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TITLE: In Vivo Imaging of Cortical Inflammation and Subpial Pathology in Multiple Sclerosis by Combined PET and MRI

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT Post-mortem studies in multiple sclerosis (MS) suggested that cortical demyelinating lesions, which are hardly detected in vivo on conventional magnetic resonance imaging (MRI) scans, are an important correlate of disability, and are driven by organized neuroinflammation with the activation of microglia. Activated microglia upregulate expression of the 18kDa translocator protein (TSPO), which can be imaged in vivo with [¹¹ C]PBR28, a second generation TSPO ligand. In this study, we combine ultra-high field 7 Tesla (T) MRI, which has demonstrated greater sensitivity to cortical lesions than conventional MRI, with [¹¹ C]PBR28 positron emission tomography (PET) imaging of activated microglia to assess whether more severe structural cortical pathology in MS is related to the presence of neuroinflammation. Our initial findings show that high-resolution [¹¹ C]-PBR28 PET imaging is able to detect in vivo diffuse inflammation in different brain tissue compartments in MS, but more prominently in in cortex, cortical lesions, as well as deep gray matter. Additionally, the degree of inflammation in cortex, deep gray matter is associated with neurological disability, impaired information processing speed, a cognitive domain frequently affected in MS, and neurodegeneration.					
15. SUBJECT TERMS Multiple sclerosis; cortex; cortical lesions; neuroinflammation; microglia; [¹¹ C]PBR28 PET imaging; ultra-high field MRI; neurological disability, cognition, deep gray matter, neurdegeneration.					
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1. Introduction

Multiple sclerosis (MS) is an immune-mediated chronic disease of the central nervous system (CNS), pathologically characterized by inflammation, demyelination and neurodegeneration, and with great variability in the clinical course of individual subjects.

Histopathological examinations of MS brains indicated that cortical demyelinating lesions are potential biomarkers of MS progression. Since cortical lesions appeared topographically related to focal meningeal inflammation in some pathological studies, it has been hypothesized that cortical demyelination in MS may be driven by organized meningeal inflammation through the activation of microglia, accompanied by a decreasing gradient of demyelination away from the pial surface (Magliozzi et al, Brain 2007; Ann Neurol 2010).

Histopathological evidence that the cortex can be the site of inflammatory demyelinating lesions near the time of MS onset (Lucchinetti et al, New Engl J Med 2011) further supports the existence of an early pathological process that primarily targets the cortex, independently from white matter (WM).

Although largely undetected on conventional magnetic resonance imaging (MRI) scans, cortical lesions, including the subpial type, have been imaged in vivo with improved sensitivity and spatial specificity at ultra high-field 7 Tesla (T) MRI. We developed a reproducible surface-based analysis for mapping cortical T_2^* relaxation rates (quantitative- T_2^* or $q-T_2^*$) as a function of cortical depth from ultra high-resolution gradient echo 7 T MRI scans. This technique has proved useful for studying the laminar architecture of the cortex in vivo, and for characterizing cortical pathological abnormalities in MS associated with changes in cortical myelin and/or iron concentration.

The purpose of this project is to evaluate inflammation and structural tissue changes in the cortex of patients with relapsing-remitting (RR) MS by combining advanced MR studies at 7 T MRI, to measure cortical lesions and diffuse subpial pathology, with positron emission tomography (PET) using the 18kDa translocator protein (TSPO)-targeting radioligand [^{11}C]-PBR28 to directly quantify microglia activation.

The overall working hypothesis is that patients with MS will show areas of cortical inflammation, topographically associated with the presence of structural cortical abnormalities (lesions) and neurodegeneration (cortical tissue loss), as suggested by post-mortem examinations. The ability to quantify in vivo MR tissue changes at different cortical depths from the pial surface, across the whole cortical mantle, and to couple these measurements with assessment of neuroinflammation, can provide insights on the biological basis of cortical degeneration in MS. This, in turn, could help to predict aggressive forms of the disease that can be susceptible to earlier and more specific therapies.

The in vivo study of the inflammatory and degenerative components of cortical disease in MS can have major implications for diagnosing, and understanding the pathogenesis of disease progression in MS.

2. Keywords

1. Multiple sclerosis (MS)
2. Relapsing-remitting (RR)
3. Cortical lesions
4. Subpial demyelination
5. 7 Tesla
6. Magnetic resonance imaging (MRI)
7. White matter (WM) lesions
8. Normal appearing white matter (NAWM)
9. Positron emission tomography
10. [^{11}C]-PBR28
11. Microglia
12. Macrophages
13. Inflammation
14. Standard uptake values (SUV)
15. Normalized standard uptake values (SUV-R)
16. Disability
17. Expanded disability status scale (EDSS)
18. MS severity score (MSSS)
19. Ambulation index (AI)
20. 9-hole peg test (9H PT)
21. Cognition
22. Information processing
23. Symbol digit modalities test (SDMT)
24. Thalamus
25. Deep gray matter (DGM)

3. Accomplishments

Current objectives

During Year 2 of the present award we have obtained IRB and HRPO renewal, and continued all study procedures initiated in YEAR 1 related to Aim1 and Aim2 of the statement of work (SOW).

The overall goal of Aim1 is to “assess in patients with relapsing-remitting multiple sclerosis (RRMS) microglia activation in the cortex, as measured by [^{11}C]-PBR28 uptake and its association with subpial pathology and cortical lesions”.

The overall goal of Aim2 is to “determine the relationship between cortical inflammation, as measured by cortical [^{11}C]-PBR28 uptake, disability and cognitive performance in patients with MS”.

Specific accomplishments

We screened about ~40 potential study participants with RRMS. We consented and enrolled in the study 13 RRMS patients, which were then genotyped for the Ala147Thr polymorphism in the TSPO gene to identify high affinity binders (HAB) and mixed affinity binders (MAB). Seven age-matched healthy controls (HC) were also consented and included in the study for 7 T protocols. One RRMS subject resulted as low affinity binder, and was therefore excluded from further study procedures. Eight RRMS subjects completed all study procedures that include: a) assessment of cortical microglia activation during a 90-min acquisition after injection of [^{11}C]-PBR28 using simultaneously acquired positron emission tomography (PET) and MR imaging (Siemens BrainPET scanner); b) assessment of structural cortical demyelinating lesions and diffuse subpial pathology using ultra-high field 7 Tesla MRI combined with multi-channel radiofrequency technology; c) administration of the 9-hole peg test, the Minimal neuropsychological assessment of MS (MACFIMS), the Expanded Disability Status Scale (EDSS), and the Ambulation Index (AI). One subject had to be excluded from data analysis because it later resulted that she might have assumed benzodiazepines, which are known to compete with [^{11}C]-PBR28 in binding the TSPO, formerly known as the peripheral benzodiazepine receptor (PBR).

We are continuing to screen potential study participants and have scheduled consenting and genotyping visits for four additional patients.

We have pooled the analyzed MR-PET and 7 T MRI data from 7 RRMS subjects of this study (6 females; mean $\pm$ SD age= 40 $\pm$ 10 years; HAB=3; MAB=4) with those (N=11; 9 females; mean $\pm$ SD age= 53 $\pm$ 8 years; HAB) from an ongoing study funded by the National MS Society, which is focused on more advanced stages of MS, namely secondary-progressive (SP) MS. Twelve HC (5 females; mean $\pm$ SD age= 52 $\pm$ 10 years; HAB=6; MAB= 6) were used for comparisons with patients' data. All subjects underwent 90 minutes of [^{11}C]-PBR28 on a unique Siemens BrainPet scanner, a dedicated brain avalanche photodiode brain PET scanner that can be operated in the bore of a 3 T whole body MR magnet. MRI anatomical scans were simultaneously collected for Freesurfer reconstruction of cortical surfaces and coregistration of PET and MR modalities. Standard uptake values (SUV) maps of [^{11}C]-PBR28 were created by averaging PET frames (1.25 mm isotropic voxel size) between 60 - 90 minutes (SUV₆₀₋₉₀). To account for signal differences across study participants, in each subject, SUV₆₀₋₉₀ were normalized by SUV₆₀₋₉₀ from a reference region (SUVR), i.e. a region devoid from glial

pathology, and showing similar uptake values in MS and HC. Briefly, we determined in each HC subgroup (HAB and MAB) mean SUV_{60-90} in WM (the brain tissue with the lowest SUV_{60-90}). In each MS patient we then identified regions of normal appearing white matter (NAWM) whose SUV_{60-90} were within ± 0.5 standard deviations (SD) of the mean WM SUV_{60-90} for HAB and MAB respectively. Figure 1 shows an example in a subject with MS of NAWM clusters with SUV_{60-90} within ± 0.5 SD from mean WM SUV from controls.

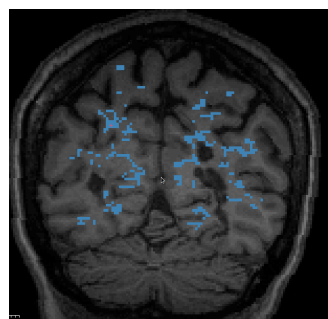


Fig. 1. Example in a subject with MS of NAWM clusters with SUV_{60-90} within ± 0.5 SD from mean WM SUV from controls. (MS= multiple sclerosis; NAWM= normal appearing white matter; SUV_{60-90} = standard uptake values 60-90 min; WM= white matter.

In MS patients, cortical lesions (subpial, intracortical and leukocortical) were segmented from T_2^* gradient echo images acquired at ultra-high resolution ($0.33 \times 0.33 \times 1$ mm voxel size) at 7 T using a 32-channel coil on a separate day. Due to motion artifacts, which affect image quality at 7 T, cortical lesions were segmented in 8 patients only. In patients, WM lesions were segmented on clinical fluid attenuated inversion recovery (FLAIR) images acquired during the MR-PET session. In all subjects (HC and MS) deep gray matter (DGM) structures including basal ganglia (BG), thalamus and hippocampus, were also segmented from 3 T anatomical scans by using FIRST-FSL. All subjects' masks were then registered to their corresponding SUVR maps to extract $[^{11}C]$ -PBR28 SUVR from

different brain tissue compartments.

In patients we also assessed neurological disability as measured by the Expanded Disability Status Scale (EDSS), ambulation index (AI), MS severity score (MSSS) and administered the 9-hole peg test (9H PT). Cognition was assessed using the MACFIMS.

Multilinear regression models were used to compare MS SUVR vs HC SUVR in different brain tissue compartments (cortex, cortical lesions, WM lesions, NAWM, thalamus, hippocampus and basal ganglia) adjusting for PBR affinity and age when appropriate, and for assessing the relationship between glial activation, clinical outcome measures, and neurodegeneration in cortex (cortical thinning) and DGM (thalamic volume). P values= 0.5 were considered significant.

Findings from this dataset will be presented at the meeting of the European Committees for Research on Multiple Sclerosis (ECTRIMS), Barcelona, Spain, October 6th-10th 2015:

1. Herranz E, Gianni C, Louapre C, Mangeat G, M. Loggia, Treaba CA, Govindarajan ST, Ward N, Sloane JA, Klawiter EC, Ouellette R, Catana C, Hooker JM, Kinkel RP, Mainero C. ^{11}C -PBR28 MR-PET imaging detects in vivo diffuse inflammation in cortex, deep gray and normal appearing white matter associated with neurodegeneration and clinical disability. Selected for Oral Presentation. ECTRIMS, Barcelona, Spain, 2015.

Results

Table 1. Percent increase in $[^{11}C]$ -PBR28 SUVR in 18 subjects with MS relative to 12 HC within different brain tissue compartments in cortex, subcortical structures and white matter.

	WM	NAWM	WM Lesions	Cortex	Cortical lesions	Thal.	Hipp.	BG
MS mean (SD)	-	1.2 (0.3)	1.1 (0.1)	1.3 (0.3)	1.5 (0.3)	1.5 (0.2)	1.4 (0.2)	1.1 (0.2)
HC mean (SD)	1.0 (0.0)	-	-	1.1 (0.1)	-	1.0 (0.1)	1.0 (0.2)	0.9 (0.2)
% Increase (MS vs HC)	-	~20%	~10%	~18%	~36%	~50%	~40%	~22%

(MS= multiple sclerosis; HC= healthy controls; WM= white matter; NAWM= normal appearing white matter; Thal.= thalamus; Hipp.= hippocampus; BG= basal ganglia).

Overall in patients, relative to HC, there was a diffuse increase PBR28 SUVR across the brain. Regions with higher uptake, however, were localized in cortical (cortical lesions, **Fig. 2**) and subcortical gray matter (thalamus, hippocampus, **Fig 3**).

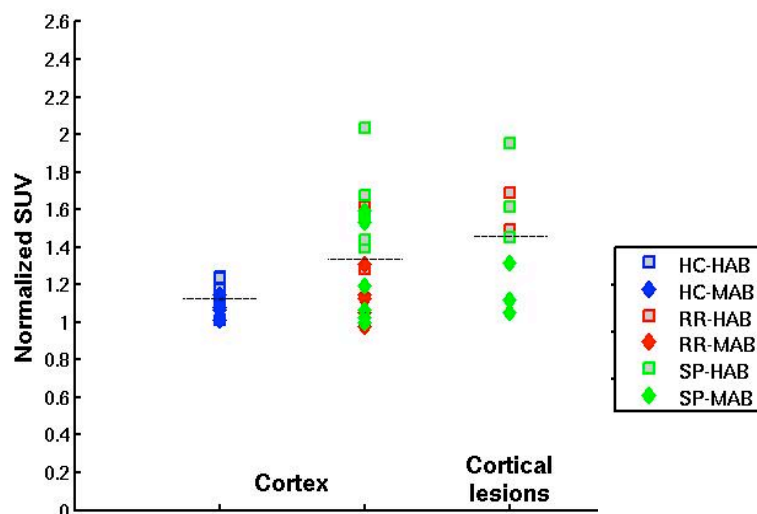


Fig. 2. Normalized [^{11}C]-PBR28 standard uptake values (SUVR) in cortex and cortical lesions for healthy controls (HC, blue), relapsing-remitting (RR, red) and secondary progressive (SP, green) multiple sclerosis (MS) patients. MS patients showed, relative to HC, higher SUVR in whole cortex ($p=0.01$) and cortical lesions ($p=0.004$) by multilinear regression adjusting for age and PBR affinity. HAB= high affinity binder; MAB= mixed affinity binder.

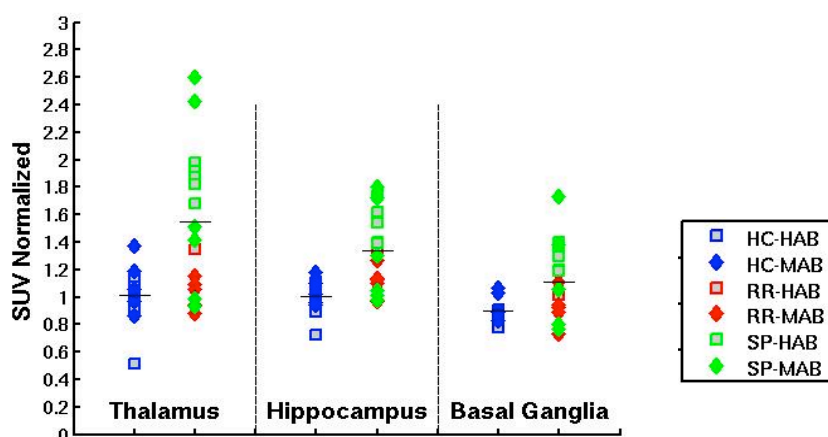


Fig. 3. Normalized [^{11}C]-PBR28 standard uptake values (SUVR) in thalamus, hippocampus, and basal ganglia for healthy controls (HC, blue), relapsing-remitting (RR, red) and secondary progressive (SP, green) multiple sclerosis (MS) patients. MS patients showed, relative to HC, higher SUVR in whole thalamus ($p=0.0005$) hippocampus ($p=0.003$) and basal ganglia ($p=0.02$) by multilinear regression adjusting for age and PBR affinity. HAB= high affinity binder; MAB= mixed affinity binder.

Although to a lesser degree than for gray matter, MS patients also exhibited, relative to HC significantly increased [^{11}C]-PBR28 SUVR in NAWM (~20% increase, $p=0.02$ by multilinear regression adjusting for age and PBR affinity). In patients, increased [^{11}C]-PBR28 uptake in WM lesions relative to HC was modest (~10%).

There was no relation between glial activation and MSSS, AI, and 9H PT.

Glial activation in cortex, but also thalamus and NAWM, positively correlated with neurological disability (EDSS), and inversely with SMDT (a measure of information processing speed from the MACFIMS battery) in both RR and SP MS ($p < 0.05$ by multilinear regression adjusting for PBR affinity). Increased [^{11}C]-PBR28 SUVR in hippocampus also correlated with SDMT test scores ($p < 0.05$).

Relative to HC, MS subjects exhibited cortical thinning (mean $\pm$ SD cortical thickness= MS 2.3 ± 0.1 vs HC 2.4 ± 0.15 mm, $p = 0.05$ adjusting for age) and decreased thalamic volume (mean $\pm$ SD thalamic volume= MS 8252 ± 2280 vs HC 9574 ± 1284 mm 3 , $p = 0.004$ adjusting for age and total intracranial volume). In MS, cortical thinning was related with diffuse inflammation in cortex, thalamus and NAWM ($p < 0.05$ by multilinear regression adjusting for PBR affinity).

Finally, we have included 7T quantitative T_2^* data from some of our RRMS patients enrolled in the present study in a larger sample of patients that includes subjects with SPMS, and very early MS (< 3 years disease duration) to quantify laminar cortical pathology as a function of cortical depth from juxtameningeal cortex towards the gray / white matter boundary, and to assess cortical lesional and perilesional pathology:

- 1) Mainero C; Louapre C; Govindarajan ST; Gianni C; Nielsen AS; Cohen-Adad J; Sloane J, Kinkel RP. A gradient in cortical pathology in multiple sclerosis by in vivo quantitative 7 Tesla imaging. *Brain*, 2015 Apr;138(Pt 4):932-45. PMID: 25681411.
- 2) Louapre C, Govindarajan ST, Gianni C, Langkammer C, Sloane JA, Kinkel RP, Mainero C. Beyond focal cortical lesions in MS: an in vivo quantitative and spatial imaging study at 7 T. *Neurology*, accepted for publication.

We plan to use the same methodology developed in this work to assess cortical lesional and perilesional microglia activation, and investigate the relationship between laminar cortical pathology, in addition to focal cortical lesions, and cortical glial activation as part of Aim1 of this award.

Discussion

Overall, our findings demonstrate in vivo the presence of diffuse microglia and macrophages activation in the brain of subjects with MS. Inflammation, however, was prominent in cortical lesions as well as subcortical gray matter (thalamus, hippocampus, basal ganglia). The use of ultra high resolution T_2^* from 7T MRI scans was crucial for identifying focal cortical lesions, which were otherwise not visible, especially for RRMS, on 3 T images. This is the first time that a specific increase in TSPO binding is reported for cortical lesions in MS, in vivo. Previous PET imaging studies reported increased TSPO expression in whole cortex of MS patients, particularly in progressive MS cases (Politis et al, *Neurology* 2012), though a direct correlation between cortical demyelinating lesions and the presence of activated microglia was not examined. Progressive MS patients compared to earlier RRMS cases tend to have more diffuse cortical pathology (Mainero et al, *Brain* 2015; Louapre et al *Neurology*, in press), not strictly confined to visible focal cortical lesions but also extending to normal appearing cortical tissue. In RRMS cortical pathological changes seem to be focal. Thereby, the finding in these MS stages of increased [^{11}C]-PBR28 uptake in cortical lesions compared to the rest of the normal appearing cortex, further supports the hypothesis that microglia activation and cortical demyelination are strictly related, as suggested by post mortem examinations. These observations need to be confirmed by studying a larger RRMS patients' dataset.

Our data also demonstrate that microglia and macrophages activation, especially in cortex, thalamus, and NAWM, is associated with neurodegeneration, and negatively impacts physical disability and cognitive performance (information processing speed).

Finally, we report increased TSPO expression in subcortical gray matter structures other than the thalamus, including basal ganglia and hippocampus. Our findings are supported by neuropathological studies that recently described extensive microglia activation in relation to demyelination and neurodegeneration in these brain regions (Haider L et al, JNNP 2014; Dutta R et al, Ann Neurol 2011).

4. Impact

A) High-resolution [^{11}C]-PBR28 MR-PET imaging is able to detect in vivo diffuse inflammation in different brain tissue compartments in MS, especially in cortical lesions and subcortical gray matter.

B) There is a close relationship between neuroinflammation, as measured by [^{11}C]-PBR28 uptake, and neurodegeneration as assessed by cortical and subcortical gray matter atrophy.

C) The degree of inflammation in cortex, as measured by [^{11}C]-PBR28 uptake, but also in subcortical gray matter and NAWM, is associated with neurological disability and impaired cognition (information processing speed).

D) High-resolution [^{11}C]-PBR28 MR-PET imaging manifests as a promising tool for assessing in vivo microglia and macrophages activation within different brain tissue compartments in MS, particularly in the cortex, cortical lesions and subcortical gray matter.

5. Changes/Problems

There are no changes in the SOW or study objectives.

6. Products

Publications and presentations:

- 1) Herranz E, Gianni C, Louapre C, Mangeat G, M. Loggia, Treaba CA, Govindarajan ST, Ward N, Sloane JA, Klawiter EC, Ouellette R, Catana C, Hooker JM, Kinkel RP, Mainero C. ^{11}C -PBR28 MR-PET imaging detects in vivo diffuse inflammation in cortex, deep gray and normal appearing white matter associated with neurodegeneration and clinical disability. Selected for Oral Presentation. ECTRIMS, Barcelona, Spain, 2015.
- 2) Mainero C; Louapre C; Govindarajan ST; Gianni C; Nielsen AS; Cohen-Adad J; Sloane J, Kinkel RP. A gradient in cortical pathology in multiple sclerosis by in vivo quantitative 7 Tesla imaging. *Brain*, 2015 Apr;138(Pt 4):932-45. PMID: 25681411.
- 3) Louapre C, Govindarajan ST, Gianni C, Langkammer C, Sloane JA, Kinkel RP, Mainero C. Beyond focal cortical lesions in MS: an in vivo quantitative and spatial imaging study at 7 T. *Neurology*, accepted for publication.

There are no inventions, patents and licenses to report.

7. Participants & Other Collaborating Organizations

Personnel	Role	Percent Effort
Caterina Mainero	Oversight responsibility for the scientific, administrative, and financial aspects of this project. Patients' screening, data collection and analysis, and supervision of post-doctoral fellows involved in these activities.	30.5 %
Celine Louapre	Post-doc, PET and MRI data acquisition, MRI data analysis	50%
Elena Herranz Muelas	Post-doc, MRI and PET data acquisition and analysis, statistical analysis	50%

8. Special Reporting Requirement

Nothing to report.

9. Appendices

Copy of the abstract presented at the ECTRIMS meeting in Barcelona, October 7th -10th 2015.
Copy of the manuscript published on Brain (Mainero et al, Brain 2015).

Abstract Preview - Step 3/4

- print version -

Topic: 20. Imaging

Title: **¹¹C-PBR28 MR-PET imaging detects in vivo diffuse inflammation in cortex, deep gray, and normal appearing white matter associated with neurodegeneration and clinical disability**

Author(s): *E. Herranz^{1,2}, C. Gianni^{1,2}, C. Louapre^{1,2}, G. Mangeat^{1,3}, M. Loggia^{1,2}, C.A. Treaba^{1,2}, S.T. Govindarajan¹, N. Ward¹, J.A. Sloane^{2,4}, E.C. Klawiter^{1,2,5}, R. Ouellette¹, C. Catana^{1,2}, J.M. Hooker^{1,2}, R.P. Kinkel⁶, C. Mainero^{1,2}*

Institute(s): ¹A. A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, ²Harvard Medical School, Boston, MA, United States, ³Institute of Biomedical Engineering, Polytechnique Montreal, Montreal, QC, Canada, ⁴Beth Israel Deaconess Medical Center, ⁵Department of Neurology, Massachusetts General Hospital, Boston, MA, ⁶University of California, San Diego, CA, United States

Text: **Background:** Neuropathology studies of multiple sclerosis (MS) report diffuse inflammation in cortex, deep gray matter (DGM) and normal appearing white matter (NAWM), which may be associated with neurodegeneration and MS progression.

Goals: To image, using ¹¹C-PBR28, expression of the translocator protein (TSPO) in activated microglia and macrophages in cortex, DGM, and WM of a heterogeneous MS cohort; to explore the relationship between inflammation, neurodegeneration, Expanded Disability Status Scale (EDSS) and Symbol Digit Modalities Test (SDMT) scores.

Methods: Seventeen patients (11 SPMS, 6 RRMS; mean±SD age=50.2±9.6 years; median, range EDSS=6, 2-7.5) and 12 age- and TSPO affinity binding matched healthy controls (HC) underwent 90-minutes of ¹¹C-PBR28 MR-PET. Anatomical MR scans were simultaneously acquired for: a) FreeSurfer cortical surface reconstruction, cortical thickness (CT) measurement; b) FIRST-FSL DGM segmentation (thalami, putamen, caudate, hippocampi) and volume estimation; c) MR-PET image registration. Standardized uptake value (SUV) maps were created for 60-90-minute PET frame (1.25 mm voxel size). In patients, WM and cortical lesions (CL, 6 MS only) were segmented on 7T T₂* images from a separate scan. Lesional, whole cortex, DGM, NAWM masks were co-registered to ¹¹C-PBR28 maps to extract SUVs. In HC, SUVs were obtained from cortex, DGM, WM. Linear regression models were used to compare SUVs, CT and DGM volumes in MS vs HC, and assess the relationship between SUVs, CT, DGM atrophy, EDSS and SDMT. Age, gender, TSPO affinity and intracranial volume were included as regressors when appropriate.

Results: Relative to controls, patients had increased ¹¹C-PBR28 SUVs in whole cortex (~19%, p=0.03), CL (~51%, p=0.01) and DGM (thalami: ~56%, p=0.005; hippocampi: ~35%, p=0.001; putamen: ~29%, p=0.004; caudate: ~22%, p=0.01). Higher SUVs were also detected in NAWM (~24% p=0.02) while increase in WM lesions was modest (~10%). Patients had decreased mean CT (p=0.05) and thalamic volume (p=0.004) vs HC. Cortical thinning correlated with higher SUVs in thalami (p=0.0006), NAWM (p=0.0009), cortex (p=0.004). The higher the EDSS, the greater SUVs in thalami (p=0.003), hippocampi (p=0.04), and NAWM (p=0.04) were. In SPMS only, lower SDMT scores related with cortical (p=0.04), and NAWM (p=0.04) increased SUVs.

Conclusions: ¹¹C-PBR28 PET shows *in vivo* that glial activation is diffuse in MS, and closely linked to neurodegeneration and poor clinical outcome.

Disclosure: This study was supported by Claflin Award; NMSS RG 4729A2/1, US Army W81XWH-13-1-0112.

EH, GM, ML, CAT, STG, NW, JAS, RO, CC, JMH, CM: no disclosure

Dr Gianni has received a fellowship from FISM 2012/B/4

Dr Louapre received a fellowship from ARSEP foundation

Dr Klawiter has received consulting fees from Biogen Idec and Mallinckrodt Pharmaceuticals and research funding from Roche and Atlas5d

Dr. Kinkel reports personal fees from Genzyme ; A Sanofi Corp, personal fees from Biogen Idec, grants from Accelerated Cure Project, personal fees from Novartis, outside the submitted work; .

Travel Grant / Young Scientific Investigator's Sessions: **I wish to apply for Travel Grant**
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I attest that I am not being sponsored or covered by any other sources
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Date of birth: 27/6/1980
Proof of age: 1160-POA-1432053763.pdf

Preferred Presentation Type: **Oral or poster presentation**

A gradient in cortical pathology in multiple sclerosis by *in vivo* quantitative 7 T imaging

Caterina Mainero,^{1,2,*} Céline Louapre,^{1,2,*} Sindhuja T. Govindarajan,¹ Costanza Gianni,^{1,2}
A. Scott Nielsen,^{2,3,4} Julien Cohen-Adad,^{1,5} Jacob Sloane^{2,3} and Revere P. Kinkel^{2,3,6}

See Barkhof for a scientific commentary on this article (doi:10.1093/brain/awv031).

*These authors contributed equally to this work.

We used a surface-based analysis of T_2^* relaxation rates at 7 T magnetic resonance imaging, which allows sampling quantitative T_2^* throughout the cortical width, to map *in vivo* the spatial distribution of intracortical pathology in multiple sclerosis. Ultra-high resolution quantitative T_2^* maps were obtained in 10 subjects with clinically isolated syndrome/early multiple sclerosis (≤ 3 years disease duration), 18 subjects with relapsing-remitting multiple sclerosis (≥ 4 years disease duration), 13 subjects with secondary progressive multiple sclerosis, and in 17 age-matched healthy controls. Quantitative T_2^* maps were registered to anatomical cortical surfaces for sampling T_2^* at 25%, 50% and 75% depth from the pial surface. Differences in laminar quantitative T_2^* between each patient group and controls were assessed using general linear model ($P < 0.05$ corrected for multiple comparisons). In all 41 multiple sclerosis cases, we tested for associations between laminar quantitative T_2^* , neurological disability, Multiple Sclerosis Severity Score, cortical thickness, and white matter lesions. In patients, we measured T_2^* in intracortical lesions and in the intracortical portion of leukocortical lesions visually detected on 7 T scans. Cortical lesional T_2^* was compared with patients' normal-appearing cortical grey matter T_2^* (paired *t*-test) and with mean cortical T_2^* in controls (linear regression using age as nuisance factor). Subjects with multiple sclerosis exhibited relative to controls, independent from cortical thickness, significantly increased T_2^* , consistent with cortical myelin and iron loss. In early disease, T_2^* changes were focal and mainly confined at 25% depth, and in cortical sulci. In later disease stages T_2^* changes involved deeper cortical laminae, multiple cortical areas and gyri. In patients, T_2^* in intracortical and leukocortical lesions was increased compared with normal-appearing cortical grey matter ($P < 10^{-10}$ and $P < 10^{-7}$), and mean cortical T_2^* in controls ($P < 10^{-5}$ and $P < 10^{-6}$). In secondary progressive multiple sclerosis, T_2^* in normal-appearing cortical grey matter was significantly increased relative to controls ($P < 0.001$). Laminar T_2^* changes may, thus, result from cortical pathology within and outside focal cortical lesions. Neurological disability and Multiple Sclerosis Severity Score correlated each with the degree of laminar quantitative T_2^* changes, independently from white matter lesions, the greatest association being at 25% depth, while they did not correlate with cortical thickness and volume. These findings demonstrate a gradient in the expression of cortical pathology throughout stages of multiple sclerosis, which was associated with worse disability and provides *in vivo* evidence for the existence of a cortical pathological process driven from the pial surface.

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Keywords: multiple sclerosis; subpial demyelination; quantitative T_2^* ; 7 T MRI; sulci; gyri

Abbreviations: EDSS = Expanded Disability Status Scale; GLM = general linear model; MSSS = Multiple Sclerosis Severity Score; NACGM = normal-appearing cortical grey matter; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis

Introduction

Multiple sclerosis is an inflammatory demyelinating and neurodegenerative disorder of the CNS, and the leading cause of non-traumatic disability in young adults in Western countries. Histopathological examinations of multiple sclerosis brains indicate that subpial demyelinating lesions, which extend intracortically from the pia mater without reaching the white matter, are potential biomarkers of multiple sclerosis progression (Peterson *et al.*, 2001; Magliozzi *et al.*, 2010; Reynolds *et al.*, 2011).

As cortical lesions appeared topographically related to focal meningeal inflammation in some pathological studies of chronic progressive multiple sclerosis (Magliozzi *et al.*, 2007, 2010; Howell *et al.*, 2011), it has been hypothesized that cortical demyelination in multiple sclerosis may be driven by organized meningeal inflammation, accompanied by a decreasing gradient of demyelination away from the pial surface. Histopathological evidence that the cortex can be the site of inflammatory demyelinating lesions near the time of multiple sclerosis onset (Lucchinetti *et al.*, 2011) further supports the existence of an early pathological process that primarily targets the cortex, independently from white matter.

Although largely undetected on conventional MRI scans, cortical lesions, including the subpial type, have been imaged *in vivo* with improved sensitivity and spatial specificity at ultra-high field 7 T MRI (Filippi *et al.*, 2014). We previously showed that a surface-based analysis of cortical T_2^* -weighted signal from 7 T T_2^* gradient-echo images combined with a multichannel radiofrequency coil can disclose subpial T_2^* signal abnormalities in subjects with multiple sclerosis relative to healthy subjects (Cohen-Adad *et al.*, 2011), providing *in vivo* evidence for the existence of diffuse subpial pathology in multiple sclerosis, previously reported only post-mortem.

Surface-based estimation of cortical T_2^* relaxation rates provides a quantitative estimate of the biophysical changes underlying tissue integrity (Cohen-Adad *et al.*, 2012). This measure is less dependent of the technical limitations that affect measurements of T_2^* -weighted signal at ultra-high field MRI, and of potential biases that arise from cortical lesion detection based on visual inspection of scans. In the healthy brain, T_2^* relaxation time ($1/R_2^*$) inversely correlates with myelin and iron content (Langkammer *et al.*, 2010; Li *et al.*, 2011). In both white matter and cortical

multiple sclerosis lesions, histopathological–magnetic resonance correlations showed that demyelination and iron loss induce an increase in T_2^* relaxation time (Yao *et al.*, 2012, 2014), whereas iron accumulation relates to shorter T_2^* (Bagnato *et al.*, 2011).

We demonstrated that surface-based mapping of quantitative T_2^* as a function of cortical depth (laminar analysis) from ultra-high resolution gradient echo 7 T MRI images is highly reproducible (Govindarajan *et al.*, 2015) and could prove useful for studying the myelo-architecture of the cortex *in vivo* (Cohen-Adad *et al.*, 2012), and for characterizing conditions associated with changes in cortical myelin and/or iron concentration.

In this study, we used a surface-based laminar analysis of 7 T quantitative T_2^* to test the following hypotheses: (i) cortical pathology in multiple sclerosis is associated with changes in quantitative T_2^* , which differ across disease stages: quantitative T_2^* abnormalities mainly involve the outer cortical layers and sulci in early disease; while they can also be detected in deeper cortical layers and gyri in later stages and progressive multiple sclerosis; and (ii) neurological disability and multiple sclerosis severity correlate with the degree of laminar quantitative T_2^* abnormalities. A further aim was to investigate the contribution of cortical laminar pathology, as measured by quantitative T_2^* , to cortical thinning in multiple sclerosis. Finally, we measured quantitative T_2^* in multiple sclerosis cortical lesions, detected visually on 7 T scans, as well as in normal-appearing cortical grey matter (NACGM), to better understand their role in determining laminar quantitative T_2^* changes in multiple sclerosis.

Materials and methods

Subjects

Forty-one patients [29 females; mean age = 43.2 ± 8.8 standard deviation (SD) years] were prospectively included in the study. Eligibility criteria were: age between 18 and 60 years, a diagnosis of clinically isolated syndrome or multiple sclerosis (McDonald *et al.*, 2001). Patients were enrolled according to three disease phenotypic categories: (i) clinically isolated syndrome - early multiple sclerosis ($n = 10$): ≤ 3 years disease duration; (ii) relapsing remitting multiple sclerosis (RRMS, $n = 18$) with ≥ 4 years disease duration; and (iii) secondary progressive multiple sclerosis (SPMS, $n = 13$) (Lublin and Reingold, 1996).

Exclusion criteria for multiple sclerosis subjects included a clinical relapse within 3 months of enrolment, corticosteroid therapy within 1 month of scanning, and other neurologic and/or significant psychiatric disease. Neurological disability in patients was assessed using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). The Multiple Sclerosis Severity Score (MSSS) was calculated on each patient using their EDSS and duration from onset of multiple sclerosis symptoms, as previously detailed (Roxburgh *et al.*, 2005). Thirty-three of 41 patients were on treatment with disease-modifying agents for at least 6 months, while the remaining eight subjects were not receiving any therapy for multiple sclerosis.

Seventeen age-matched healthy volunteers (nine females; mean age = 39.3 ± 8.8 SD years) served as controls. General exclusion criteria included significant psychiatric and/or neurological disease (other than multiple sclerosis for patients), major medical comorbidity, pregnancy, and contraindications for MRI.

Subjects gave their written informed consent to participate in the study and the local Ethics Committee of our Institution approved the study procedures.

MRI data acquisition

All subjects underwent, on a 7 T Siemens whole-body scanner using a custom-built 32-channel phased array coil, acquisition of: (i) multi-echo 2D FLASH T_2^* -weighted spoiled gradient-echo imaging, repetition time = 2210 ms, echo time = $6.44 + 3.32n$ [$n = 0, \dots, 11$] ms, flip angle = 55° , two slabs of 40 slices each to cover the supratentorial brain, field of view = $192 \times 168 \text{ mm}^2$, resolution = $0.33 \times 0.33 \times 1 \text{ mm}^3$ (25% gap), bandwidth = 335 Hz/pixel, acquisition time for each slab = ~ 10 min; (ii) a T_1 -weighted 3D magnetization-prepared rapid acquisition gradient echo sequence (MPRAGE, repetition time/inversion time/echo time = 2600/1100/3.26 ms, flip angle = 9° , field of view = $174 \times 192 \text{ mm}^2$, resolution = $0.60 \times 0.60 \times 1.5 \text{ mm}^3$, bandwidth = 200 Hz/pixel, acquisition time = 5.5 min) for co-registration of 7 T gradient-echo data with cortical surfaces; and (iii) a single-echo 2D FLASH T_2^* -weighted spoiled gradient-echo pulse sequence (repetition time/echo time = 1700/21.8 ms, the other parameters being identical to the multi-echo 2D FLASH T_2^* sequence).

In addition to the 7 T session, all subjects were scanned once on a 3 T Siemens scanner (Tim Trio) using the Siemens 32-channel coil to acquire a structural scan with a 3D magnetization-prepared rapid acquisition with multiple gradient echoes (MEMPR) [repetition time/inversion time = 2530/1200 ms, echo time = (1.7, 3.6, 5.4, 7.3) ms, flip angle = 7° , field of view = $230 \times 230 \text{ mm}^2$, resolution = $0.9 \times 0.9 \times 0.9 \text{ mm}^3$, bandwidth = 651 Hz/pixel, acquisition time = ~ 6.5 min] for cortical surface reconstruction, co-registration with 7 T data, and cortical thickness and cortical volume estimation.

MRI data processing

White matter lesion volume

White matter lesion volume (mm^3) was assessed from white matter lesions segmented on magnitude images from 7 T single-echo FLASH T_2^* scans using a semi-automated

method implemented in 3D Slicer version 4.2.0 (<http://www.slicer.org>).

Cortical surface reconstruction, cortical thickness and volume estimation

Pial and white matter surfaces, cortical thickness maps, and cortical volumes were obtained using the software FreeSurfer, version 5.3.0 (<http://surfer.nmr.mgh.harvard.edu/>), according to a multi-step procedure that calculates the grey matter/white matter border and the CSF/grey matter (pial) border in the 3D MEMPR volume (Dale *et al.*, 1999). Topological defects in cortical surfaces due to white matter and leukocortical lesions were corrected using a semi-automated procedure with lesions filling.

Mean cortical thickness was measured in each subject as the distance (mm) between the grey matter/white matter boundary and the pial surface (Fischl and Dale, 2000). For each subject, we also estimated the mean normalized cortical volume defined as the ratio between mean cortical volume and total intracranial volume as assessed in FreeSurfer.

For vertex-by-vertex surface-based cortical thickness analyses across subjects' groups, each individual subject's surface was smoothed using a 10 mm full-width at half-maximum Gaussian kernel, and subsequently registered to a surface template 'fsaverage' using FreeSurfer.

Quantitative T_2^* mapping along the cortex

T_2^* signal was corrected at each voxel for susceptibility-induced through-slice dephasing as described previously (Cohen-Adad *et al.*, 2012). A Levenberg–Marquardt non-linear regression model was then used to fit voxel-wise the corrected T_2^* signal versus echo time; R^2 goodness of fit was measured, and voxels with poor fit ($R^2 < 0.8$) were excluded from further analyses. Poor fits were typically present in lower brain regions including the temporal pole, and in regions at the tissue/air interface (close to the sinuses).

Each individual T_2^* map was registered to the corresponding 3 T cortical surface using a boundary-based registration algorithm (Greve and Fischl, 2009), as previously detailed (Cohen-Adad *et al.*, 2012). The registered T_2^* data were concatenated into a whole brain volume using FreeSurfer tools and resampled at 0.33 mm^3 isotropic resolution.

T_2^* was sampled along the entire cortex in right and left hemispheres at 25%, 50% and 75% depth from the pial surface (0% depth) towards grey matter/white matter boundary (100% depth) over the surface of each individual subject, and smoothed along the cortical surface using a 5 mm full-width at half-maximum Gaussian kernel. Given that cortical thickness varies throughout the cortex, depth was not defined as an absolute distance between the pial and the grey matter/white matter boundary, but rather as a relative distance between the pial and white matter surface. We used an equidistant model for sampling quantitative T_2^* within the cortex as it has shown excellent reproducibility (Govindarajan *et al.*, 2015), and has been found comparable to equivolume modelling when investigating *in vivo* data with spatial resolution similar to those used in our study (Waehnert *et al.*, 2014).

Because it is unclear whether leukocortical lesions, which extend across cortex and white matter but do not reach the pial surface, originate from cortex or white matter, such lesions were not masked in quantitative T_2^* maps.

For group analyses, all subjects' surfaces were then registered to the surface template 'fsaverage' using FreeSurfer.

Quantitative T_2^* in focal cortical lesions

Using Slicer, two raters, blinded to patients' demographic and clinical data, segmented by consensus, on magnitude images from 7 T single-echo FLASH T_2^* , focal cortical multiple sclerosis lesions that appeared as focal cortical hyperintensities extending for at least three voxels and across two consecutive slices. In subjects with multiple sclerosis, focal cortical lesions were characterized as (i) intracortical lesions including lesions originating from the pial surface and extending at different depths throughout the cortical width (type III-IV lesions), and type II lesions (Peterson *et al.*, 2001; Bo *et al.*, 2003b); and (ii) leukocortical lesions extending through grey matter/white matter without reaching the pial surface. Areas of NACGM were also identified in each multiple sclerosis subject throughout the cortex.

Subsequently, in each subject, intracortical lesions, leukocortical lesions and NACGM masks were created and coregistered, using a boundary-based registration method (Greve and Fischl, 2009), to the corresponding cortical quantitative T_2^* maps for estimating mean quantitative T_2^* (ms) in each mask. Given that the focus of the study was the cortex, for leukocortical lesions quantitative T_2^* was measured only in the intracortical portion of the mask.

Statistical methods

A general linear model (GLM) was run on a vertex-by-vertex basis across the whole cortex, using FreeSurfer tools, to assess: (i) differences in quantitative T_2^* at each depth from the pial surface (25%, 50%, 75%) between controls and the three subgroups of patients (early multiple sclerosis, RRMS, SPMS); (ii) the relationship, in all patients, between quantitative T_2^* at 25%, 50% and 75% depth and EDSS and MSSS; (iii) cortical thickness differences between patients and controls; and (iv) the relationship between quantitative T_2^* at each depth and cortical thickness across the whole cortex using the per-vertex regressor option in FreeSurfer, which allows testing at the vertex level for possible correlations between two imaging modalities. Age was used as a nuisance factor (covariate of no interest) in all GLM analyses.

For all surface-based group analyses we performed a clusterwise correction for multiple comparisons using a Monte-Carlo simulation with 10 000 iterations (Hagler *et al.*, 2006). Localization of significant clusters across the cortex was performed using the Desikan atlas in FreeSurfer.

For comparisons of conventional MRI metrics (whole mean cortical thickness, normalized cortical volume, and white matter lesion volume) across patients' groups relative to controls and correlations with clinical variables, analysis of covariance (ANCOVA) controlled for age, and Spearman rank correlation coefficient were performed using the software R (version 2.13.1). In patients, differences between mean quantitative T_2^* in intracortical lesions, and leukocortical lesions relative to mean quantitative T_2^* in NACGM were assessed using paired *t*-test; differences between mean quantitative T_2^* in intracortical lesions, leukocortical lesions, NACGM in patients and mean cortical quantitative T_2^* in healthy subjects were assessed using linear regression using age as covariate of

no interest (R software, version 2.13.1). For all analyses, statistically significant threshold was set at P -value < 0.05

Sulci and gyri analysis

Using the FreeSurfer masks of gyri and sulci, we assessed whether quantitative T_2^* changes in patients preferentially involved cortical sulci or cortical gyri. The masks of gyri and sulci were applied to all clusters that exhibited significant differences in quantitative T_2^* at the GLM (corrected $P < 0.05$) in each patient subgroup (early multiple sclerosis, RRMS, SPMS) relative to controls, at each depth (25%, 50%, 75%). In each mask the surface area (mm^2) of vertices exhibiting significant quantitative T_2^* changes was computed. For comparison, given that the surface area of sulci is $\sim 14\%$ smaller than the surface area of gyri, the surface areas (mm^2) of significant quantitative T_2^* changes in sulci and gyri were normalized by the total cortical surface area.

Results

Participants' demographics, including EDSS and MSSS in patients, and conventional MRI metrics are reported in Table 1.

Laminar quantitative T_2^* in multiple sclerosis across the cortex

Figure 1 and Supplementary Table 1 illustrate the results of the GLM laminar analysis comparing 7 T quantitative T_2^* at 25%, 50% and 75% depth from the pial surface in the earliest disease stages, RRMS and SPMS relative to healthy subjects, and the overlap of significant clusters across the three patients' groups. In all subgroups of patients we observed, relative to controls and in both hemispheres, a significant increase in T_2^* relaxation time (clusterwise $P < 0.05$, corrected for multiple comparisons), consistent with myelin and iron loss. Scattered small clusters of shorter quantitative T_2^* , suggestive of increased susceptibility effects and/or increased iron content, were seen in each disease group mainly in frontal and temporal areas (Supplementary Table 1).

In early multiple sclerosis, significant clusters of longer T_2^* were mainly located in the outer cortical layers, at 25% depth from the pial surface, in the rostral anterior cingulate and parietal regions, as well as in the precentral and postcentral cortex. Fewer clusters of longer T_2^* were present in deeper cortical laminae, specifically in the postcentral, and occipital cortex at 50% depth, whereas there was only one cluster of increased quantitative T_2^* in the calcarine cortex at 75% depth (Supplementary Table 1). Subjects with RRMS and longer disease duration showed a discrete involvement of all cortical layers across frontal, parietal, occipital regions, as well as in the isthmus cingulate and temporal cortex (Supplementary Table 1). Subjects with SPMS showed diffuse cortical involvement at all depths throughout the cortical mantle (Supplementary Table 1).

Table 1 Demographic and conventional MRI characteristics of study subjects

	Controls	MS patients	Early MS	Late RRMS	SPMS
<i>n</i>	17	41	10	18	13
Gender (M/F)	8/9	12/29	2/8	5/13	5/8
Age, years mean (SD)	39.3 (8.8)	43.2 (8.8)	38.3 (10.5)	44.3 (7.6)	45.5 (8.1)
Disease duration, years, mean (SD)	–	11.5 (8.0)	2.8 (1.1)	11.4 (4.3)	18.5 (8.4)
EDSS score, mean (SD)	–	3.3 (1.8)	2.2 (0.8)	2.5 (1.2)	5.2 (1.6)
MSSS, mean (SD)	–	4.3 (2.4)	5.0 (1.8)	2.9 (2.0)	5.5 (2.4)
CT, mm mean (SD)	2.5 (0.09)	2.40 (0.11) ^a	2.43 (0.09)	2.42 (0.10)	2.35 (0.14) ^b
NCV, mean (SD)	0.33 (0.06)	0.29 (0.02) ^c	0.30 (0.02)	0.29 (0.02) ^d	0.28 (0.03) ^e
WMLV, cm ³ mean (SD)	–	5.7 (9.1)	1.7 (2.2)	2.5 (2.4)	13.4 (13.3)
ICL, <i>n</i> median (range)	–	6 (0–55)	5 (0–22)	4.5 (0–23)	30 (2–55)
LCL, <i>n</i> median (range)	–	3 (0–137)	0.5 (0–18)	1.5 (0–18)	37 (0–137)

MS = multiple sclerosis; CT = cortical thickness; NCV = normalized cortical volume; ICL = intracortical lesions; LCL = leukocortical lesions; WMLV = white matter lesion volume.
^a $P < 0.02$ = all multiple sclerosis versus controls; ^b $P < 0.02$ = SPMS versus controls; ^c $P < 0.005$ = all multiple sclerosis versus controls; ^d $P = 0.08$, RRMS versus controls; ^e $P = 0.07$, SPMS versus controls, by ANCOVA.

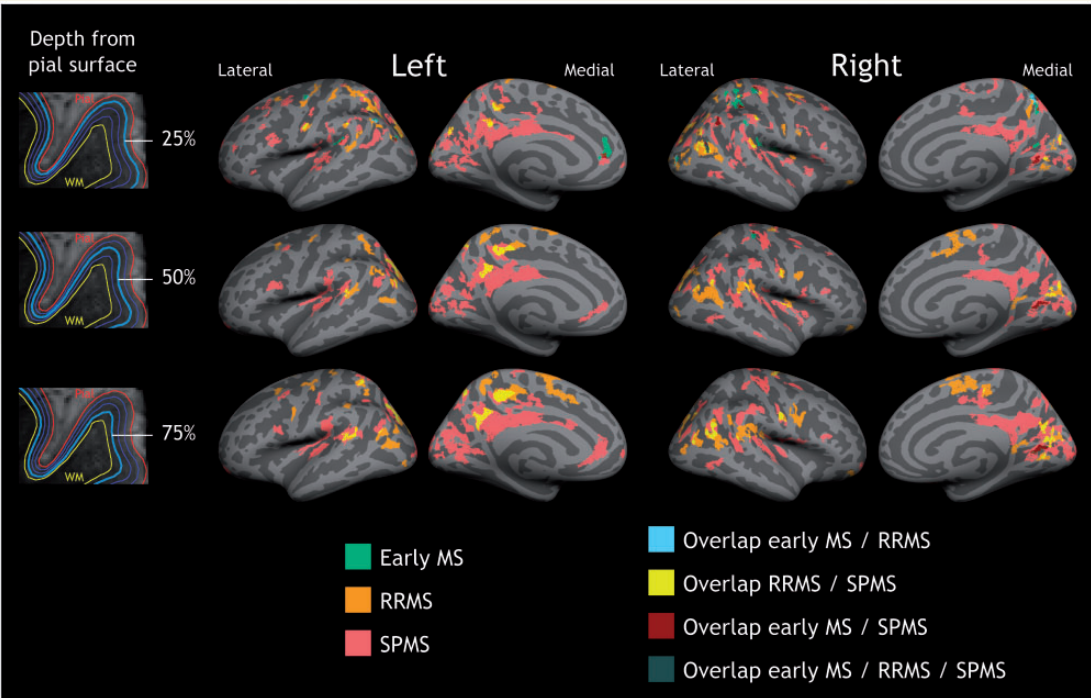


Figure 1 Quantitative T_2^* differences between multiple sclerosis patients and controls. Overlay of the GLM significance maps ($P < 0.05$ corrected for multiple comparisons) on the average pial surface showing in the three subgroups of patients, early multiple sclerosis (MS), RRMS and SPMS, clusters of increased T_2^* relaxation time relative to healthy controls at 25% (top row), 50% (middle row), and 75% depth (bottom row) from the pial surface, as well as overlap of significant clusters across patients' subgroups. In early disease, quantitative T_2^* changes were focal and mainly confined to the juxtameningeal cortex, and cortical sulci (darker grey areas); in RRMS and SPMS quantitative T_2^* changes involved deeper cortical laminae, and multiple cortical areas. WM = white matter.

In patients, the surface area of increased quantitative T_2^* relative to controls (normalized by the total cortical surface area) was greater in cortical sulci than in gyri, across all cortical depths in early multiple sclerosis and SPMS, and at 25% depth in RRMS (Fig. 2). The greater involvement of cortical sulci relative to gyri was prominent in early disease.

Quantitative T_2^* in focal cortical lesions and normal-appearing cortical grey matter

Cortical lesions counts in all multiple sclerosis subjects and in each multiple sclerosis subgroup are shown in Table 1.

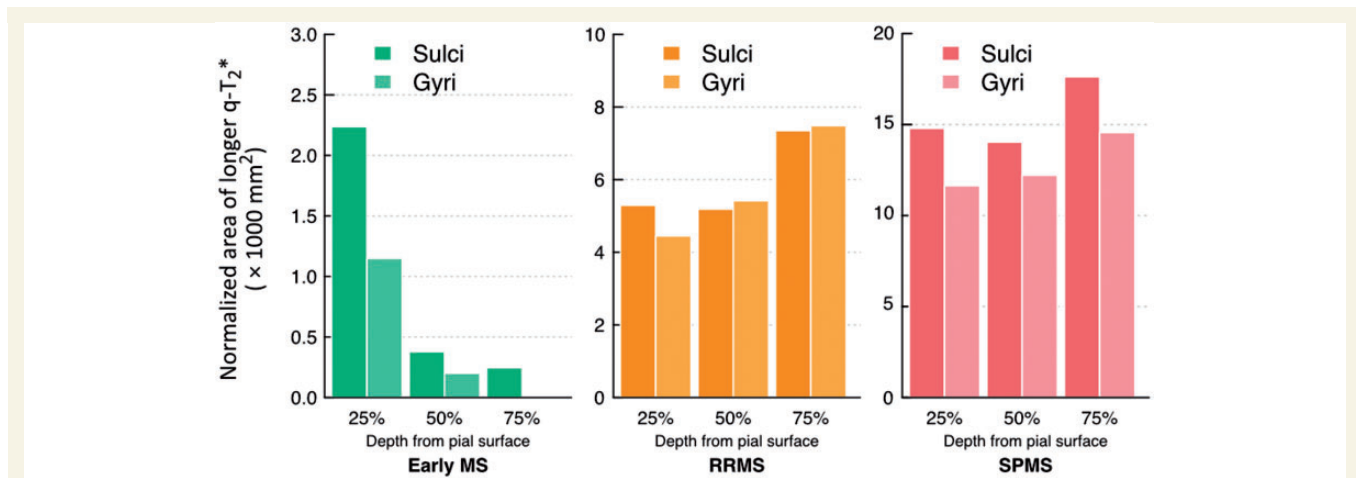


Figure 2 Distribution of T_2^* changes in patients in cortical sulci and gyri. Diagram illustrating the distribution of vertices exhibiting increased T_2^* relaxation time in subjects with early multiple sclerosis, RRMS and SPMS, relative to controls, at 25%, 50% and 75% depth from the pial surface, across cortical sulci and cortical gyri, and expressed as the total surface area (mm^2) in significant sulcal or gyri vertices normalized by the total cortical surface area. $q\text{-}T_2^*$ = quantitative T_2^* .

In all patients, mean quantitative T_2^* in intracortical lesions (40.2 ± 5.1 ms) and leukocortical lesions (40.1 ± 4.7 ms) was significantly higher than mean NACGM quantitative T_2^* (33.5 ± 2.5 ms) ($P < 10^{-10}$ and $P < 10^{-7}$ by paired t -test), and than mean cortical quantitative T_2^* (33.03 ± 1.6 ms) in controls ($P < 10^{-5}$ and $P < 10^{-6}$ by linear regression). This comparison remained significant in each multiple sclerosis subgroup (Supplementary Table 2). Although there were no significant differences between NACGM quantitative T_2^* in all patients and mean cortical quantitative T_2^* in controls, NACGM quantitative T_2^* in SPMS cases was significantly increased relative to cortical quantitative T_2^* from healthy subjects (Supplementary Table 2).

Cortical thickness and laminar quantitative T_2^*

Overall, patients ($n = 41$) showed significantly decreased mean cortical thickness and normalized cortical volume (ie. entire cortex) relative to healthy subjects ($P < 0.02$ and $P < 0.005$ by ANCOVA, Table 1). Significant cortical thinning was also observed in SPMS subjects ($P < 0.02$ by ANCOVA, Table 1), and there was a trend towards significance for decreased normalized cortical volume in the RRMS and SPMS subgroups ($P < 0.08$ and $P < 0.07$ by ANCOVA, Table 1). The observed decrease in mean global cortical thickness and normalized cortical volume in the entire multiple sclerosis group ($n = 41$) relative to controls was not exclusively driven by SPMS subjects, as we also observed significant cortical thinning and cortical volume loss in early multiple sclerosis and RRMS when grouped together ($P < 0.02$ and $P < 0.05$ by ANCOVA, data not shown).

The GLM analysis between patients and controls did not disclose significant regional changes in cortical thickness after correction for multiple comparisons. We found that in patients, in several regions of both hemispheres, thinning of the cortex corresponded to longer T_2^* ($P < 0.05$ corrected) at all three depths from the pial surface (Fig. 3 and Table 2). Because of the negative correlation in subjects with multiple sclerosis between laminar quantitative T_2^* and cortical thickness across several cortical areas, we assessed differences in laminar quantitative T_2^* (25%, 50% and 75% depth) between each patients' subgroup and controls using a GLM with per-vertex regression of cortical thickness as a covariate of no interest (nuisance factor), along with age. Increase in quantitative T_2^* across disease stages and depths remained significant ($P < 0.05$ corrected) and substantially unchanged after regressing out cortical thickness (Fig. 4).

Correlation with clinical measures

We did not find any relation between either regional or global cortical thickness and normalized cortical volume and either EDSS or MSSS, whereas white matter lesion volume was positively associated with EDSS ($P < 0.008$ by Spearman test) but not with MSSS.

Figure 5 shows the results of the GLM analysis investigating the relationship between both EDSS and MSSS in all patients and laminar quantitative T_2^* at all three depths (25%, 50%, 75%) from the pial surface. There was a positive correlation ($P < 0.05$ corrected) between quantitative T_2^* and both EDSS and MSSS at all depths, the greatest association being observed at 25% depth from the pial surface for both EDSS and MSSS (Table 3). Across all cortical depths, clusters showing a significant correlation between quantitative T_2^* and EDSS and MSSS were mostly

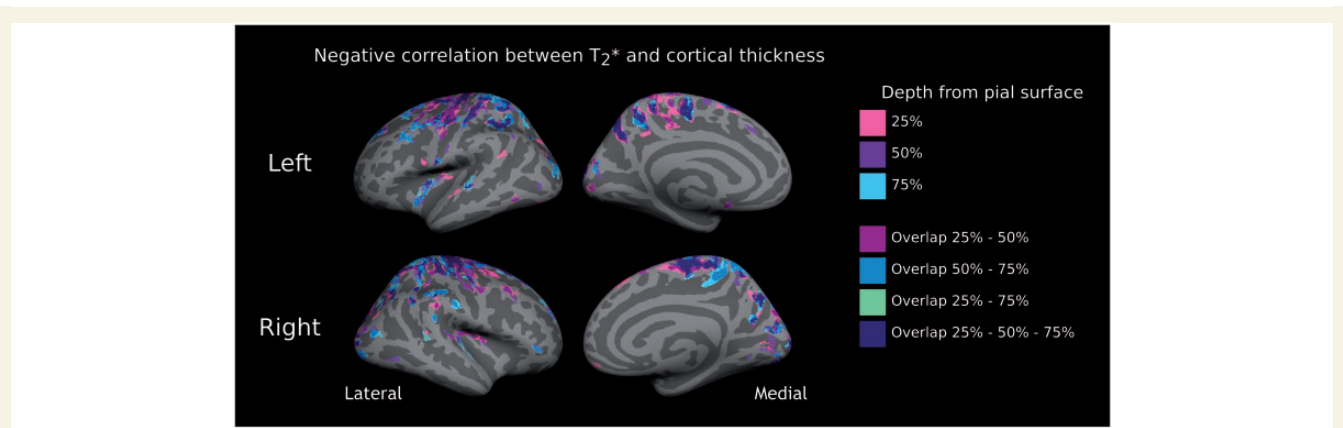


Figure 3 Relationship between laminar T_2^* and cortical thickness in patients. Overlay of the GLM significance maps ($P < 0.05$ corrected for multiple comparisons) on the average pial surface for left (top) and right (bottom) hemispheres showing, in the whole group of subjects with multiple sclerosis ($n = 41$), clusters exhibiting a negative correlation between T_2^* relaxation time at 25%, 50% and 75% depth from the pial surface and cortical thickness, as well as significant clusters overlapping across the three depths.

Table 2 Location of clusters exhibiting a significant negative correlation ($P < 0.05$, corrected) between 7 T T_2^* relaxation time at 25%, 50% and 75% depth from the pial surface and cortical thickness in 41 subjects with multiple sclerosis

	Left hemisphere	Right hemisphere
T_2^* 25%	Medial orbitofrontal - caudal middle frontal - superior frontal Precentral - postcentral Insula - superior temporal - inferior temporal Superior parietal - inferior parietal - supramarginal - precuneus - posterior cingulate Lateral occipital - cuneus - fusiform	Medial orbitofrontal - caudal middle frontal - superior frontal Paracentral - precentral - postcentral Superior temporal - bankssts Superior parietal - inferior parietal Supramarginal - precuneus Lateral occipital - cuneus - pericalcarine - lingual
T_2^* 50%	Medial orbitofrontal - caudal middle frontal - superior frontal Paracentral - precentral - postcentral Insula - superior temporal - inferior temporal Superior parietal - inferior parietal Supramarginal - precuneus Lateral occipital - cuneus - lingual	Pars triangularis - rostral middle frontal Caudal middle frontal Paracentral Insula - superior temporal - transverse temporal Superior parietal - inferior parietal - supramarginal - precuneus Lateral occipital - pericalcarine - lingual
T_2^* 75%	Caudal middle frontal - superior frontal Paracentral - precentral - postcentral Insula - superior temporal - inferior temporal Superior parietal - inferior parietal - supramarginal Precuneus - cuneus - lateral occipital	Pars triangularis - rostral middle frontal Superior frontal Precentral - postcentral Superior temporal - bankssts Superior parietal - inferior parietal - supramarginal - precuneus Lateral occipital - cuneus

Bankssts = banks of the superior temporal sulcus.

located in the sensorimotor cortex, though other significant clusters were observed in the insula, cingulate, prefrontal, parietal, and temporal cortex, and this was mainly observed at 25% depth. The significant association between EDSS and laminar quantitative T_2^* remained significant after including in the GLM analysis, in addition to age, white matter lesion volume as a nuisance variable (covariate of no interest) (Fig. 5 and Table 3).

Discussion

Subpial lesions are thought to constitute a major pathological substrate for disease progression in multiple sclerosis. Ultra-high field MRI enables improved detection and classification of focal cortical lesions in multiple sclerosis relative to lower field (≤ 3 T) MRI (Filippi *et al.*, 2014), but *in vivo* quantification of disseminated subpial

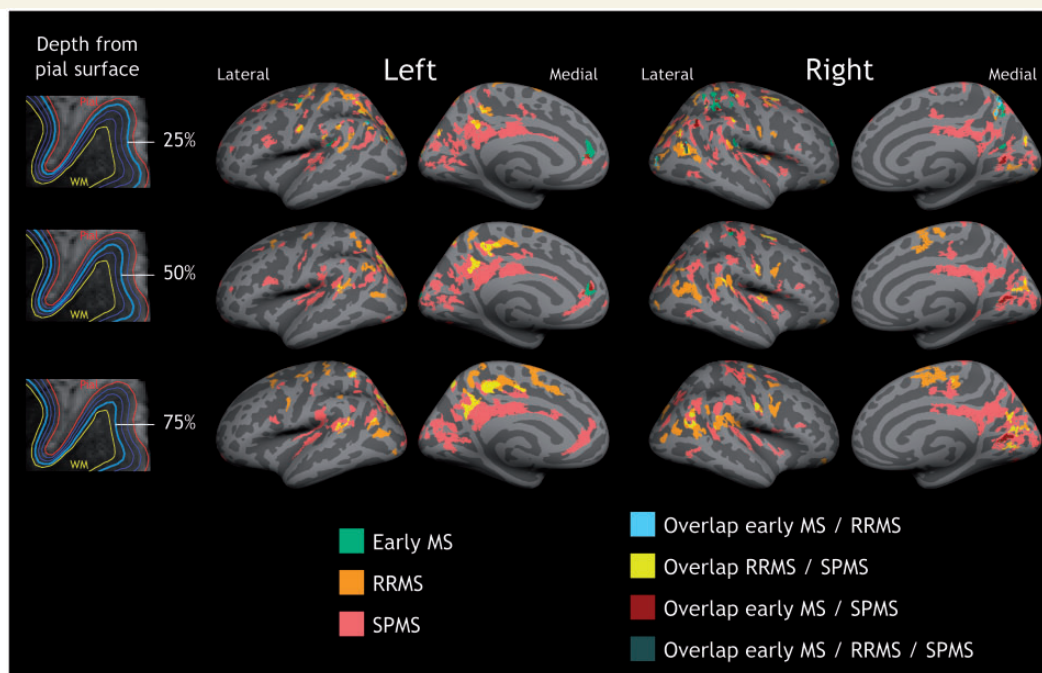


Figure 4 Quantitative T_2^* differences between multiple sclerosis patients and controls independent from cortical thickness. Overlay of the GLM significance maps ($P < 0.05$ corrected for multiple comparisons) on the average pial surface showing in early multiple sclerosis (MS), RRMS and SPMS, clusters of increased T_2^* relaxation time relative to healthy controls at 25%, 50% and 75% depth from the pial surface, after including in the GLM cortical thickness at the vertex level as a covariate of no interest, along with age. WM = white matter.

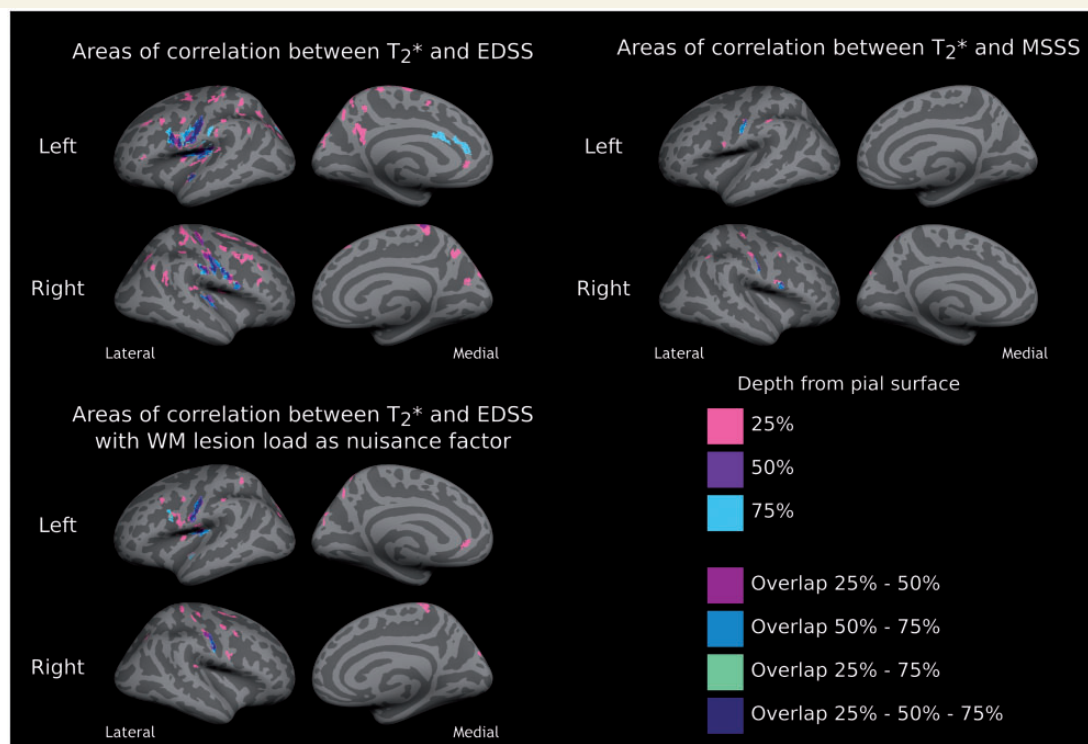


Figure 5 Relationship between quantitative T_2^* , disability and disease severity. Overlay of the GLM significance maps ($P < 0.05$ corrected for multiple comparisons) showing in 41 subjects with multiple sclerosis clusters exhibiting a positive correlation between T_2^* relaxation time at 25%, 50% and 75% depth from the pial surface and neurological disability, as measured by EDSS, and disease severity, as measured by the MSSS. Overlaid are also significant clusters overlapping across the three depths. The positive correlation between EDSS and quantitative T_2^* remained significant even after including white matter (WM) lesion load as a covariate of no interest (nuisance variable) in the GLM (bottom).

Table 3 Clusters of significant positive correlation between quantitative T_2^* at 25%, 50% and 75% depth from the pial surface and EDSS and MSSS in 41 subjects with multiple sclerosis

	EDSS score						MSSS score					
	25%		50%		75%		25%		50%		75%	
	P-value	S-area (mm ²)	P-value	S-area (mm ²)	P-value	S-area (mm ²)	P-value	S-area (mm ²)	P-value	S-area (mm ²)	P-value	S-area (mm ²)
LEFT HEMISPHERE												
Pars triangularis	0.02	38.98										
Rostral middle frontal	0.0001	88.57										
Caudal middle frontal	0.0001	80.6										
Superior frontal	0.02	39.31										
Rostral ant. cingulate	0.04	35.45			0.0001	132.58						
Caudal ant. cingulate					0.009	89.31						
Precentral	0.0001	996.28	0.0001	227.7	0.0001	152.53	0.03	37.93				
	0.0006	58.57										
Post central	0.0001	730.19	0.0001	584.04	0.0001	700.09	0.03	37.77	0.0007	120.31	0.0001	156.88
					0.009	89.4						
Paracentral	0.0002	67.12										
	0.0001	103.84										
Superior parietal	0.0001	618.05			0.01	84.31	0.0005	63.87				
	0.04	36.18					0.03	37.44				
Supramarginal	0.001	54.58			0.05	69.84	0.004	48.98				
	0.01	43.41										
	0.0001	84.86										
Inferior parietal	0.004	48.71										
	0.01	41.9										
	0.0001	160.57										
Superior temporal	0.0001	96.99	0.01	87.22	0.004	98.12						
	0.006	46.06										
Insula	0.0001	70.27	0.0001	722.81	0.0001	764.52						
Precuneus	0.0001	548.42										
	0.02	77.54										
RIGHT HEMISPHERE												
Pars opercularis			0.002	105.49	0.002	105.81			0.03	73.99		
Rostral middle frontal	0.008	45.02										
	0.01	43.86										
Caudal middle frontal	0.0001	281.48					0.04	36.16				
Superior frontal	0.004	47.7										
	0.0002	66.55										
	0.009	43.77										
	0.0001	83.62										
Precentral	0.0001	506.47			0.0001	197.61	0.001	53.33			0.01	84.02
	0.02	40.1					0.003	49.99				
	0.0002	66.39										
Post central	0.003	50.36	0.0001	620.82	0.0001	256.13	0.05	35.66	0.005	95.68	0.01	80.4
	0.0001	576.83	0.0002	131.78			0.0001	74.65				
Paracentral	0.0001	172.44	0.05	69.9								
Supramarginal	0.0007	115.2	0.0005	119.69	0.0004	121.36						
	0.0001	102.32										
Inferior parietal	0.0001	225.63					0.02	39.43				
	0.001	56.19										
Insula			0.0001	181.95								
Superior temporal	0.02	40.87			0.0003	126.94						
Transverse temporal	0.003	51.14										
Precuneus	0.0002	225.82										
Cuneus	0.0001	226.37										

Clusters in bold represent clusters that remained significant after including in the GLM white matter lesions as a nuisance factor (covariate of no interest).

S-area = surface area; ant = anterior.

demyelination remains challenging. In this study, using a surface-based technique on ultra-high resolution 7 T quantitative T_2^* maps ($\sim 0.13 \text{ mm}^3$ voxel size), we demonstrated *in vivo* the presence of pathological changes that involve predominantly the outer layers of the cerebral cortex and cortical sulci in the earliest stages of multiple sclerosis, and extend to deeper cortical laminae and gyri in later disease, becoming diffuse across the whole cortical width and mantle in SPMS.

Comparative post-mortem MRI and histopathology data suggested that conventional imaging methods are not able to reliably detect cortical disease cases in which pathology is confined to the juxtameningeal cortex, based on the observation that visibility of multiple sclerosis cortical lesions at lower strength field MRI depended uniquely on lesions size and involvement of several cortical layers (Seewann *et al.*, 2011). The surface-based technique that allows quantitative T_2^* at 7 T to be estimated vertex-wise across the whole cortical mantle and width could, thus, prove to be a powerful alternative imaging tool for assessing *in vivo* juxtameningeal cortical pathology in such multiple sclerosis cases.

Histopathological studies suggested that all brain lobes can be the site of cortical lesions, although some pathological findings reported a preferential distribution of cortical demyelination in the insula, cingulate and the temporobasal cortex (Bo *et al.*, 2003b; Kutzelnigg and Lassmann, 2006). In our multiple sclerosis cohort, changes in cortical quantitative T_2^* , particularly in SPMS, were distributed across the cortical mantle. Frontal, sensorimotor and parietal areas seemed to be preferred sites of pathological changes at all disease stages.

Quantitative T_2^* changes in cortical sulci and gyri

There is evidence that the histopathological and immunological characteristics of subpial demyelination differ significantly from those in white matter lesions, which suggests a location-dependent expression of the multiple sclerosis immunopathological process (Peterson *et al.*, 2001; Bo *et al.*, 2003a, 2007). Autopsy studies of progressive multiple sclerosis showed that more aggressive subpial pathology was associated with the presence of ectopic meningeal B cell follicular-like structures that were located along and in the depth of the cerebral sulci (Magliozzi *et al.*, 2007, 2010; Howell *et al.*, 2011), and which are thought to trigger cortical demyelination through the activation of microglia (Lassmann and Lucchinetti, 2008). The role of ectopic meningeal B cell follicles in early multiple sclerosis has not been elucidated yet, and, in general, evidence of meningeal inflammation in the early stage of multiple sclerosis is limited. Biopsies on atypical multiple sclerosis cases at disease onset described meningeal inflammation by means of perivascular cells infiltration, in association with cortical demyelinating lesions (Lucchinetti

et al., 2011). *In vivo* studies assessing at lower field strength MRI magnetization transfer imaging (MTR) in the cortex of patients with multiple sclerosis failed to observe a disease effect on cerebral sulci (Samson *et al.*, 2013), possibly due to partial volume effects between the cortex and adjacent white matter and CSF (1 mm^3 voxel size). The authors, however, were able to detect global changes in MTR in the outer portion of the cortex in RRMS and SPMS patients relative to healthy subjects (Samson *et al.*, 2014). Other findings using a surface-based analysis on MTR images at 1.5 T found decreased MTR in the mid-cortical surface (50% depth from the pial surface) in a small cohort of multiple sclerosis subjects relative to healthy controls, however, a quantitative assessment of MTR differences across cortical layers and in sulci versus gyri was not performed (Derakhshan *et al.*, 2014). We found that, in early disease and in SPMS across all cortical depths and in RRMS at 25% depth, quantitative T_2^* changes preferentially involved cortical sulci rather than cortical gyri, corroborating the hypothesis that subpial demyelination is a process likely facilitated by the adjacent meningeal inflammatory milieu. The preferential localization of subpial demyelination in cortical sulci could be related to the tendency of meningeal inflammatory cells and soluble mediators to collect and concentrate in sulci, while being diluted at the outer gyral brain surface due to physiological flow variations of CSF. As disease progresses, persistence of inflammatory changes, and lack of effective repair mechanisms can induce further demyelination that spreads to cortical gyri leading to the phenomenon termed 'general subpial demyelination' (Bo *et al.*, 2003b), described in late stage multiple sclerosis. Interestingly, in our study, involvement of cerebral sulci was prominent in early multiple sclerosis, whereas involvement of cortical gyri increased in later disease stages.

Cortical quantitative T_2^* and clinical measures

Post-mortem studies in chronic progressive multiple sclerosis demonstrated an association between a decreasing gradient of intracortical demyelination and neuronal loss away from the pial surface and disease severity as measured by age at which patients became wheelchair-dependent (Magliozzi *et al.*, 2007, 2010).

We previously reported in different multiple sclerosis cohorts an association between neurological disability and the number of focal subpial lesions on 7 T T_2^* -weighted images, which also included type IV cortical lesions that extend from the pial surface through the entire cortical width without reaching the subcortical white matter (Mainero *et al.*, 2009; Nielsen *et al.*, 2013). Here, the laminar analysis of quantitative T_2^* revealed a significant positive relation between T_2^* relaxation time at all three cortical depths (25%, 50%, 75%) and both EDSS and MSSS, the greatest effect being in the outer cortical layers

(25% depth). This association was independent of underlying white matter lesions, indicating that subpial pathology has a significant and unique contribution to disability and disease severity in multiple sclerosis.

The greatest association in our multiple sclerosis cohort between EDSS and MSSS and quantitative T_2^* at 25% depth is in line with neuropathological examinations that observed that multiple sclerosis brains with a decreasing gradient of demyelination and neuronal loss away from pial surface (likely linked to meningeal inflammation) could be distinguished from other multiple sclerosis cases that lacked a gradient in the expression of cortical pathology, despite the presence of neuronal loss and translaminal cortical demyelination (Magliozzi *et al.*, 2010). Clinically, these patients exhibited a milder disease course compared to the former group suggesting that a pathogenic mechanism for cortical demyelination driven from the cortical surface, and possibly associated with meningeal inflammation, is related with worse clinical outcome.

In our early multiple sclerosis cohort, cortical quantitative T_2^* changes were mainly found in the outer portion of the cortex. Interestingly, early patients showed levels of neurological disability similar to subjects with late RRMS. This, given the short disease duration, translated into higher MSSS, thus implying the presence of a clinically aggressive early disease course. It is also possible that some patients with a relatively benign disease course have been included in the late RRMS group. The term 'benign', however, is still controversial as the course of multiple sclerosis can worsen at any time, even after many years of apparent stability (Lublin, 2014), and frequently does not take into account cognitive deficits that may occur in multiple sclerosis in the presence of mild physical disability. Indeed our data demonstrate the presence of distributed cortical quantitative T_2^* at different depths from the pial surface in the RRMS group relative to healthy individuals. Longitudinal evaluations are needed to better elucidate the role of intra-cortical quantitative T_2^* to disease progression and cognitive impairment in multiple sclerosis.

Cortical quantitative T_2^* and cortical thickness

We investigated the relationship between laminar quantitative T_2^* and cortical thickness in multiple sclerosis. Although the clinical relevance of cortical tissue loss assessment in multiple sclerosis has been consistently reported in cross-sectional and longitudinal studies, the underlying mechanisms are still unknown (Geurts *et al.*, 2012). Cortical thinning may be the consequence of axonal transection by white matter lesions leading to neuronal loss or may underlie a degenerative process that primarily targets the cortical grey matter. The knowledge of the relationship between subpial demyelination and cortical atrophy has been hampered by the lack of tools able to image *in vivo* subpial pathology. In our multiple sclerosis cohort, longer

quantitative T_2^* , at all three depths, was associated with cortical thinning across several cortical areas. However, a clear preferential distribution through the cortical width in the correlation between these two measures was not evident, and in multiple sclerosis subjects changes in laminar quantitative T_2^* relative to controls were independent of cortical thickness. Although cortical thickness and normalized cortical volume were globally decreased in the cohort of subjects with multiple sclerosis relative to controls, and significant cortical thinning was also observed in SPMS cases, we did not find regional differences in cortical thickness between patients and controls. We calculated that for the patients with early and RRMS to have 80% power to detect a patient versus control difference in cortical thickness (which is ~80% of the standard deviation) at 5% significance, ~26 subjects per group would be necessary.

We did not find any correlation between cortical thickness or normalized cortical volume and either EDSS or MSSS; rather, quantitative T_2^* proved to be a marker of neurological disability more sensitive than cortical tissue loss. This suggests that taking into account the spatial variation of tissue integrity measures in the cortex can greatly improve the ability to find multiple sclerosis-related pathological changes. Laminar quantitative T_2^* and cortical thickness may also reflect distinctive aspects of a degenerative process that targets the cortex, and as such different measures of clinical disability. Pathological data indeed suggested that neuronal and axonal loss seem to contribute more than demyelination to cortical atrophy in multiple sclerosis (Klaver *et al.*, 2013). Longitudinal studies could help to elucidate the spatiotemporal events leading to cortical tissue loss in multiple sclerosis, as well as the contribution of white matter lesions.

Origin of cortical quantitative T_2^* changes in multiple sclerosis

In this study we detected an overall increase in quantitative T_2^* in patients, at all disease stages, relative to controls. Cortical T_2^* contrast has been associated predominantly with non-heme iron (Haacke *et al.*, 2005; Fukunaga *et al.*, 2010), which is stored in ferritin particles and provides a substrate for oligodendrocytes for producing and sustaining myelin sheaths surrounding axons. In healthy cortical laminae non-heme iron has been shown to co-localize with myelin on cellular and molecular levels (Connor and Menzies, 1990). In multiple sclerosis, in both white matter and cortical multiple sclerosis lesions longer T_2^* (or shorter R_2^*) has been pathologically related to iron and myelin loss (Yao *et al.*, 2012, 2014). Other histopathological-magnetic resonance correlations of *ex vivo* multiple sclerosis brains using gradient echo T_2^* -weighted imaging at 7T found increased T_2^* signal in demyelinating lesions, and decreased T_2^* in focal areas characterized by the presence of iron rich microglia and/or macrophages (Pitt *et al.*, 2010). Findings from our study,

thus, likely reflect the prevailing effects of a pathological process that underlies a decrease in iron and/or myelin content rather than changes due to iron accumulation in the cortex.

In our multiple sclerosis cohort, quantitative T_2^* measured in focal cortical lesions was significantly increased (longer T_2^*) compared with NACGM quantitative T_2^* and mean cortical quantitative T_2^* in healthy controls, suggesting that focal cortical lesions may contribute, at least in part, to the observed changes in laminar quantitative T_2^* in multiple sclerosis. Pathological-magnetic resonance correlations on gradient echo images at 7 T highlighted, however, that a consistent subset of cortical lesions can be missed at prospective visual inspection of magnetic resonance scans as cortical lesion counts greatly improved with retrospective scoring (i.e. after comparison of histological sections) (Pitt et al., 2010; Yao et al., 2014). Indeed in our study, in SPMS cases, quantitative T_2^* changes relative to healthy controls also involved the NACGM. We previously reported in another multiple sclerosis cohort that in some patients, FLASH- T_2^* magnitude images were characterized, in addition to focal subpial lesions, by the presence of diffuse band-like areas of subtle hyperintensity mainly involving the outer cortical laminae and extending over an entire gyrus or multiple gyri (Mainero et al., 2009). These observations suggest that even at ultra-high field MRI visual characterization of focal cortical lesions does not account for the full spectrum of cortical pathology in multiple sclerosis, and that quantitative methods able to assess cortical damage voxel-wise could better depict the extent and pattern of cortical pathological changes in the disease.

Heme-bound iron, which may underlie normal vascularization, can also affect T_2^* contrast. The contribution of intracortical vascular changes to cortical demyelination in multiple sclerosis, however, is still uncertain. Autopsy studies of late stage multiple sclerosis showed that cortical lesions lack the blood-brain barrier breakdown that characterizes active white matter lesions (Peterson et al., 2001; Bo et al., 2003a). Other histopathological examinations found blood-brain barrier breakdown and perivascular inflammation in the cortex of multiple sclerosis brains at disease onset (Lucchinetti et al., 2011). *In vivo* assessments described a significant reduction in cerebral blood flow and cerebral blood volume in cortical lesions compared with the normal-appearing grey matter, suggesting reduced metabolism due to loss of cortical neurons. A subset of cortical lesions showing an increased cerebral blood flow and/or cerebral blood volume, however, was also detected, possibly implying that perfusion could evolve during inflammation (Peruzzo et al., 2013). Other studies found, in early RRMS, decreased grey matter perfusion in the absence of volume loss, consistent with neuronal metabolic dysfunction (Debernard et al., 2014). Preliminary findings using gadolinium-enhanced T_2 -fluid-attenuated-inversion-recovery (FLAIR) MRI reported, in a subset of multiple sclerosis cases, leptomeningeal contrast enhancement that was unrelated to contrast-enhancement in white matter

lesions (Reich et al., 2014). Taken together, these observations lead to speculation that the time course of cortical blood-brain barrier breakdown could differ from that in white matter lesions and across disease stages. Future studies aimed at assessing intracortical vascular changes in multiple sclerosis could help to clarify its role in cortical pathology. In addition, quantitative T_2^* measurements can be combined with other techniques specific to myelin such as T_1 mapping, quantitative magnetization transfer imaging (Dortch et al., 2013), and to iron such as quantitative susceptibility mapping (Deistung et al., 2013) to better characterize the contribution of myelin and iron to cortical multiple sclerosis pathology.

Conclusion

This study demonstrates that a surface-based analysis of ultra-high resolution quantitative T_2^* MRI acquisition at 7 T with a highly parallelized radiofrequency coil can facilitate the *in vivo* characterization of cortical pathology at distinct depths from the pial surface in multiple sclerosis. We were able to detect *in vivo* a gradient in the expression of intracortical multiple sclerosis pathology across disease stages, which supports the hypothesis that cortical pathology in multiple sclerosis may be, at least in part, the consequence of a pathogenic process driven from the pial surface. Because we cannot exclude that changes in deeper cortical laminae (75% depth) could also be driven by white matter lesions extending into the cortex, longitudinal evaluations are needed to confirm our preliminary observations. Nevertheless, the significant association between laminar quantitative T_2^* , neurological disability and disease severity, prominent in the outer cortical layers and independent from white matter lesions, provides *in vivo* evidence that this pattern of cortical disease can be the pathological basis for disease progression in many multiple sclerosis cases.

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Supplementary material

Supplementary material is available at *Brain* online.

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