Webinar – 12 July 2022

‘Importance of autopsies in leukodystrophies ’

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European Reference Network
for rare or low prevalence complex diseases
Network Neurological Diseases (ERN-RND)

ean european academy of neurology

European Reference Network
for rare or low prevalence complex diseases
Network Neuromuscular Diseases (ERN EURO-NMD)

Speaker

Marianna Bugiani

- Training: child neurologist, pathologist
- Current position: child pathologist and neuropathologist
- Research: leukodystrophies, children brain tumors, developmental disorders

Nothing to disclose

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Learning objectives

By the end of this webinar you will be able to:

- Articulate the current definition of leukodystrophy
- Summarize the multicellular involvement as related to leukodystrophy disease mechanisms
- Explain how neuropathology can unravel the effects of current treatments for leukodystrophies

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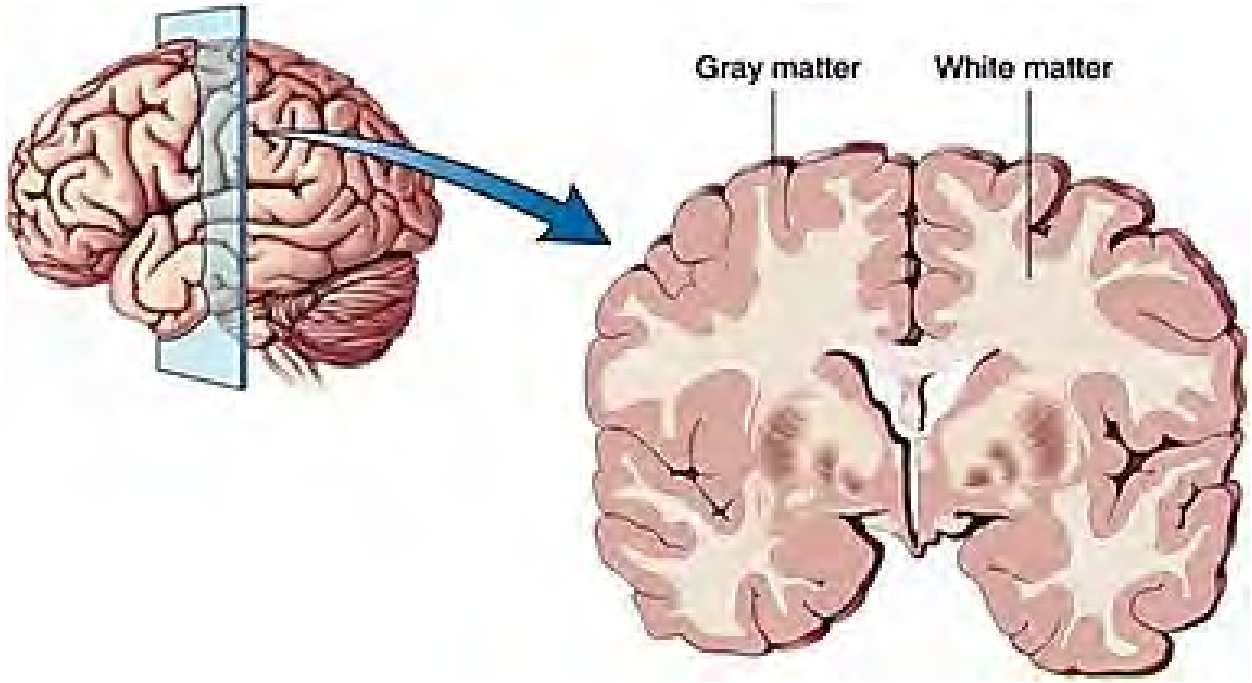
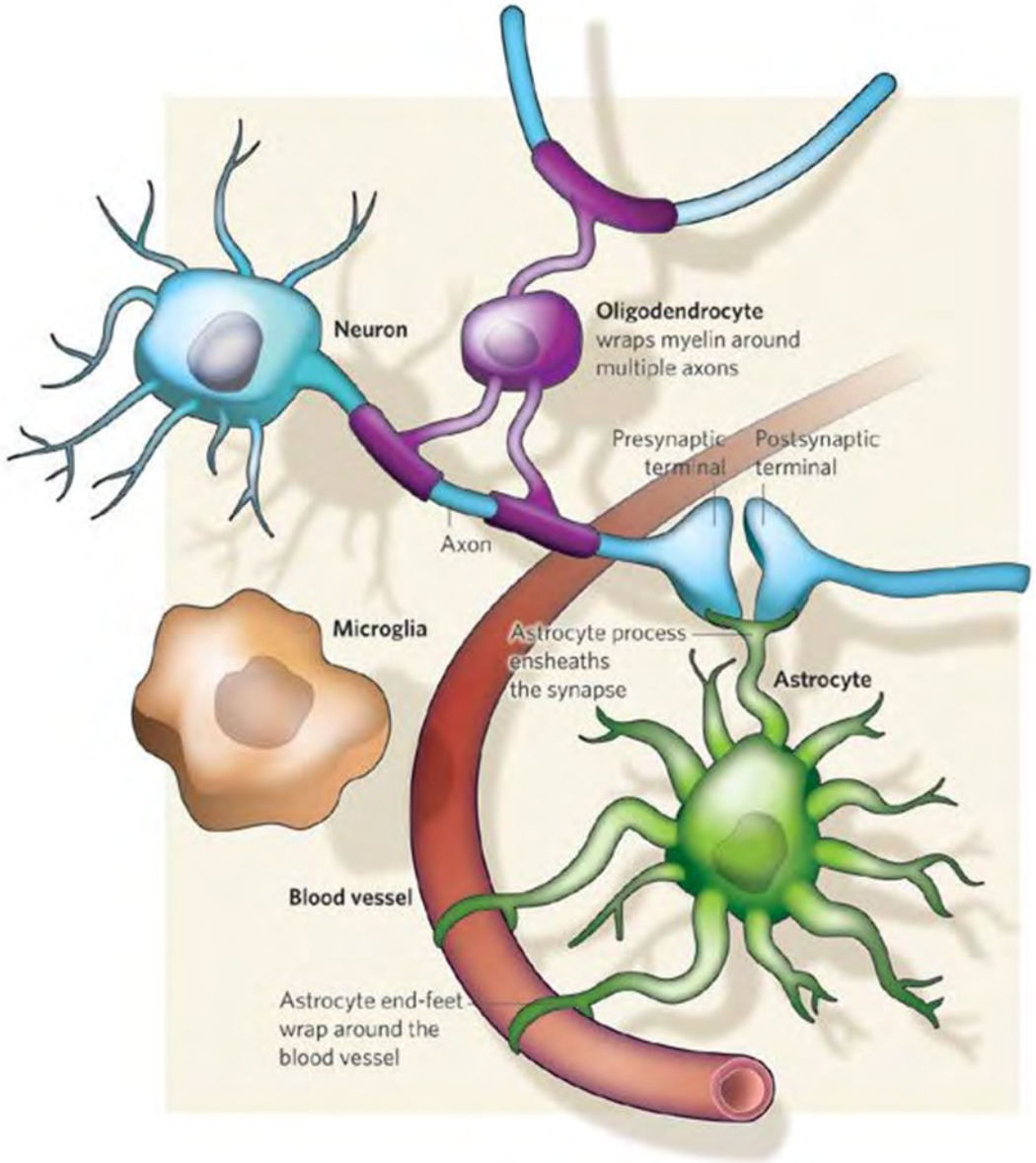
Webinar outline

- Introduction: what is a leukodystrophy?
- Evolution of the concept over time: the impact of basic research and NGS
- How neuropathology helps evaluating the effects of treatment
- How neuropathology explains disease mechansism
- Conclusions
- Acknowledgements

The brain white matter





What is a leukodystrophy?

- 1980’s
- genetic, progressive disorders primarily affecting myelin (myelin loss or insufficient myelination), either directly or through oligodendrocytes

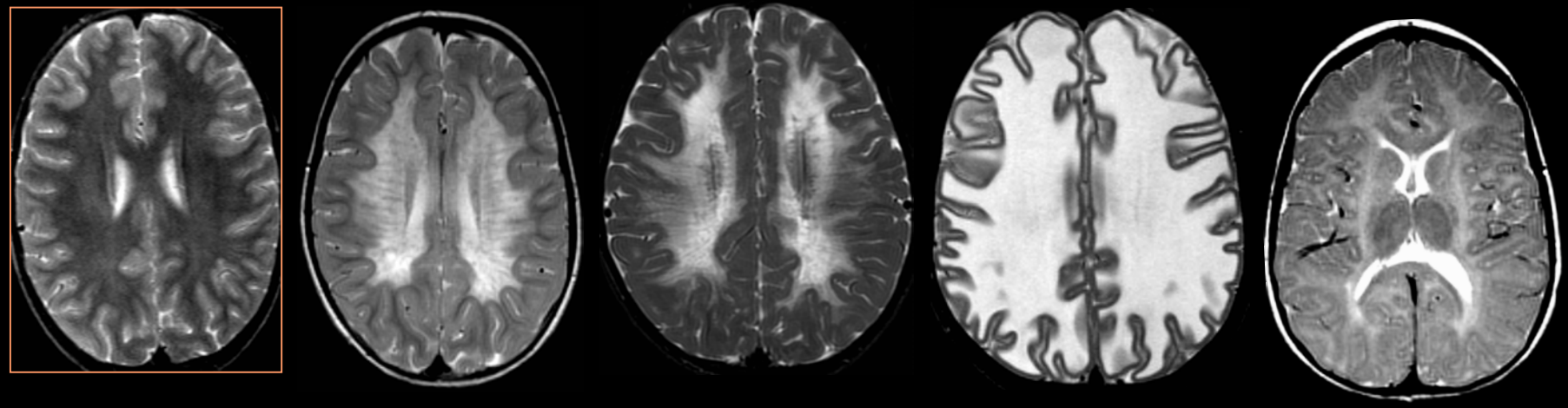
Morell & Wiesmann, Neuropediatrics 1984; 15 (suppl): 62
Seitelberger, Neuropediatrics 1984; 15 (suppl): 53

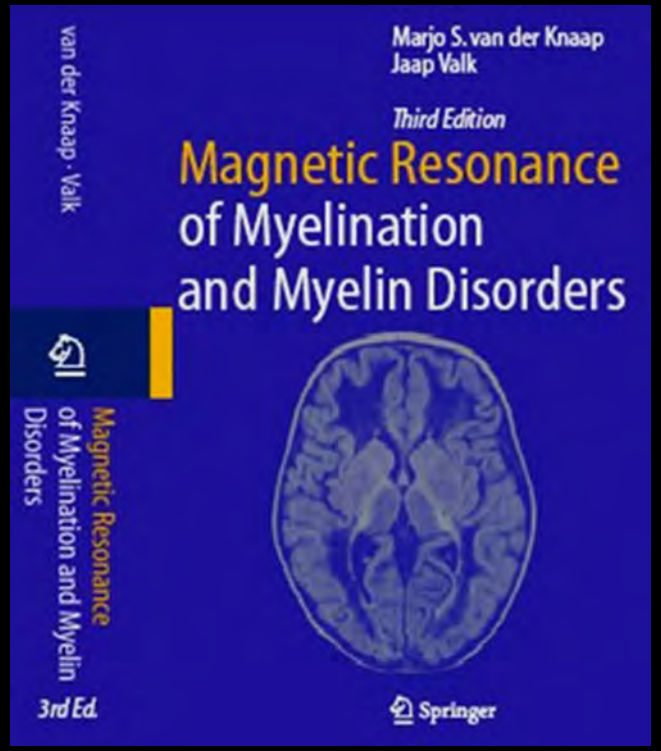
- No known gene defects
- MRI had not entered clinical practice
- Data available from pathology, biochemical analyses of brain tissue and knowledge of several metabolic and enzymatic defects

- Curative treatment focused on stopping myelin loss and on remyelination

1980's: introduction of MRI

- Very high sensitivity for white matter abnormalities
- Replaced neuropathology



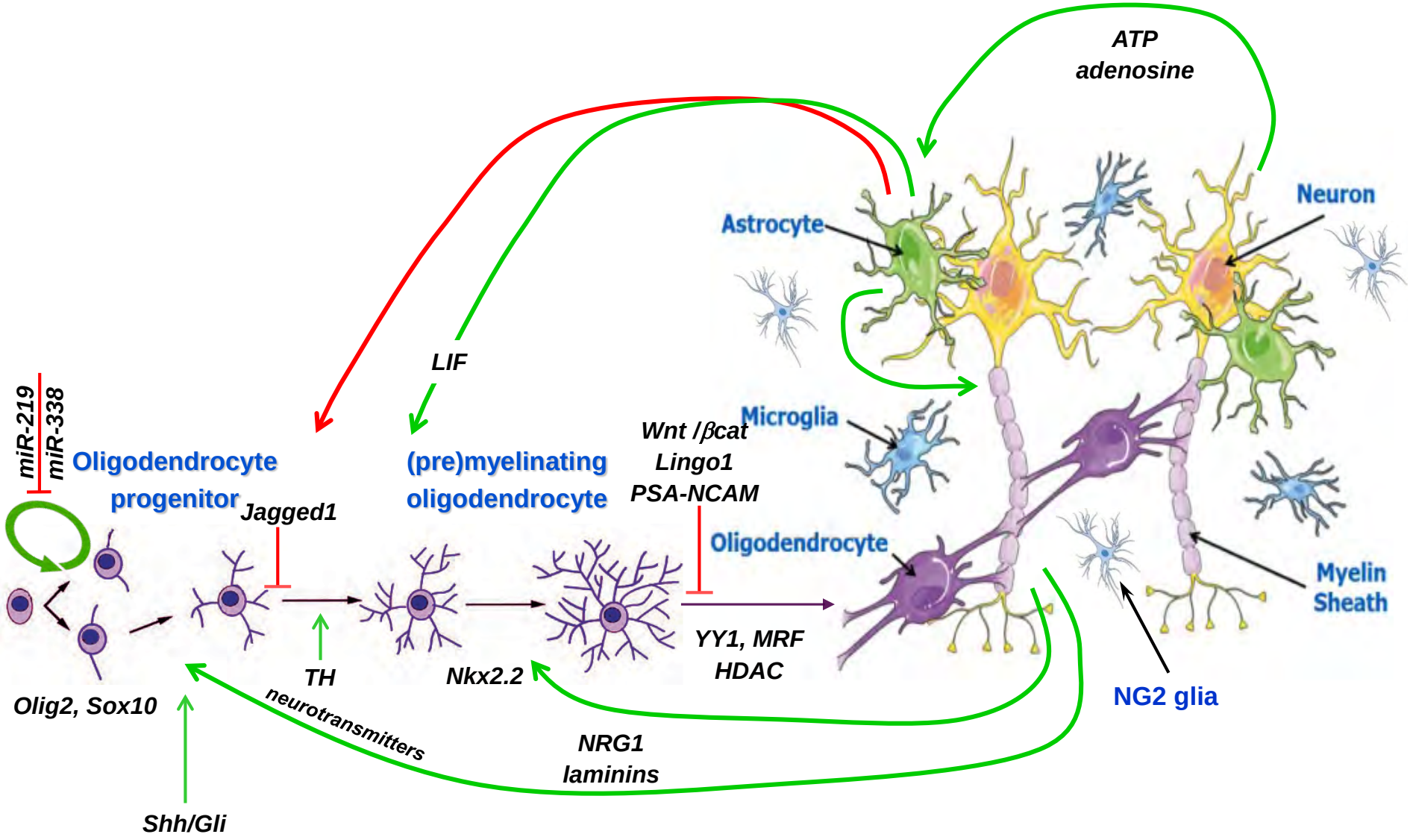


MRI pattern recognition

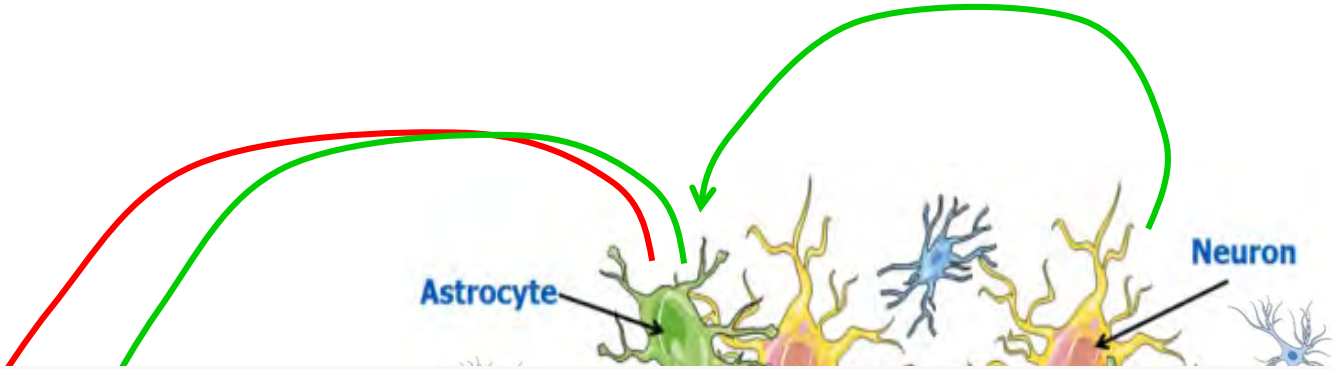
Next generation sequencing

Most leukodystrophies are due to defects in gene encoding proteins specific for cell types other than the oligodendrocytes

Oligodendrocyte development, myelination, myelin maintenance and regeneration: teamwork required

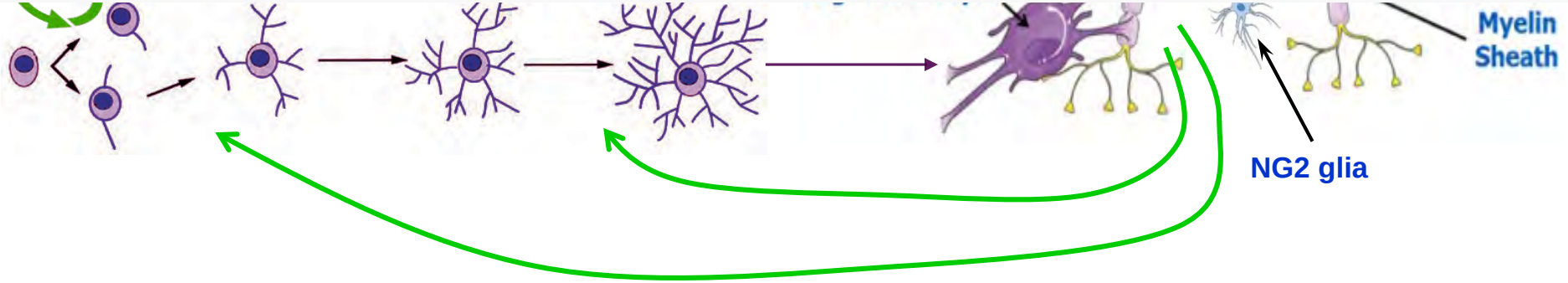


Oligodendrocyte development, myelination, myelin maintenance and regeneration: teamwork required



New definition of leukodystrophy:

genetic white matter disorder due to a defect in any of the white matter structural components



A new classification of leukodystrophies

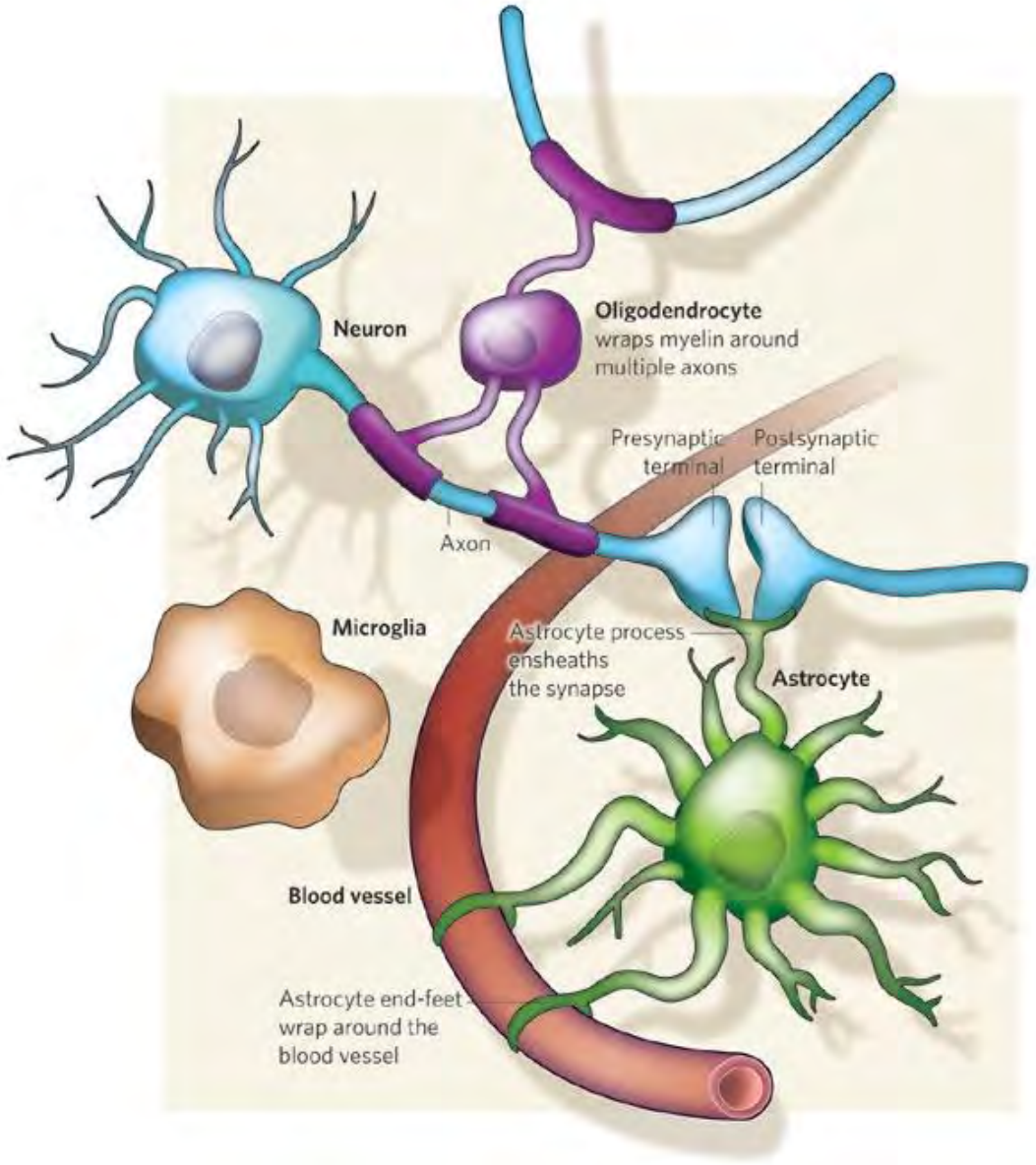
Myelin disorders

Astrocytopathies

Leuko-axonopathies

Leuko-microgliopathies

Leukovasculopathies



What is a leukodystrophy?

1. A genetic disease of the brain white matter due to defects in any of its structural components
2. A disease of the myelin only
3. A disease of the oligodendrocytes only
4. A disease of the neurons only

Metachromatic leukodystrophy

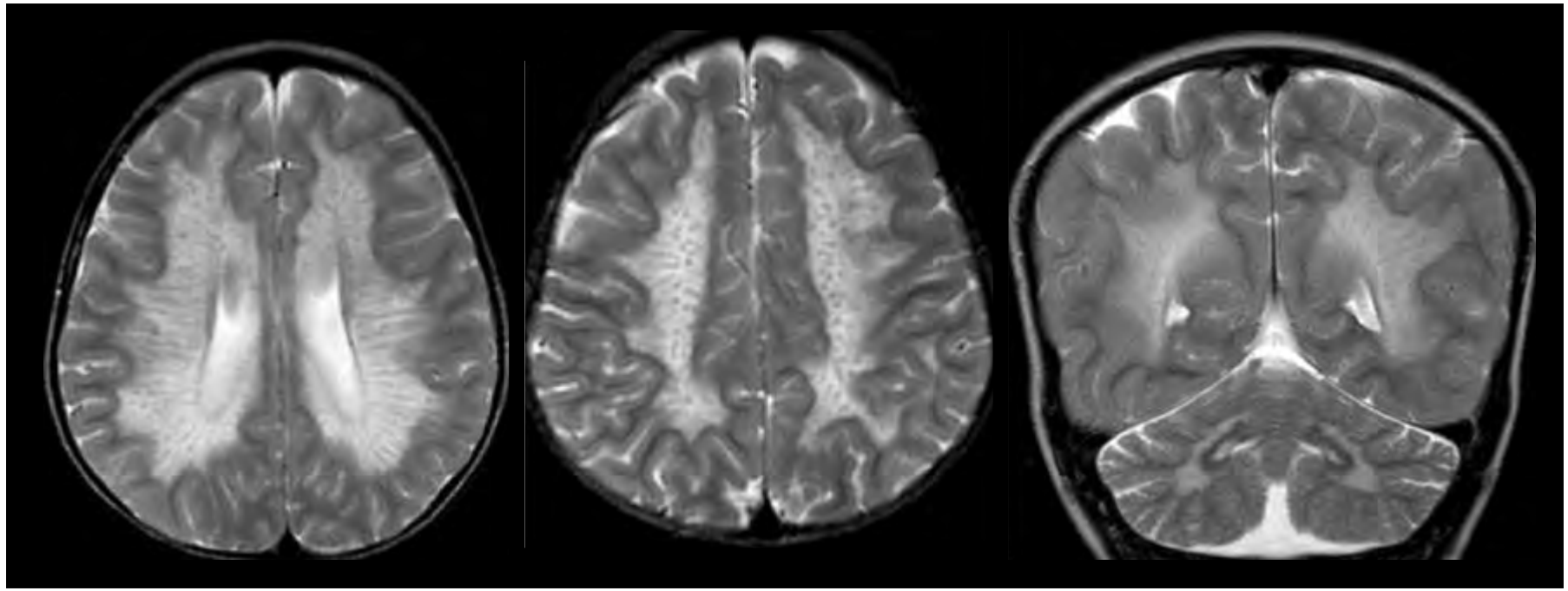
Autosomal recessive

ARSA (ASA defect, sulfatide storage), *PSAP* (sphingolipid activator protein saposin B)

Three subtypes: late infantile, juvenile, adult

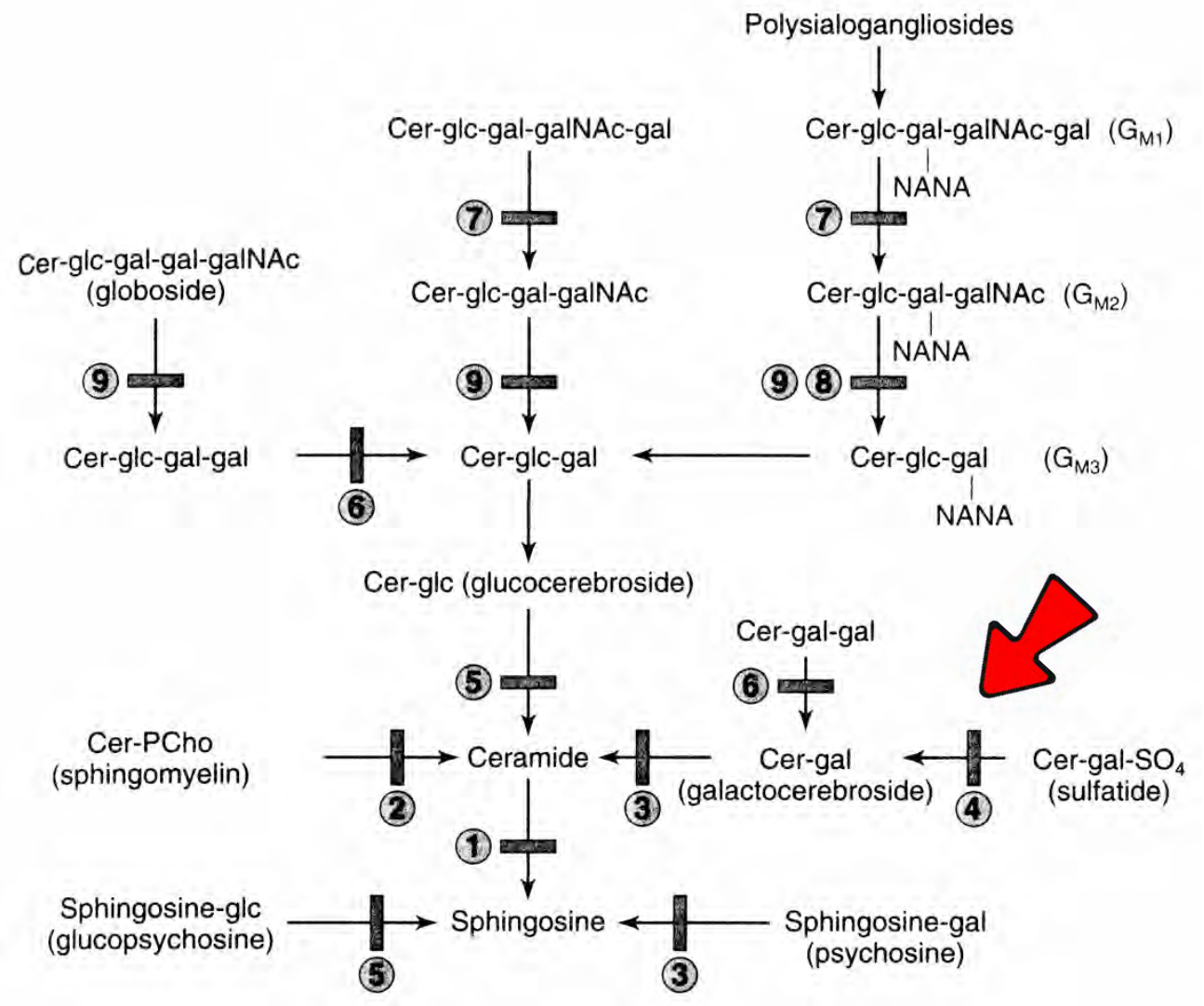
Ethnic and private mutations; reduced or abolished enzyme activity

Some degree of genotype-phenotype correlation (e.g., homozygous null = late infantile)



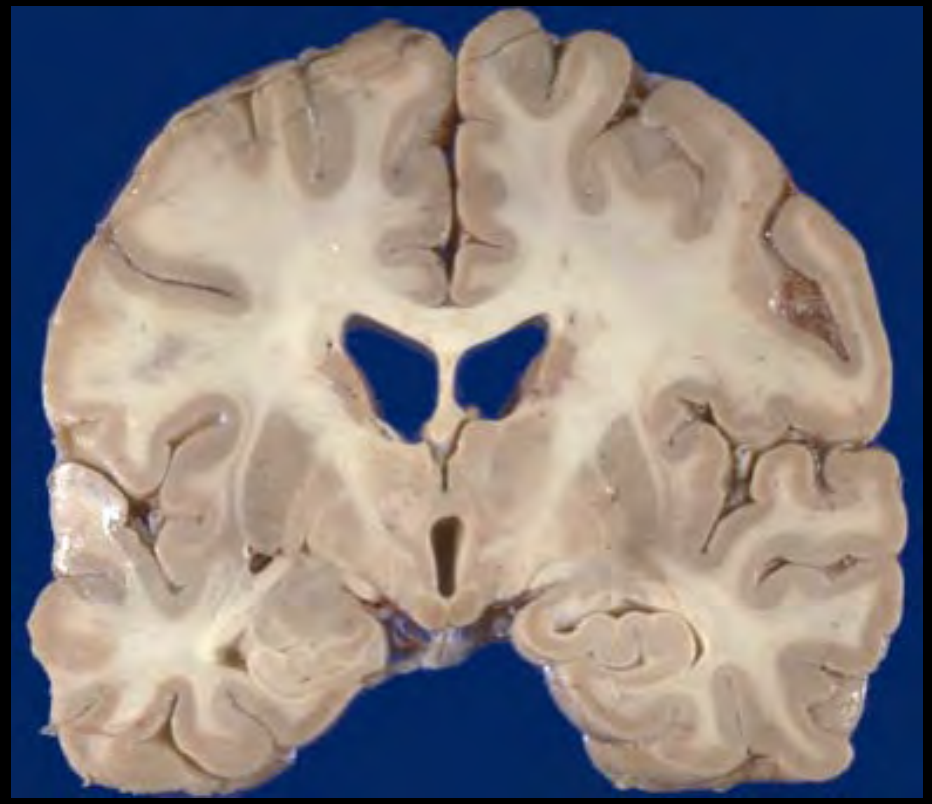
Lysosomal disorders:

enzymatic defect → storage material → cell death

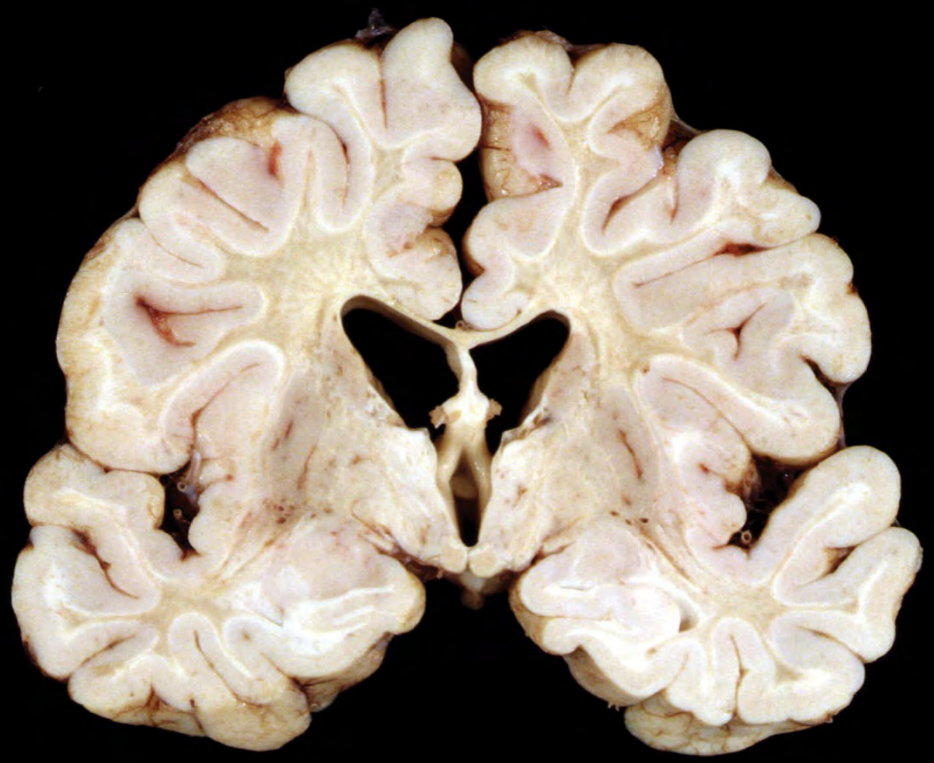


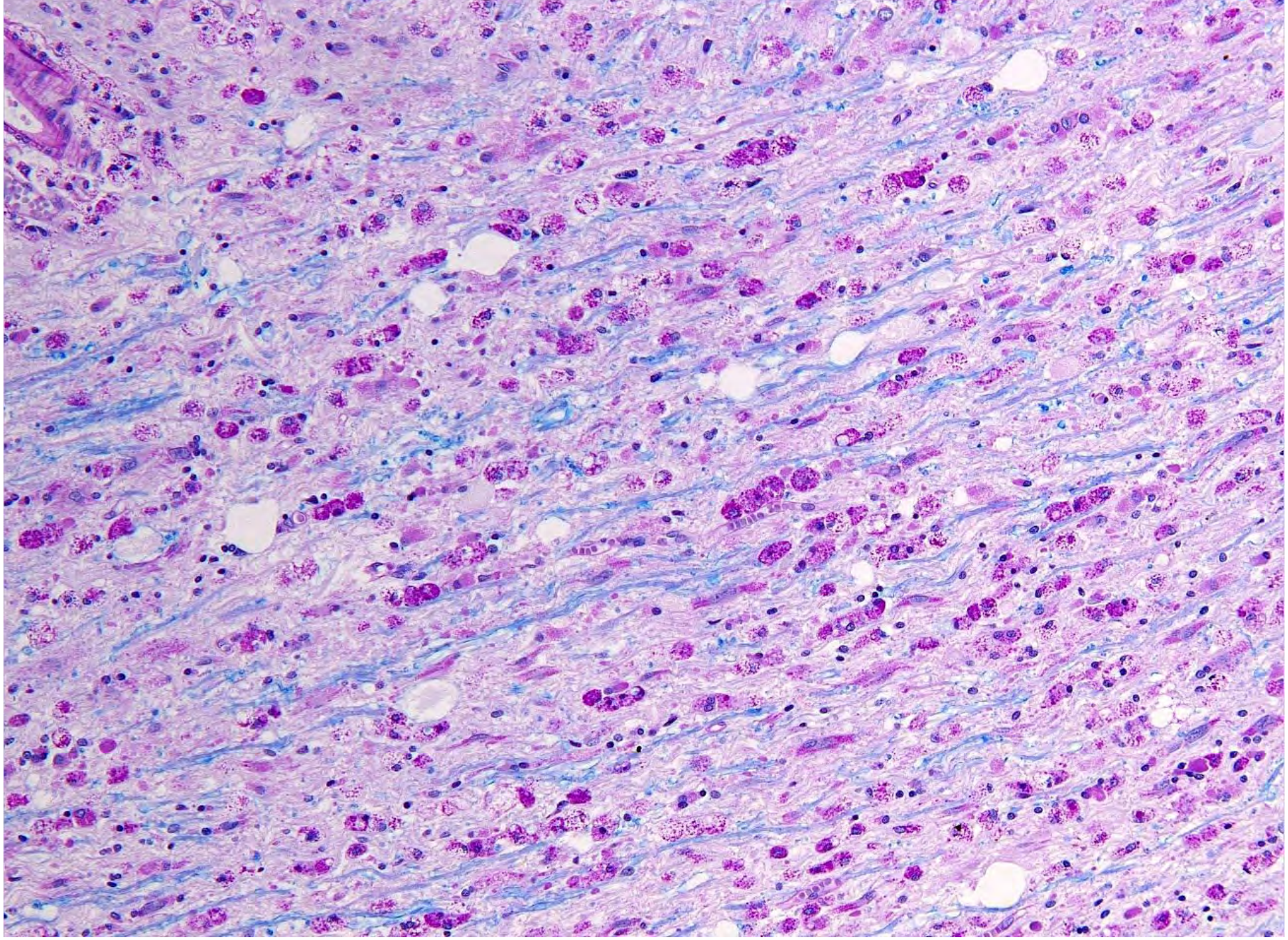
Metachromatic leukodystrophy (MLD)

normal brain



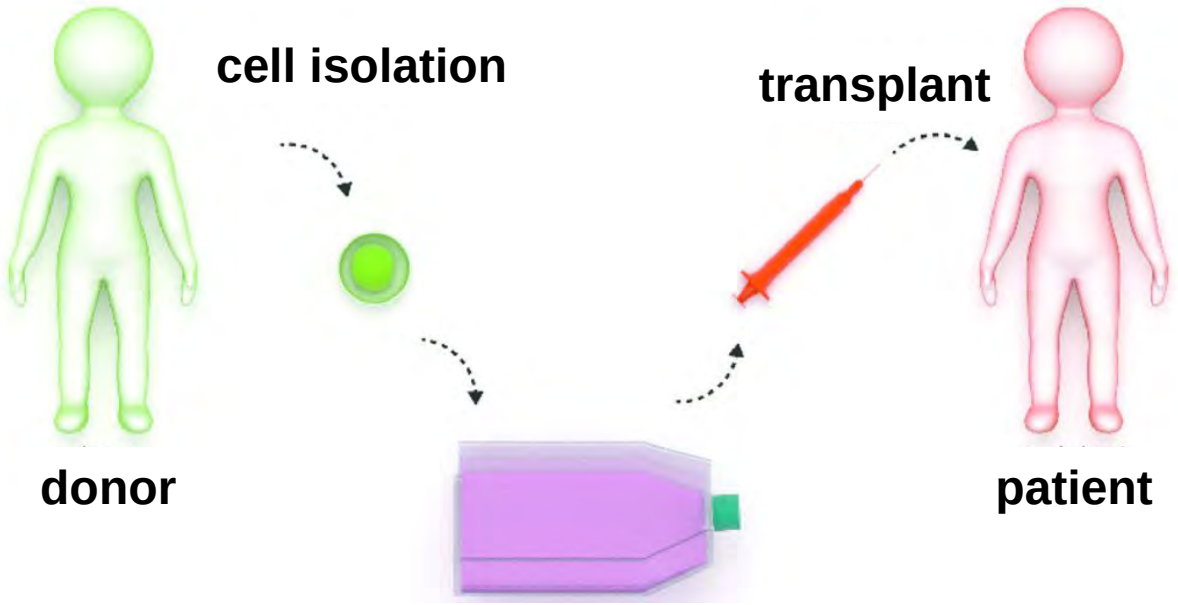
MLD brain



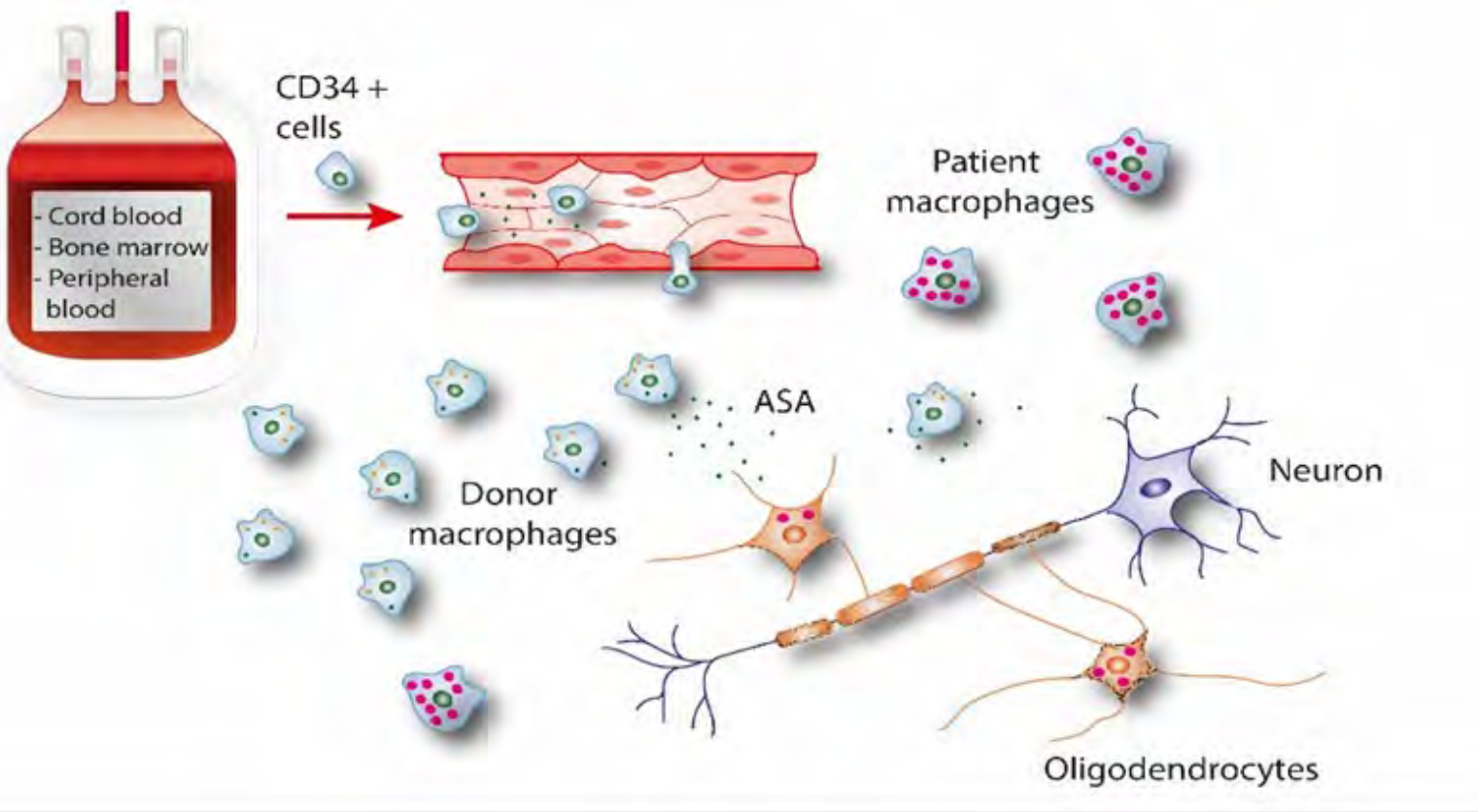


Myelin stain **Macrophages full of storage**

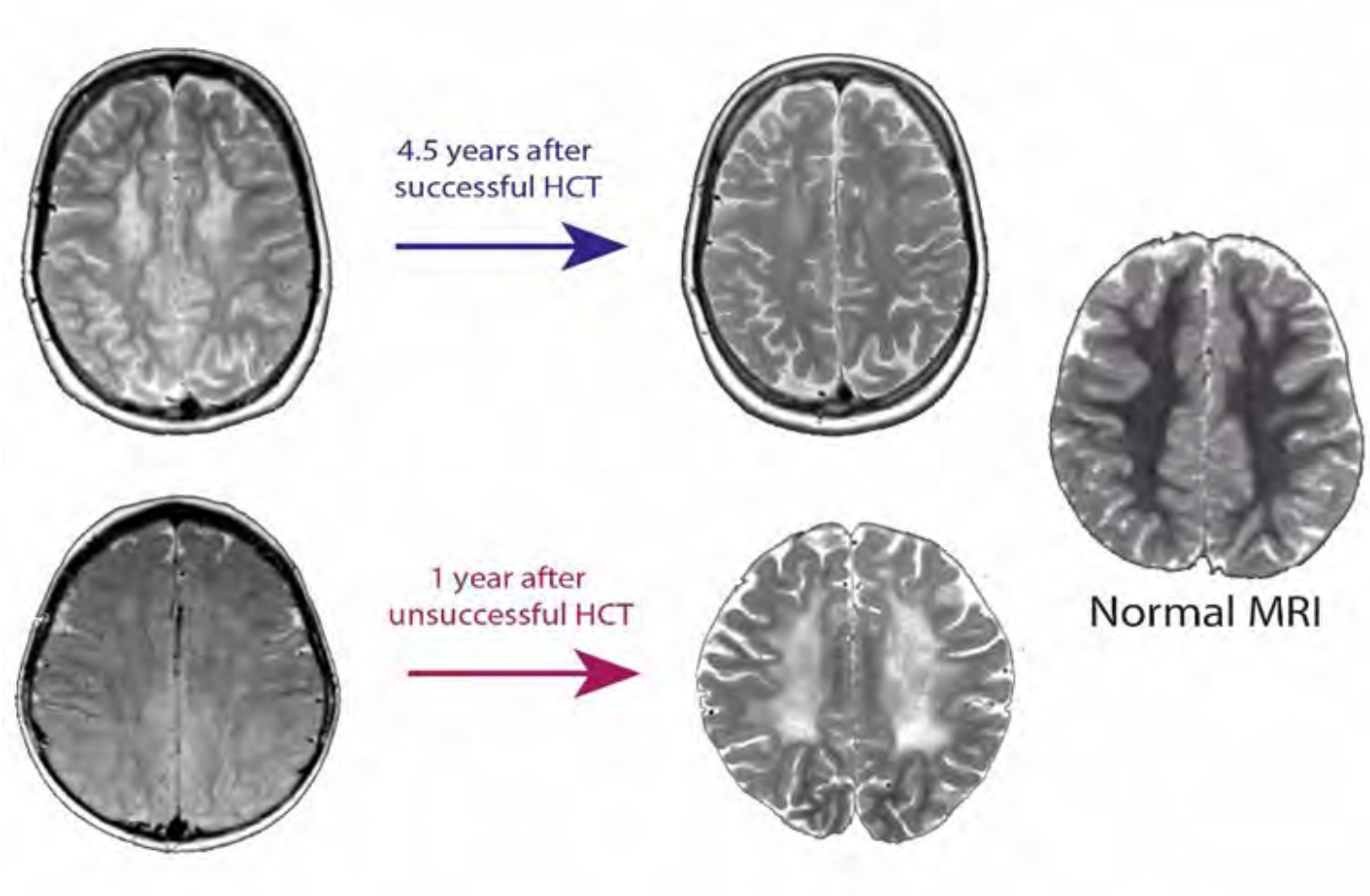
Metachromatic leukodystrophy: hematopoietic stem cell transplantation



Metachromatic leukodystrophy and hematopoietic stem cell transplantation: supposed mechanism of action

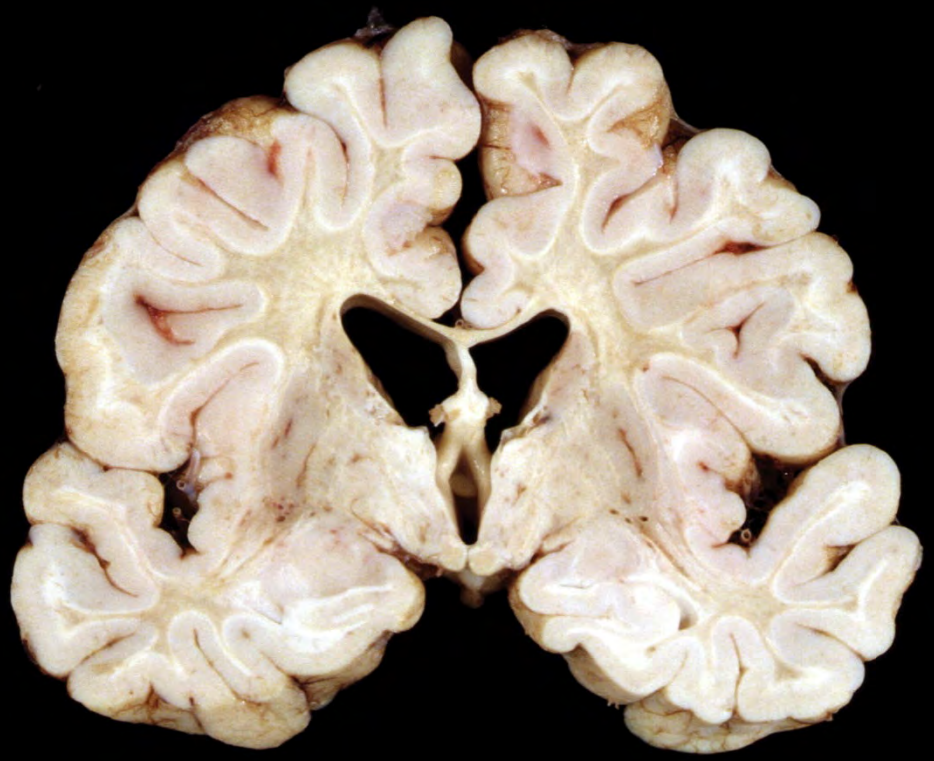


Metachromatic leukodystrophy and hematopoietic stem cell transplantation: clinical evolution

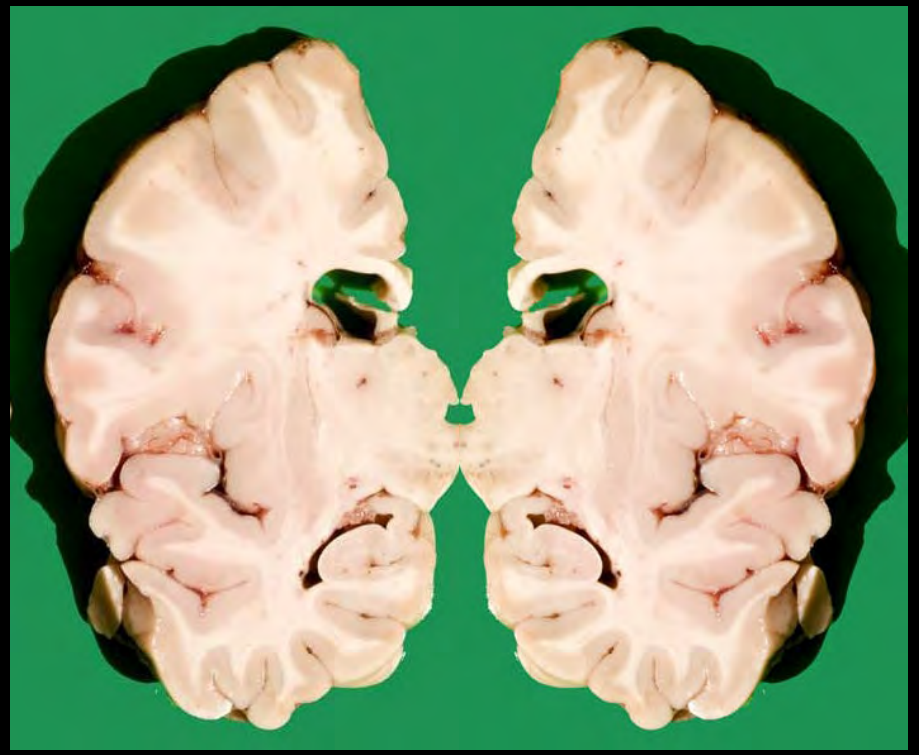


Metachromatic leukodystrophy

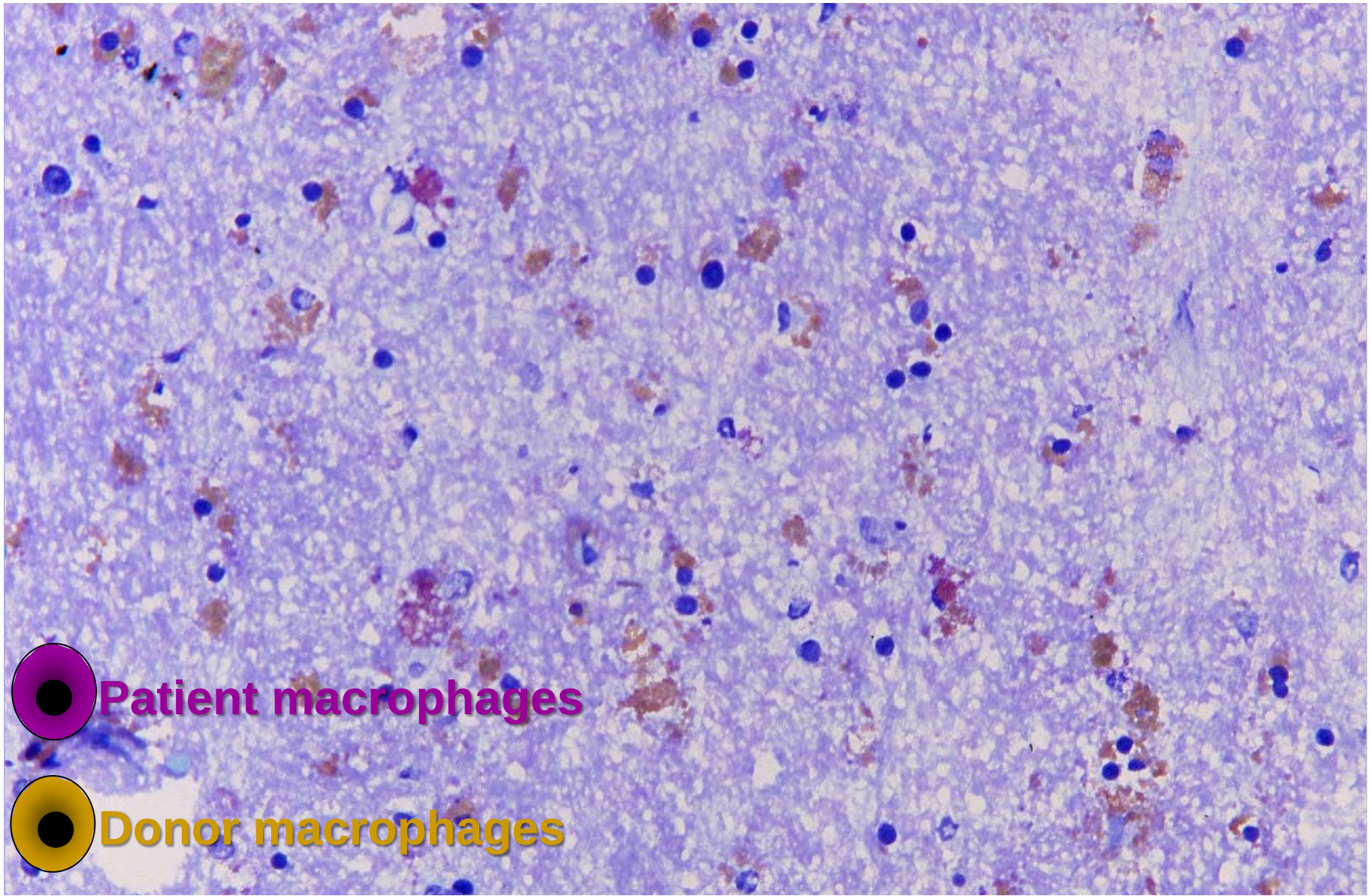
no transplantation



after transplantation

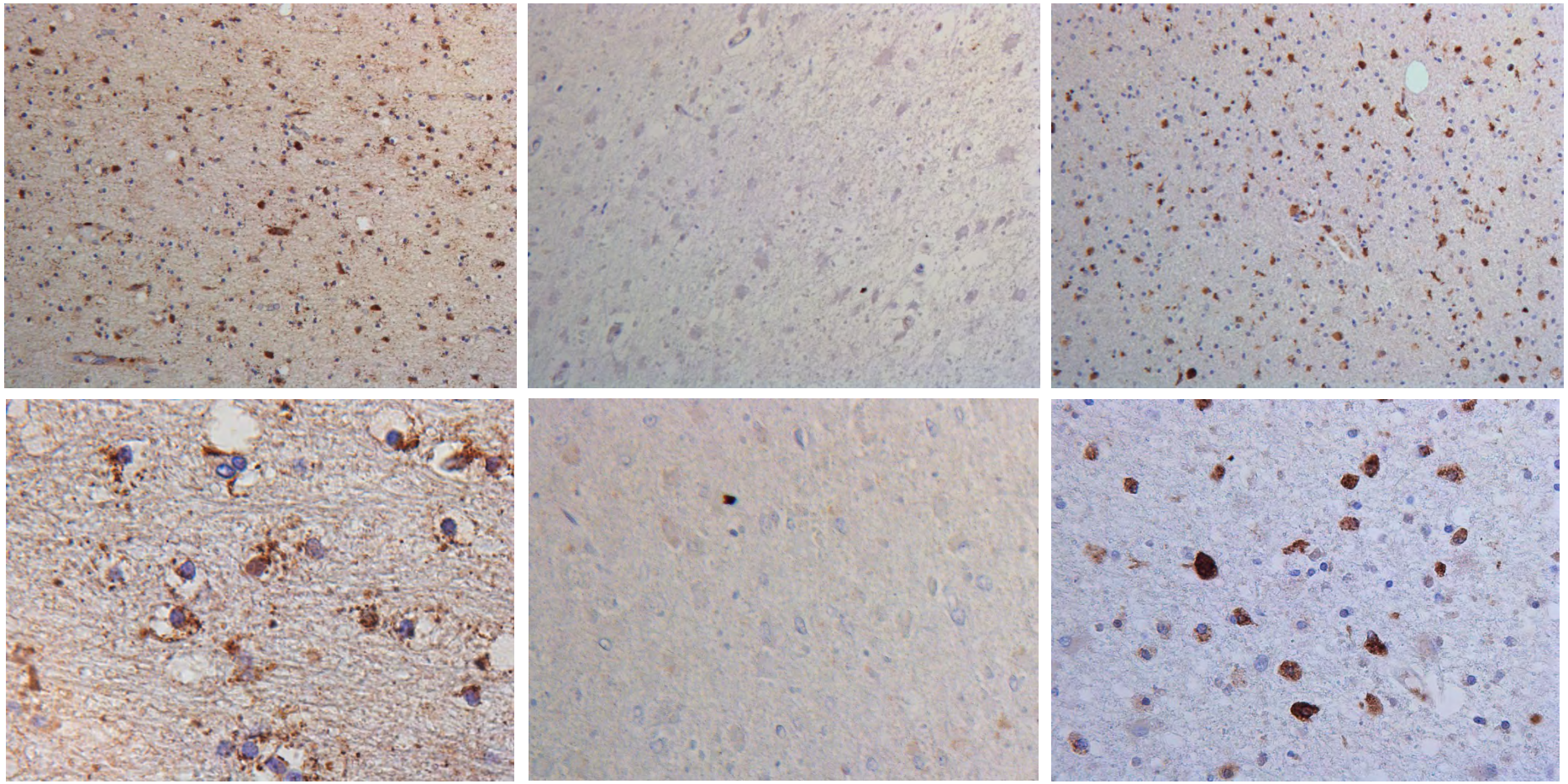


Donor cells reach the brain of transplanted MLD patients, carry the enzyme and digest the storage material



Wolf et al., 2020

ASA Immunopositivity in the frontal white matter

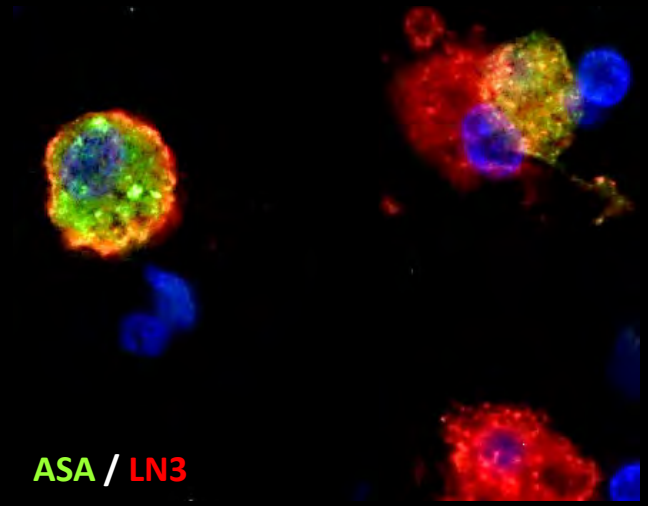


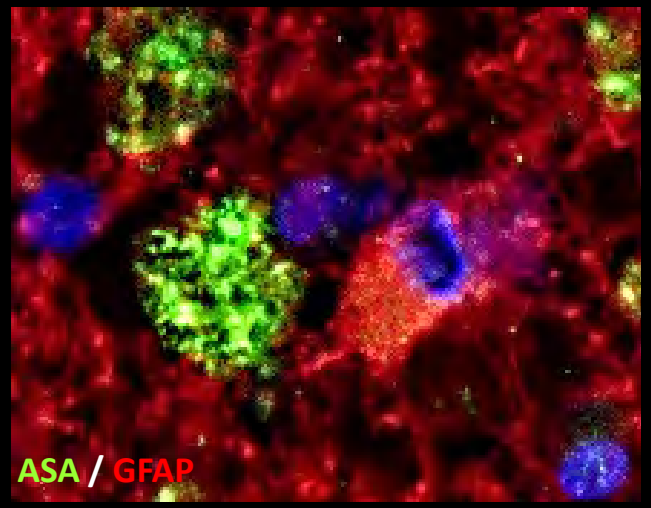
Control | Non Transplanted | Transplanted

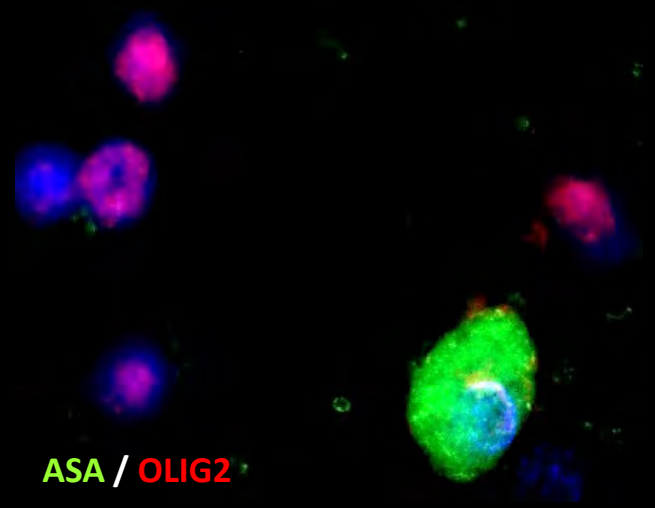
Wolf et al., 2020

NO enzymatic cross correction

from donor macrophages to resident (neural) cells





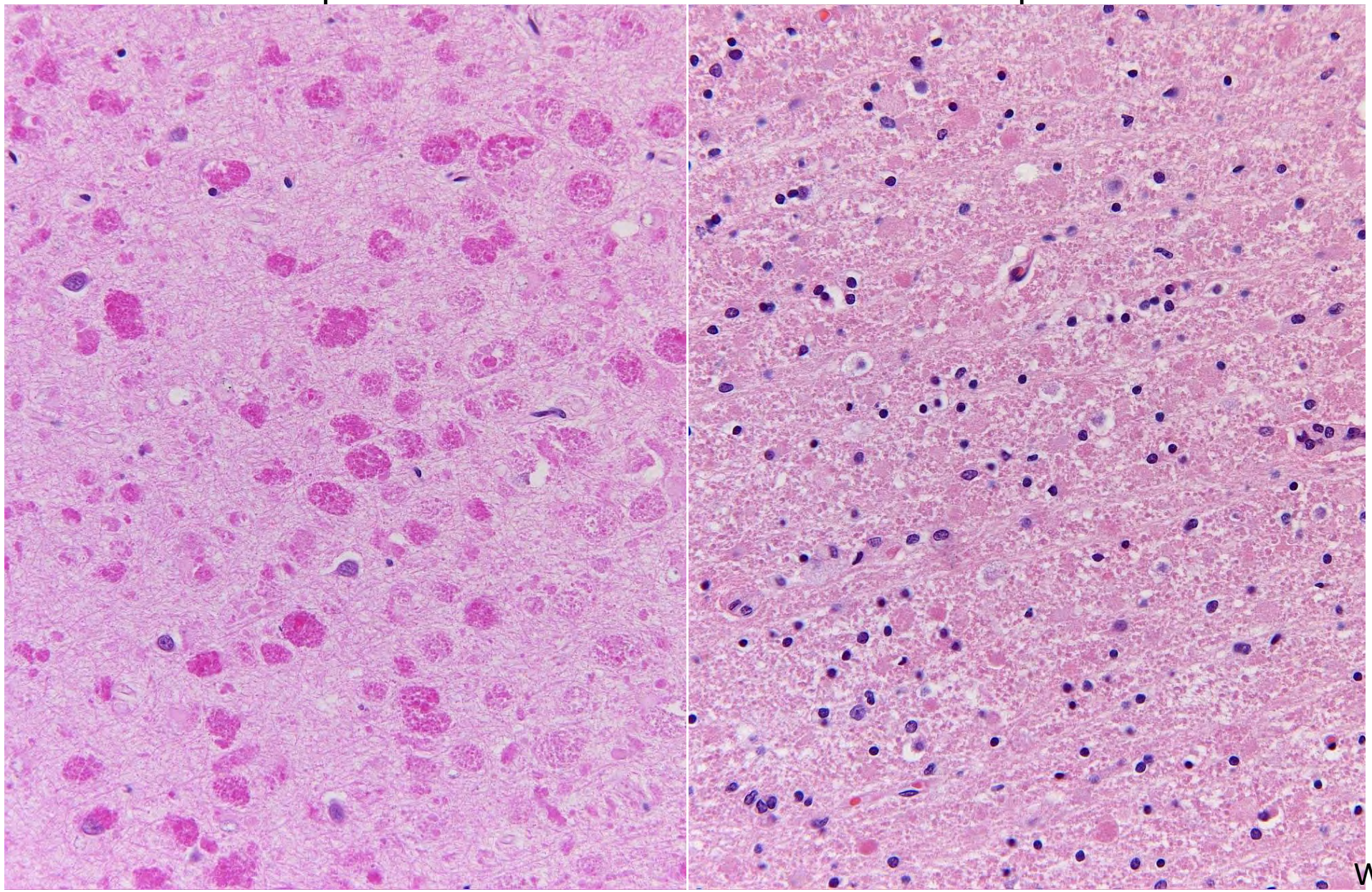


Wolf et al., 2020

Transplantation promotes survival of the oligodendrocytes

no transplantation

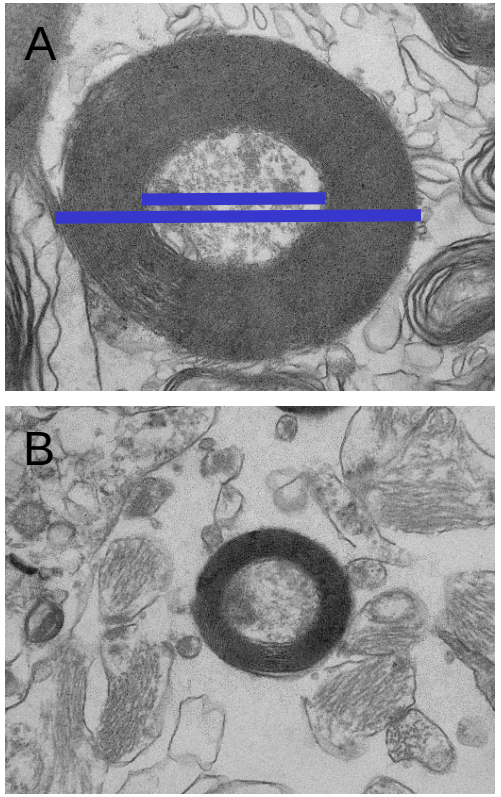
after transplantation

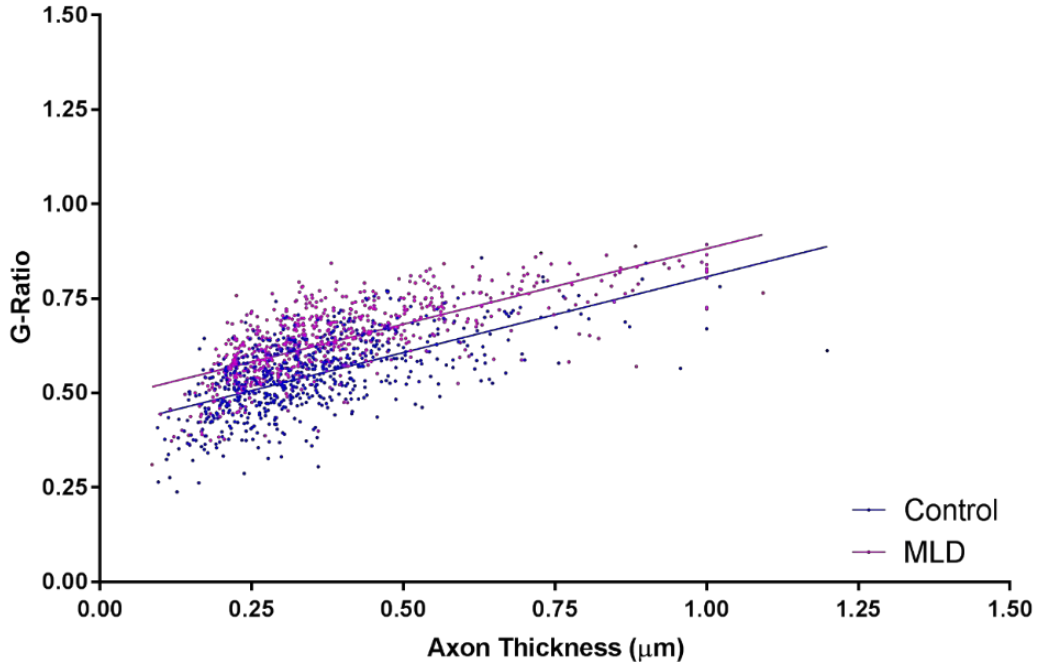


Wolf et al., 2020

Transplantation is associated with remyelination

(“normalization” of white matter signal changes on MRI)

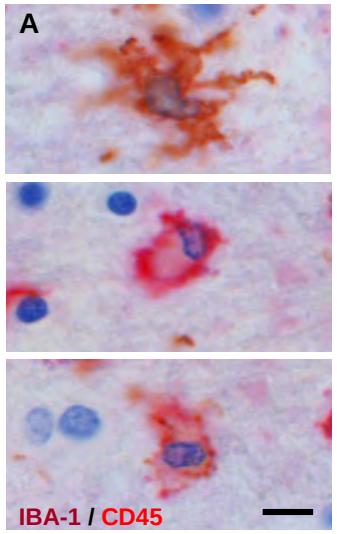


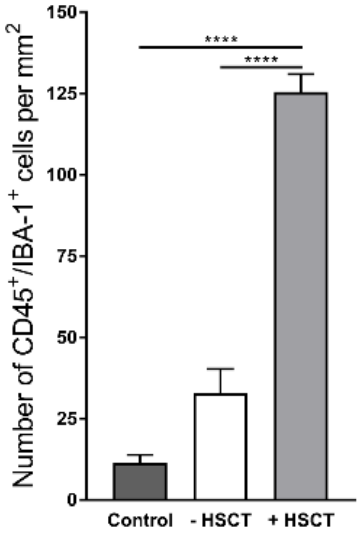


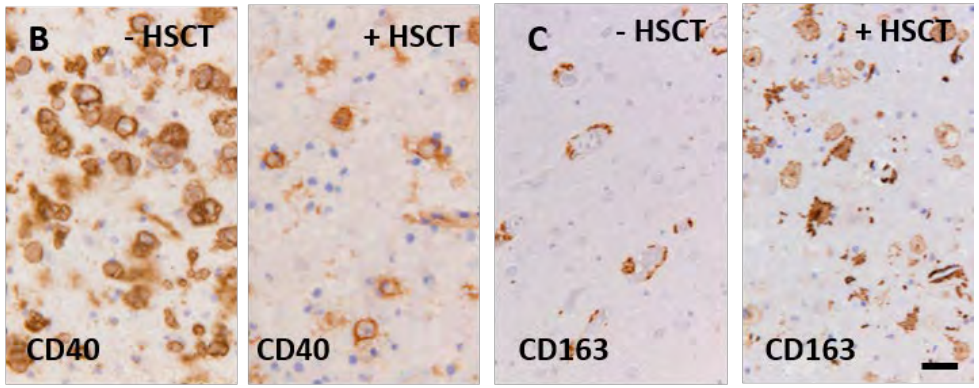
G-Ratio = The ratio of the inner axonal diameter to the total outer diameter

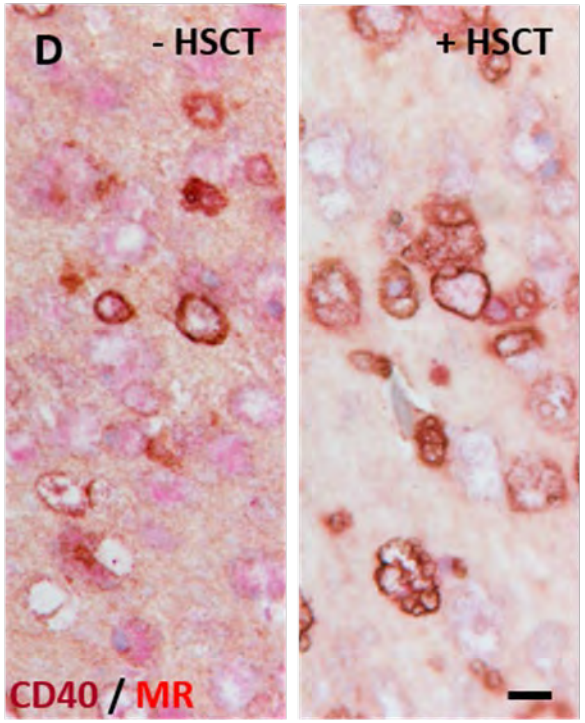
Wolf et al., 2020

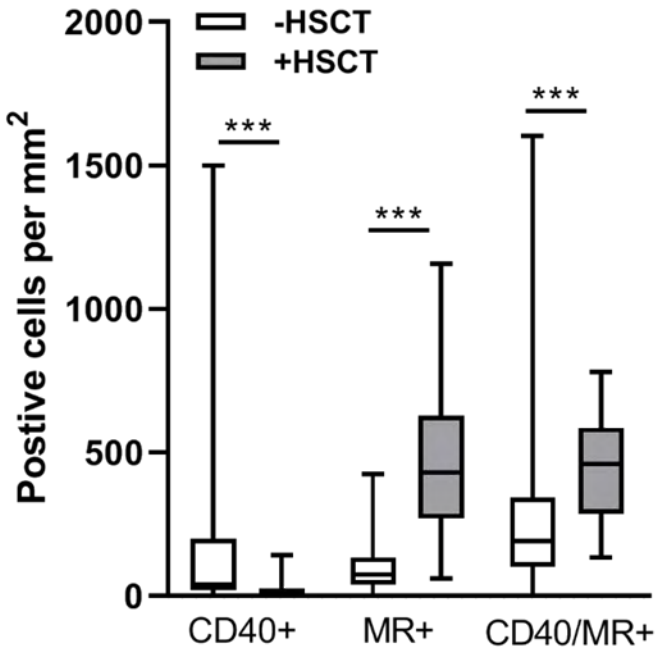
Transplantation modulates microglia activation status





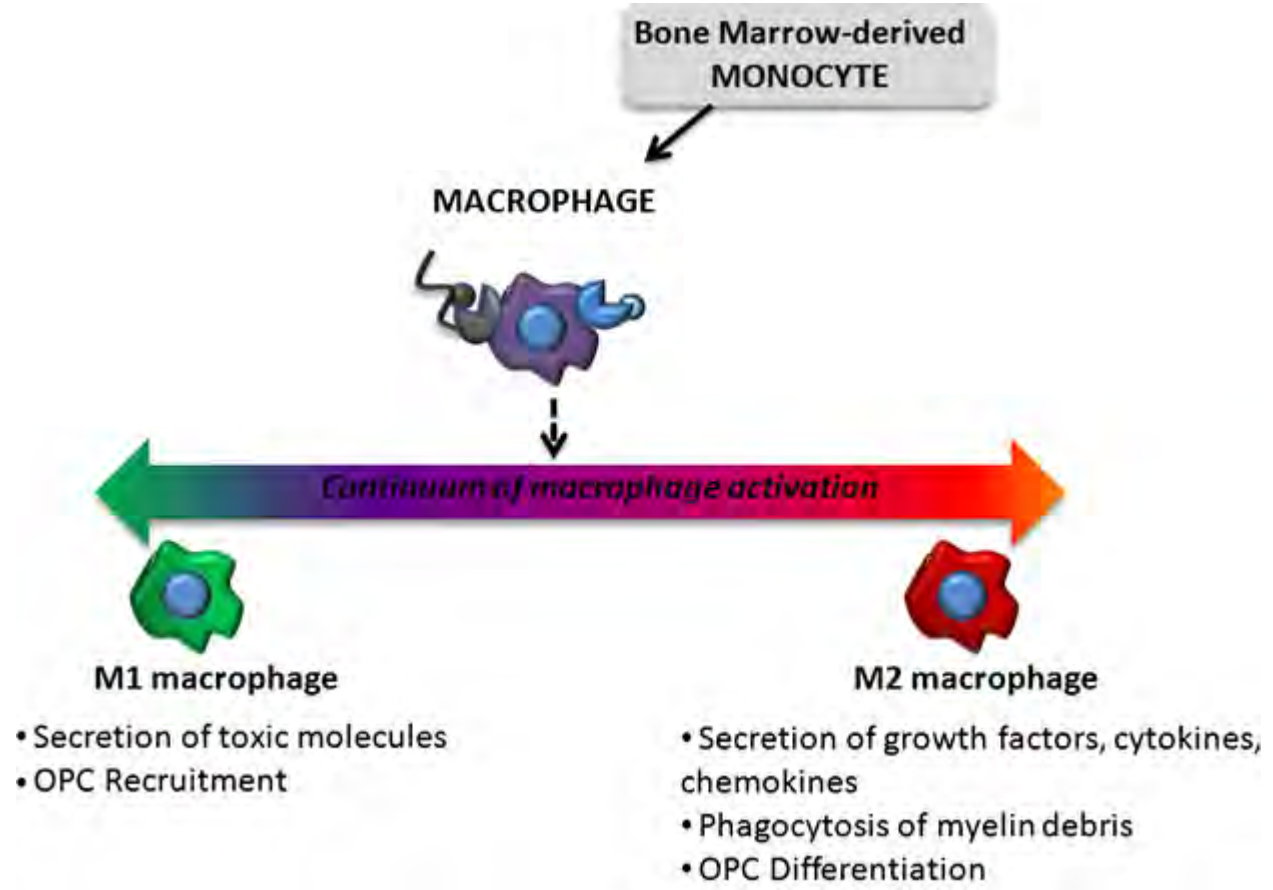






Wolf et al., 2020

Transplantation skews the macrophages towards an anti-inflammatory phenotype that supports remyelination

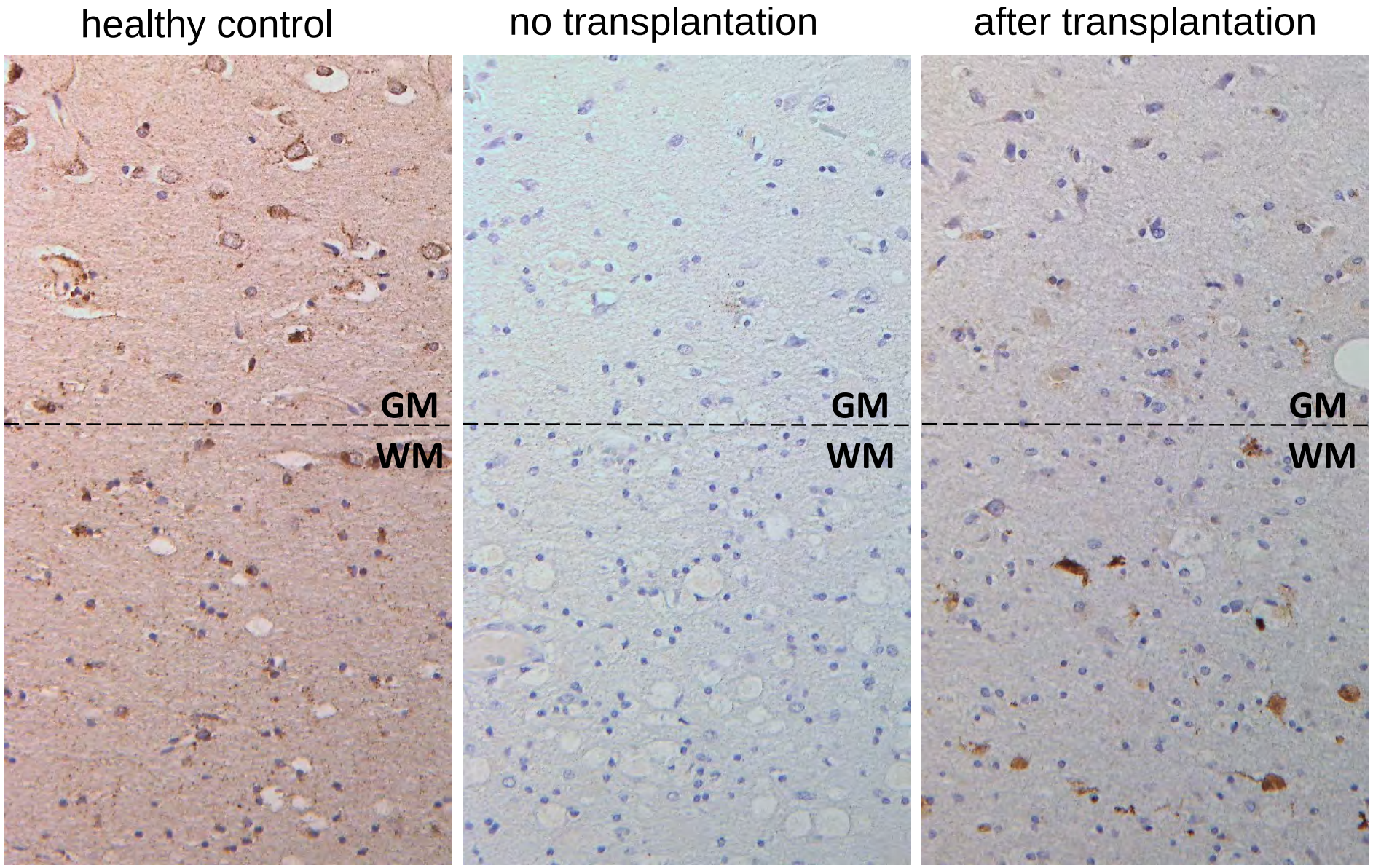


Hematopoietic stem cell transplantation

may repair the leukodystrophy.

But, why do patients may become demented afterwards?

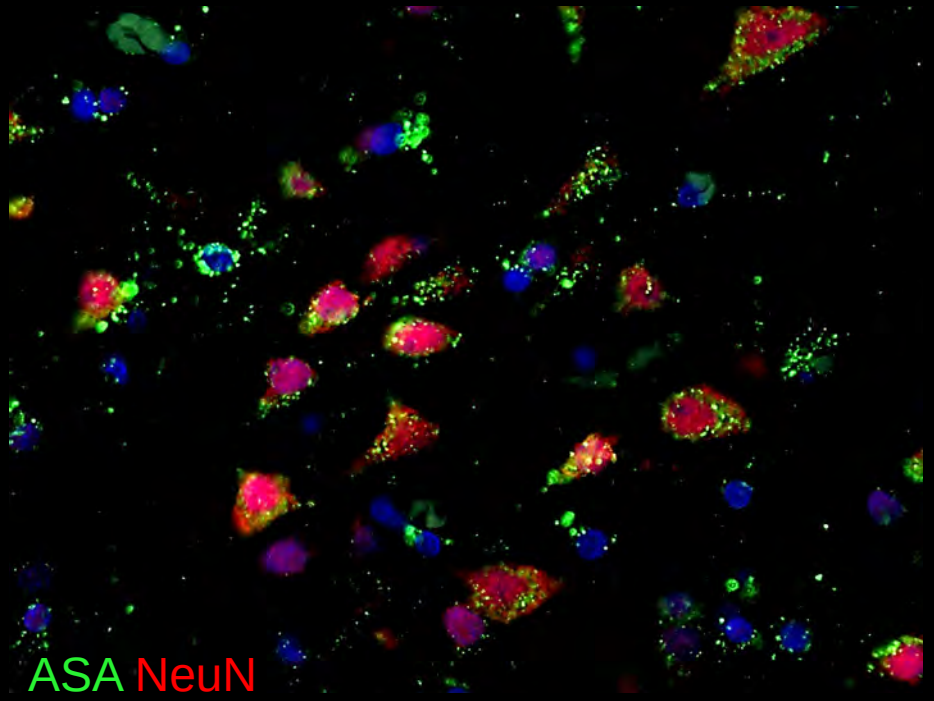
Donor macrophages do NOT reach the grey matter



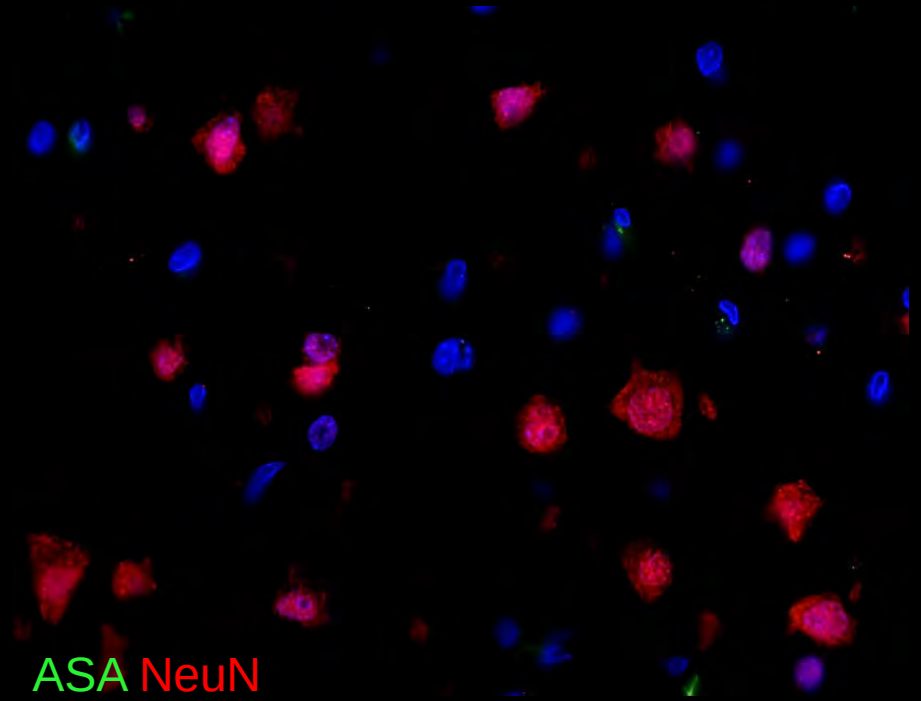
Donor macrophages do NOT reach the grey matter:

and the neuronal pathology goes on…

Control



Transplanted



Does hematopoietic stem cell transplantation always work in metachromatic leukodystrophy?

1. Never, it has no effects
2. Seldom, only in advanced cases
3. Sometimes, and often only on the white matter pathology
4. Always

A new classification of leukodystrophies

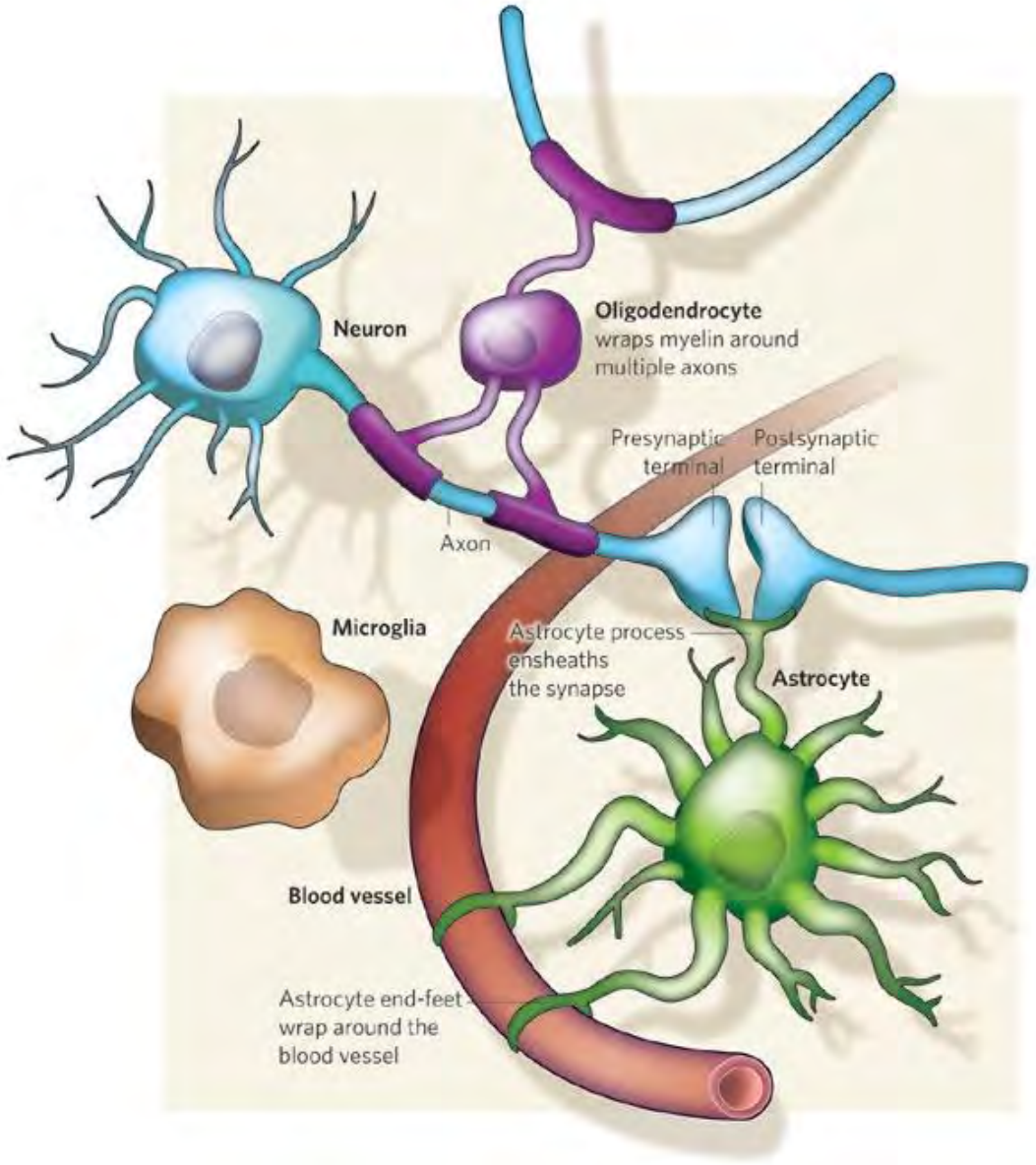
Myelin disorders

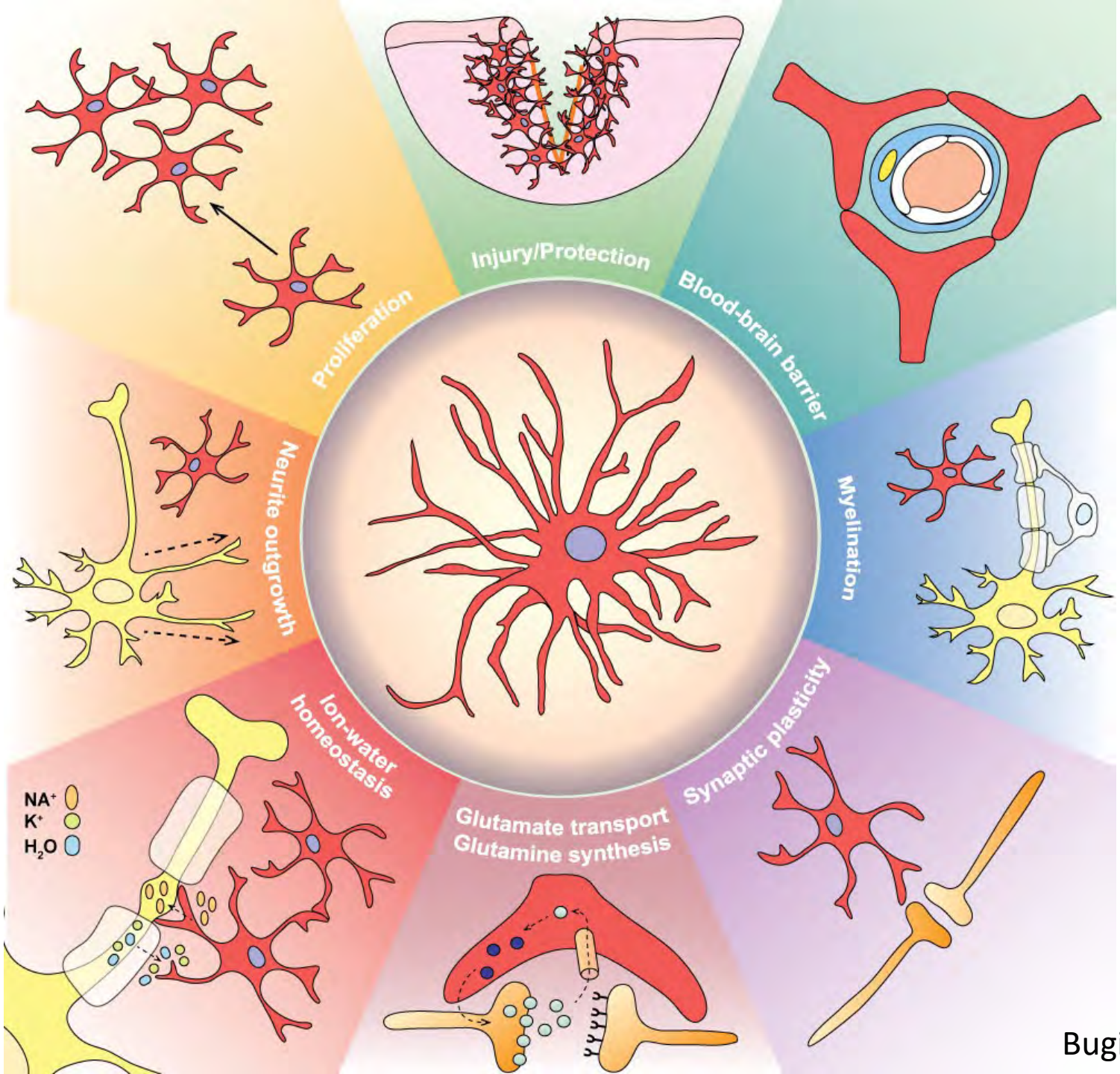
Astrocytopathies

Leuko-axonopathies

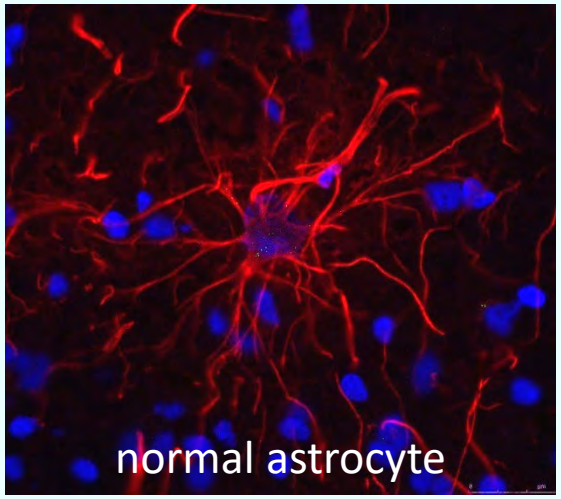
Leuko-microgliopathies

Leukovasculopathies



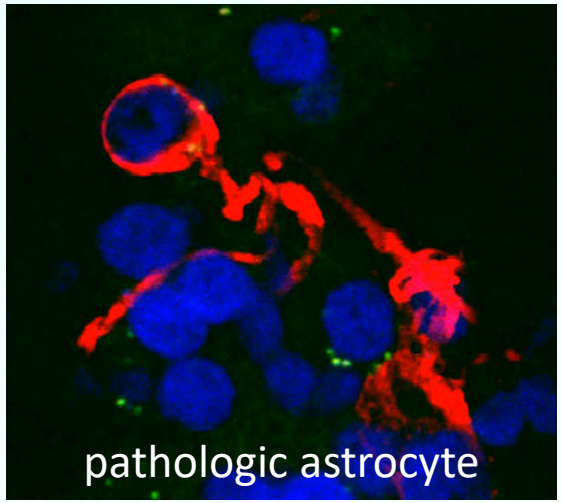


Bugiani & Breur, 2018



DISEASE

↑ and ↓ of specific proteins



LOSS OF PHYSIOLOGIC FUNCTIONS

GAIN OF PATHOLOGIC FUNCTIONS

chronic glial scarring

lack of normal gliotic repair

impact on the ECM

impact on myelin deposition and maintenance

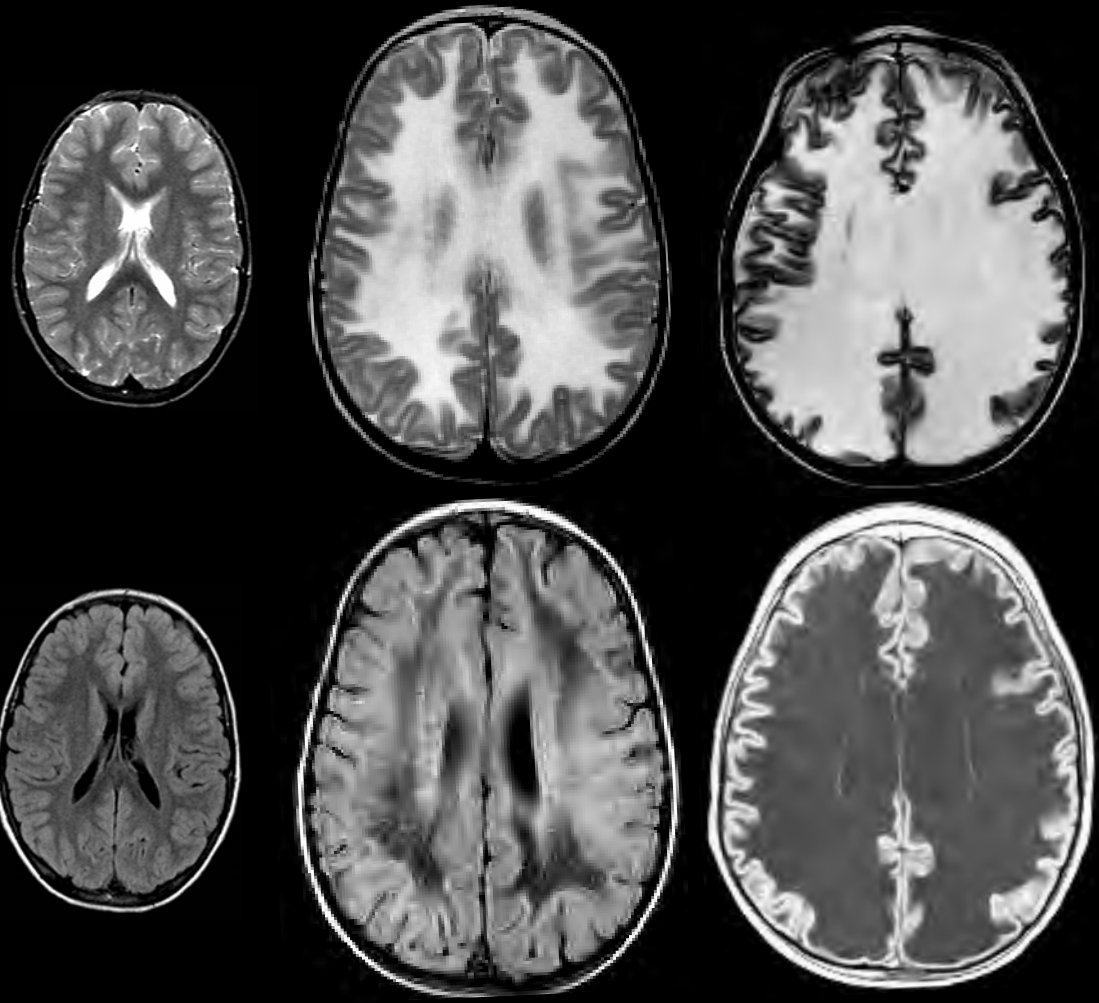
impact on axons

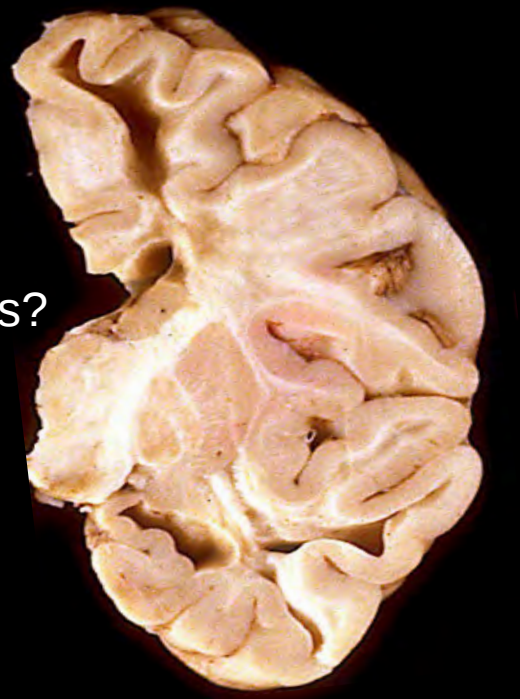
inhibition of oligodendrocyte maturation

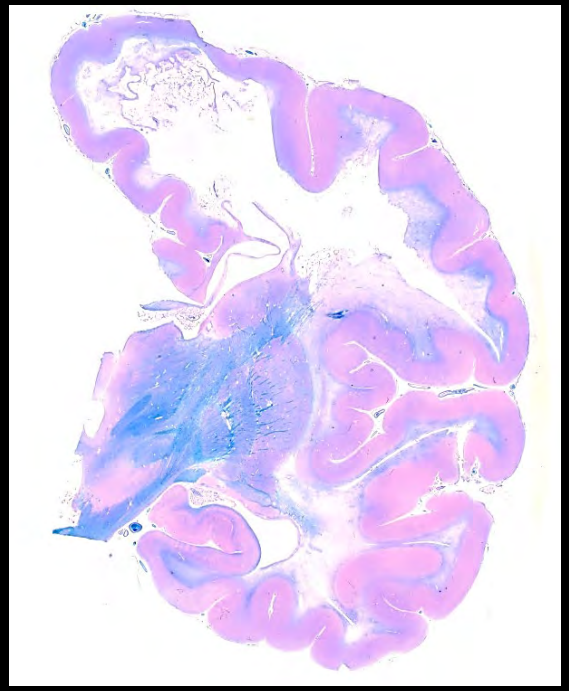
lack of myelin

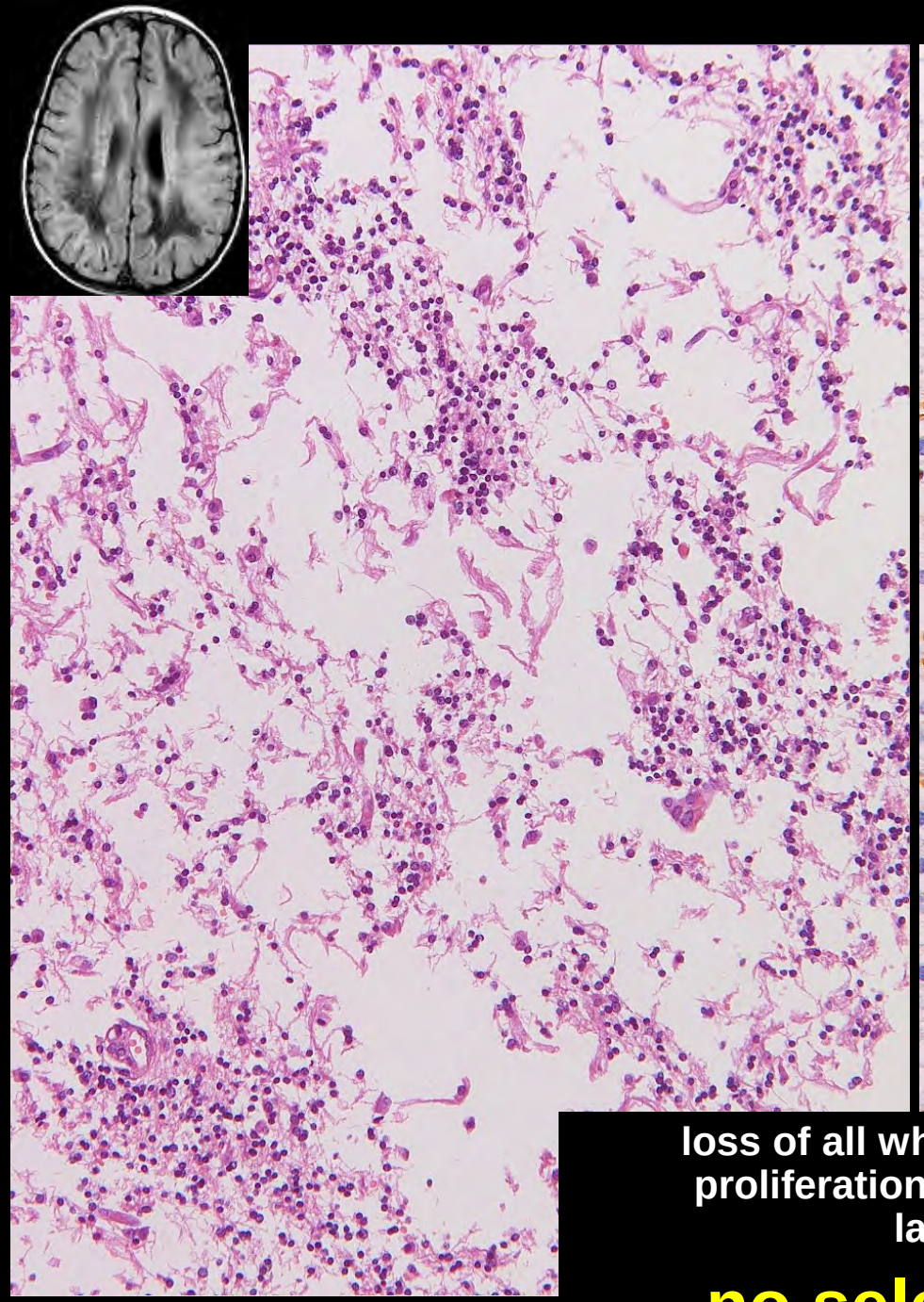
Vanishing White Matter

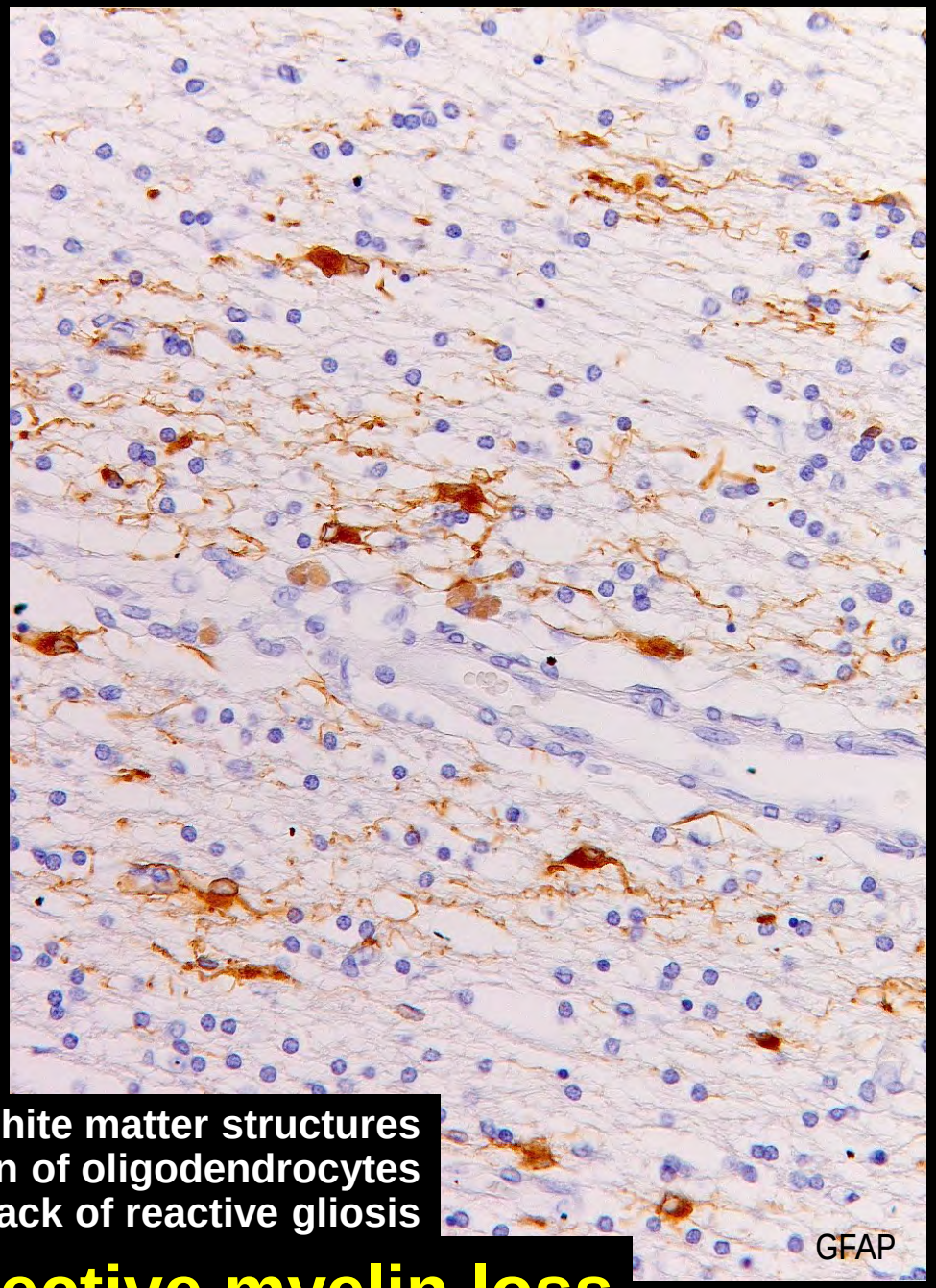
- Mutations in *EIF2B1-5*, encoding the 5 eIF2B subunits
- eIF2B: initiation of translation of all mRNAs
  regulation of general mRNA translation rate
- Disease mechanisms? Altered expression of specific proteins?







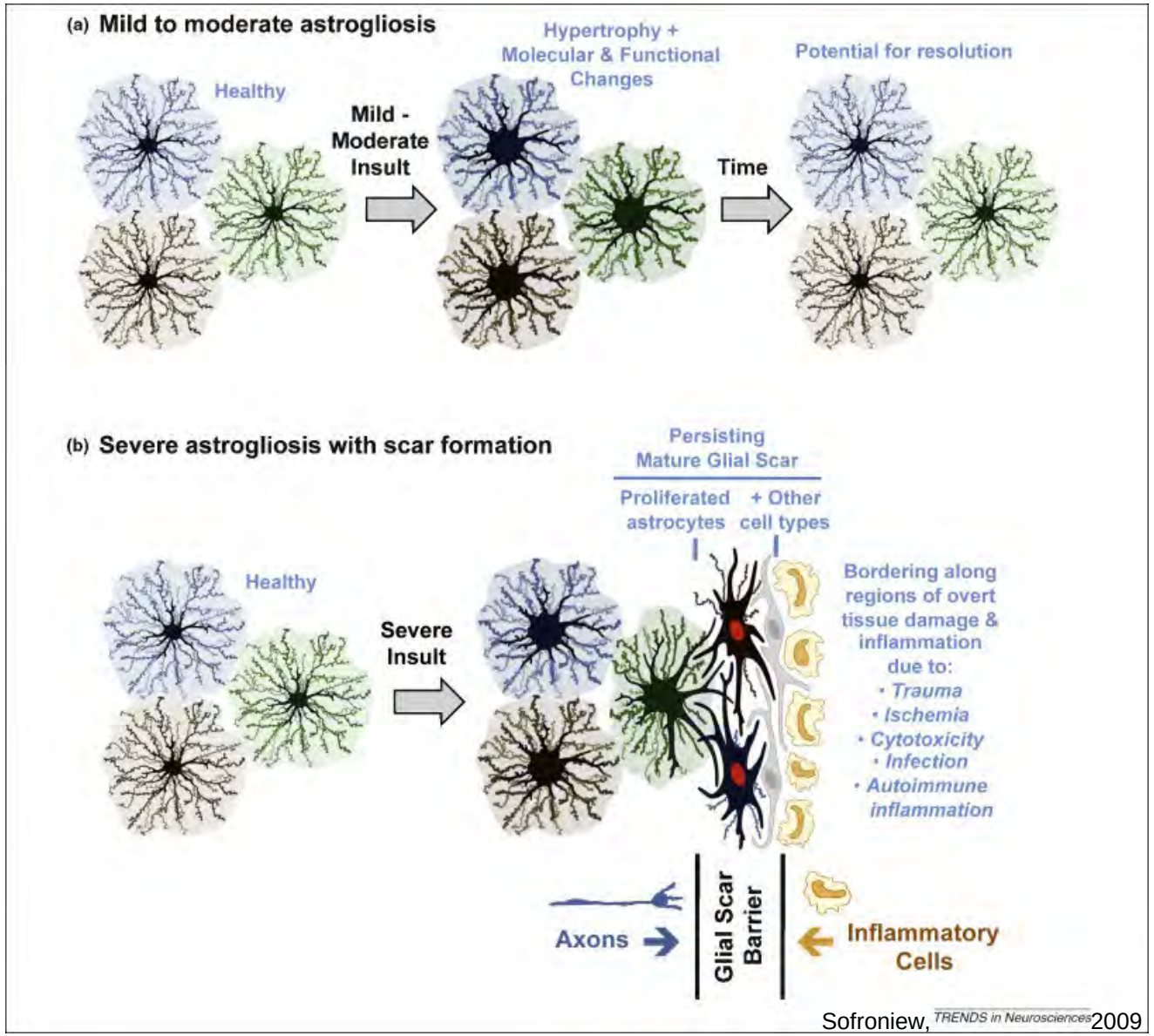




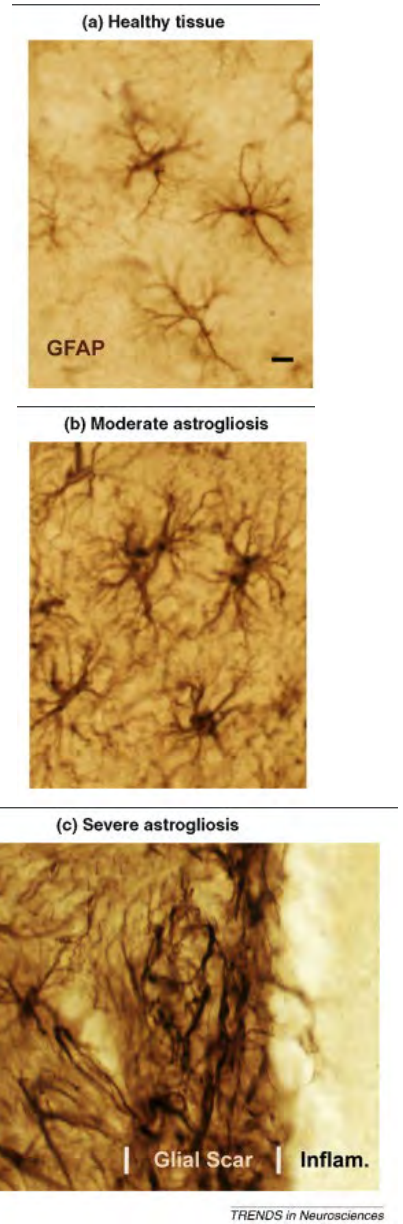
loss of all white matter structures
proliferation of oligodendrocytes
lack of reactive gliosis

no selective myelin loss

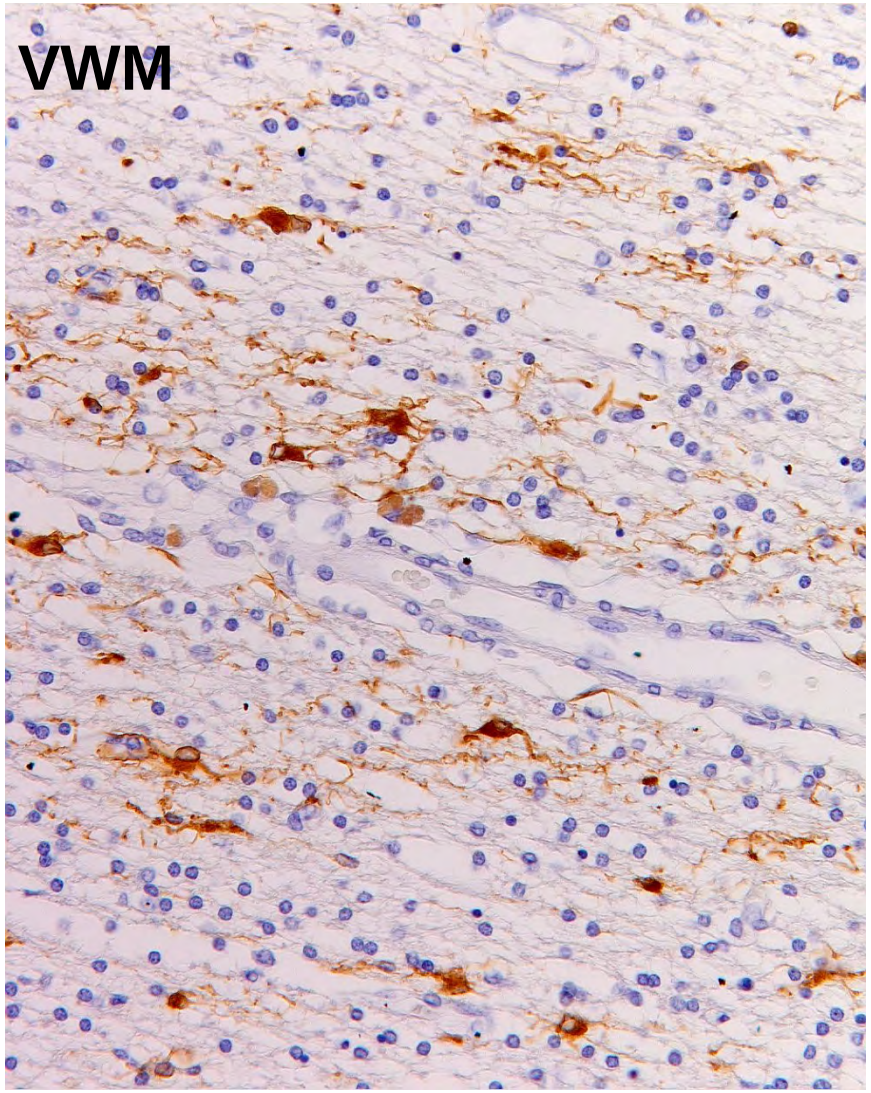
Reactive gliosis

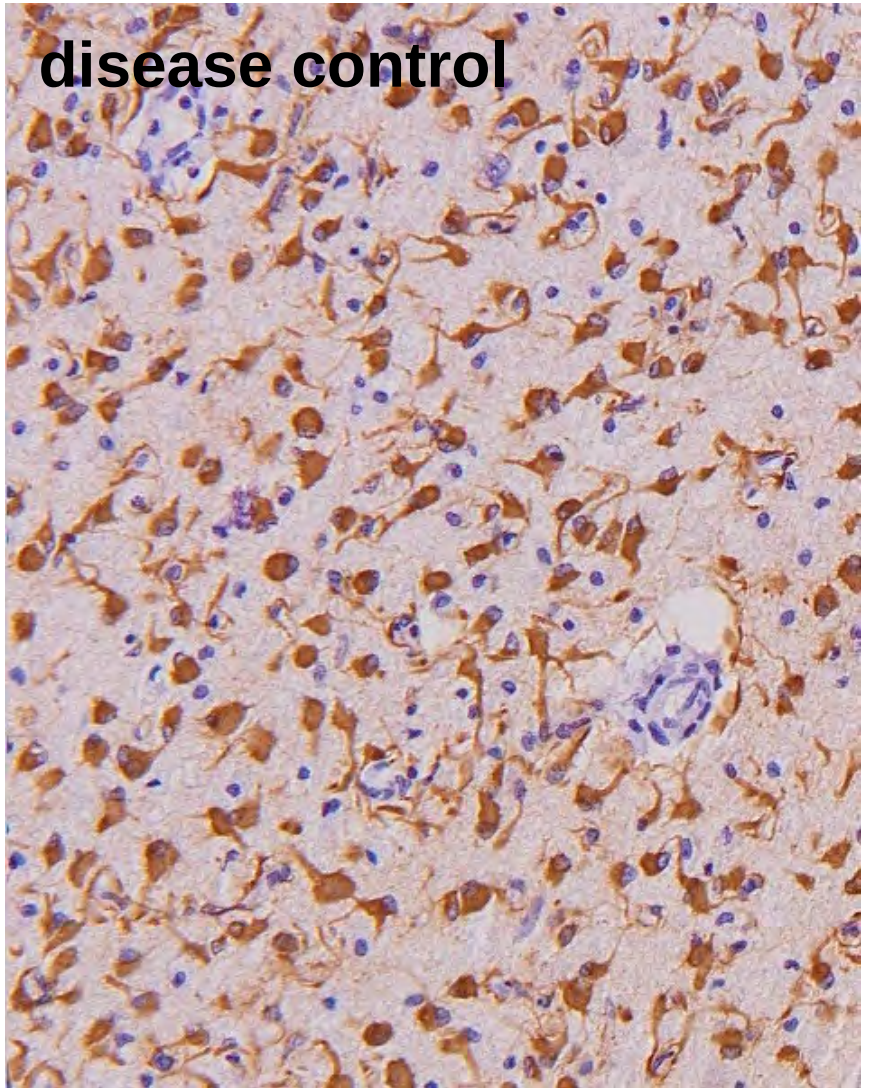


Sofroniew, TRENDS in Neurosciences 2009



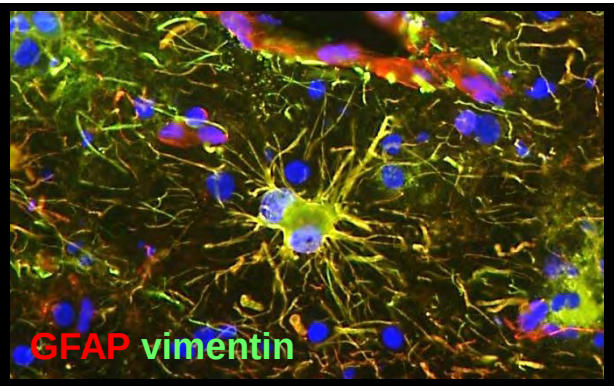
VWM white matter astrocytes proliferate, remain immature and lack mature function (e.g. astrogliotic scar tissue formation)

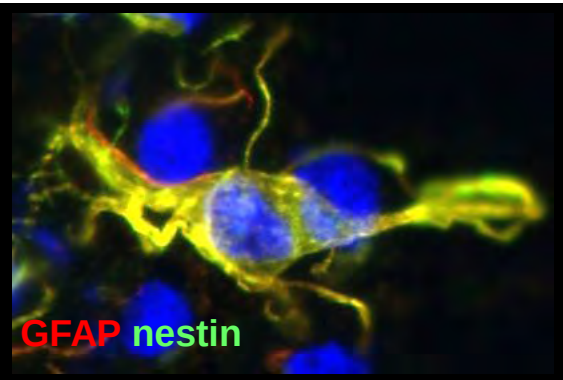


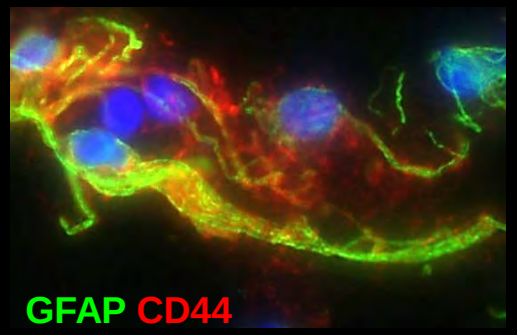


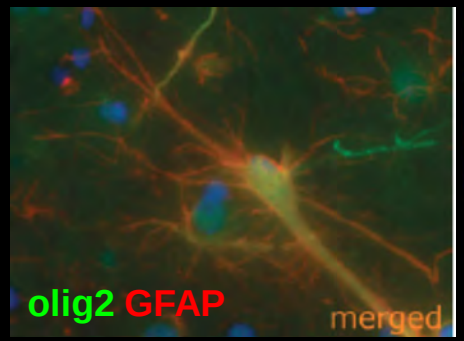
Bugiani *et al*., 2010, 2011

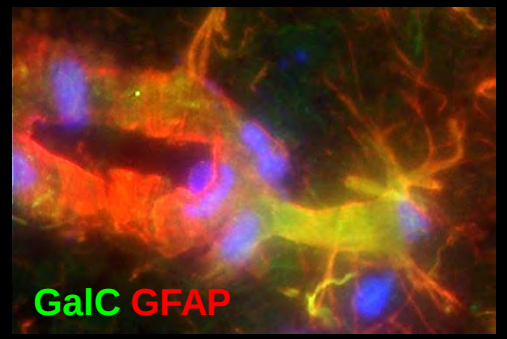
VWM white matter astrocytes remain immature

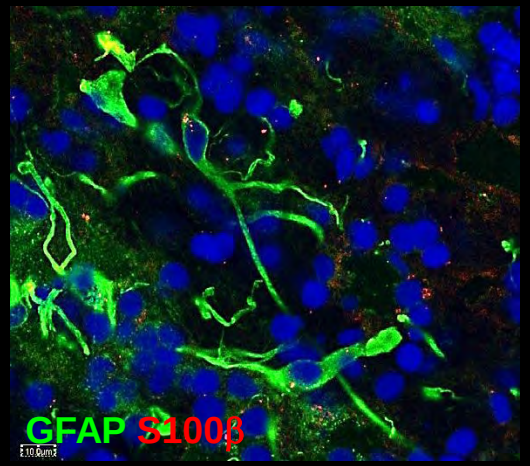


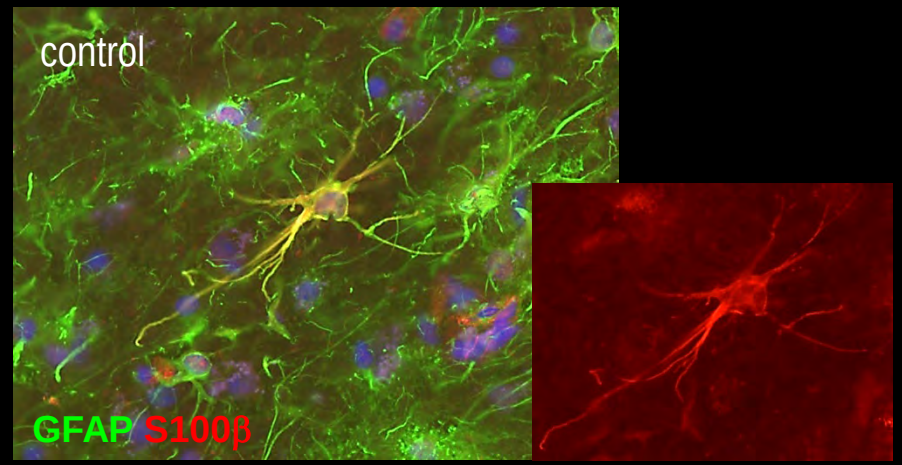






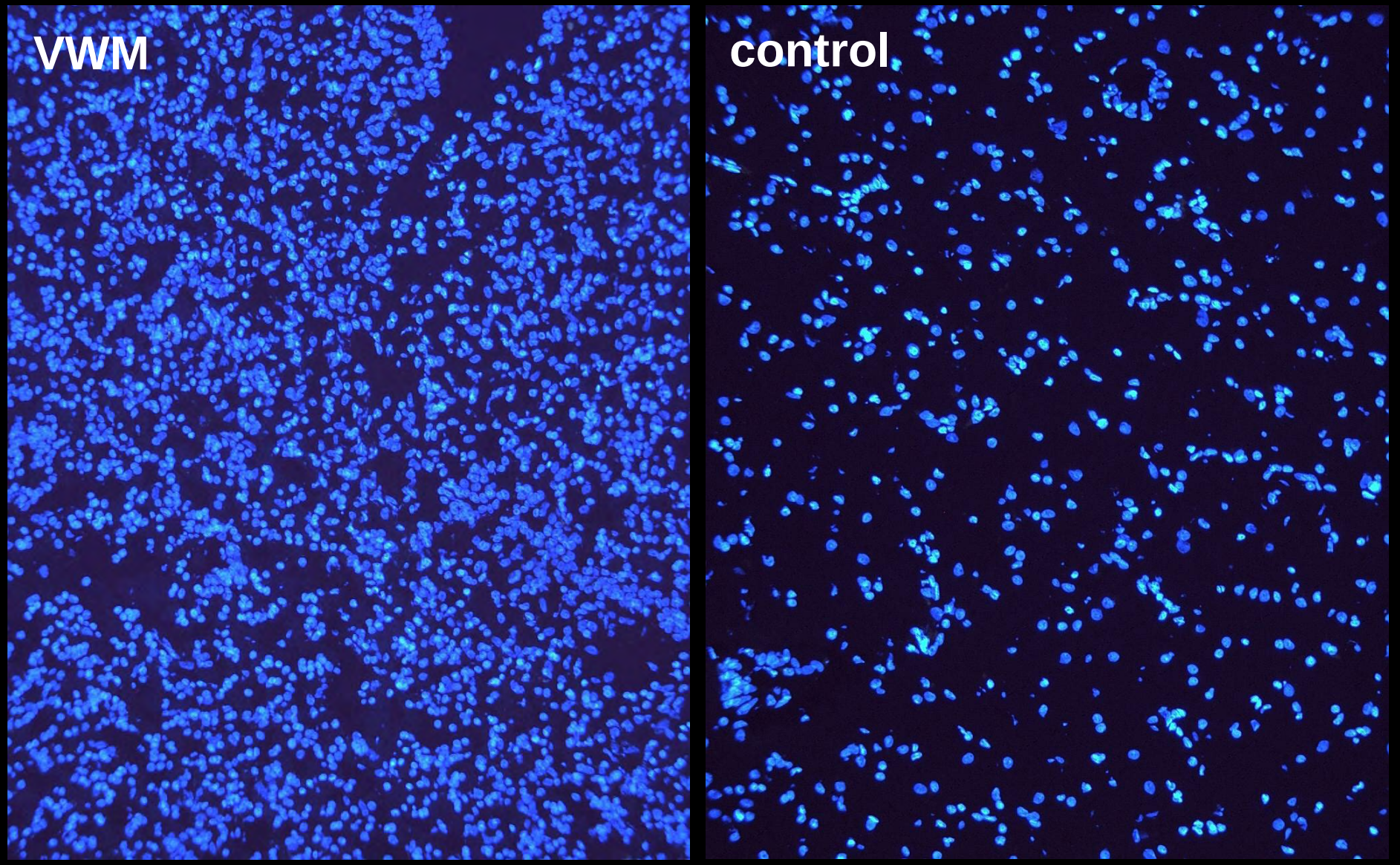




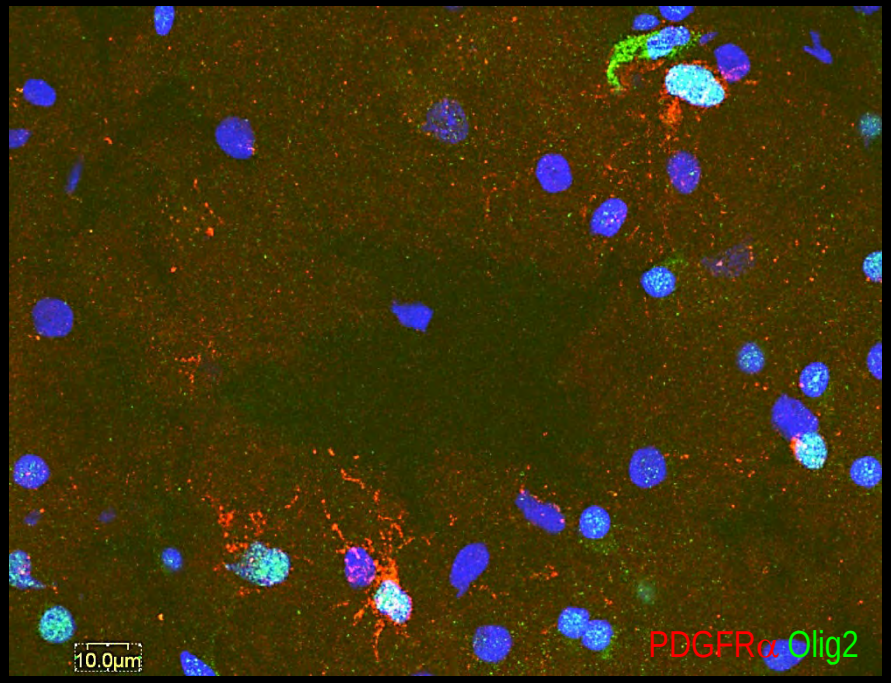


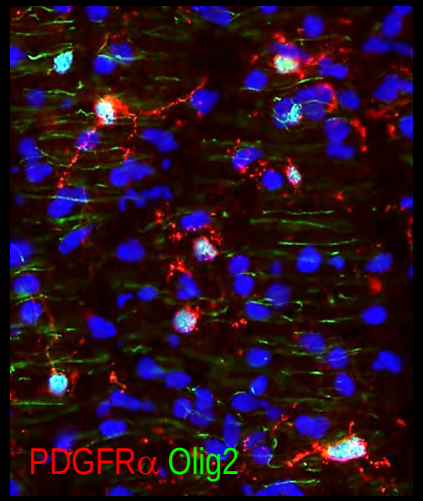
Bugiani *et al*., 2010, 2011

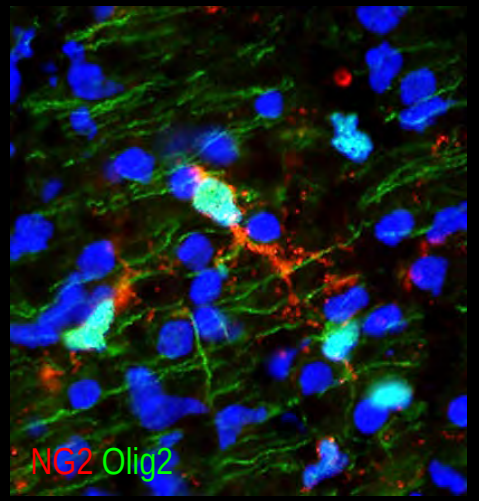
VWM white matter:
lack of myelin, but too many oligodendrocytes

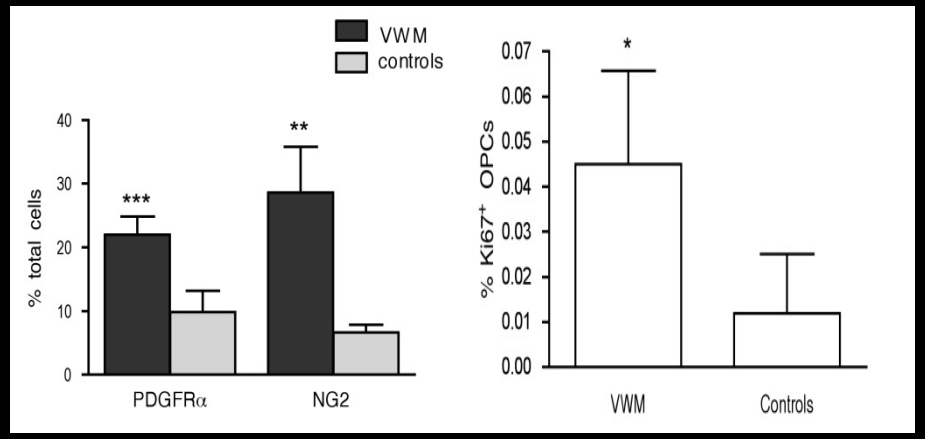


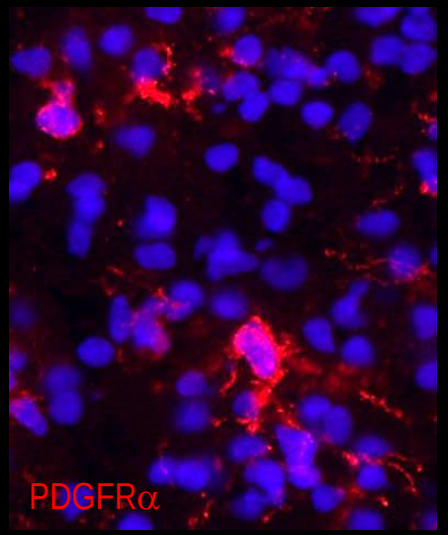
Oligodendrocytes proliferate and are increased in number, but they remain immature and lack of mature myelination function

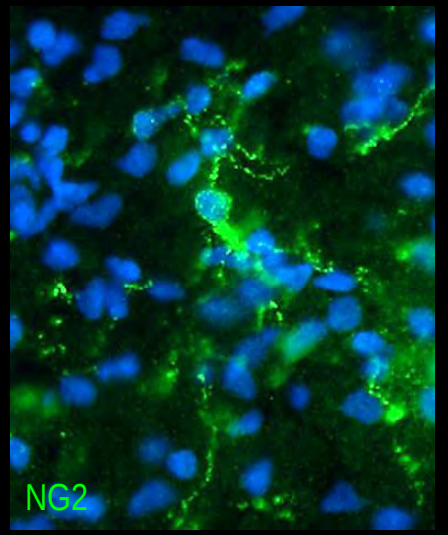






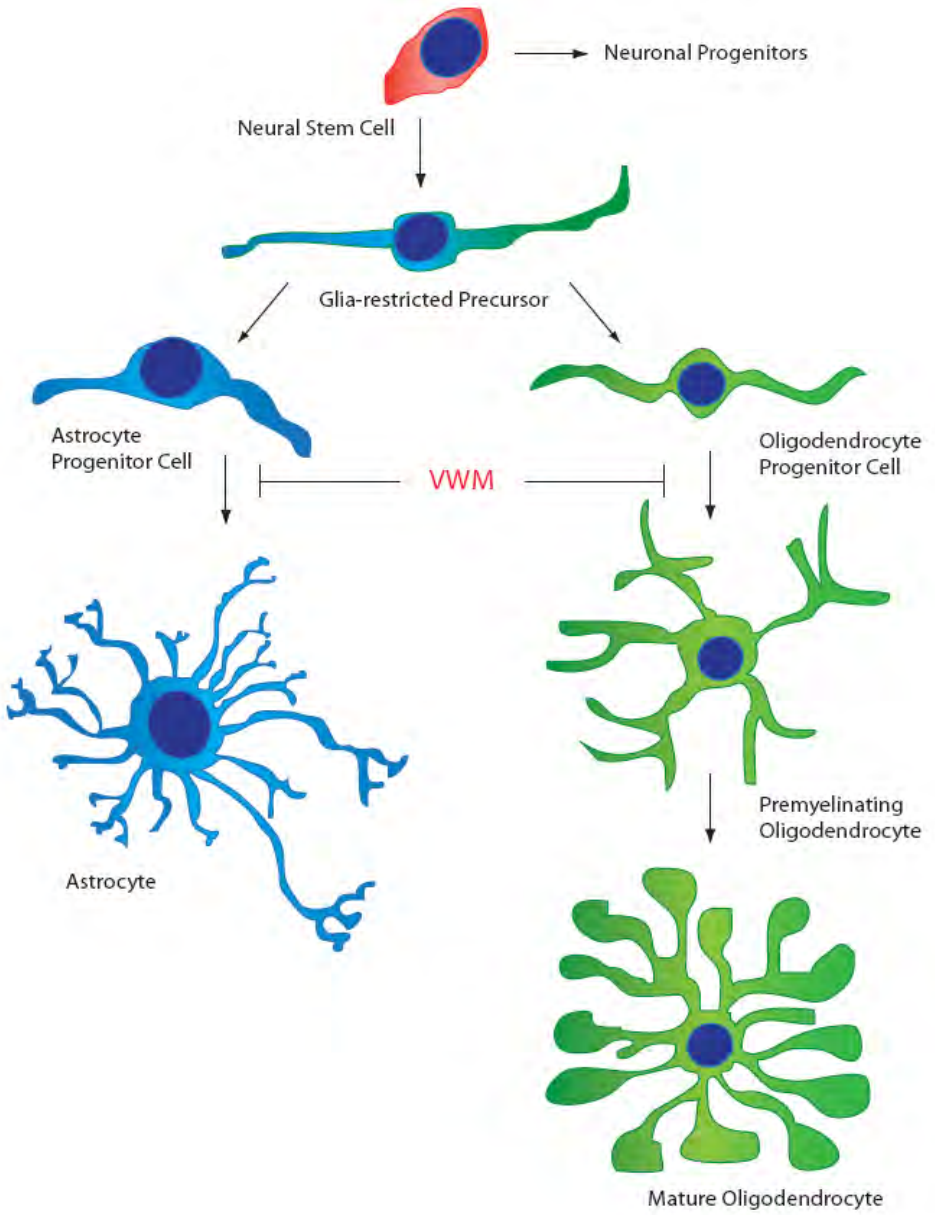






Bugiani *et al*., 2011

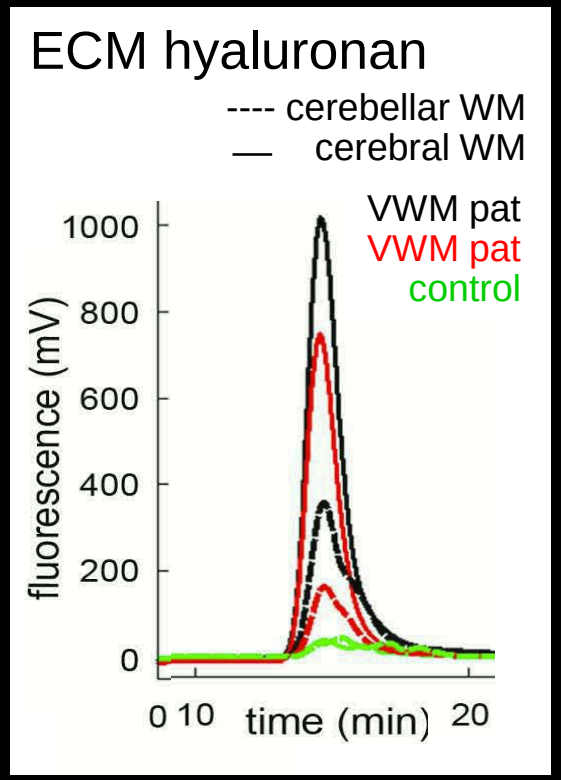
Deficient maturation of macroglial cells in VWM white matter driven by astrocytic dysfunction

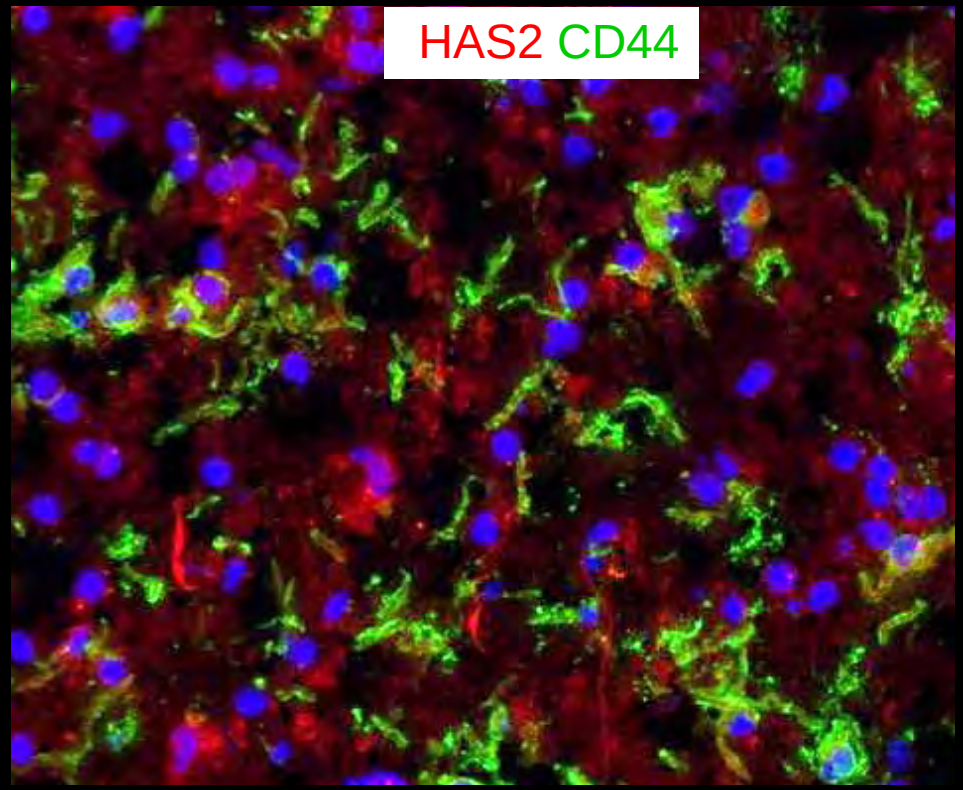


Courtesy of GC Scheper

Highly elevated high molecular weight hyaluronan in VWM white matter

- Produced by astrocytes
- Known to inhibit oligodendrocyte precursor maturation
- Its level correlates with the severity of white matter abnormality
- Hyaluronan synthase2 (HAS2) increased in immature ($CD44^{+}$) white matter astrocytes

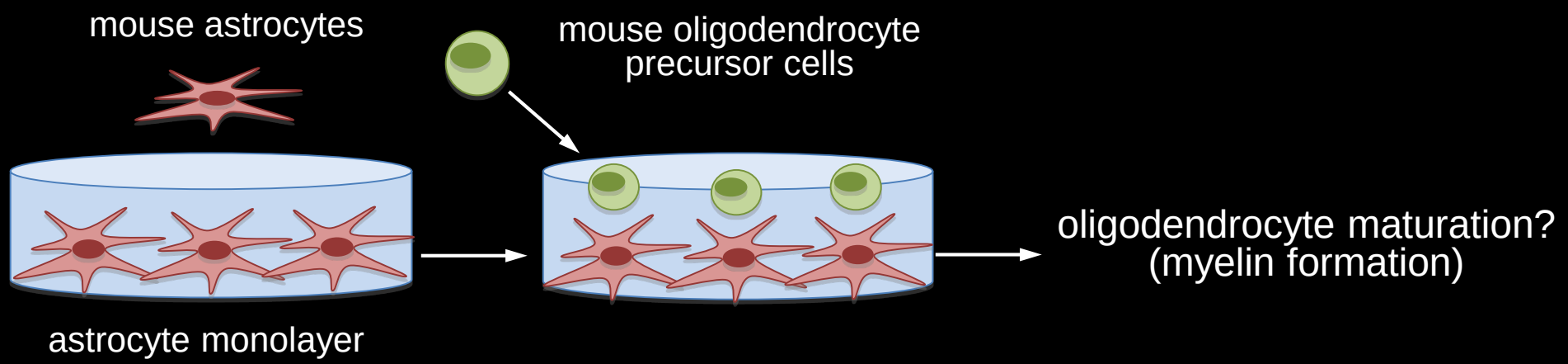




Bugiani M, *et al.*, 2013

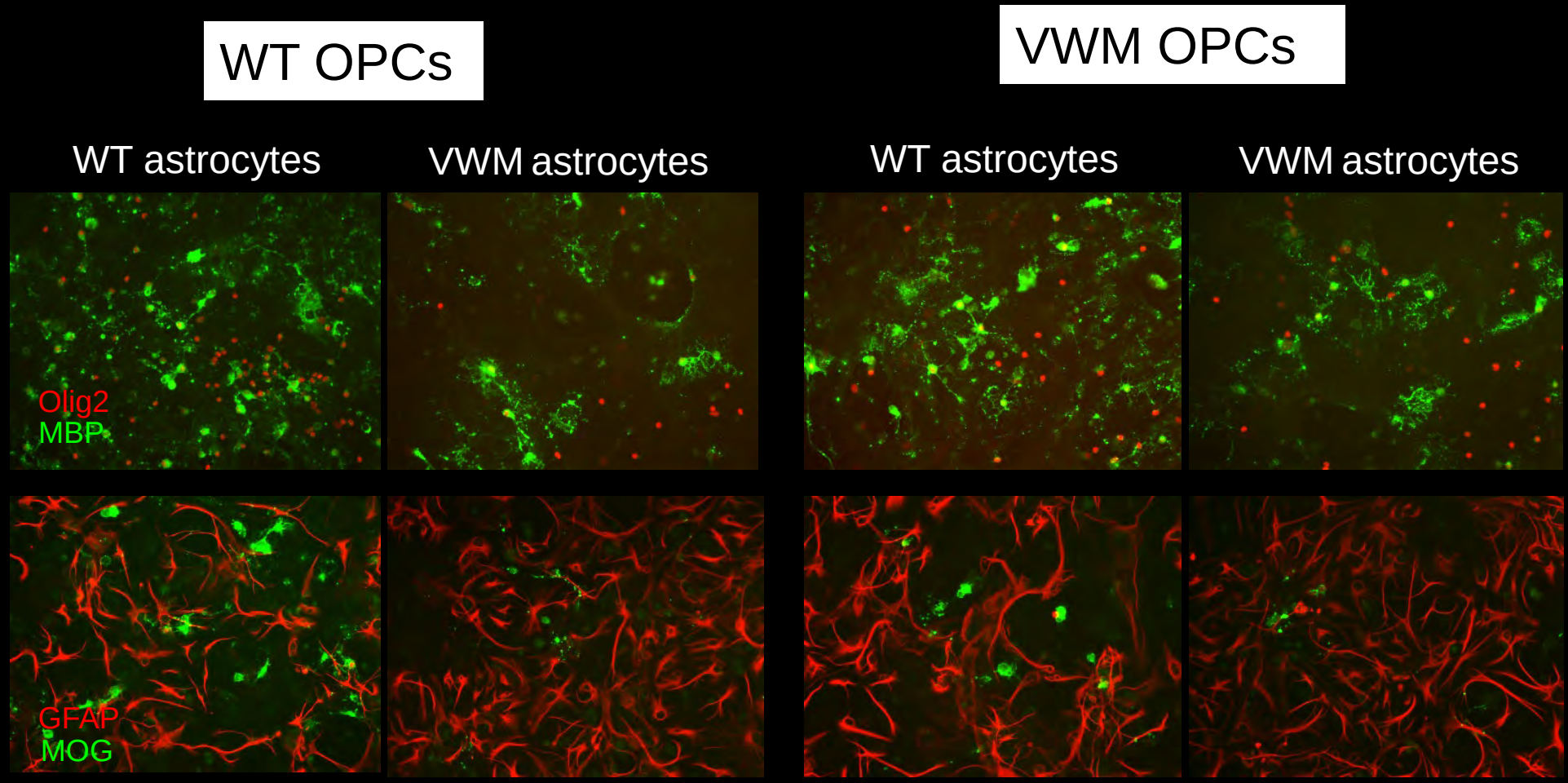
Does the VWM defect impact oligodendrocytes and astrocytes at the same time or is one causing the dysfunction of the other?

Studies in cocultures, using VWM mouse cells



Dooves S & Bugiani M, *et al.,* 2016

VWM astrocytes have a negative impact on both WT and VWM oligodendrocytes, but VWM oligodendrocytes display normal myelin production with WT astrocytes

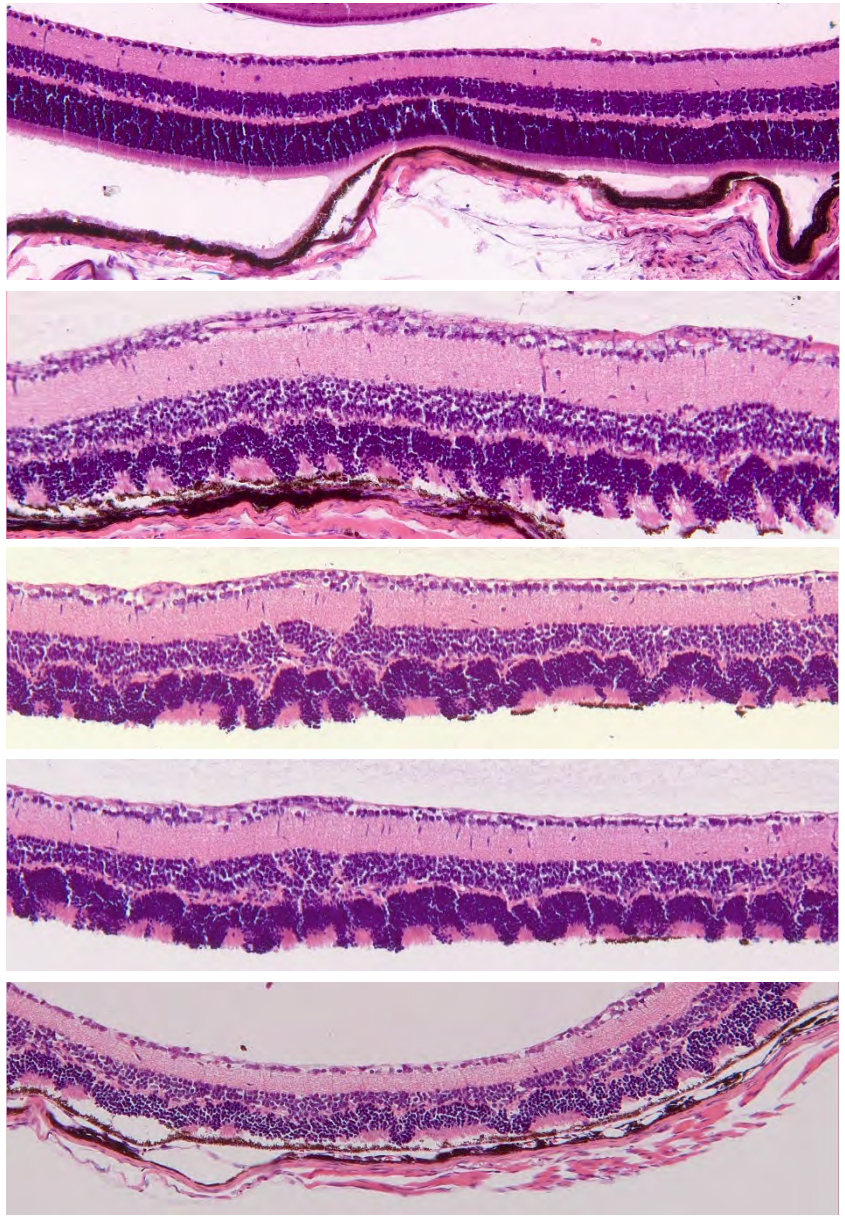


- So, VWM OPCs do not have an intrinsic problem

VWM mice: the eye pathology

Wild-type

VWM mice



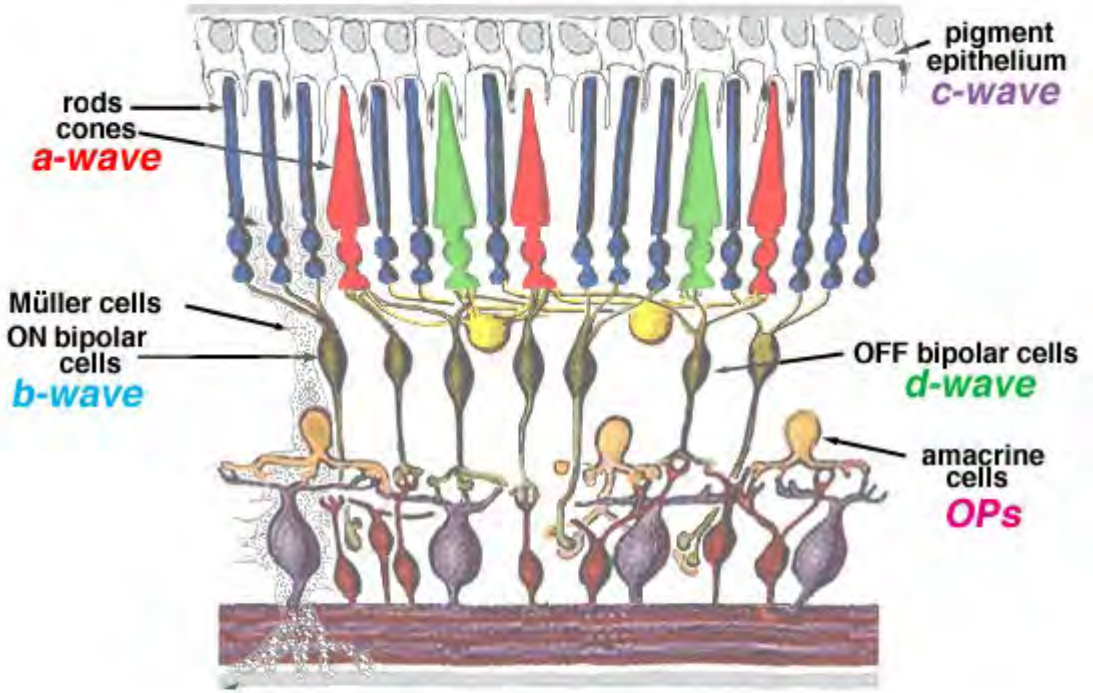


Fig.3 Cartoon of the retina to show where the major components of the ERG originate.

Dooves S & Bugiani M, *et al.*, 2016

Vanishing white matter is?

1. A disease of the oligodendrocytes, the myelin-forming cells
2. A disease of the astrocytes
3. A disease of the microglia, the brain immune cells
4. Not a brain disease

European Reference Network
for rare or low prevalence complex diseases
Network Neurological Diseases (ERN-RND)

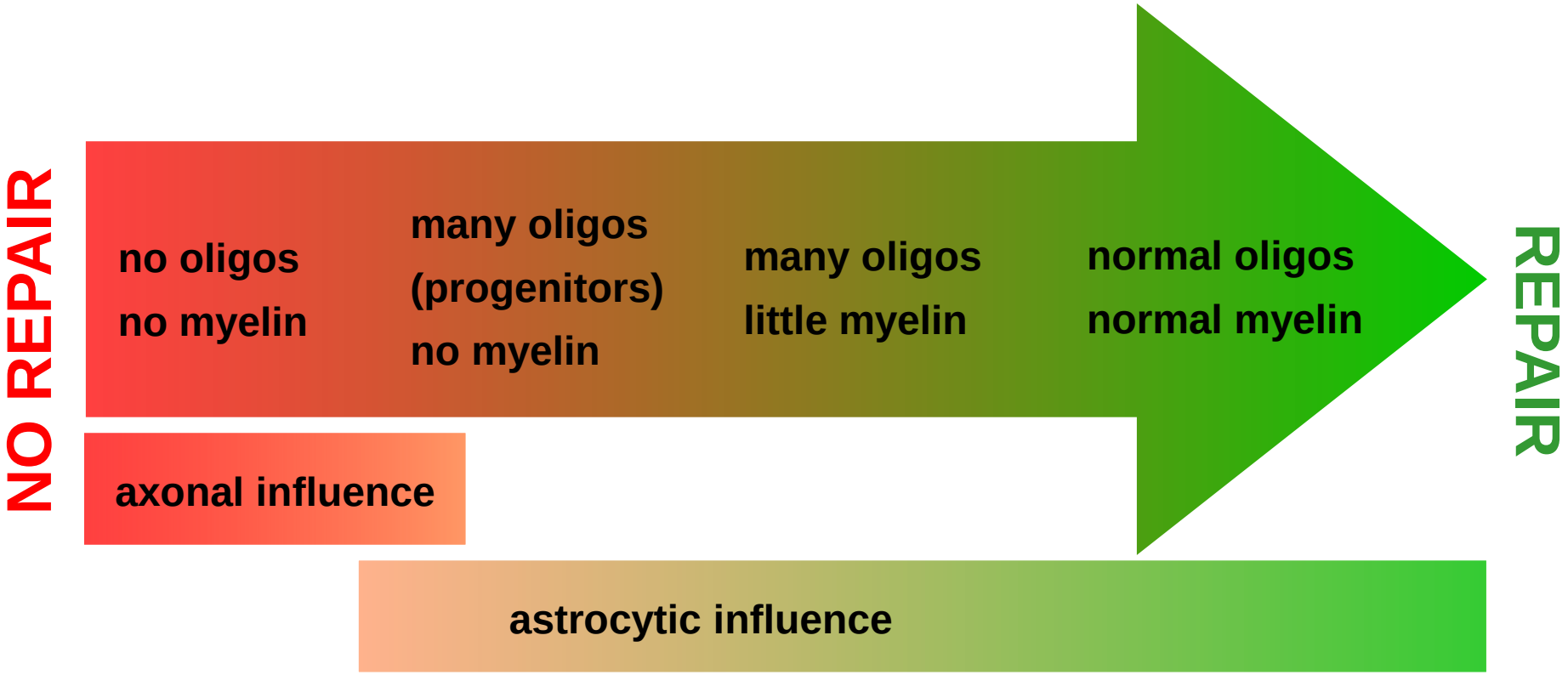
ean european academy of neurology

European Reference Network
for rare or low prevalence complex diseases
Network Neuromuscular Diseases (ERN EURO-NMD)

Key conclusions

- The definition of leukodystrophies had to be revised
- Genetic disorders in which any white matter structural component is primarily affected
- Better understanding of the complexity of the brain white matter
- When treating patients with leukodystrophies, we need to repair more than myelin alone

The intrinsic repair potential of leukodystrophies



Does neuropathology help in unraveling the leukodystrophies disease mecahnisms and the effects of treatment?

1. Absolutely not, autopsies are useless
2. Often, autopsies however are too much of a burden for families and doctors
3. Always, autopsies should always be performed
4. I don’t care…

Amsterdam Leukodystrophy Centre

Thanks to
- patients and families
- all our PhD students and technicians
- numerous collaborators with different backgrounds
- funding agencies
- supporting departments and institutes