ON-LINE APPENDIX

Image Processing

Coregistration and averaging of MP2RAGE images were performed using a custom pipeline developed in an open-source neuroimaging software environment (Neuroimaging in Python: Pipelines and Interfaces [NIPYPE]; https://github.com/nipy/nipype). Linear coregistration was performed using the Advanced Normalization Tools software package (http://stnava.github.io/ANTs) (downsampled registration using ANTs affine initializer with 6 *df* and a mutual information cost function).

Ex Vivo MR Imaging and Immunohistochemistry

A brain from a patient with MS, not included in the in vivo study, was imaged postmortem at 7T with T2*GRE and MP2RAGE (On-line Table 3). Lesions were identified independently on each sequence and compared. Tissue 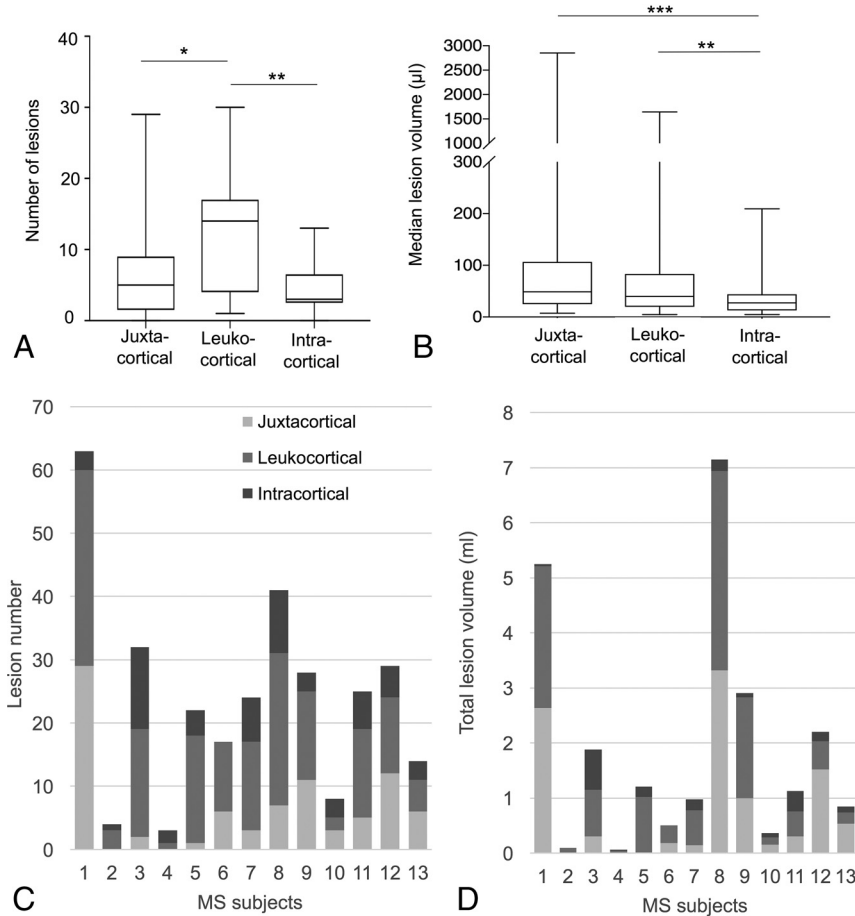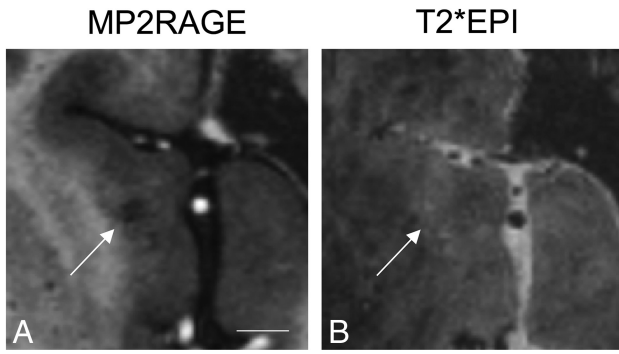at the locations of 4 lesions seen on MP2RAGE was isolated and embedded in paraffin. Ten-micrometer sections were cut on a sliding microtome and mounted on 1 × 3 inch glass slides. Immunostaining was performed as previously described[1] using mouse anti-myelin proteolipid protein (1:200) (Abcam; http://www.abcam.com/index.html), visualized with diaminobenzidine, and counterstained with hematoxylin. All sections were imaged by using a Zeiss Axio-Observer Z1 microscope (https://www.zeiss.com/microscopy/us/products/light-microscope/axio-observer-for-biology.html).

REFERENCE

1. Absinta M, Vuolo L, Rao A, et al. **Gadolinium-based MRI characterization of leptomeningeal inflammation in multiple sclerosis.** *Neurology* 2015;85:18–28 CrossRef Medline
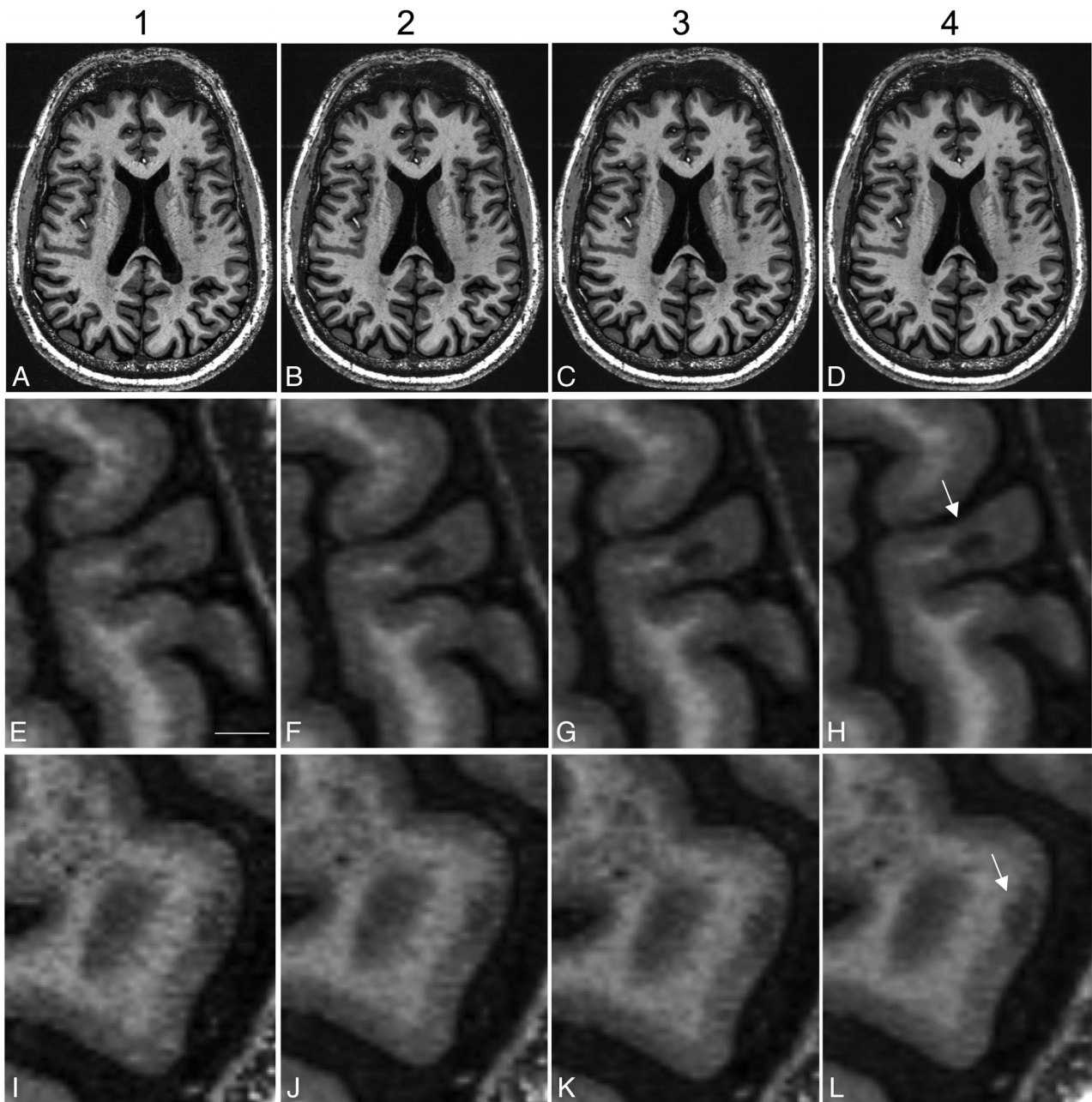
ON-LINE FIG 1. Comparison of different numbers of MP2RAGE repetitions. Image quality increases with increasing number of MP2RAGE repetitions (*A*, *E*, and *I* are a single repetition; *B*, *F*, and *J* are the median of 2 repetitions; *C*, *G*, and *K* are the median of 3 repetitions; and *D*, *H*, and *L* are the median of 4 repetitions). *E–H*, An example of a leukocortical lesion identified prospectively on all 4 images. *I–L*, An example of a leukocortical lesion predominantly involving the cortex identified prospectively only when 3 (*K*) or 4 (*L*) repetitions were averaged. Scale bar = 5 mm.

MP2RAGE

T2*EPI

ON-LINE FIG 2. Cortical lesion in a healthy control. A single leuko-cortical lesion was identified on MP2RAGE (*A*) in 1 of 5 healthy controls. This lesion is not seen on T2*EPI (*B*) and was not included in the FOV on the T2*GRE images collected. Scale bar = 5 mm.

ON-LINE FIG 3. Cortical lesion number and volume distribution. *A*, There are more leukocortical than intracortical or juxtacortical lesions observed across all cases. *B*, Median juxtacortical lesion volume exceeds median leukocortical or intracortical volume; median leukocortical lesion volume also exceeds median intracortical volume. Total cortical lesion number (*C*) and volume (*D*) for the 3 lesion subtypes are highly variable across MS cases. *Three asterisks* indicate $P < .001$; *double asterisks*, $P < .01$; *asterisk*, $P < .05$.
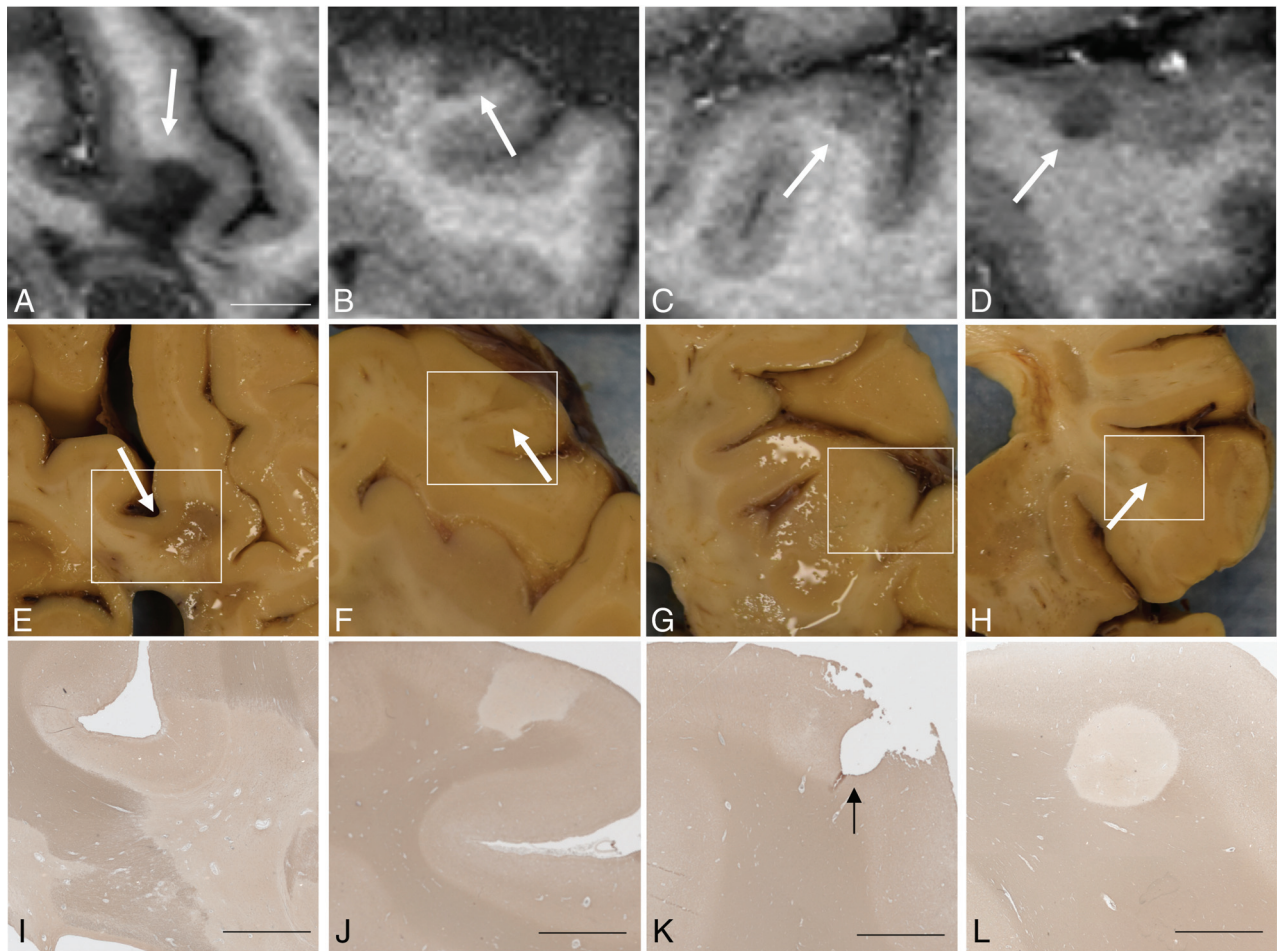
ON-LINE FIG 4. Postmortem MR imaging and histopathology. Regions corresponding to the location of 4 lesions (*white arrows*) seen on postmortem MP2RAGE (*A–D*) were identified grossly (*E–H*), and the corresponding tissue was examined for demyelination using an antibody against myelin proteolipid protein (*I–L*, brown). Lesions in *A*, *B*, and *D* are visible grossly and correspond to demyelinated lesions (*I, J*, and *L*). The lesion in *C* corresponds to a tear in the cortex (*K, black arrow*). Scale bar = 5 mm (*A*), 1 mm (*I–L*).

On-line Table 1: Cohort characteristics

	MS (*n* = 13)	Healthy Controls (*n* = 5)
Age (yr)		
Mean	54	57
SD	10	17
Sex (No.)		
Female	8	2
Male	5	3
Years since symptom onset		
Mean	24	
SD	11	
Clinical subtype (No.) (%)		
Relapsing-remitting MS	9 (69%)	
Secondary-progressive MS	3 (23%)	
Primary-progressive MS	1 (8%)	
Expanded Disability Status Scale score		
Minimum, maximum	1, 7	
25th Percentile, 75th percentile	1.5, 3.5	

On-line Table 2: MRI acquisition parameters for in vivo 3T sequences

	In Vivo 3T 3D-MP2RAGE[a]	In Vivo 3T 3D-FLAIR[a]
Orientation	Sagittal	Sagittal
Voxel size (mm)	1 mm isotropic	1 mm isotropic
FOV (mm)	240 (AP) × 176 (RL) × 256 (SI)	256 (AP) × 176 (RL) × 256 (SI)
TI (ms)	700/2500	1800
TR (ms)	5000	4800
TE (ms)	2.96	353
Flip angle	4°/5°	120
No. of averages	1	1
Scan time (hr:min:sec)	0:08:16	0:06:53

Note:—AP indicates anterior-posterior axis; RL, right-left axis; SI, superior-inferior axis.
[a] 3T images were acquired on average 8 months from the 7T MP2RAGE session. Range, −5 to +30 mo.

On-line Table 3: MRI acquisition parameters for ex vivo 7T sequences

	Ex Vivo 7T MP2RAGE	Ex Vivo 7T 3D-T2*GRE
Orientation	Coronal	Sagittal
Voxel size (mm)	0.4 mm isotropic	0.42 mm isotropic
FOV (mm)	57.6 (AP) × 179 (RL) × 134 (SI)	159 (AP) × 37 (RL) × 159 (SI)
TI (ms)	300/1350	NA
TR (ms)	4000	60
TE (ms)	4.49	15.99
Flip angle	4°/5°	10°
No. of averages	1	4
Scan time (hr:min:sec)	0:08:47	2:15:42

Note:—AP indicates anterior-posterior axis; RL, right-left axis; SI, superior-inferior axis; NA, not applicable.

On-line Table 4: Comparison of lesions identified on T2*GRE versus lesions identified on T2*EPI

	T2*GRE Only	T2*EPI Only	P Value
Total cortical lesions (median per patient, range)	44 (3, 0–15)	33 (3, 0–7)	.60
Juxtacortical (No.)	11 (25%)	7 (21%)	NA
Leukocortical (No.)	23 (52%)	14 (42%)	NA
Intracortical (No.)	10 (23%)	12 (36%)	NA
Mean lesion volume	130 ± 425 μL	70 ± 95 μL	.43

Note:—NA, not applicable.

On-line Table 5: Lesion number and volume by lesion type

	Lesion No.					
		Per Patient				
	Total	Median	Range, IQR	Significance	Mean Lesion Volume	Significance
All lesions	309	24	3–62, 15		79 ± 201 μL	
Juxtacortical	60	4	0–29, 5		115 ± 311 μL	
Leukocortical	164	14	1–30, 12	* vs JC, ** vs IC	76 ± 154 μL	NS vs JC, ** vs IC
Intracortical	85	3	0–13, 3	NS vs JC	37 ± 36 μL	*** vs JC

Note:—JC indicates juxtacortical; IC, intracortical; NS, not significant; asterisk, $P < .05$; double asterisks, $P < .01$; 3 asterisks, $P < .001$.