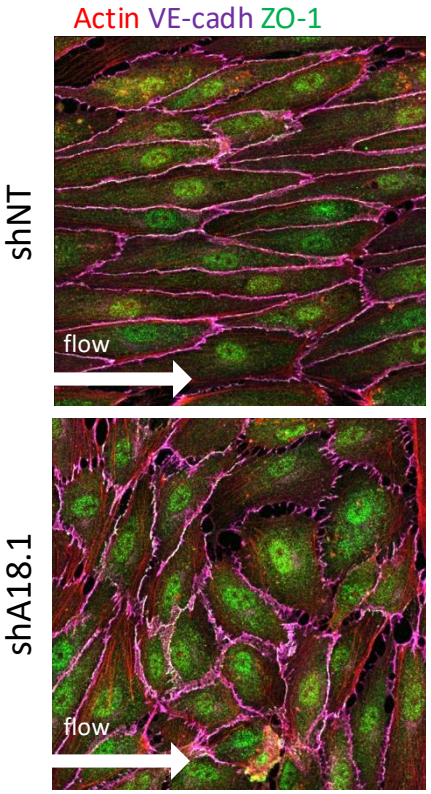


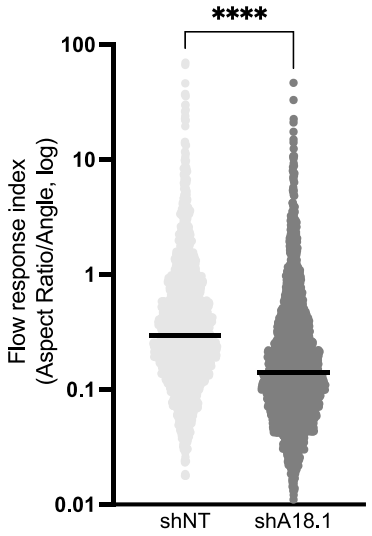
ARHGEF18 bounds to RhoA
its activity is downregulated by pathological shear stress

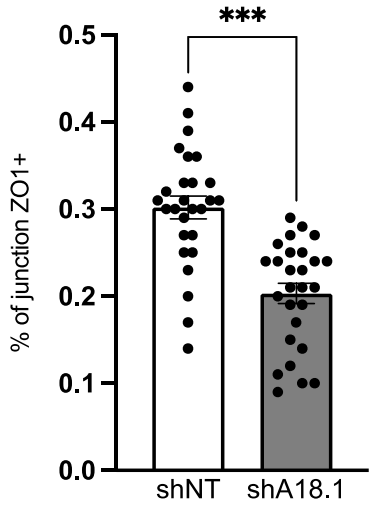
one-way ANOVA

https://www.biorxiv.org/content/10.1101/2022.09.10.507283v1

ARHGEF18 contributes in ECs alignment with the flow

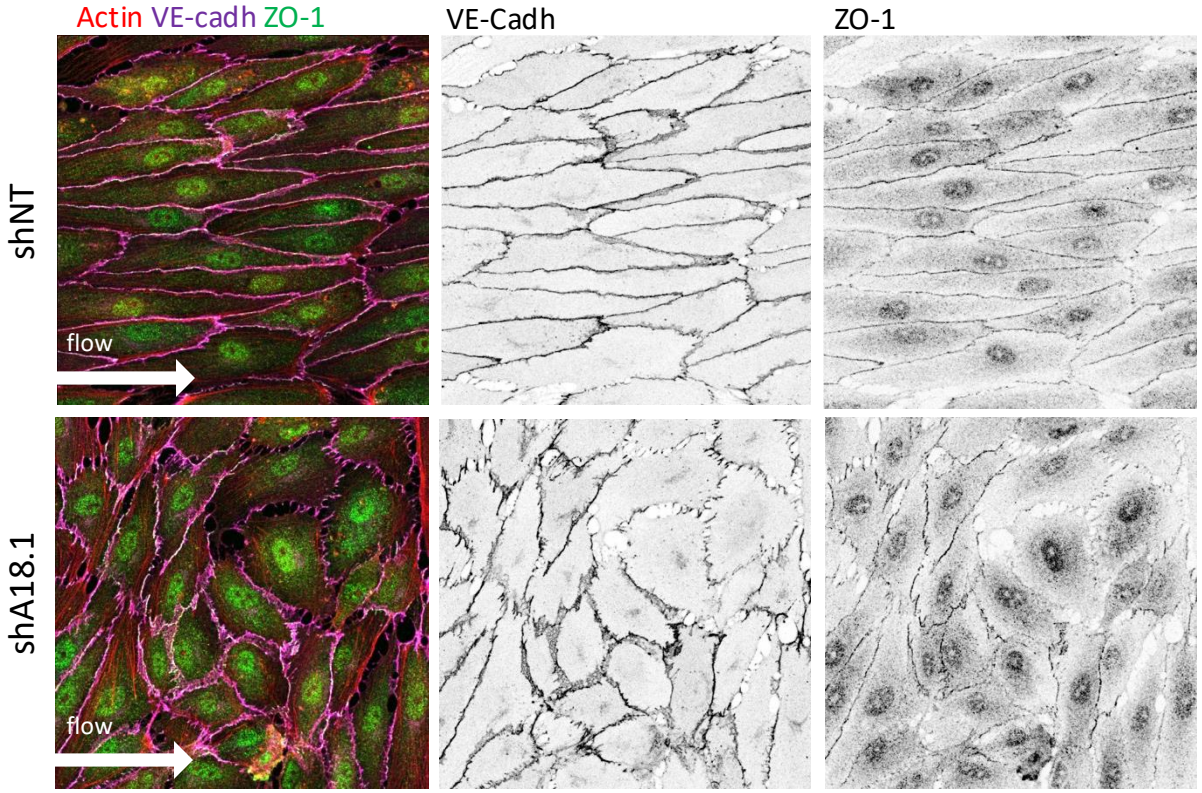


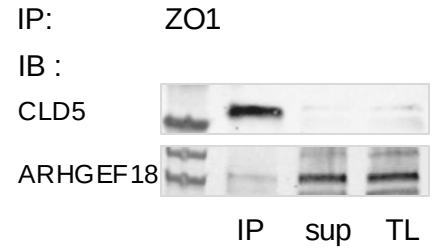


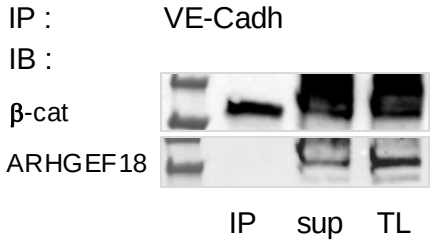


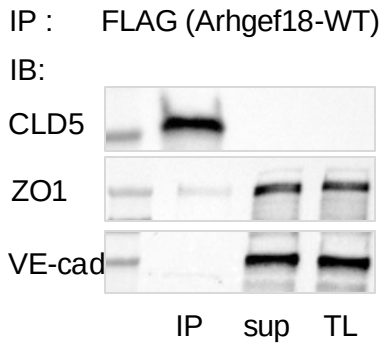
https://www.biorxiv.org/content/10.1101/2022.09.10.507283v1

ARHGEF18 interact with Tight Junction proteins





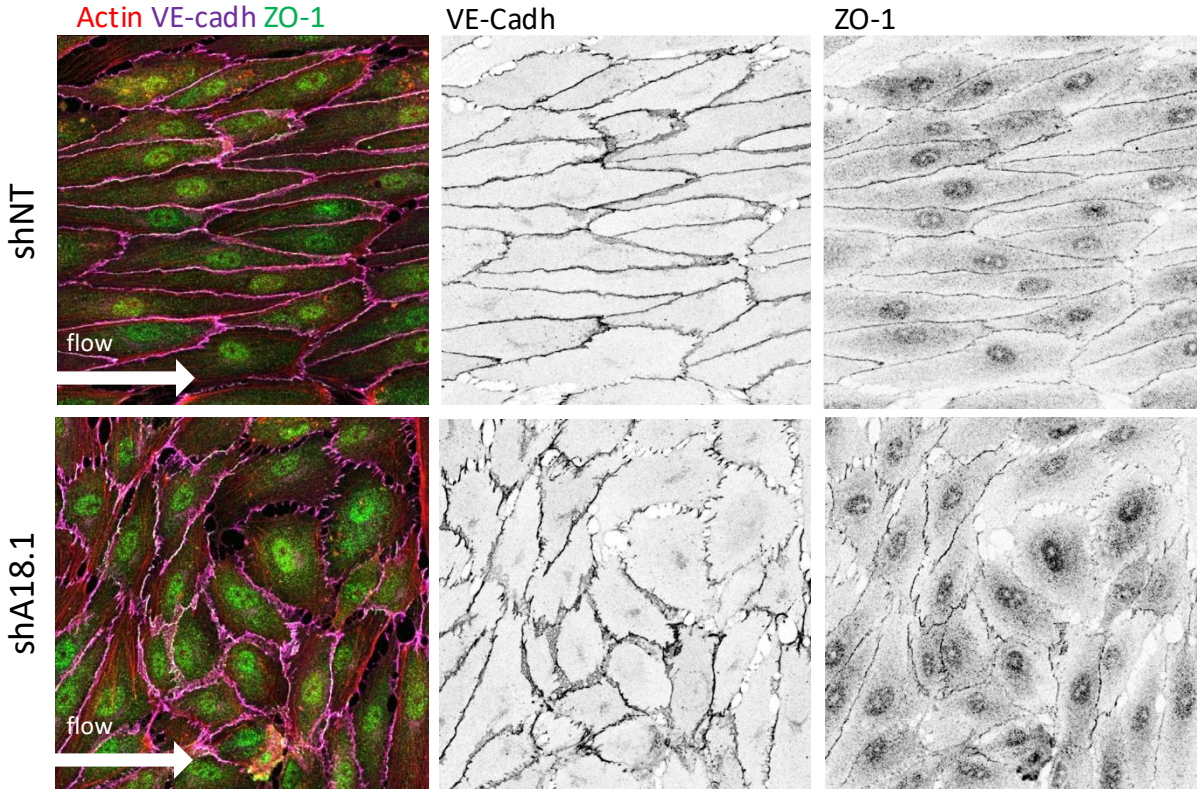


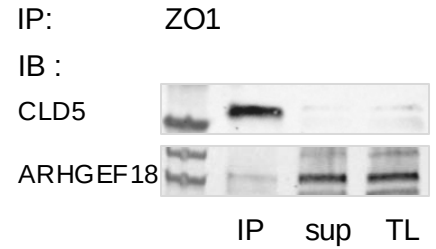


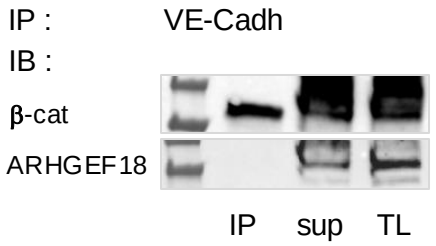
ARHGEF18 interacts with ZO-1 and Claudin5 but not VE-cadherin

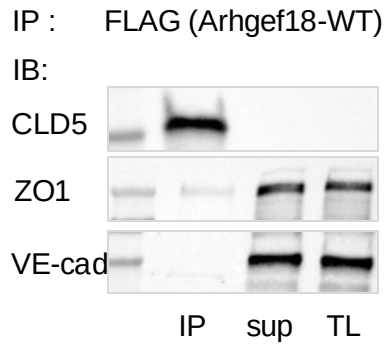
https://www.biorxiv.org/content/10.1101/2022.09.10.507283v1

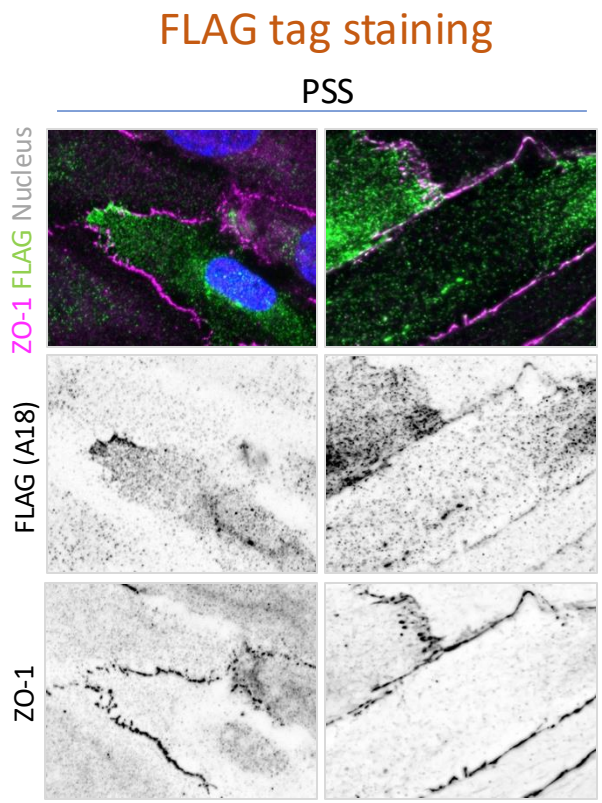
ARHGEF18 interact with Tight Junction proteins









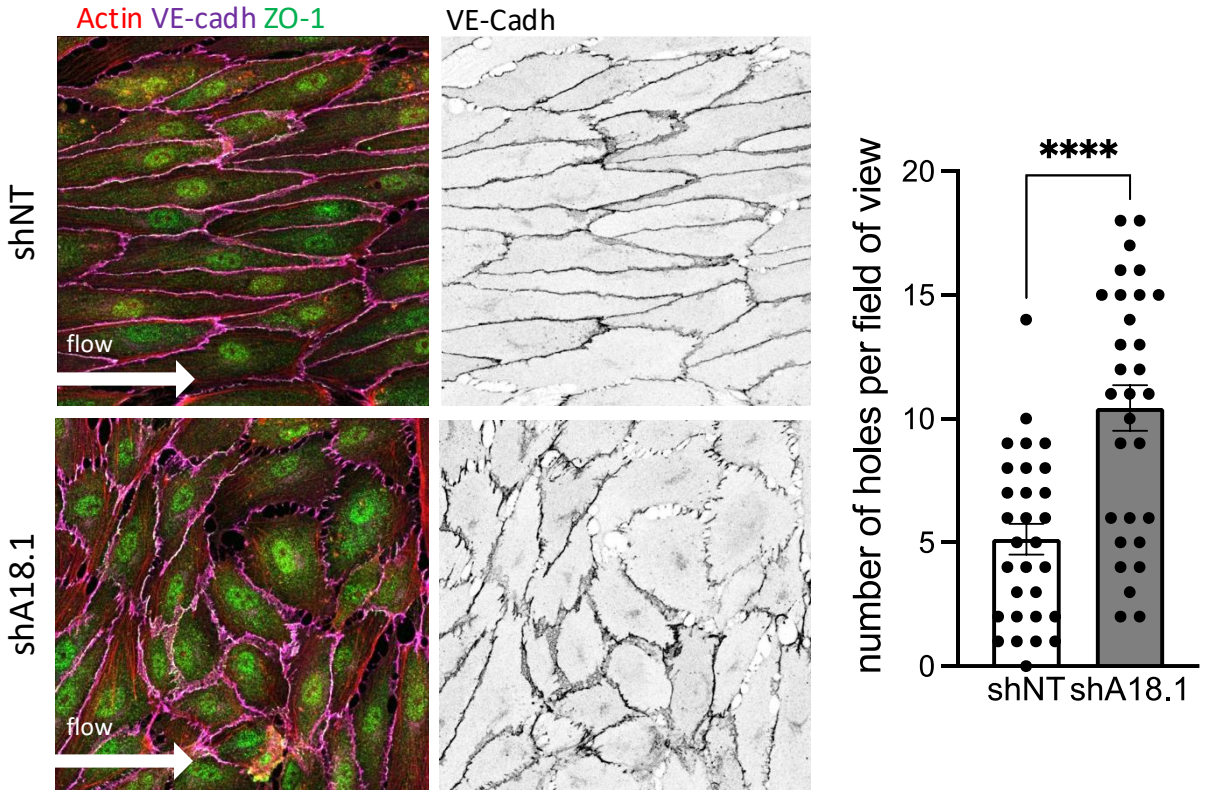


ARHGEF18 interacts with ZO-1 and Claudin5 but not VE-cadherin

Its expression seems localized near to the junction

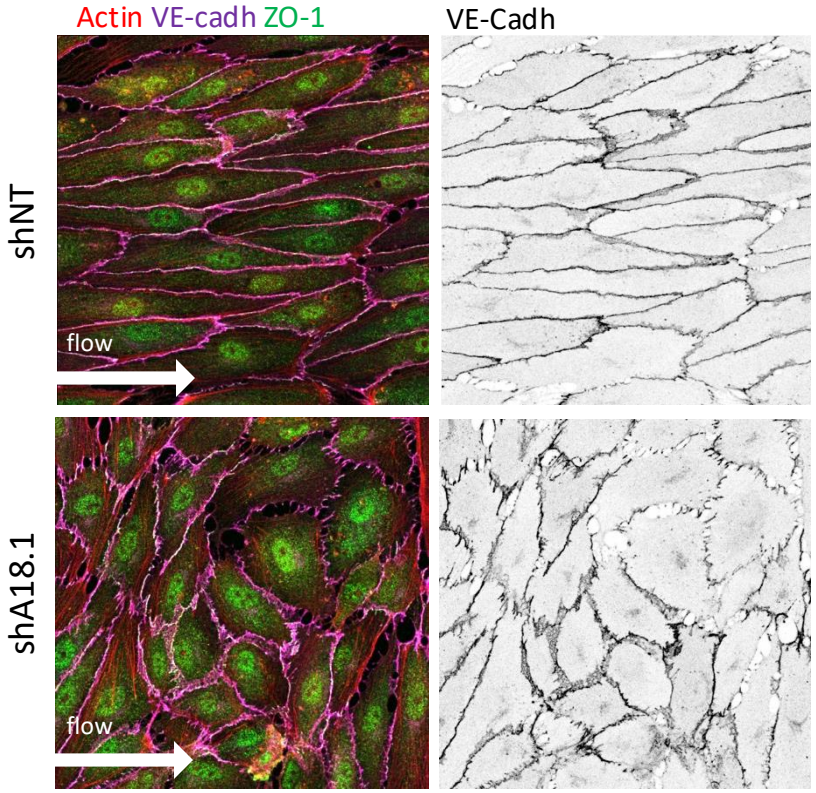
https://www.biorxiv.org/content/10.1101/2022.09.10.507283v1

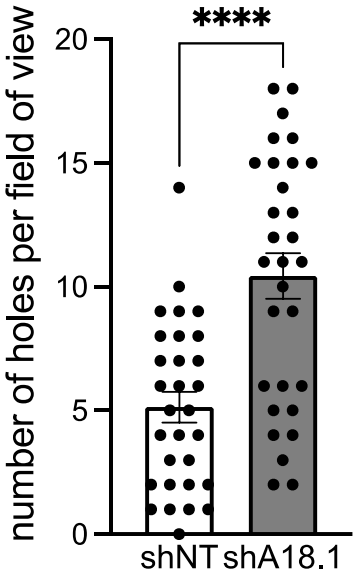
ARHGEF18 favors cell-cell junctions integrity



ARHGEF18 deficient ECs present holes in between cells

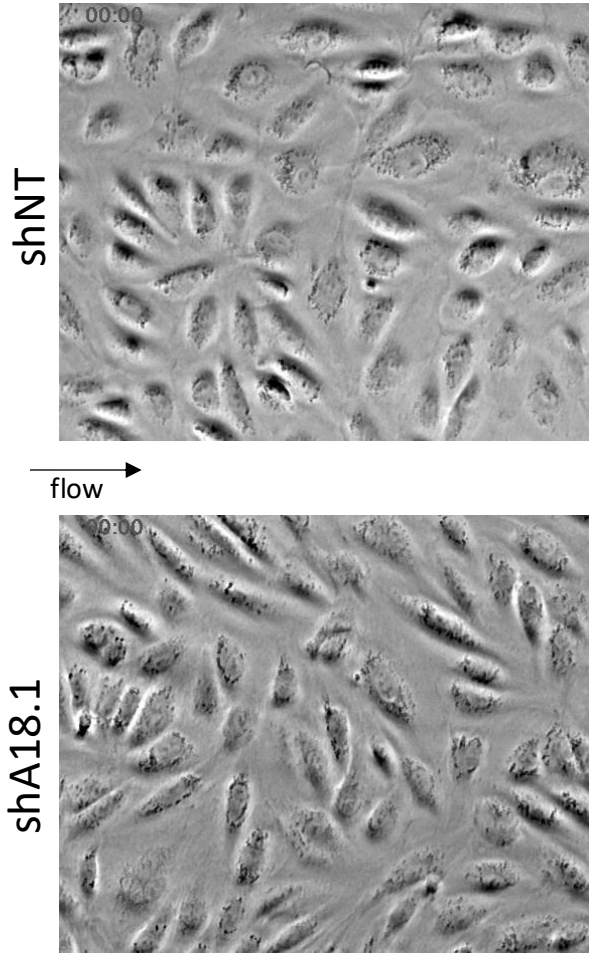
ARHGEF18 favors cell-cell junctions integrity

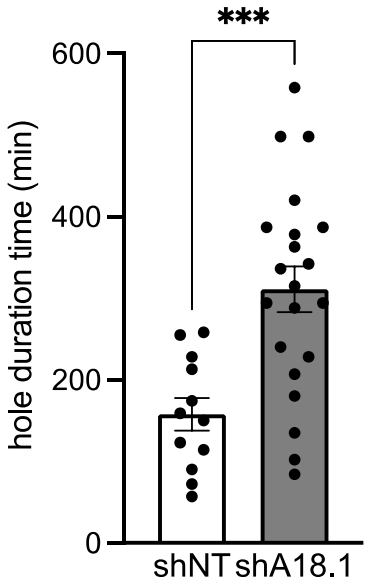




ARHGEF18 deficient ECs present holes in between cells

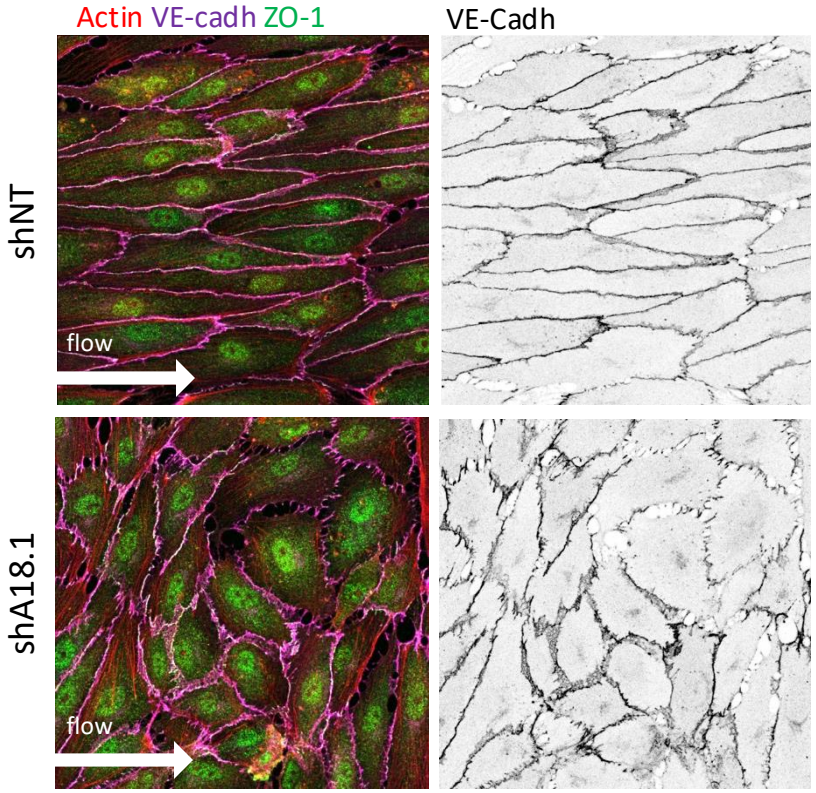
Dynamics of holes formation under flow

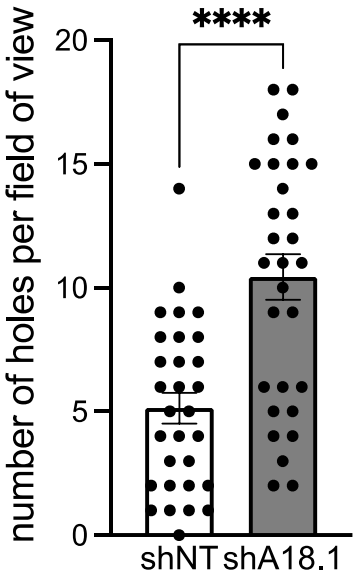




ARHGEF18 deficient ECs fail to close holes under flow
... And to migrate in the direction of the flow

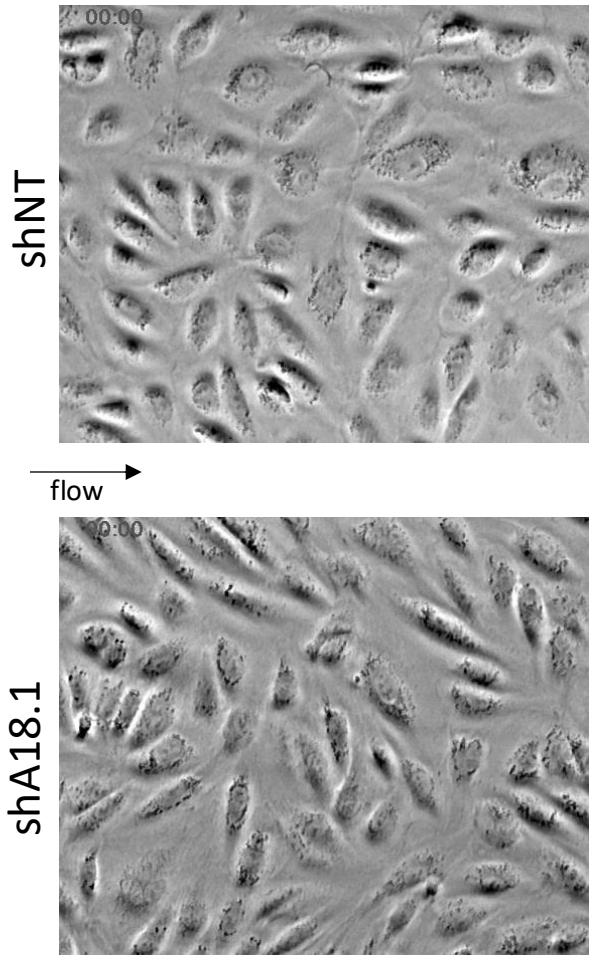
ARHGEF18 favors cell-cell junctions integrity

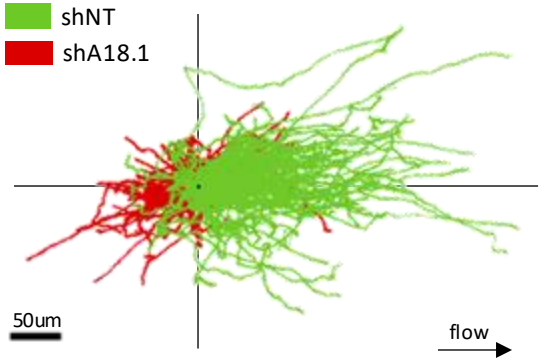




ARHGEF18 deficient ECs present holes in between cells

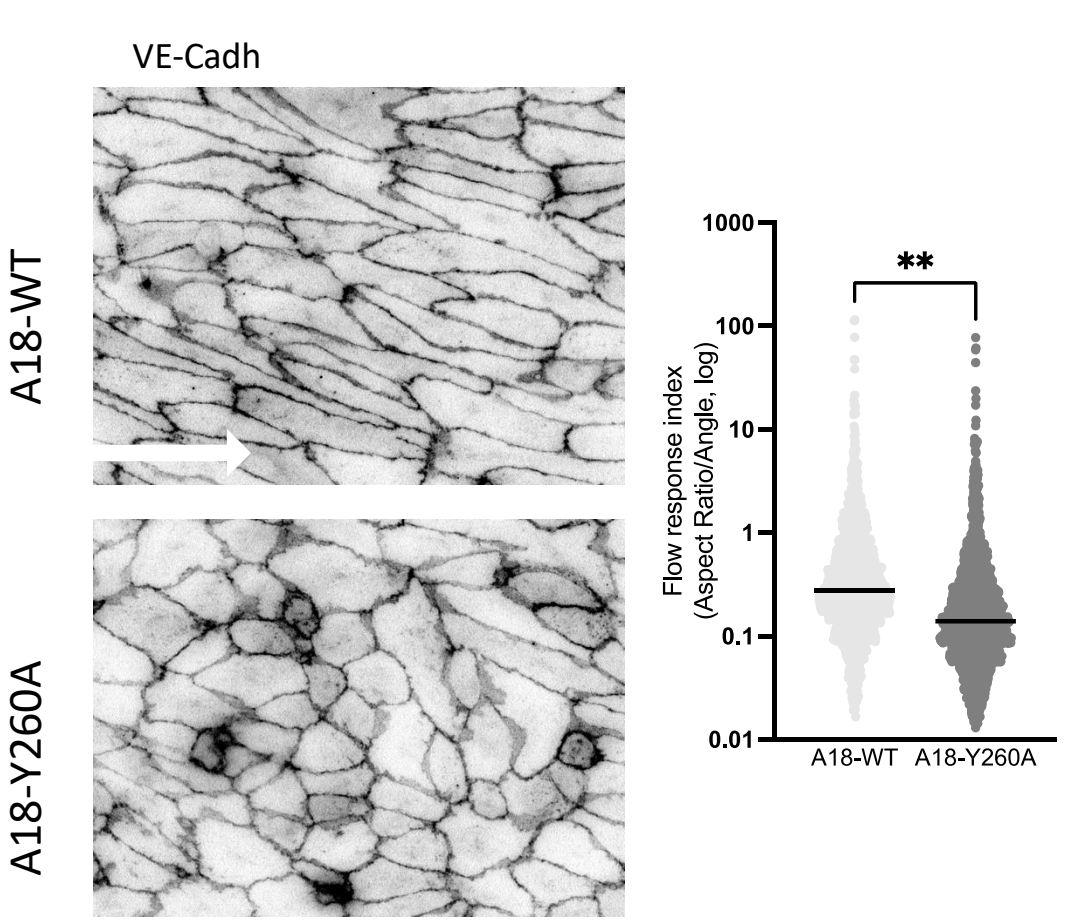
Dynamics of holes formation under flow



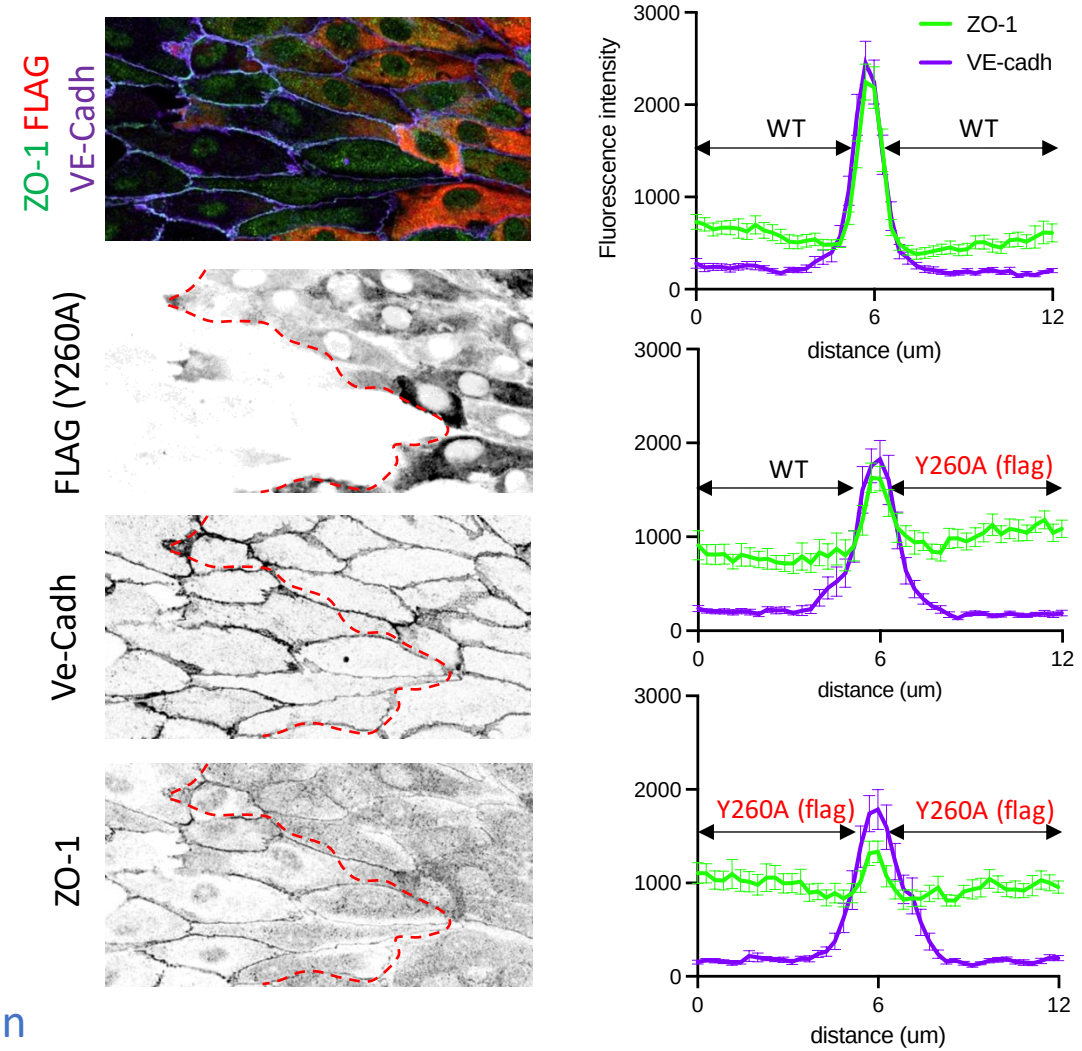


ARHGEF18 deficient ECs fail to close holes under flow

Nucleotide exchange activity contributes to alignment and tight junctions

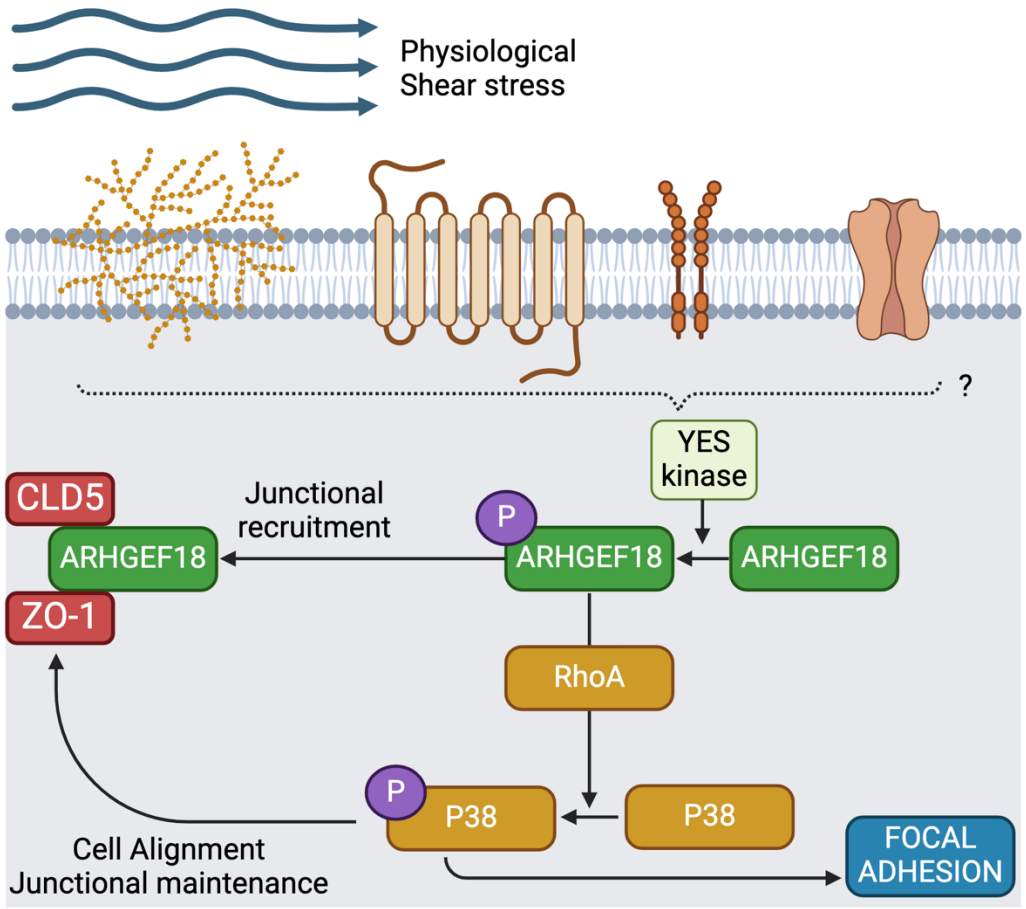


ECs lacking ARHGEF18 guanin exchange activity failed to align



... And to recruit ZO-1 at tight junction

https://www.biorxiv.org/content/10.1101/2022.09.10.507283v1



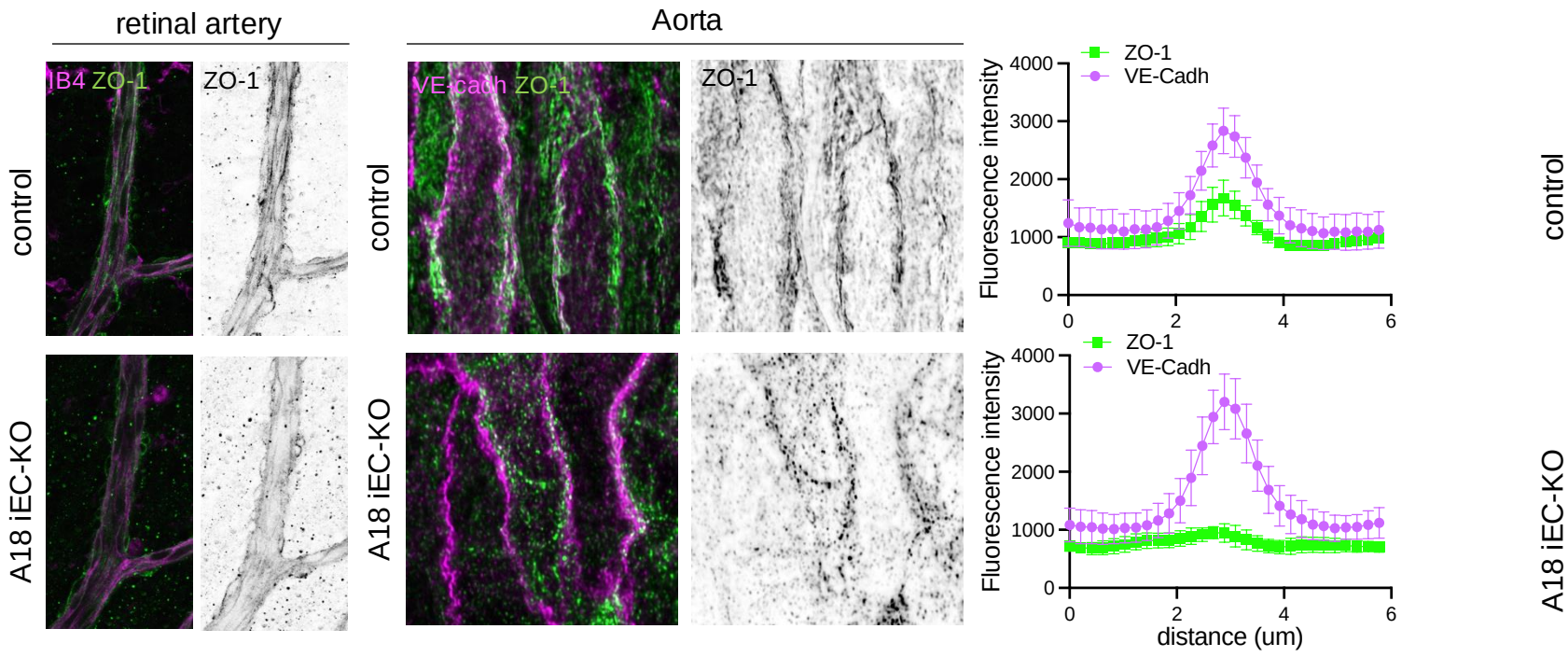
In vitro

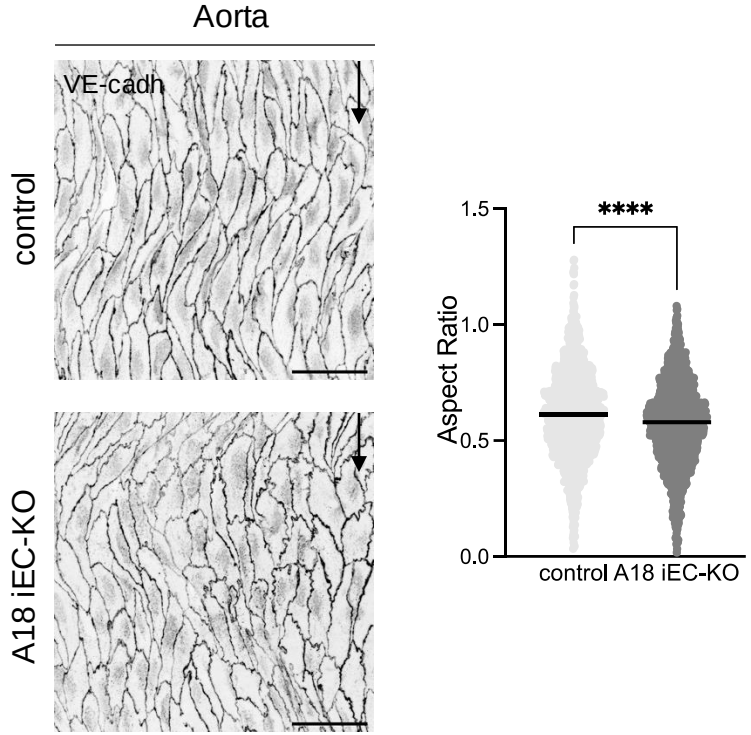
Orientation and elongation
Migration
Tight Junction / permeability
Basal adhesion

ARHGEF18 contributes to Tight Junction formation in vivo

Arhgef18$^{lox/lox}$; CDH5-CRE^{ERT2}

P6 retinas/aortas





ZO-1 localization at TJs is reduced in Arhgef18-iEC mice compared to control

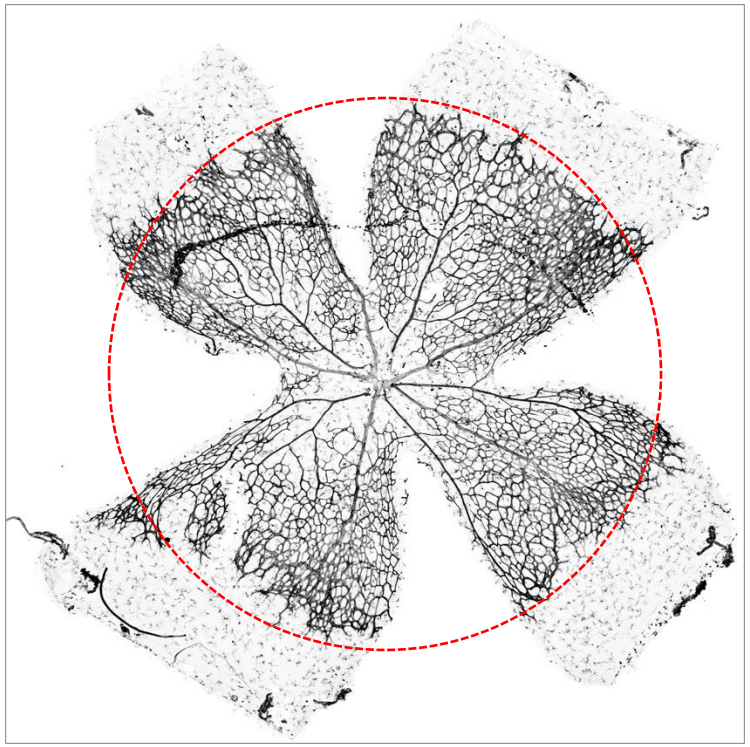
Elongation in response to flow is altered

ARHGEF18 participates in developmental angiogenesis

Arhgef18$^{lox/lox}$; CDH5-CRE^{ERT2}

Arhgef18$^{lox/lox}$

P6 retinas

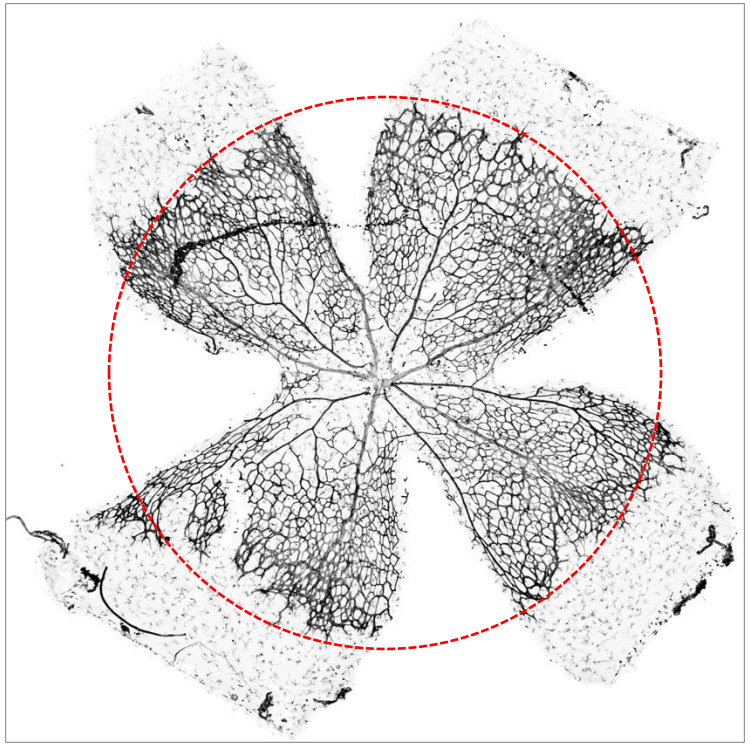


ARHGEF18 participates in developmental angiogenesis

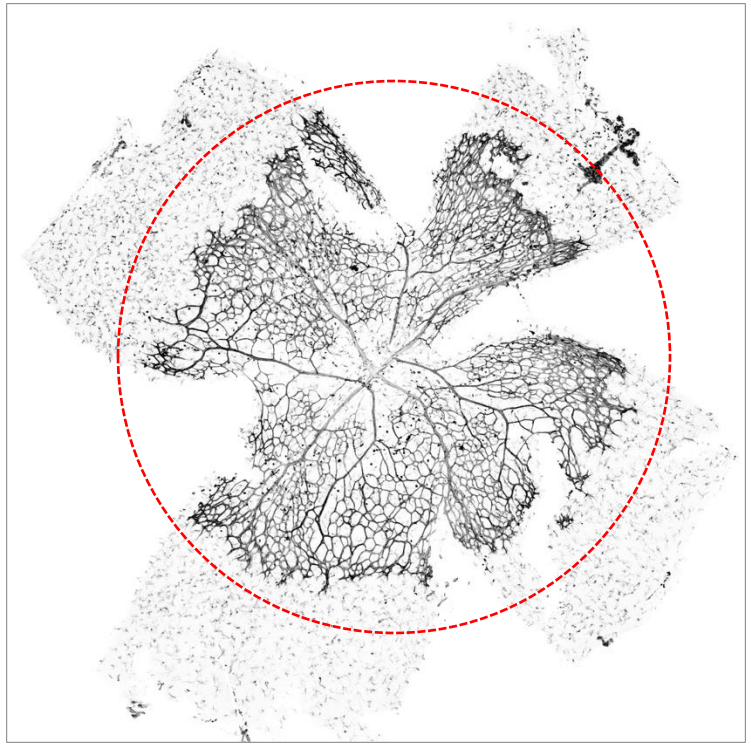
Arhgef18$^{lox/lox}$; CDH5-CRE^{ERT2}

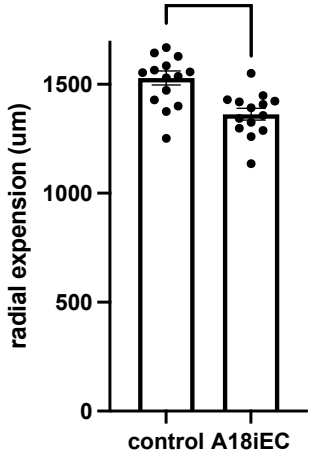
P6 retinas

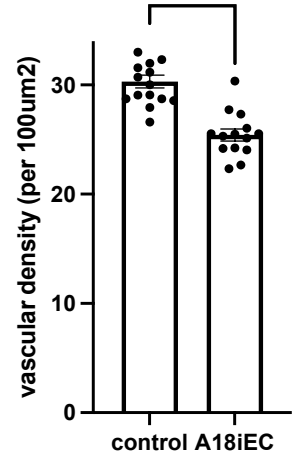
Arhgef18$^{lox/lox}$



Arhgef18$^{lox/lox}$; CDH5-CRE^{ERT2}







Vascular expansion is reduced in Arhgef18-iEC mice compared to control

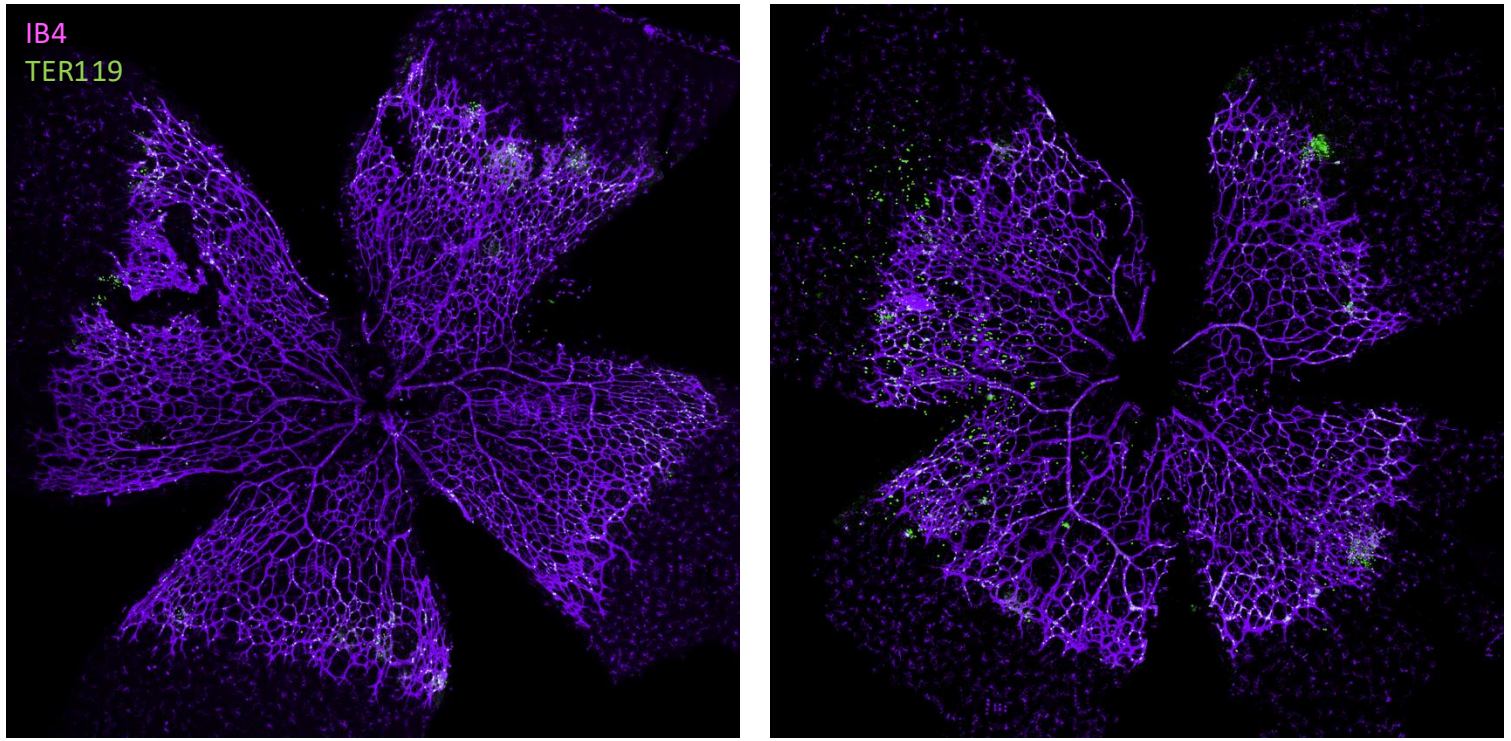
ARHGEF18 prevent retinal hemorrhage in vivo

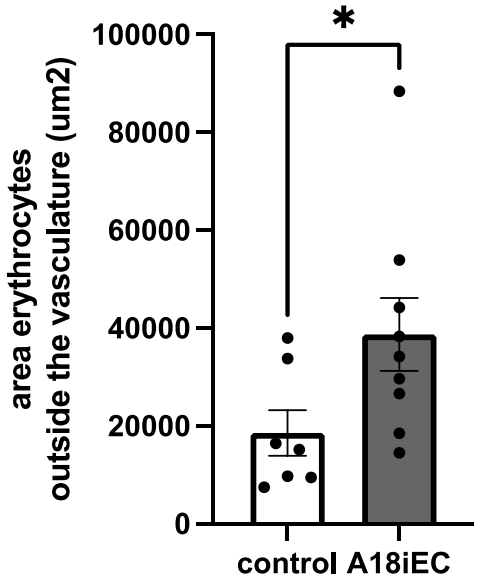
Arhgef18$^{lox/lox}$; CDH5-CRE^{ERT2}

Arhgef18$^{lox/lox}$

Arhgef18$^{lox/lox}$; CDH5-CRE^{ERT2}

P6 retinas



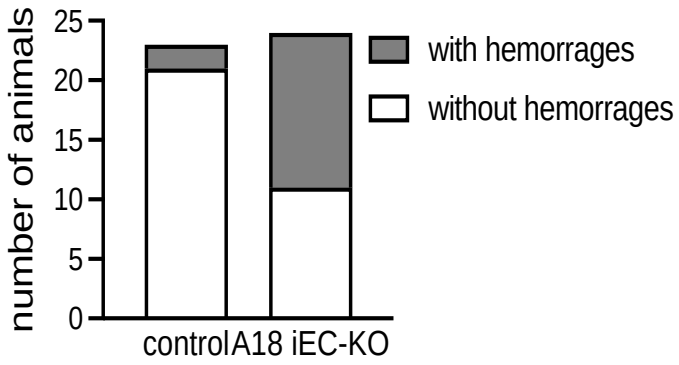


The Area of Erythrocytes outside the vasculature is reduced in Arhgef18-iEC retinas compared to control

ARHGEF18 prevents brain hemorrhage in vivo

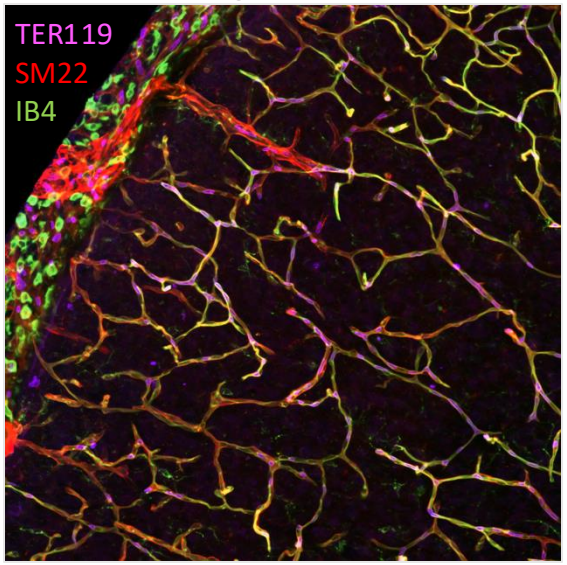
Arhgef18$^{lox/lox}$; CDH5-CRE^{ERT2}

P6 brains

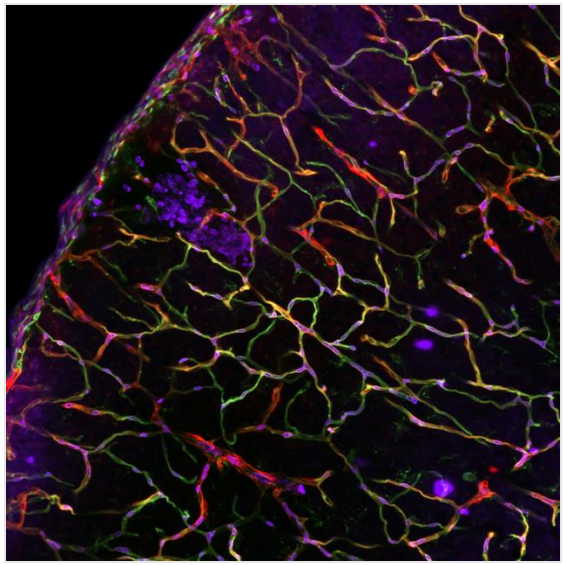


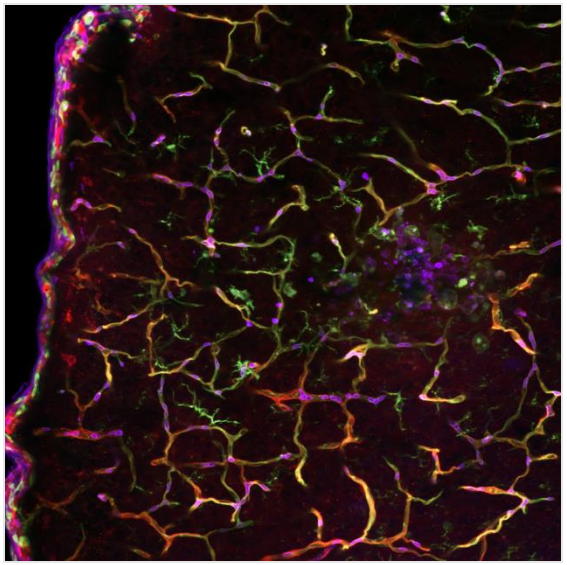
Brains of Arhgef18-iEC pups harbors more visual focal hemorrages than control

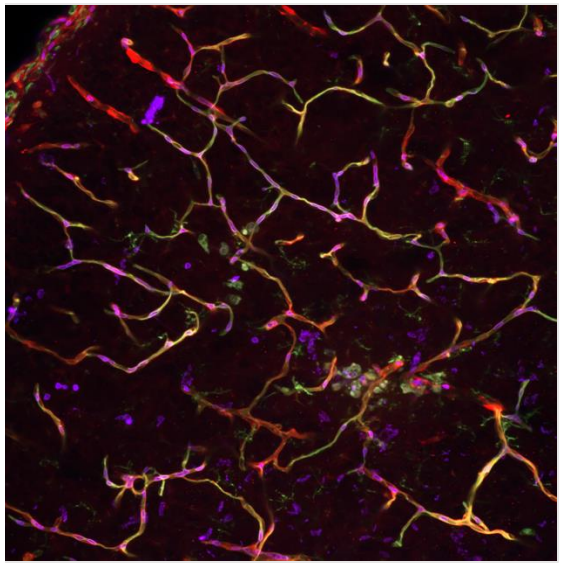
Arhgef18$^{lox/lox}$



Arhgef18$^{lox/lox}$; CDH5-CRE^{ERT2}





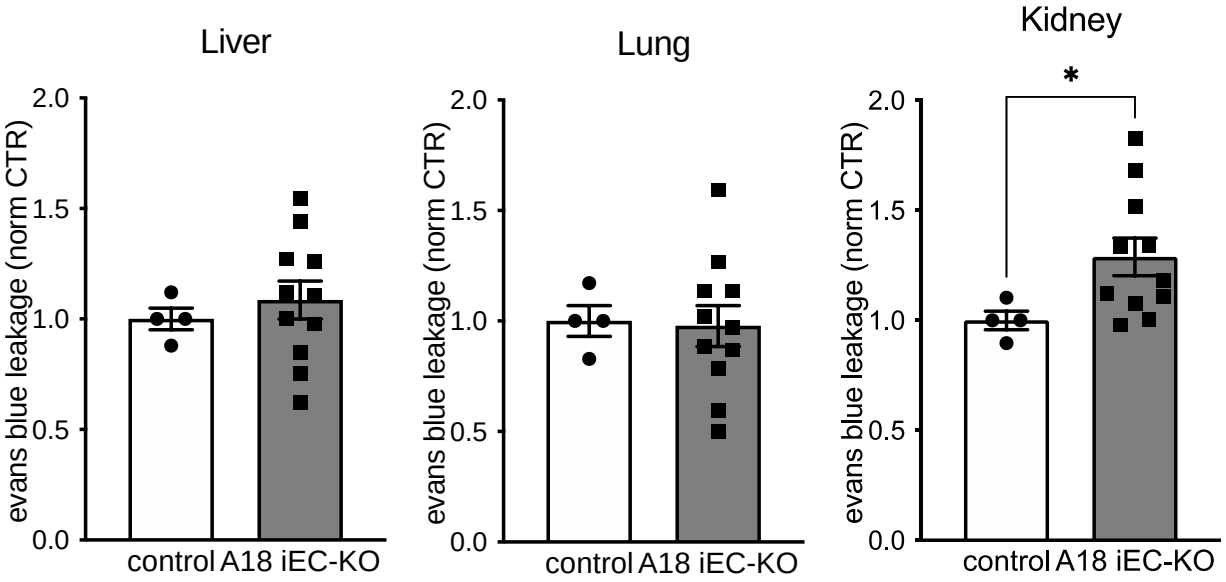


ARHGEF18 prevents vascular leakage in vivo

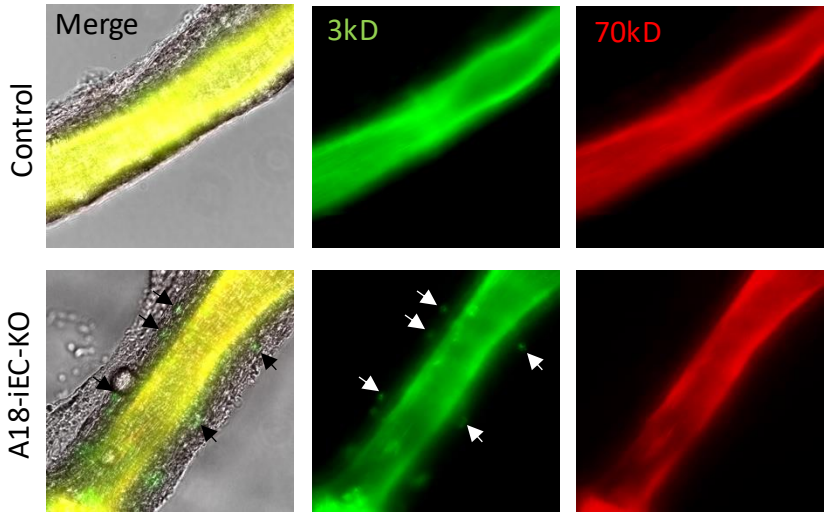
Arhgef18$^{lox/lox}$; CDH5-CRE^{ERT2}

P6 Brains

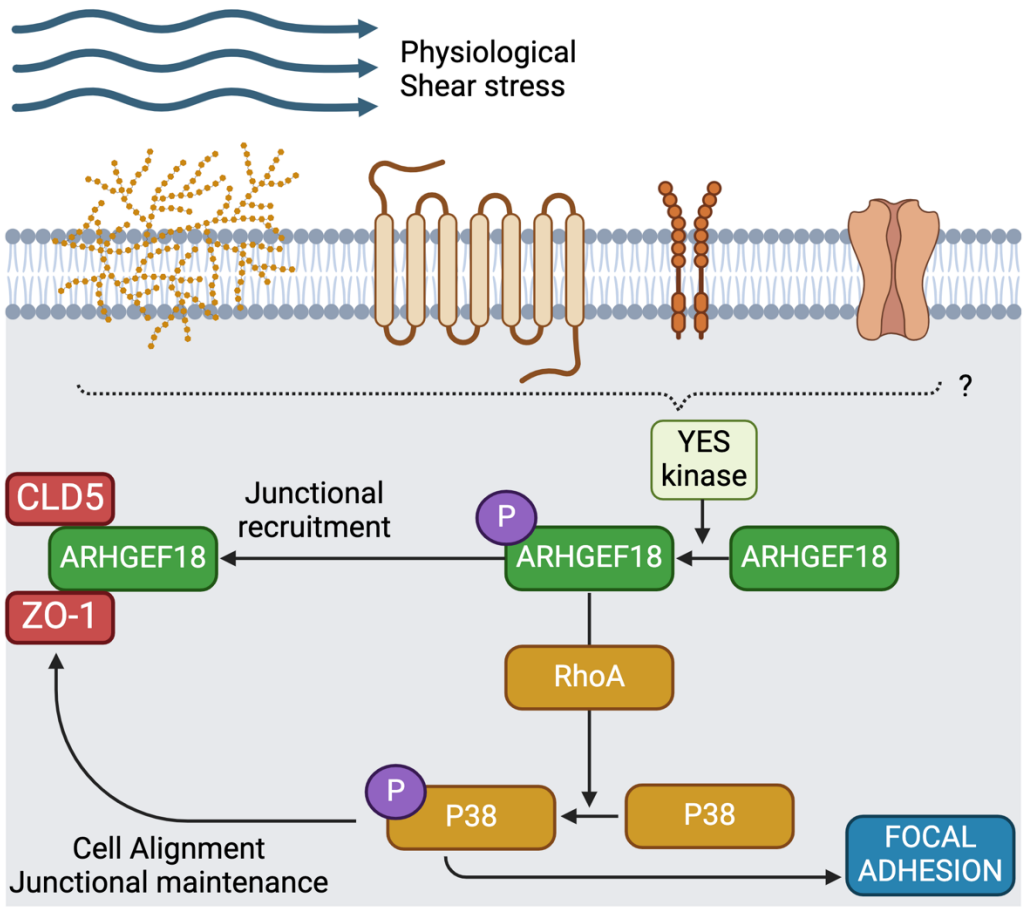
albumin extravasation (Evans Blue)



dextran extravasation



Brains of Arhgef18-iEC pups have increase vascular leakage in the kidney and in the mesenteric arteries



In vitro

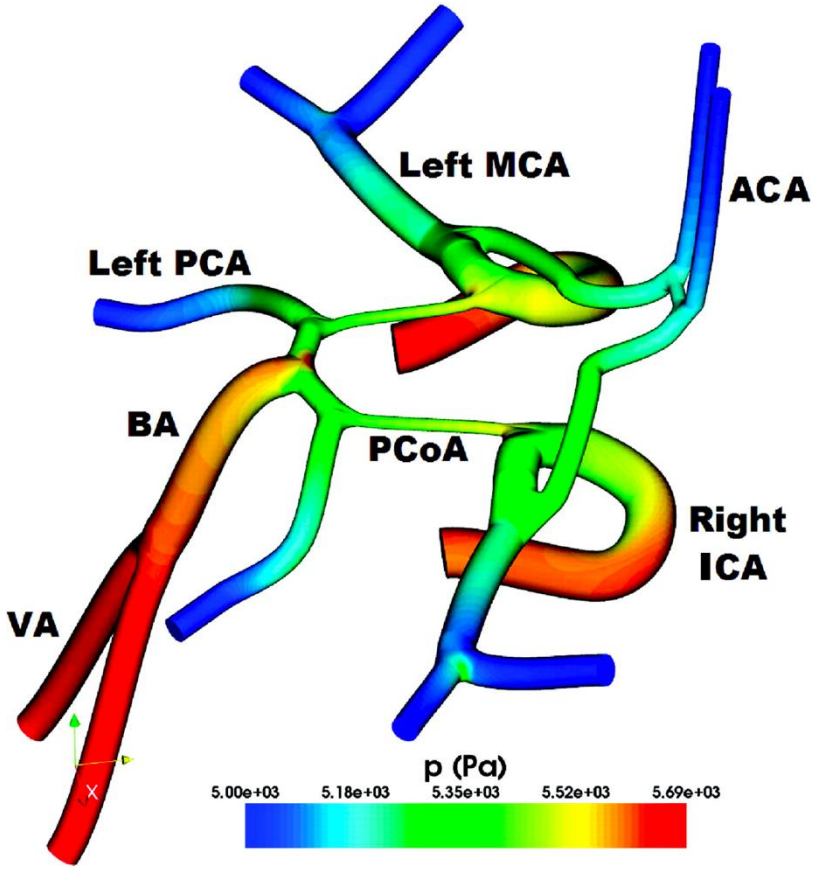
Orientation and elongation
Migration
Tight Junction / permeability
Basal adhesion

In vivo

conducting arteries :
Elongation/tight junctions

Resistance arteries :
Permeability /vascular leakage

Intracranial Aneurysm : causes and consequences



Sandve Alnæs. *Stroke*. 2007

- 3% of the general population
- unpredictable rupture
- 50% of death in case of rupture

- **Risk factors :**
  - Smocking, alcohol consumption, **hypertension**
  - female sex, age, **familial history**

- **physiopathology :**
  - endothelial dysfunction
  - inflammation
  - remodeling of the vascular wall
  - molecular mechanisms at play are mostly unknown
  - associated with altered hemodynamics

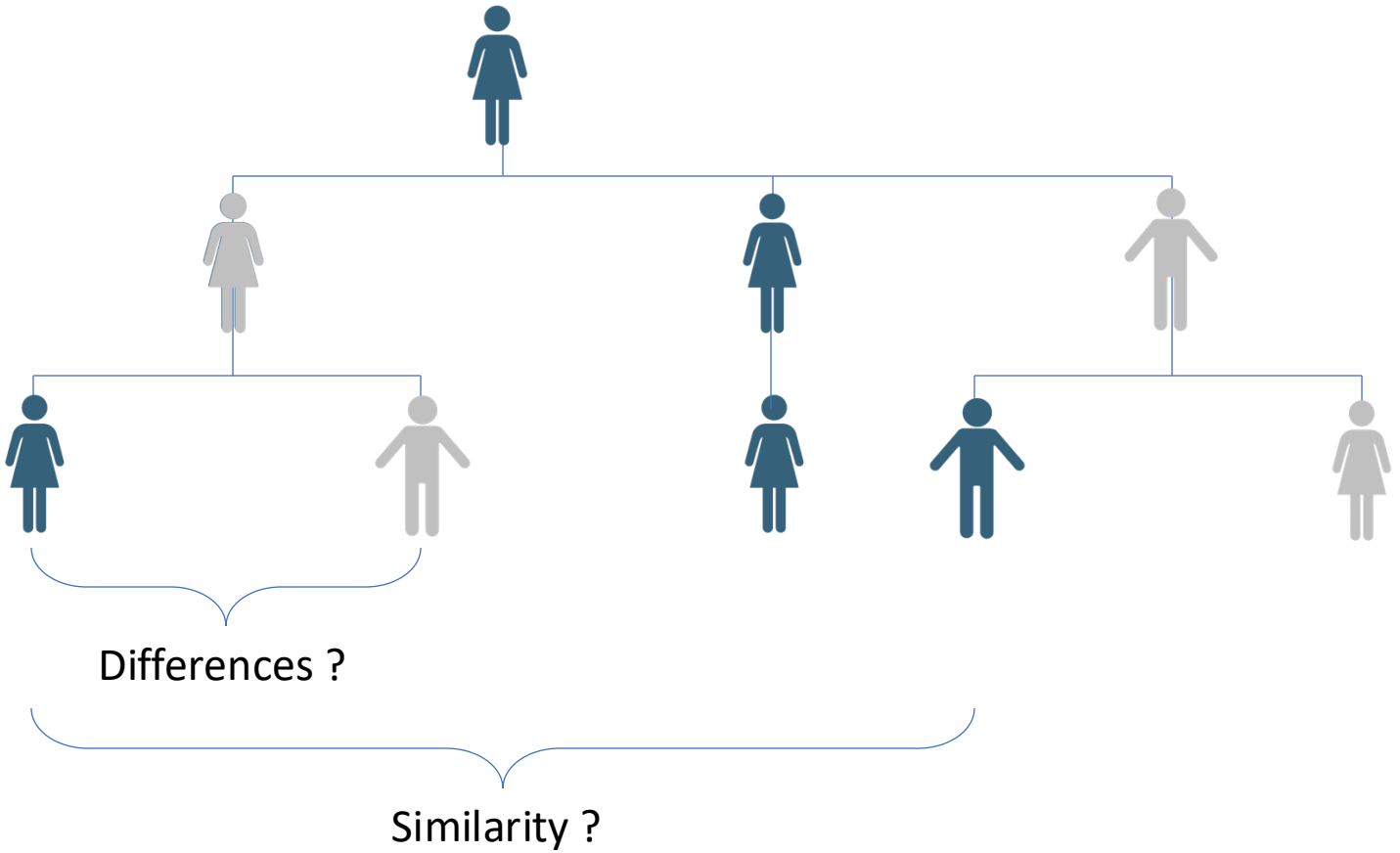
Using of the familial form ?

What is a familial form ?

A patient with at least two close relatives with the disease

Why are they suffering from the disease ?
How can we use their peculiarity ?

This forms represent 10% of the total of ICA

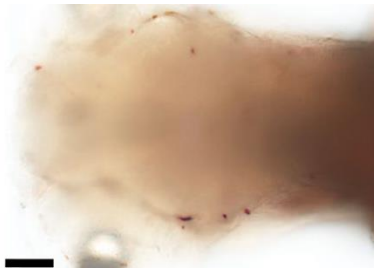


IA and genetics

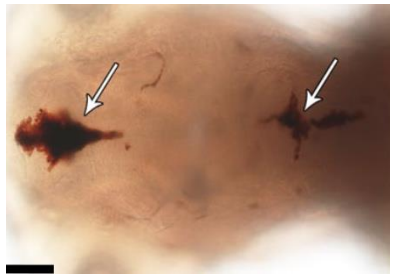
ADAMTS15
RNF213
THSD1
ARHGEF17
PPIL4
…

zebrafish brain after adrenaline exposure

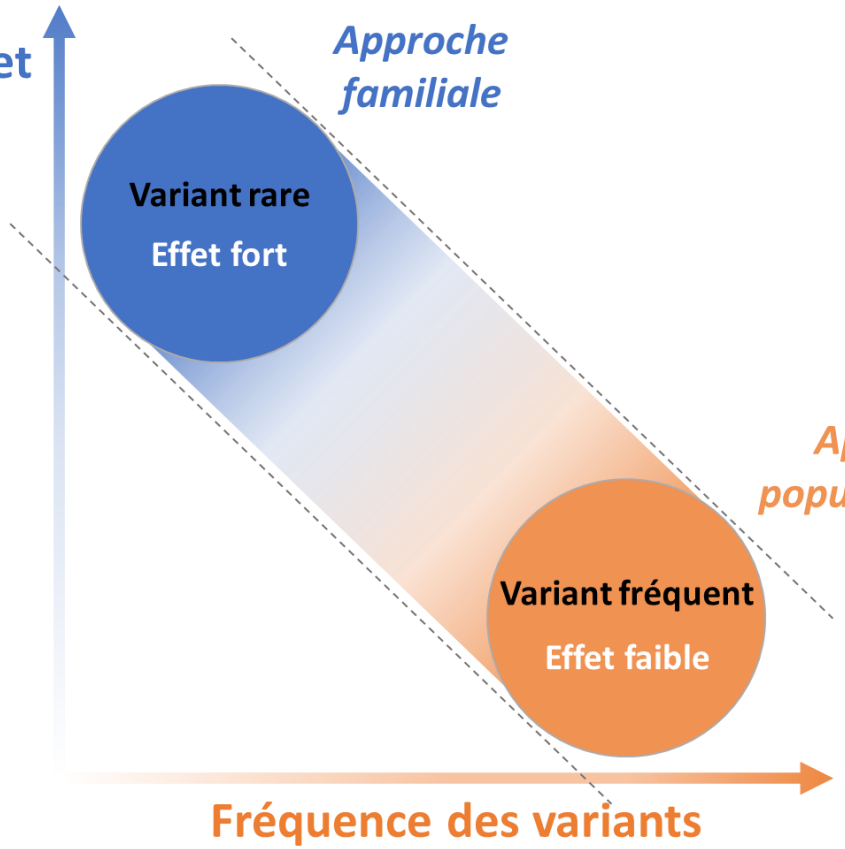
ppil4$^{+/+}$



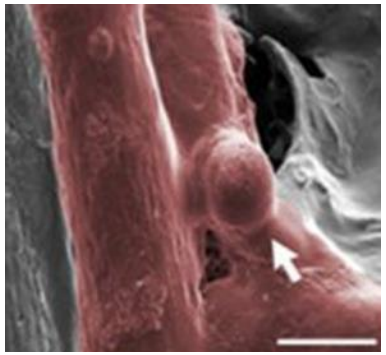
ppil4$^{-/-}$



Barak *et al.*, Nat Med, 2021



CoW artery of *Sox17*-KO^{EC} after angiotensin treatment



Lee *et al.*, Circulation, 2015

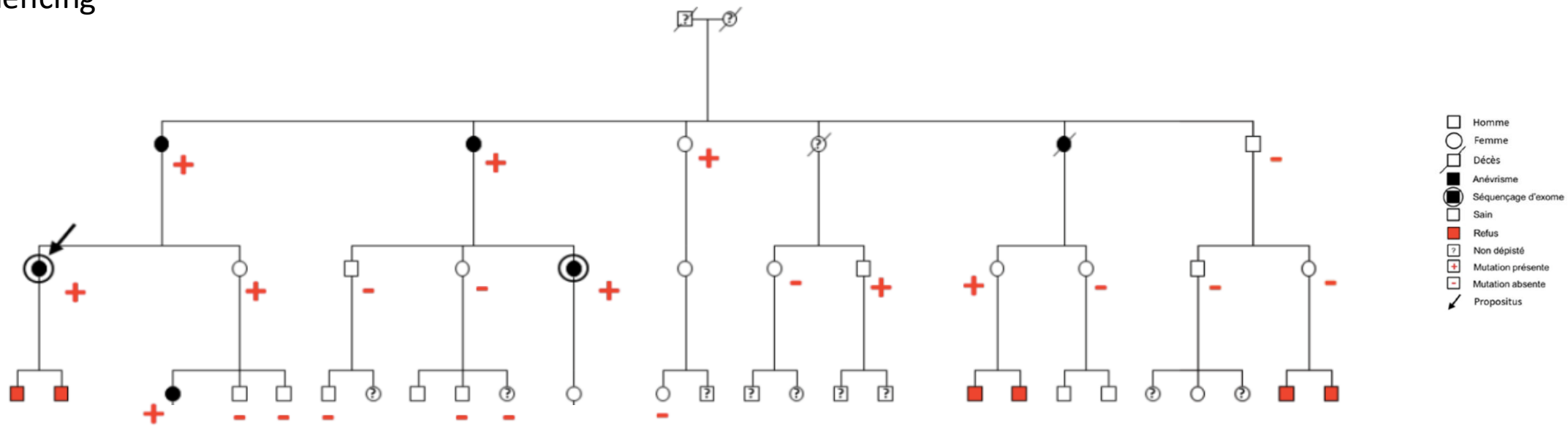
EDNRA
CDKN2A/B/BAS
SOX17
…

Manolio *et al.*, Nature, 2009 ; Bakker et Ruigrok, Stroke , 2021

Using of the familial form ?

Bourcier *et al.*, Am. J. Hum. Genet., 2018

Exome sequencing



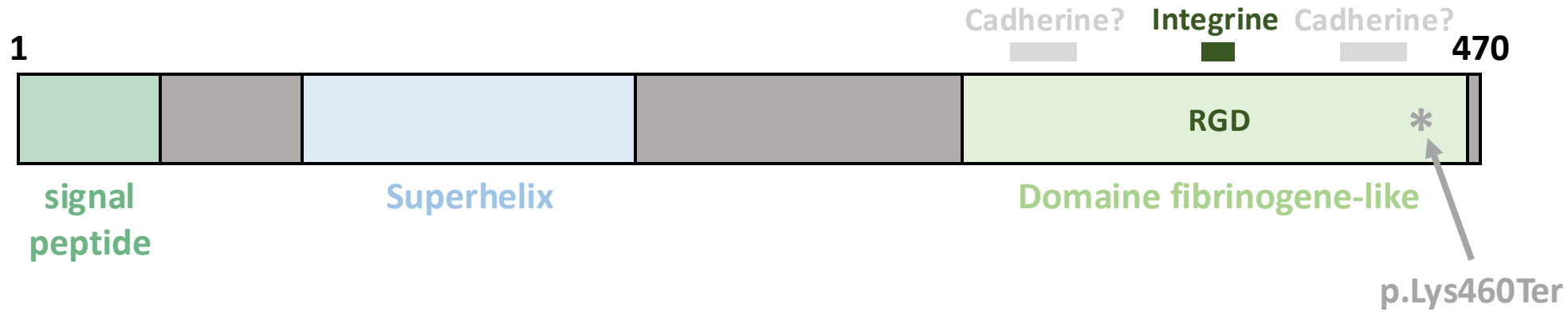
Identification of a mutation in the gene coding for the ANGPTL6 protein:

- **Reduced liver production**
- **less protein in the blood**

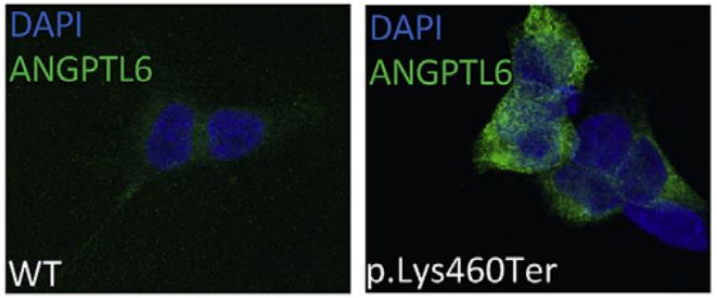
How does this mutation contribute to the formation of intracranial aneurysms?

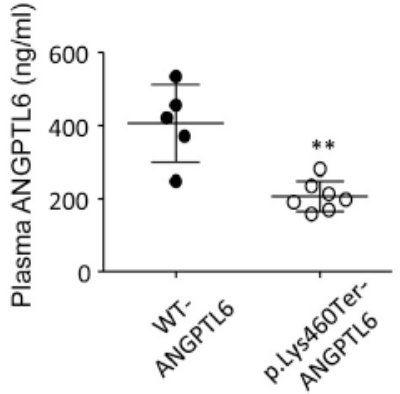
what would you first do ?

the p.Lys460Ter-ANGPTL6 variant



➡ Rare variant p.Lys460Ter-ANGPTL6 predicted as loss of function



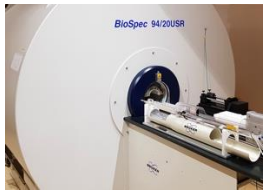


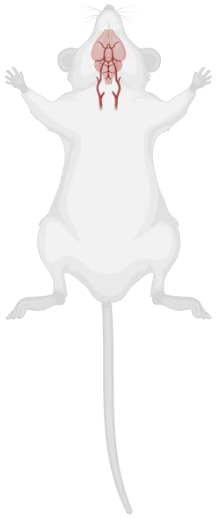
➡ Plasma concentration decreased by half in patients carrying the variant

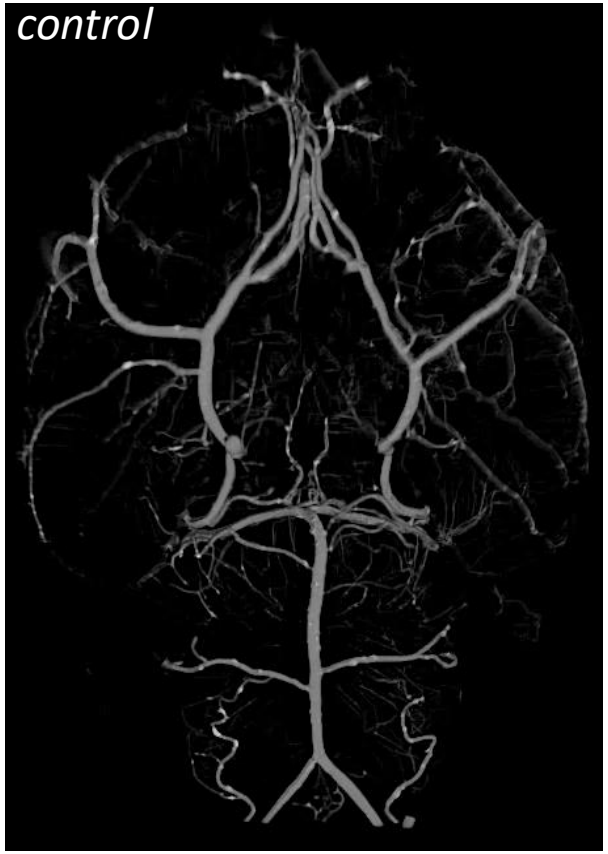
Bourcier *et al.*, Am J Hum Gen, 2018

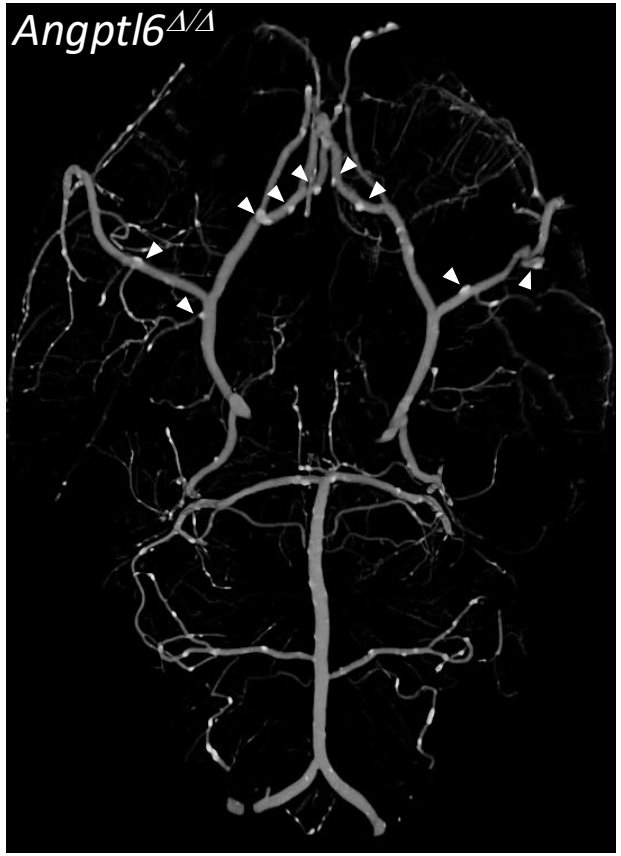
How are the cerebral arteries of mutant mice?

Hypertension
CT scan

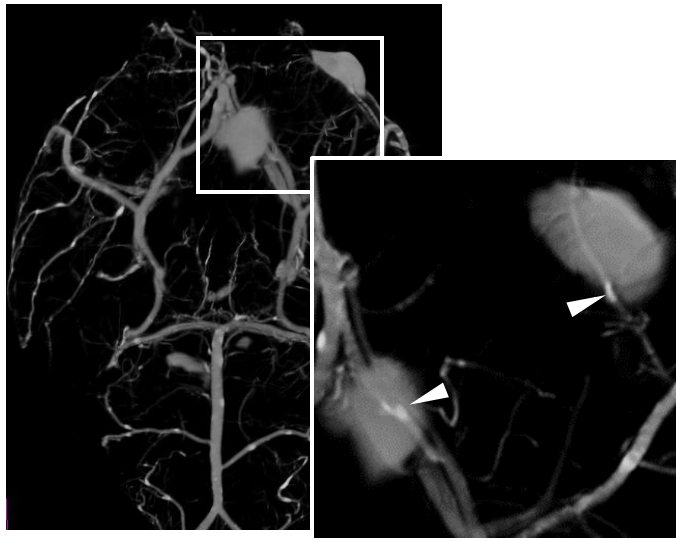








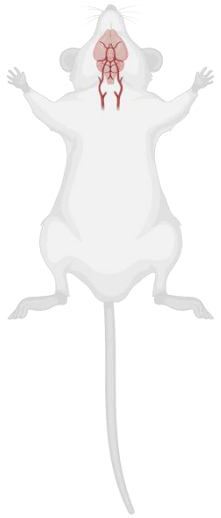
Angptl6$^{\Delta/\Delta}$

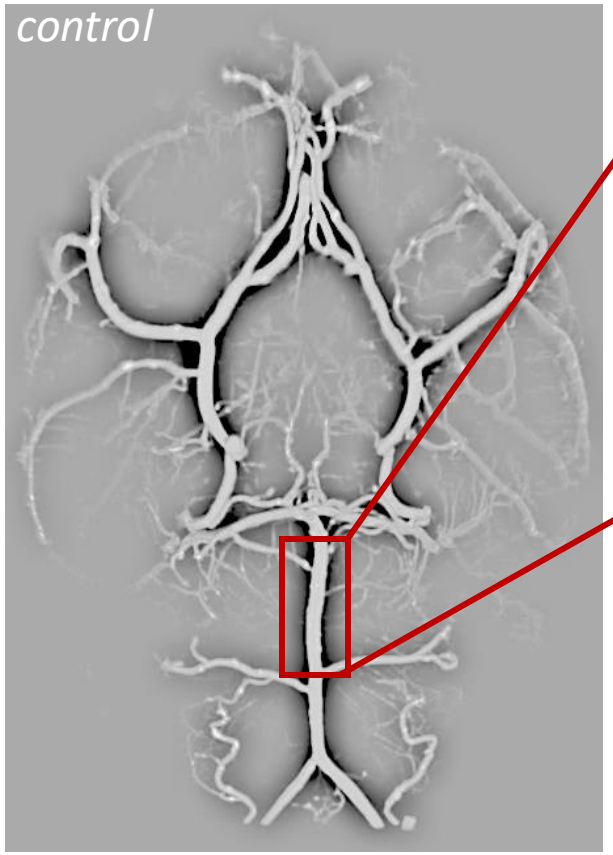


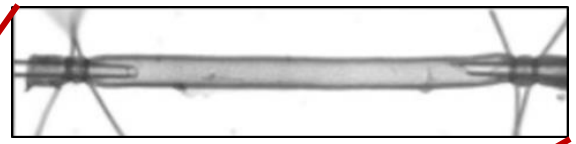
Arteries in mutant mice show local dilatation

unpublished data

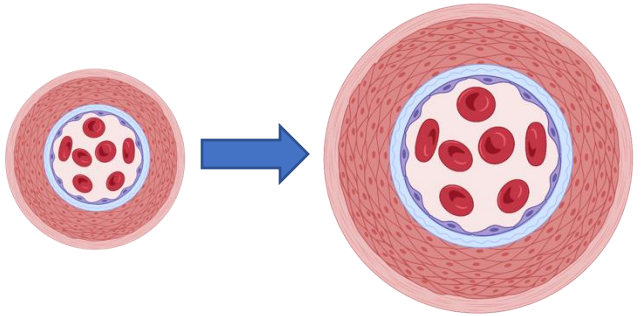
Are the cerebral arteries of those mice able to dilate properly ?

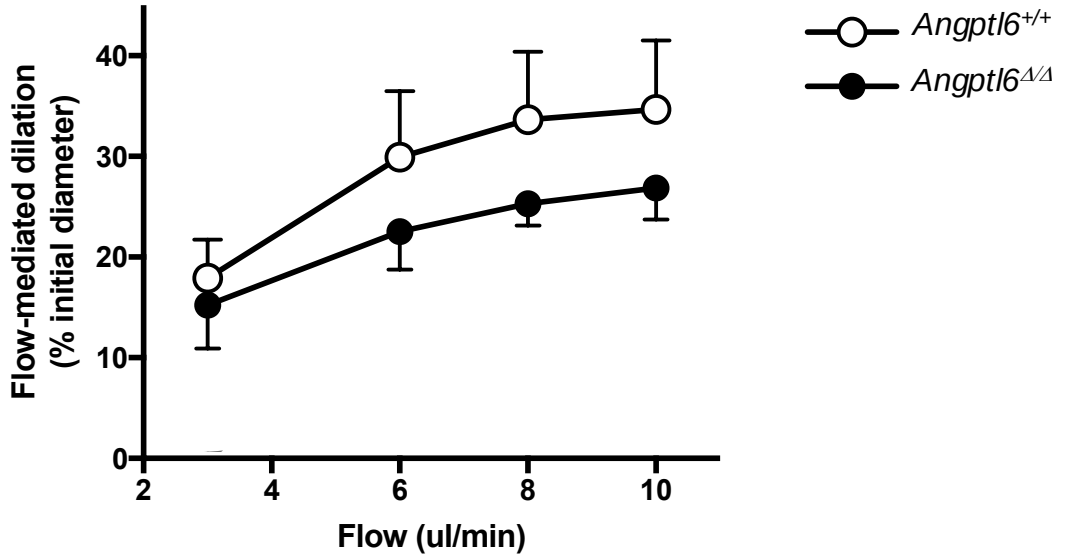






Flow mediate dilation



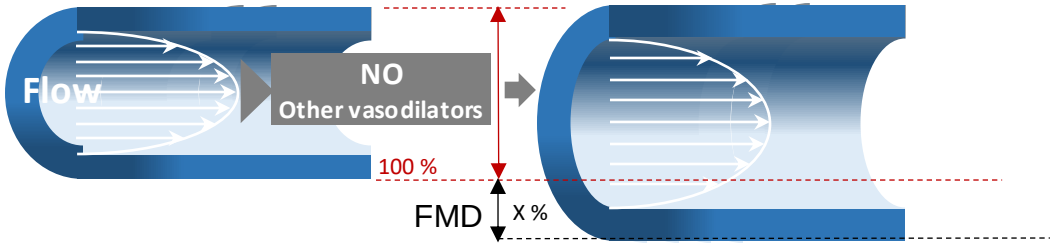


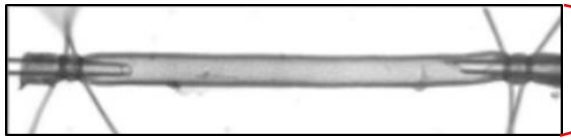
Arteries in mutant mice dilate less in response to blood flow than control

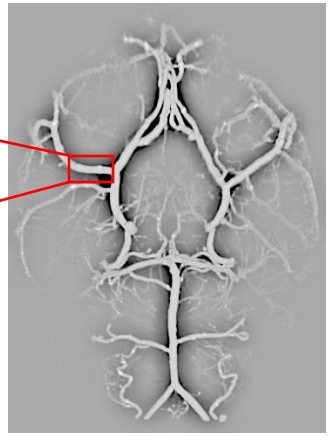
unpublished data

Does *Angptl6* mutation affect brain arteries dilation ?

Flow mediated dilation of brain arteries







unpublished data

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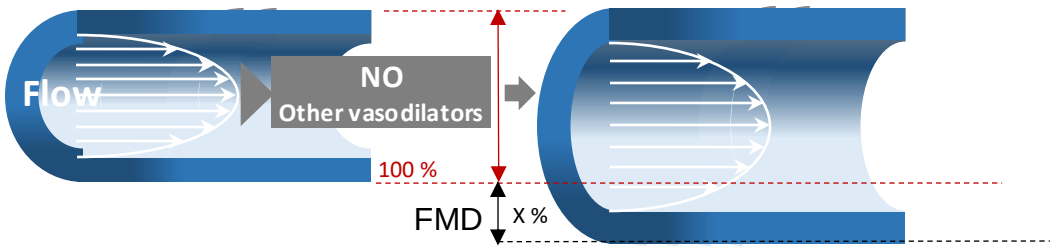
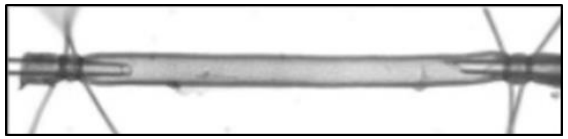
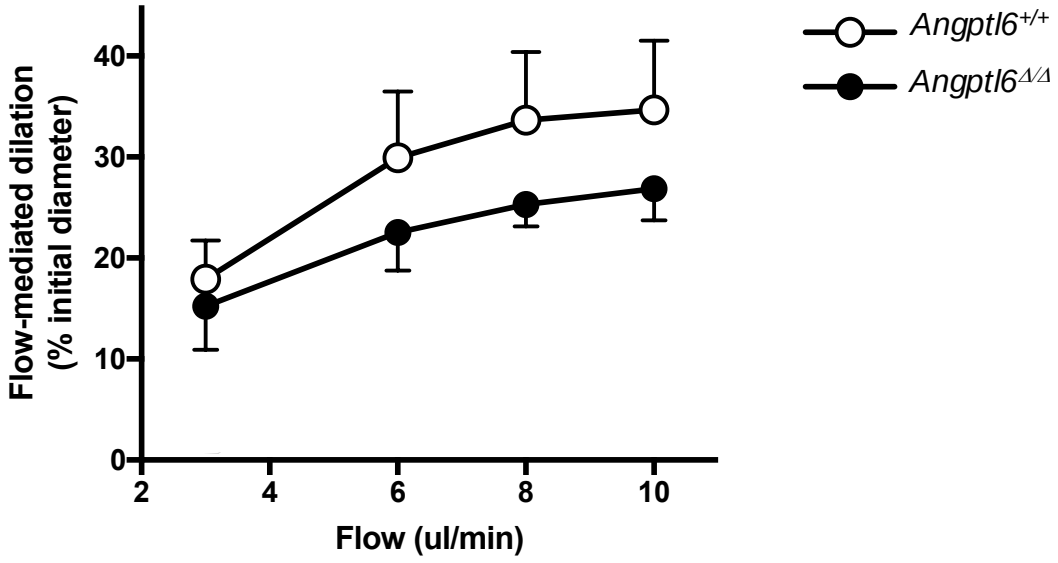
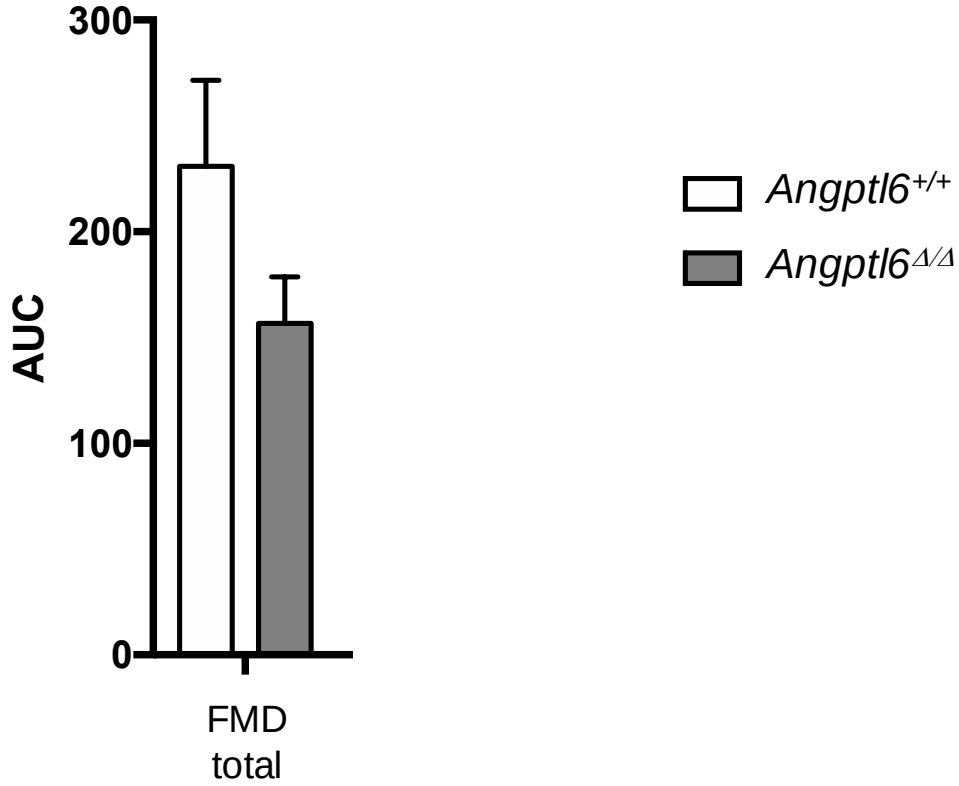
Flow mediated dilation of brain arteries









unpublished data

Does *Angptl6* mutation affect brain arteries dilation ?

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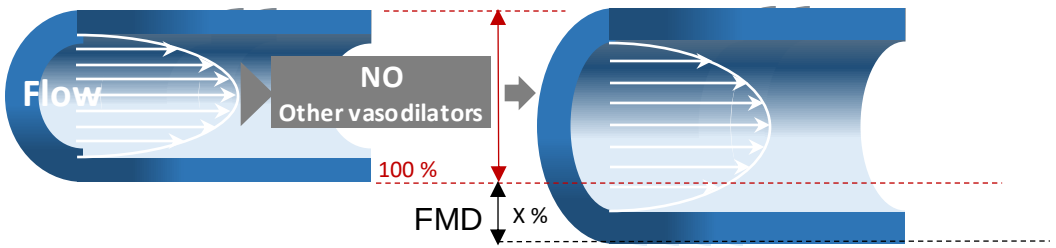

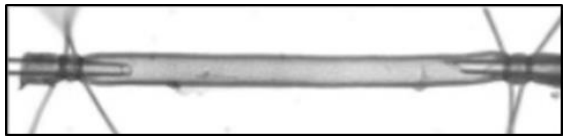

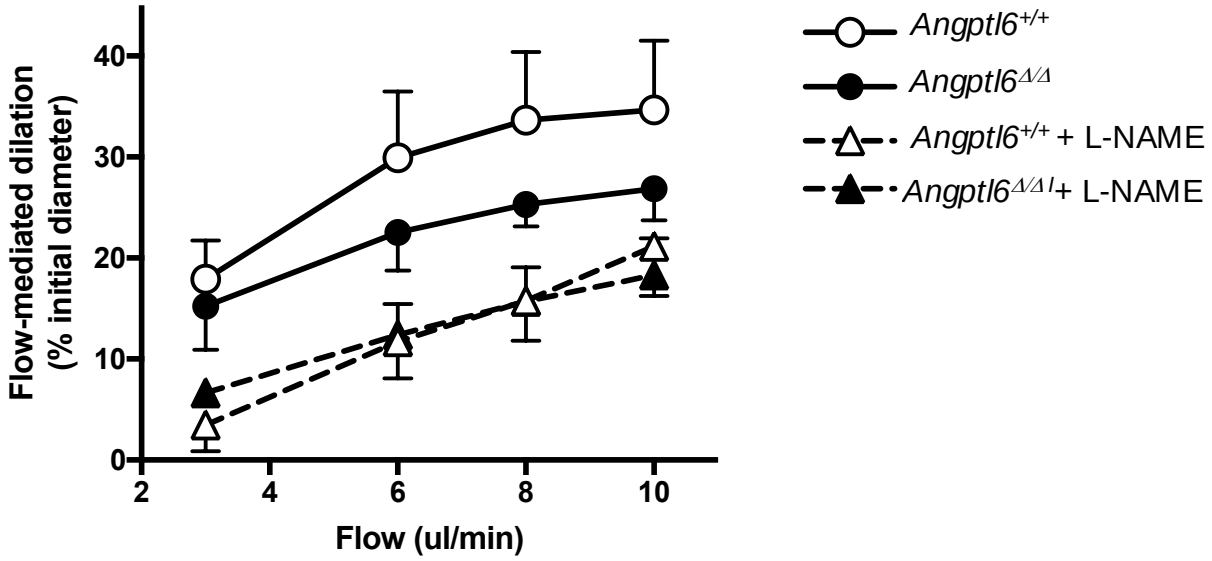

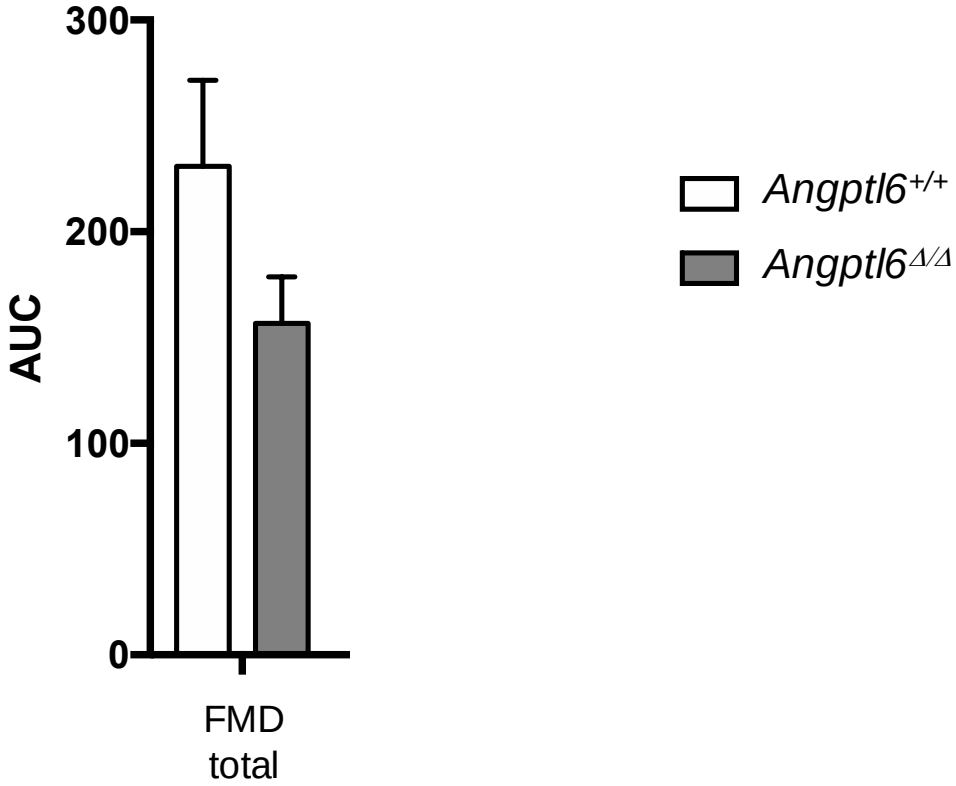

unpublished data

Does *Angptl6* mutation affect brain arteries dilation ?

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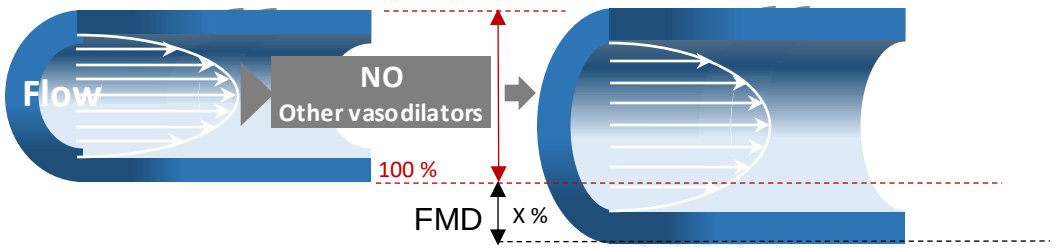
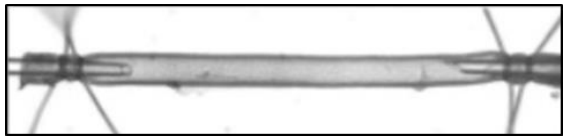
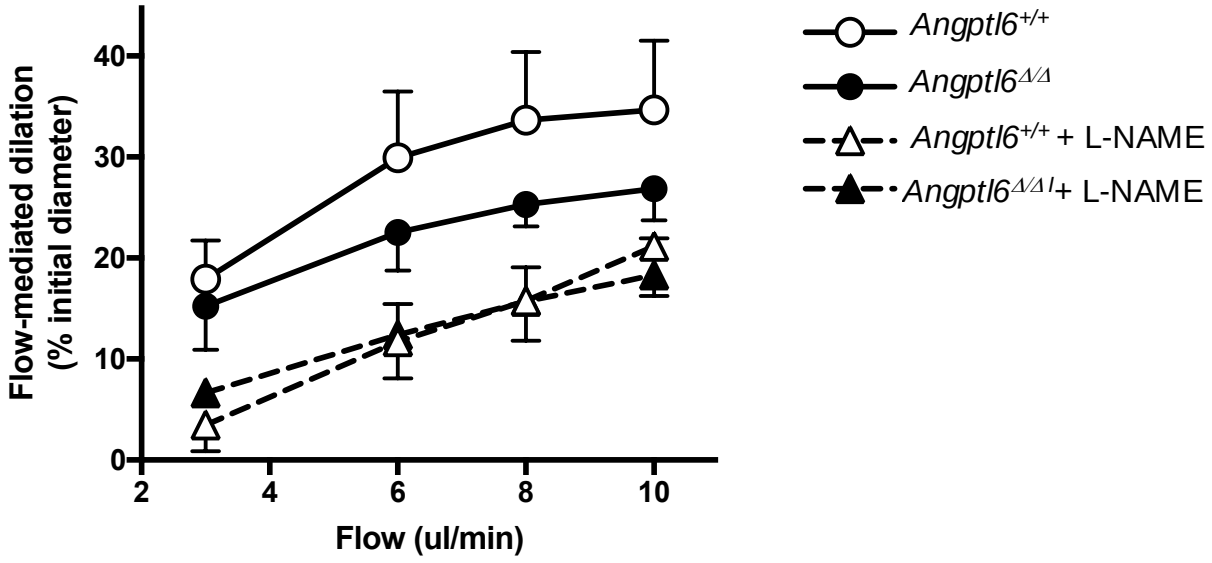
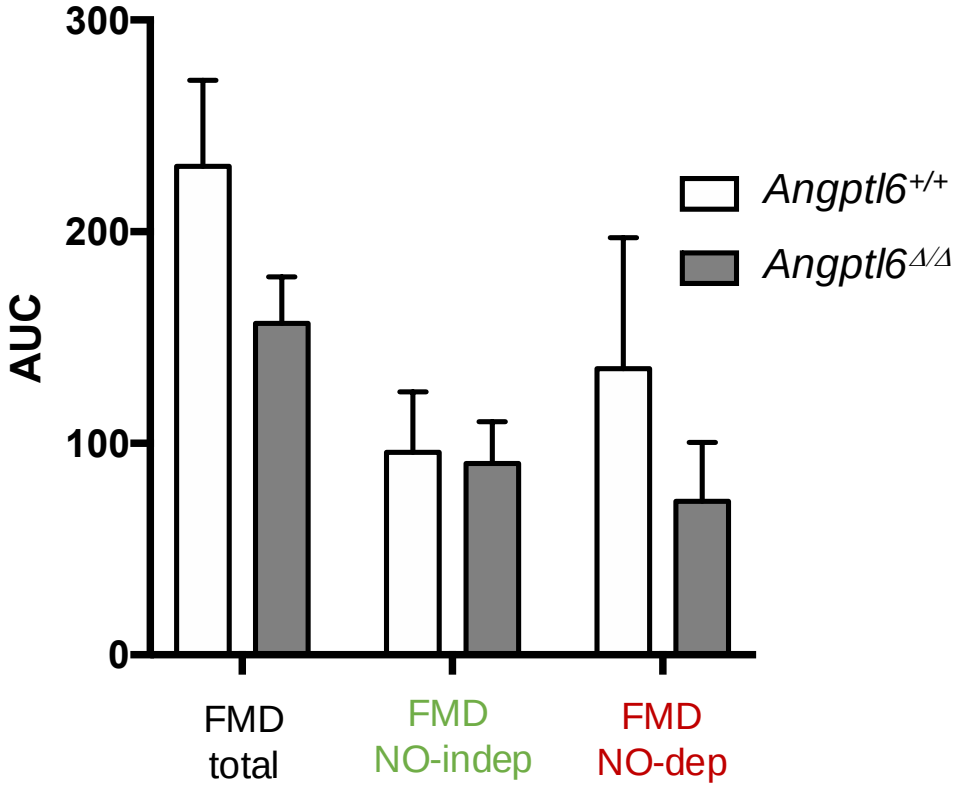






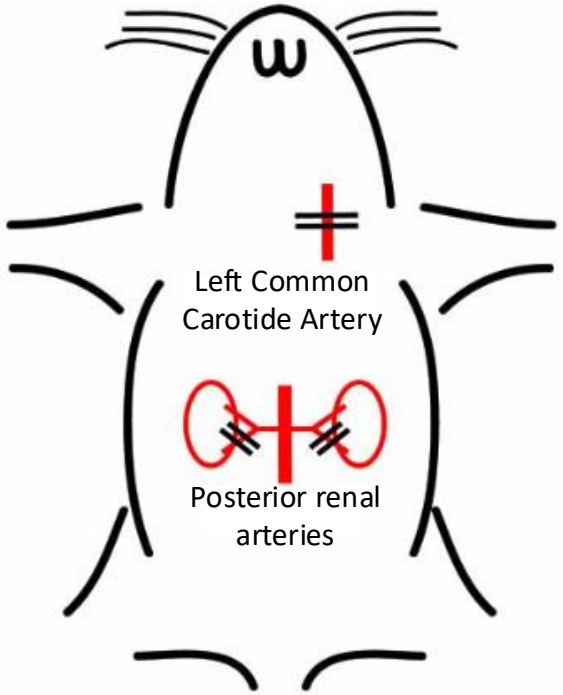


***Angptl6*$^{\Delta/\Delta}$ mice have an endothelial dysfunction**

unpublished data

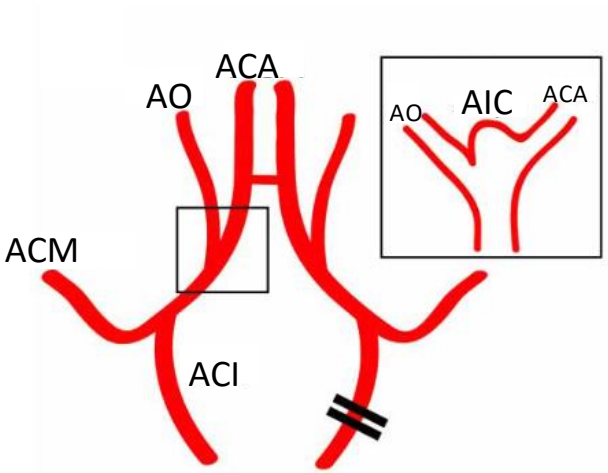
What is the role of the hemodynamics ?

Morimoto model



+ salt in drinking water

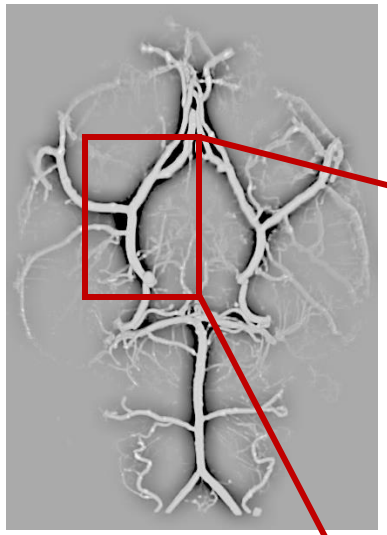
4 months later



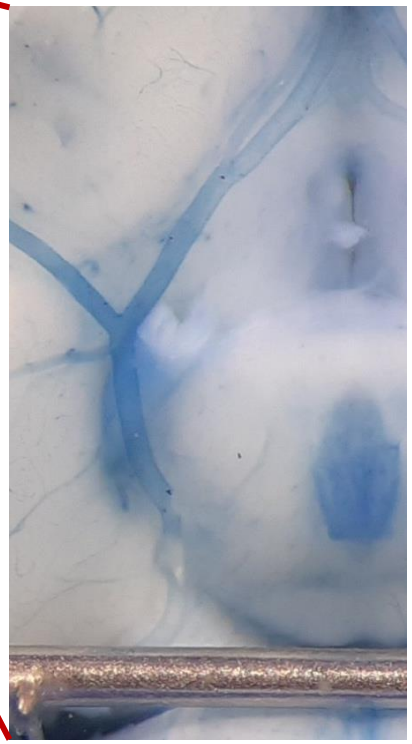
Morimoto et al., Stroke, 2002 ; Ishibashi *et al.*, CNR, 2010

Do mutant mice develop intracranial aneurysms?

Morimoto model



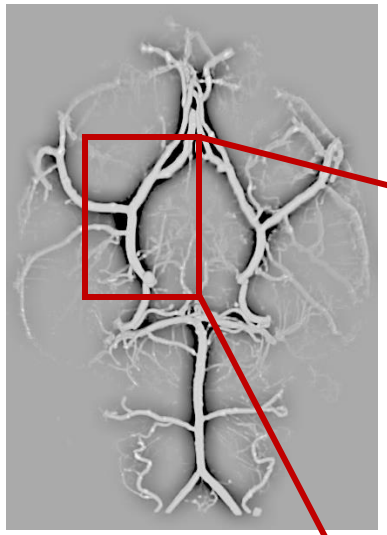
No surgery



unpublished data

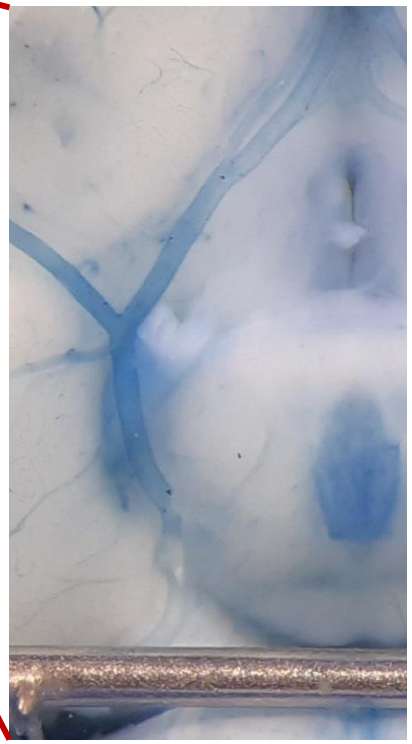
Do mutant mice develop intracranial aneurysms?

Morimoto model



Surgery

No surgery

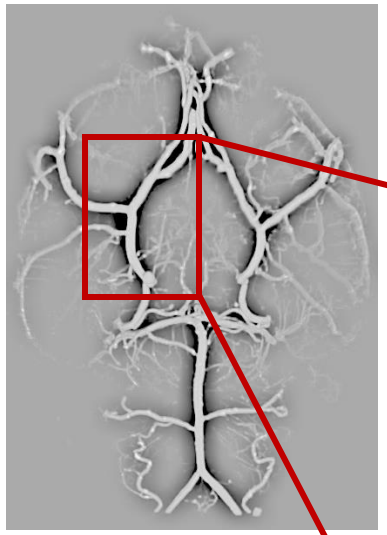


control

unpublished data

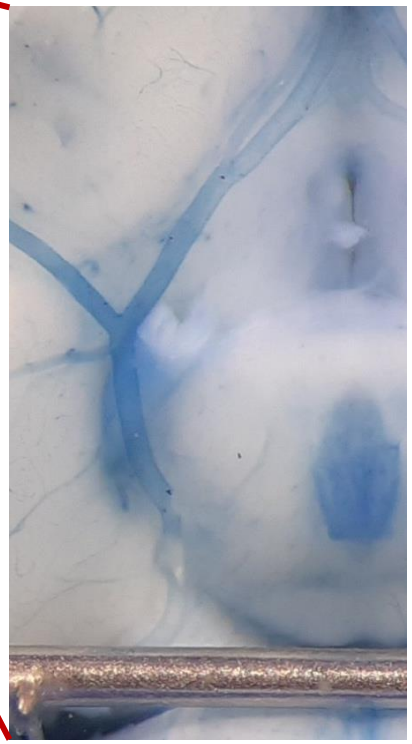
Do mutant mice develop intracranial aneurysms?

Morimoto model



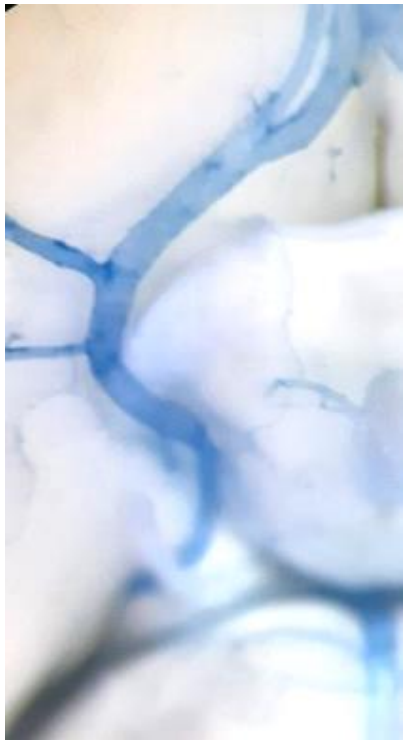
Surgery

No surgery



control

Angptl6$^{\Delta/\Delta}$

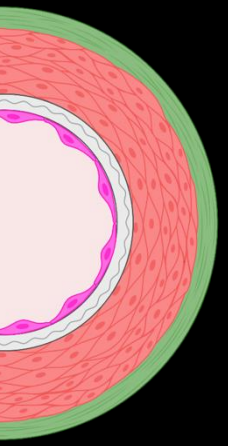


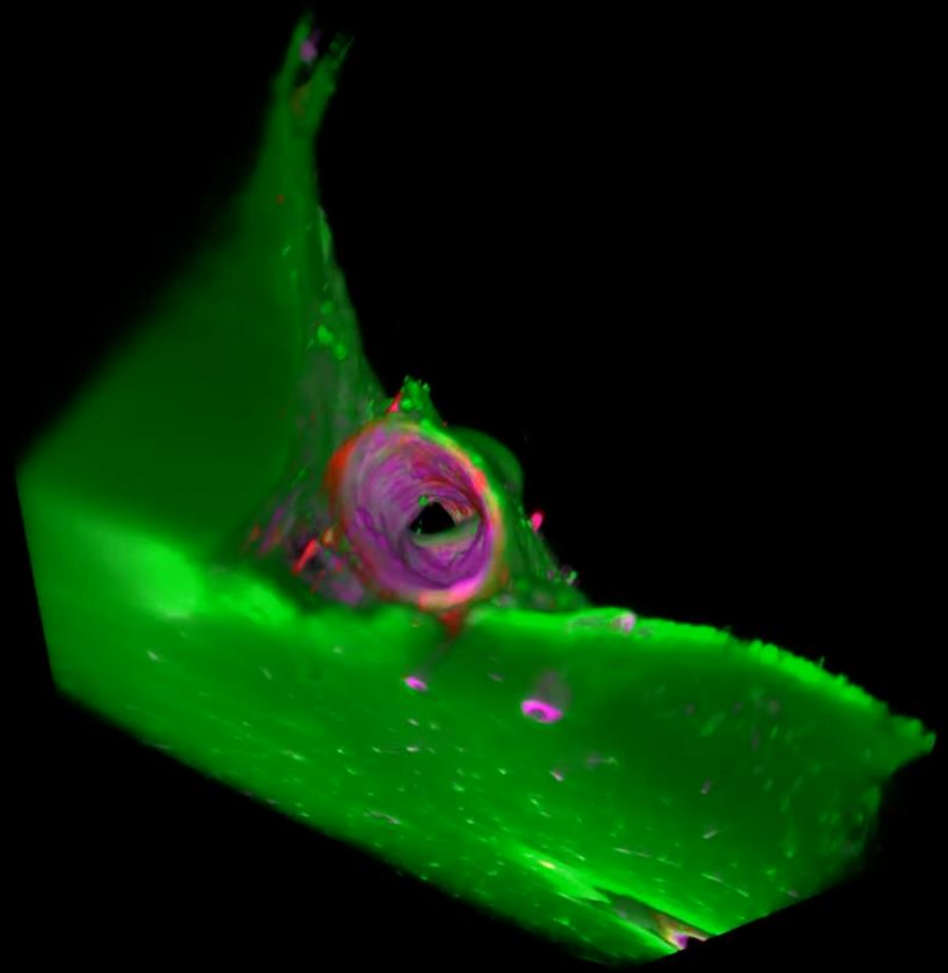
no aneurysms, but arteries wider and more tortuous than controls

unpublished data

Do mutant mice develop intracranial aneurysms?

using a more precise method: light sheet microscopy

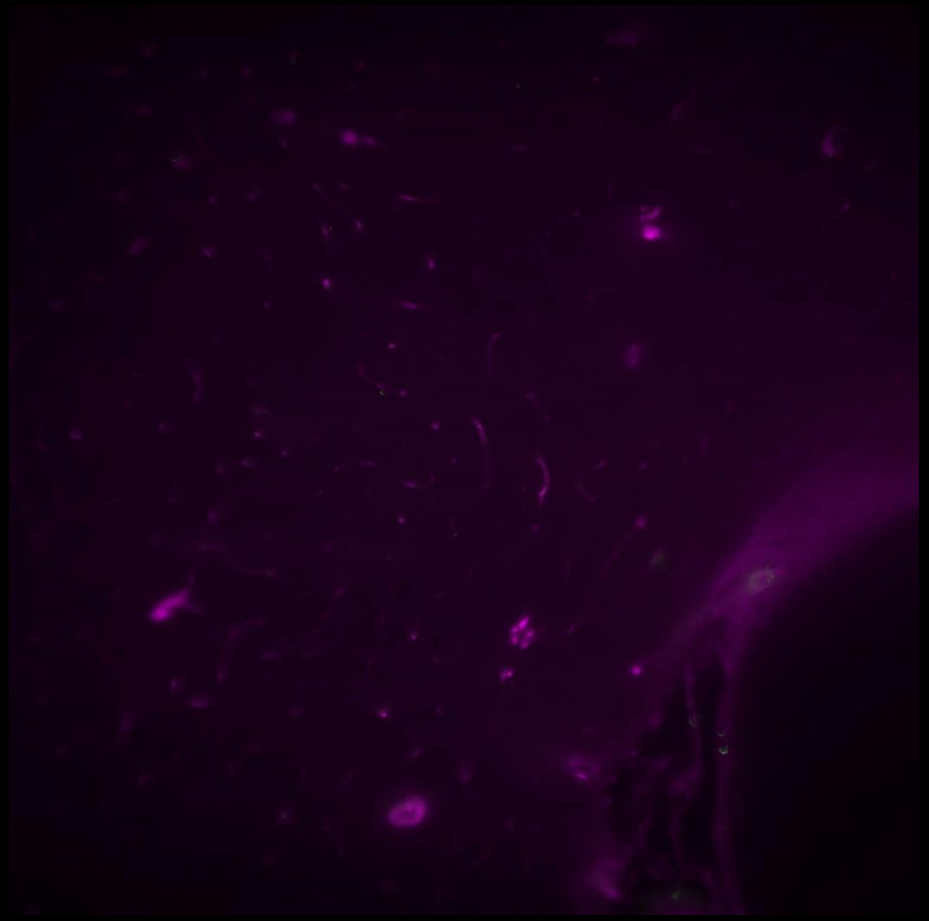




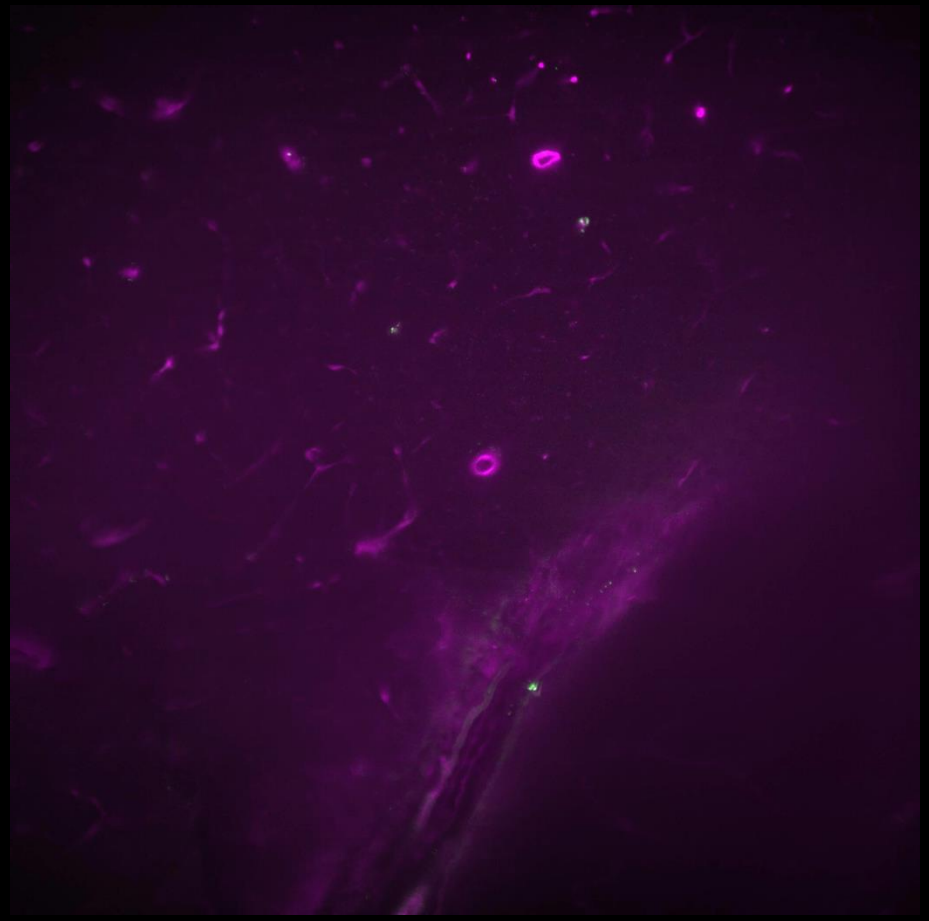
Do mutant mice develop intracranial aneurysms?

using a more precise method: light sheet microscopy

control



Angptl6$^{\Delta/\Delta}$



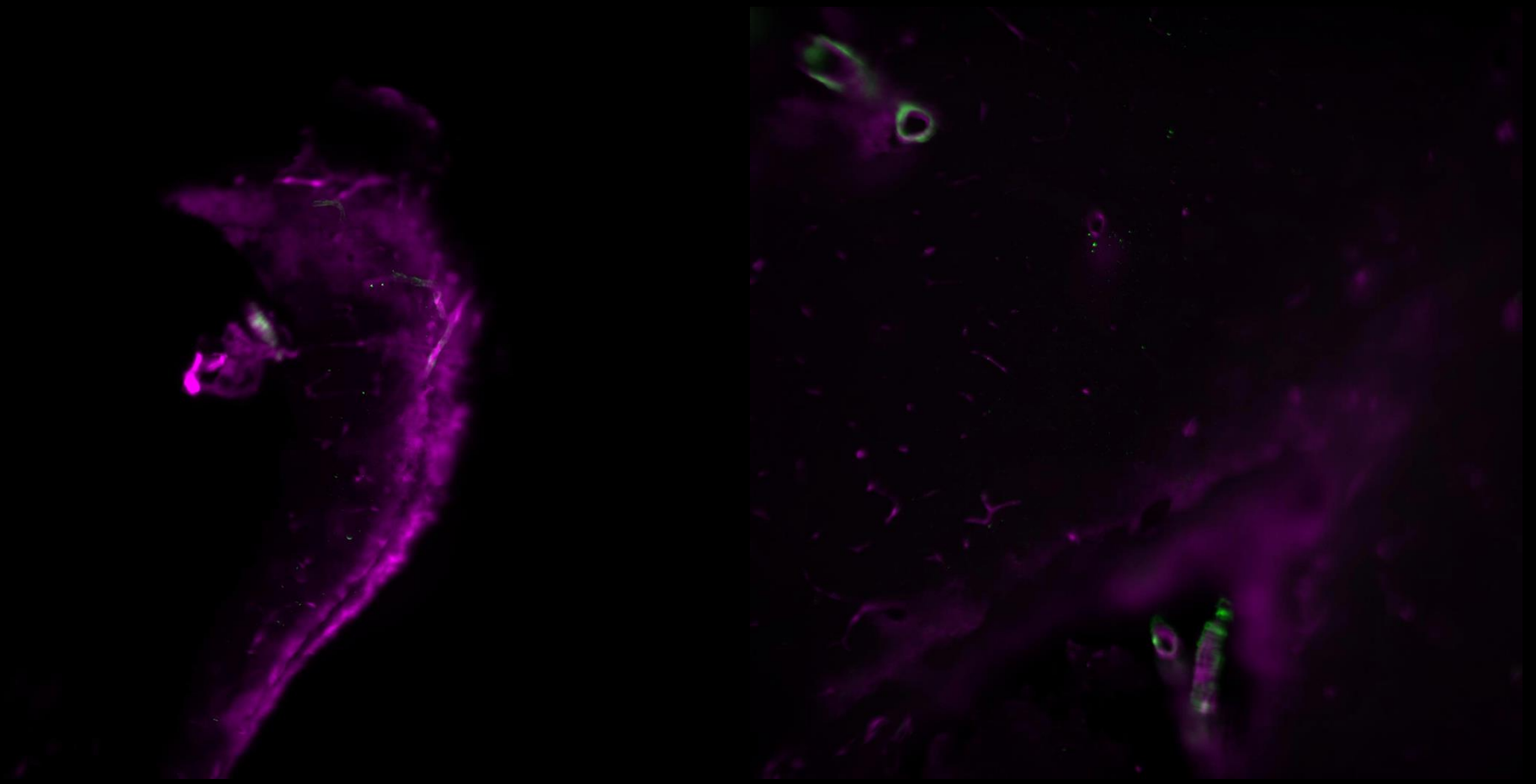
The bifurcations of mutant arteries display some distortions

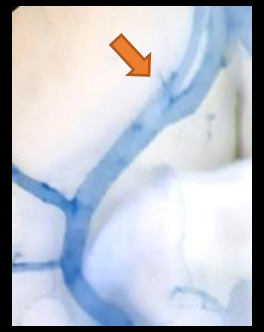
Do mutant mice develop intracranial aneurysms?

using a more precise method: light sheet microscopy

control

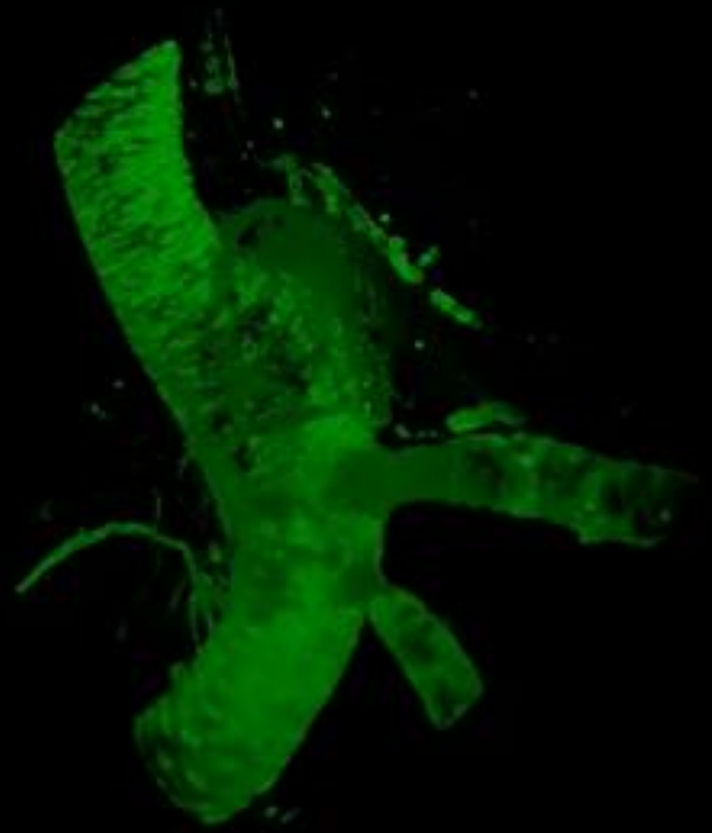
Angptl6$^{\Delta/\Delta}$





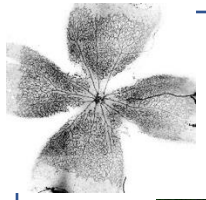
The bifurcations of mutant arteries present a thickening of the wall

Do mutant mice develop intracranial aneurysms?

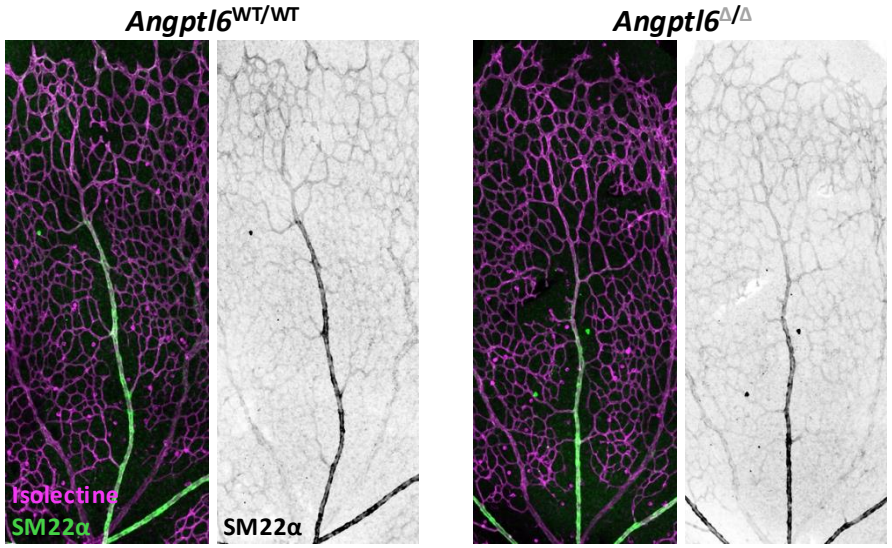


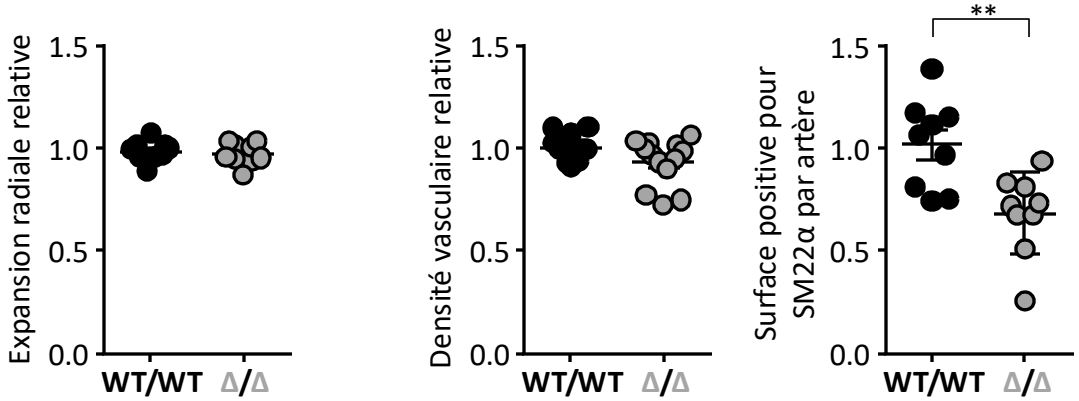
Yes, some mice form aneurysms similar to the patient's ones.

Is developmental angiogenesis in Angptl6-KI mice affected?



Immunofluorescence on newborn retinas





➡ Angptl6-KI mice lack MLC coverage during vascular network formation

Using of the familial form ? YES ☺

Angptl6$^{\Delta/\Delta}$

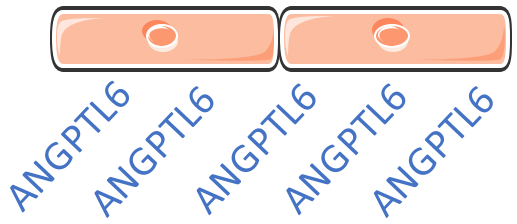
Developmental : Delayed VSMC coverage

Adulthood :

- Hyper-density signal under hypertensive treatment
- Arteries enlargement and tortuosity
- Endothelial dysfunction / Impaired flow mediated dilation

Surgical IA :

- Locale vascular wall deformation
- Neo-Intima / IA



In vitro : ANGPTL6 enhances SMC adhesion
prevents SMC transmigration

Surya BATTA

Corentin Lebot

Celine Menguy

Marc Rio

Céline Menguy

Milène Freneau

Mary-Adel Mrad

Corentin Lebot

Laurent Beck

Sarah Beck-Cormier

Hugo Bergereau

Nathan Chate

Marie Guicheteau

Dorian Hassoun

Reda Moutaukil

Thibault Quillard

Morgane Rousselle

Team leaders:

Gervaise Loirand

Vincent Sauzeau

Open for students ;)

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