Pathological correlates of MRI-measured GM damage in MS

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Overview

- Introduction: Grey Matter (GM) pathology in MS
  - Cortical grey matter pathology
- Cortical grey matter lesion detection
- Quantitative MRI-histology studies of cortical grey matter
- Grey matter damage beyond the cortex
- Summary and Conclusions

Introduction: GM pathology in MS

- Widespread grey matter (GM) damage occurs (especially in the cortex), even in the earliest phases of the disease

  (Lucchinetti *et al.*, 2011)
- Progresses with time

  (Bo *et al.,* 2003; Kutzelnigg *et al.,* 2005; Peterson *et al.,* 2001)
- Atrophy, lesions & 'normal-appearing grey matter' (NAGM) damage
- In both the brain and spinal cord
- Results in significant physical and cognitive disability

Introduction: Cortical GM pathology in MS

- Cortical GM pathology:
  - Demyelination
  - Neuronal loss
  - Synapse and neurite/axon density reductions
  - Microglial activation
  - Damage to the glia limitans

(Peterson *et al.,* 2001; Wegner *et al.,* 2006)

  - Remyelination

(Albert *et al.,* 2007)

Introduction: Cortical GM pathology in MS

- High frequency of demyelinating lesions in outer, subpial, cortex, especially in secondary progressive (SP) MS

  (Bo *et al.,* 2003; Kutzelnigg *et al.,* 2007; Kutzelnigg & Lassmann, 2006; Kutzelnigg *et al.,* 2005)

- Gradient of neuronal loss identified in SPMS
  - greatest loss seen in outer cortical layers

  (Magliozzi *et al.,* 2010)

- Inflammatory changes with B-cell follicles in adjacent meninges observed in SPMS

  (Choi *et al.,* 2012; Howell *et al.,* 2011; Magliozzi *et al.,* 2007)

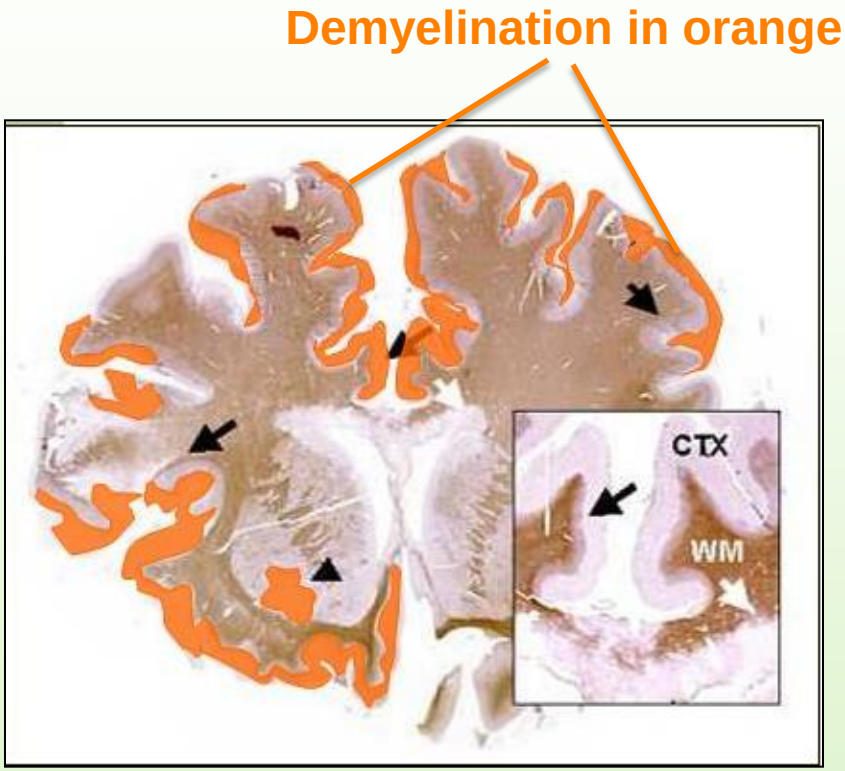


Image courtesy of Prof J Geurts, VU Medical Center, Amsterdam

Cortical GM lesions in MS: why are they so much harder to detect than WM lesions?

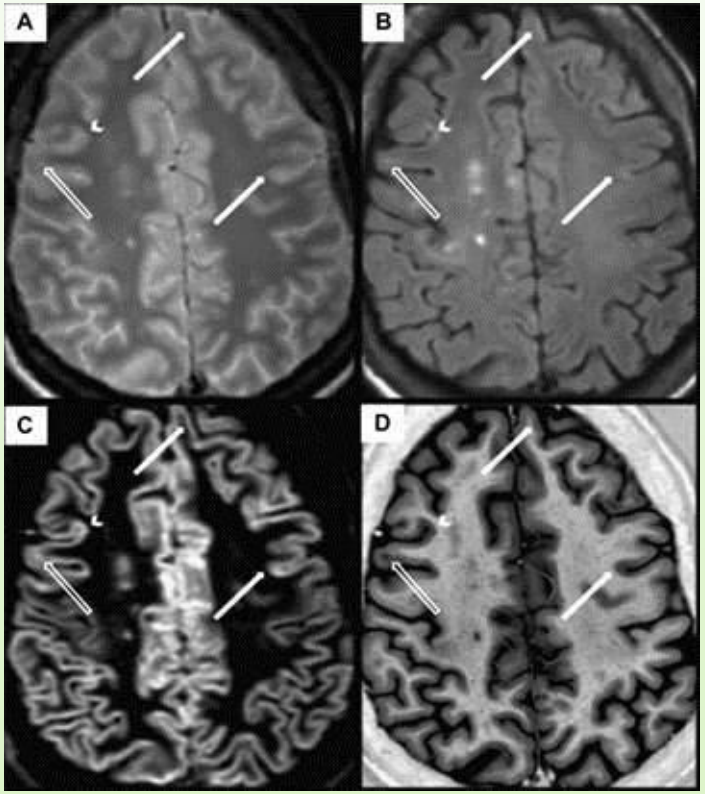
- Smaller difference in relaxation times between lesions and adjacent GM (Bainbridge *et al.,* 2003; Bo *et al.,* 2003)
- Smaller in size and often located adjacent to Cerebrospinal Fluid (CSF)
  - Misclassification due to partial volume effects possible
- GM lesions less inflammatory than WM lesions (Lassmann *et al.,* 2007; Peterson *et al.,* 2001) and the blood-brain barrier (BBB) is not disrupted (van Horssen *et al.,* 2007)
- Quantitative MRI methods may enable detection of unseen cortical GM damage
  - Undetected lesions and/or NAGM damage

Sensitivity of MRI to cortical GM lesions

- Higher field strength improves sensitivity (Kangarlu *et al.,* 2007)
- BUT ~80% of intracortical lesions may remain undetected at 4.7T (Geurts *et al.,* 2008)
- Combination of complementary sequences? (Bagnato *et al.,* 2009)
- Double Inversion Recovery (DIR) improved detection (Geurts *et al.*, 2005)
  - WM and CSF signals suppressed
- BUT majority of GM lesions were still not detected, even retrospectively (Seewann *et al.*, 2012)
  - Low contrast-to-noise ratio (GM vs. lesions)
- Phase-Sensitive Inversion Recovery (PSIR) increased detection rate *in vivo* (Sethi *et al.,* 2012)

Corresponding *in vivo* T_2-weighted (A), FLAIR (B), DIR (C) and PSIR (D) images (from Sethi *et al.,* J Neurol Neurosurg Psychiatry 83, 877-882 (2012)

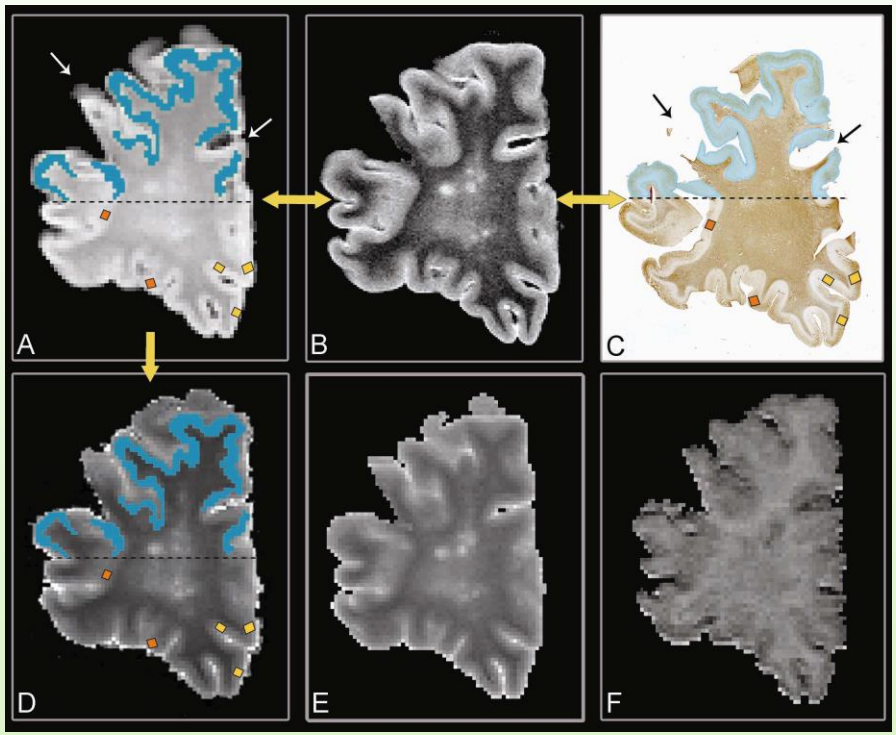
T_2w **FLAIR**



DIR **PSIR**

Correlations of MRI parameters with histology in lesions at 1.5T (Seewan *et al.* 2011)

- 16 brain slices from 10 MS patients scanned at 1.5T
- Measured T_1, T_2 and Magnetisation Transfer Ratio (MTR) in ROIs in lesions and NAGM, and globally in the whole cortex



- MRI and histology matched via PDw image
- Quantitative histology
- Explored regional and global correlations with quantitative MRI parameters

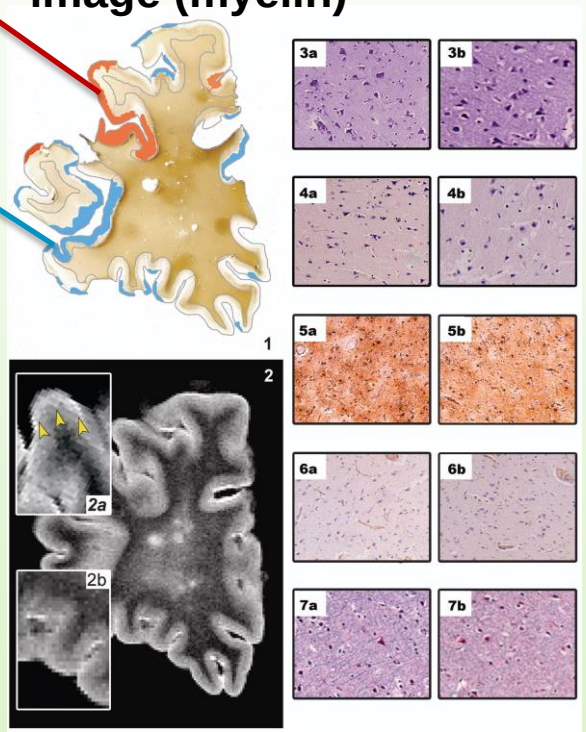
Images from Seewann, *et al*., Multiple Sclerosis 17, 1202-1210 (2011)

Correlations of MRI parameters with histology in lesions at 1.5T (Seewan *et al.* 2011)

MR-visible lesions

MR-invisible lesions

Lesions marked on PLP-stained image (myelin)



PDw image

- **MRI-visible lesions did not differ from MRI-invisible lesions in terms of histopathology or quantitative MRI measures**
- **Only in lesion size**

Nissl stain (neurons)

HLA-DR (antigen presenting cells/ microglia)

GFAP (glial fibrillary acidic protein; gliosis)

Fibrinogen (BBB leakage)

Bodian silver stain (neurons)

Images from Seewann, *et al*., Multiple Sclerosis 17, 1202-1210 (2011)

Regional analysis of MRI at 1.5T and histology in cortical GM (Seewan *et al.* 2011)

Measurement (Mean (SE))	Non-lesional cortex (n=49)	Cortical lesions (n=42)
T_1 (ms)	**323.7 (22.1)**	**349.9 (22.4)***
T_2 (ms)	**72.1 (2.6)**	**77.1 (2.7)***
MTR (pu)	15.6 (0.6)	14.8 (0.6)
T (Bodian)	**190.4 (2.3)**	**198.8 (2.4)*****
T (Nissl)	**155.8 (4.6)**	**177.4 (4.7)*****
T (GFAP)	210.0 (4.9)	213.9 (5.3)
T (fibrinogen)	235.0 (1.9)	234.3 (1.9)

*= p<0.05; ***p<0.001

T=Transmittance

MTR is sensitive to cortical demyelination in MS at 3T (Chen *et al* 2013)

- 7 MS patients scanned *in situ*
- Mean normalised MTR in cortical tissue (normalised to mean WM MTR)
- Compared cortical tissue with any demyelination (***intermediately demyelinated [IDctx]*** or ***mostly demyelinated [MDctx]***) with ***mostly myelinated [MMctx]*** tissue
  - Determined using proteolipid protein (PLP) staining
- Found that demyelinated cortical tissue had a significantly lower normalised MTR value when compared to mostly myelinated tissue

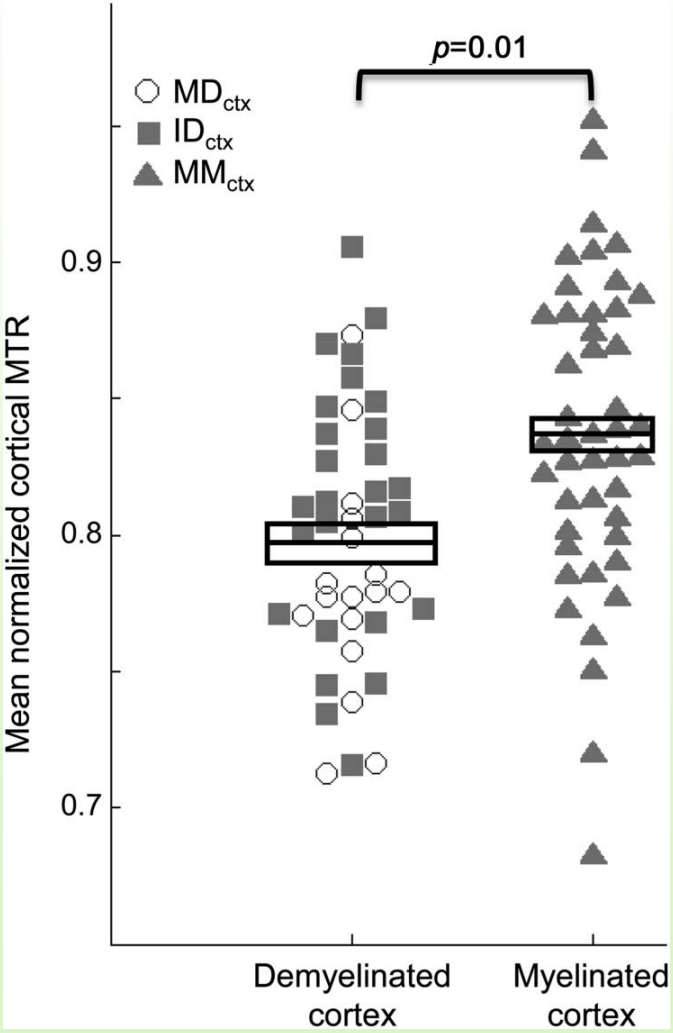


Figure from Chen *et al.*, Neurology 80, 246-252 (2013)

Correlations of focal MRI parameters with histology at 1.5T (Seewan *et al.* 2011)

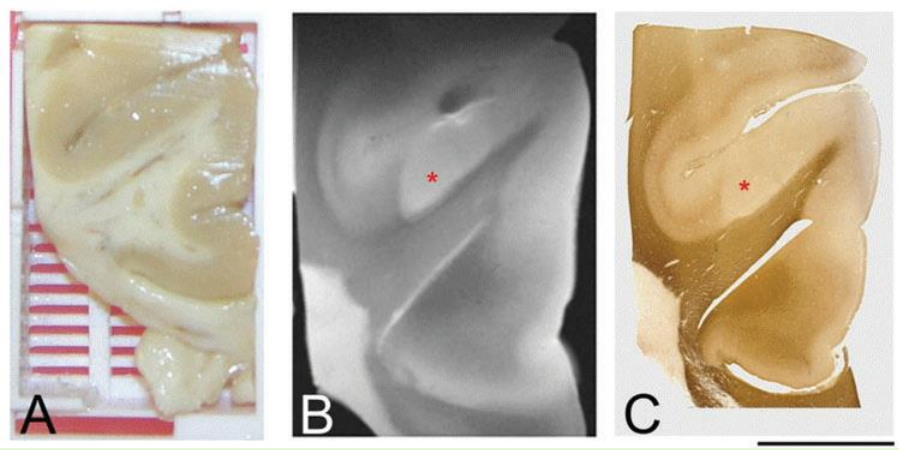
- **Longer T_2** relaxation times ($r=0.30$, $p<0.01$) and **lower MTR** values ($r=-0.27$, $p<0.05$) correlated with **demyelination**
- **Higher T_1** relaxation times correlated with lower Nissl but not Bodian staining intensities (for **axonal density**) ($r=0.27$, $p<0.05$)
- No significant associations of T_1, T_2 and MTR with microglial activation (HLA-DR), gliosis (GFAP) and BBB leakage (fibrinogen)

Correlations of global cortical MRI parameters with histology at 1.5T (Seewan *et al.* 2011)

- **Peak T_2** location correlated with **myelin** as measured by PLP transmittance ($r=0.56$, $p<0.05$)
- **MTR peak** location and **mean MTR** values correlated with the percentage of cortical **demyelination** ($r=0.76$, $p<0.01$, and $r=0.77$, $p<0.05$)
- BUT no associations were found between gliosis, BBB damage and axonal density and T_1, T_2 and MTR histogram parameters

Correlations of MRI parameters with histology in lesions at 9.4T (Schmierer *et al.* 2010)

- Fixed post mortem motor cortex from 21 MS patients scanned at 9.4T & measured T_1, T_2 and MTR in lesions and NAGM
- Quantitative histology:
  - H & E and cresyl-violet (neurons)
  - Luxol fast blue (myelin)
  - Immunohistochemistry using the following antibodies:
    - MBP (myelin content)
    - GFAP (astrocytes/gliosis)
    - SMI31 and SMI32 (phosphorylated and non-phosphorylated neurofilaments of axons, respectively)
- Demonstrated higher lesion detection rate with T_2w images
- Correlations with MRI measures explored



Type IV cortical grey matter lesion (indicated by red asterisk) in post-mortem brain of a subject with multiple sclerosis. Dissected tissue block (A) with corresponding T_2-weighted image (B) and tissue section immuno-stained for MBP (C).

Image from Schmierer *et al*., Brain 133, 858-867 (2010)

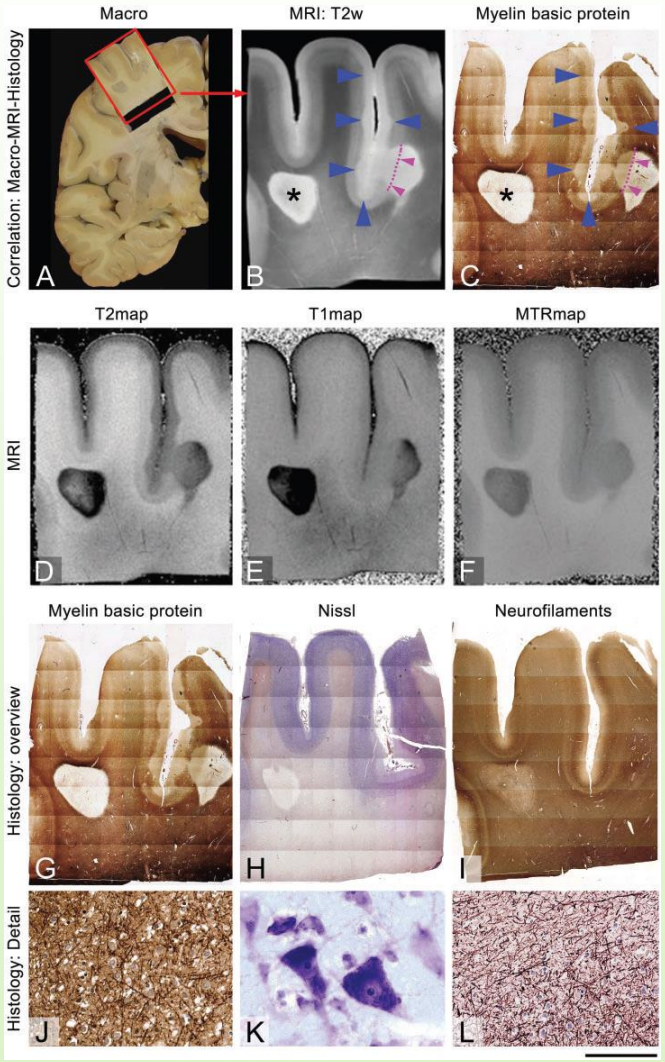
Comparison of cortical GM lesions and non-lesional cortex in MS at 9.4T

	Cortical grey matter lesions	Non-lesional cortex	P-value
ND (n/mm^2)	**98 (34)**	**129 (44)**	**<0.01**
iNF (1/T)	**1.16 (0.09)**	**1.24 (0.1)**	**<0.01**
iMBP (1/T)	**1.2 (0.08)**	**1.37 (0.13)**	**<0.01**
T_1 (ms)	768 (229)	725 (186)	0.13
T_2 (ms)	**25.9 (5)**	**22.6 (4.7)**	**<0.01**
MTR (pu)	**31.1 (11.9)**	**37.5 (8.7)**	**0.01**

ND= neuronal density; T = transmittance; iNF = intensity of immuno-staining for phosphorylated neurofilament; iMBP = intensity of immuno-staining for myelin basic protein.

Correlation of MRI parameters with histology in lesions at 9.4T (Schmierer *et al.* 2010)

Matching of MRI to histology data for correlative analysis



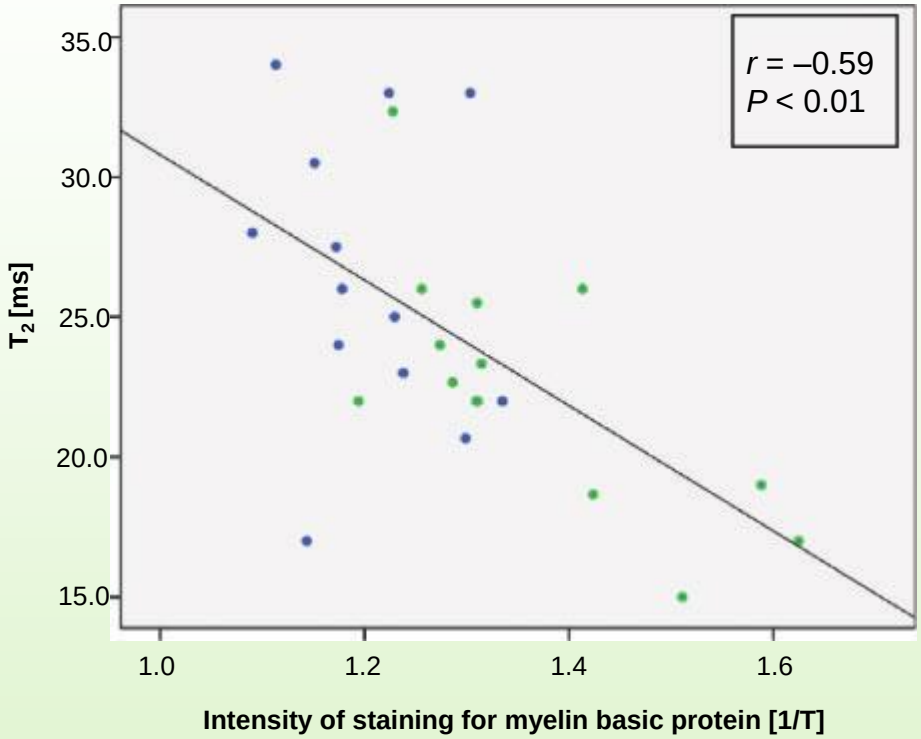
Blue arrows = type III lesion, Red arrows = type I lesion

Red line = WM/GM border

*** = MS WM lesion**

Image from Schmierer *et al.*, Brain 133, 858-867 (2010)

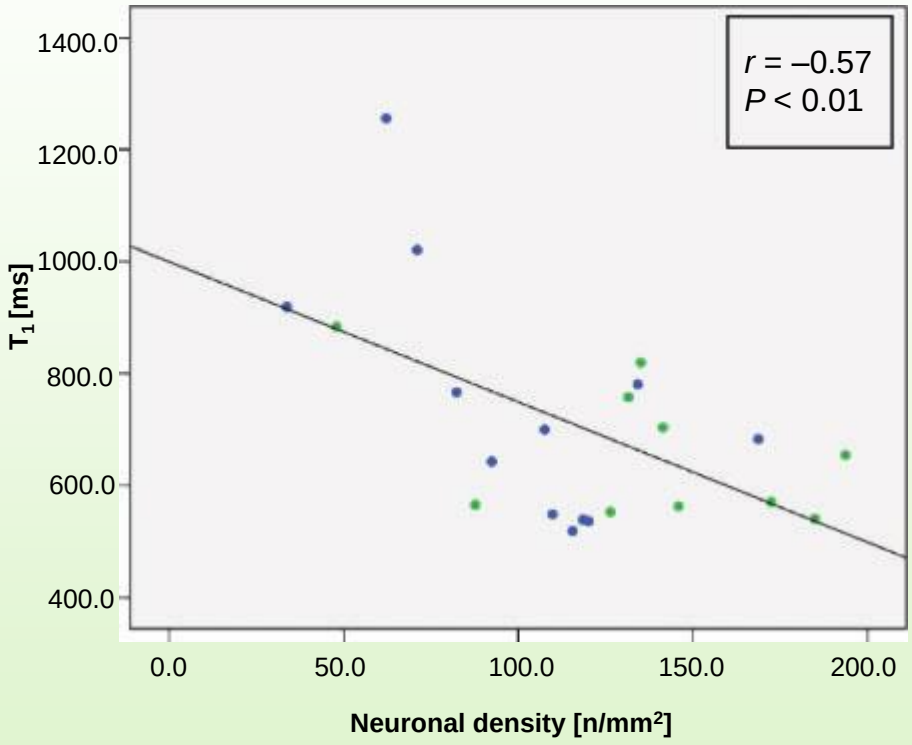
T_2 vs Myelin Content (iMBP)



Blue markers = cortical grey matter lesions; green markers = non-lesional cortex; T = transmittance.

Image from Schmierer *et al*., Brain 133, 858-867 (2010)

T_1 vs neuronal density



Blue markers = cortical grey matter lesions; green markers = non-lesional cortex; T = transmittance.

Image from Schmierer *et al*., Brain 133, 858-867 (2010)

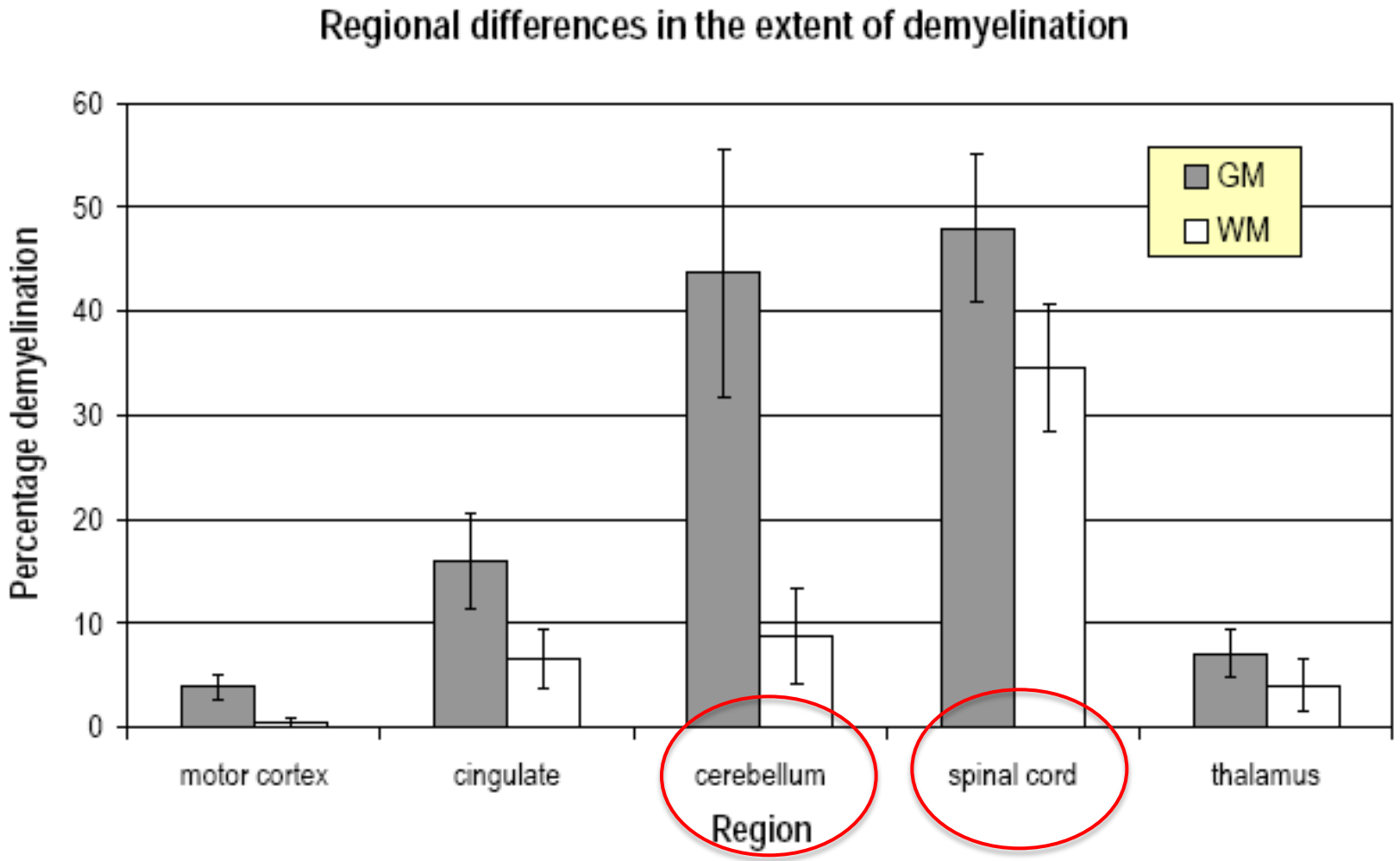
Other quantitative histology-MRI correlations (Shmierer *et al.*, 2010)

- Phosphorylated neurofilaments ('axonal area'; iNF) vs. iMBP ($r=0.58$, $p<0.01$)
- iNF vs. T_2 ($r= -0.55$, $p<0.01$)
- iMBP vs. MTR ($r=0.52$, $p=0.02$)
- T_2 vs Neuronal Density ($r= -0.51$, $p=0.02$)

<u>Multivariate analysis</u>

- iNF vs T_2 association secondary to that between iMBP and T_2
- AND iMBP vs T_2 association more robust than that between iMBP and MTR

Regional variations in extent of GM damage



Gilmore *et al* JNNP 80, 182-187

Courtesy of N Evangelou (Nottingham University , UK)

MRI-histopathology studies in the spinal cord

- No quantitative histology-MRI correlative studies performed in spinal cord GM

- Areas of abnormality in GM observed on PD-weighted images at 4.7T corresponded to myelin staining patterns observed using MBP (Gilmore *et al.*, 2009)

- Multi-component (mc) DESPOT technique applied in 3 complete formalin-fixed spinal cords on both a 3T and a 7T scanner
  - Good correspondence between the myelin water fraction and myelination status (McDowell *et al*., Proc ISMRM 2015)

Limitations of MRI-histopathology studies

- Tissue fixation affects quantitative MRI parameters measured in MRI-histology studies (Bainbridge *et al.;* Birkl *et al.,* 2015; Schmierer *et al.,* jMRI 2010; Schmierer *et al.*, 2008)
- Temperature changes will also affect MRI indices, e.g. T_1 shortens with decreasing temperature (Bottomley *et al.,* 1984)
- It may be difficult to achieve accurate registration between MRI and pathology
- The size of spinal cord specimens requires high resolution data to enable accurate co-registration
- Difficult to assess whole brain/cord histopathologically
- It is likely that MS patients studied histopathologically have longstanding progressive disease and are therefore not necessarily representative of all aspects of the disease (a lot of lesions are more likely to be chronic)

Summary: MRI-histopathological *correlates* in MS GM

Pathological substrate & change	Histological measure	Change in qMRI measure	References
Myelin ↓	PLP/MBP staining intensity	T_1 ↑ T_2 ↑ MTR ↓	Schmierer *et al* 2010 Seewan *et al* 2011/Schmierer *et al* 2010 Seewan *et al* 2011
Neuronal density ↓	Nissl/ SM131 staining intensity	T_1 ↑	Seewan *et al* 2011/ Schmierer *et al* 2010
Astrocytes/ gliosis	GFAP staining intensity	No correlations found	Seewan *et al* 2011 & Schmierer *et al* 2010
BBB leakage	Fibrinogen staining intensity	No correlations found	Seewan *et al* 2011

Summary

- Quantitative MRI measures provide a promising tool to evaluate components of grey matter MS pathology
  - Important for validation of markers of disease processes *in vivo*
- Further studies performed at higher field strength needed for higher SNR & resolution
- Studies of larger numbers of subjects with different clinical phenotypes, and examining all substrates of the disease via different staining methods, are required
  - In addition to other quantitative MRI methods, such as Diffusion Tensor Imaging (DTI) and quantitative MT
- Whole brain and spinal cord
- Where possible, future studies performed prior to fixation may provide a better insight into the mechanisms of pathology *in vivo*

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