# Longitudinal Assessment of Multiple Sclerosis with the Brain-Age Paradigm

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**Objective:** During the natural course of multiple sclerosis (MS), the brain is exposed to aging as well as disease effects. Brain aging can be modeled statistically; the so-called "brain-age" paradigm. Here, we evaluated whether brain-predicted age difference (brain-PAD) was sensitive to the presence of MS, clinical progression, and future outcomes.
**Methods:** In a longitudinal, multicenter sample of 3,565 magnetic resonance imaging (MRI) scans, in 1,204 patients with MS and clinically isolated syndrome (CIS) and 150 healthy controls (mean follow-up time: patients 3.41 years, healthy controls 1.97 years), we measured "brain-predicted age" using T1-weighted MRI. We compared brain-PAD among patients with MS and patients with CIS and healthy controls, and between disease subtypes. Relationships between brain-PAD and Expanded Disability Status Scale (EDSS) were explored.

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**Results:** Patients with MS had markedly higher brain-PAD than healthy controls (mean brain-PAD +10.3 years; 95% confidence interval [CI] = 8.5–12.1] versus 4.3 years; 95% CI = 2.1 to 6.4; $p < 0.001$). The highest brain-PADs were in secondary-progressive MS (+13.3 years; 95% CI = 11.3–15.3). Brain-PAD at study entry predicted time-to-disability progression (hazard ratio 1.02; 95% CI = 1.01–1.03; $p < 0.001$); although normalized brain volume was a stronger predictor. Greater annualized brain-PAD increases were associated with greater annualized EDSS score ($r = 0.26$; $p < 0.001$).
**Interpretation:** The brain-age paradigm is sensitive to MS-related atrophy and clinical progression. A higher brain-PAD at baseline was associated with more rapid disability progression and the rate of change in brain-PAD related to worsening disability. Potentially, "brain-age" could be used as a prognostic biomarker in early-stage MS, to track disease progression or stratify patients for clinical trial enrollment.

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Age has long been implicated as the dominant driver of disease progression in multiple sclerosis (MS).[1] Older age increases the risk of progression,[2] irrespective of disease duration; once progression starts, disability accrual is independent of the previous evolution of the disease, the presence of relapses, or relapse rates.[3–7] Some people with MS experience faster rates of brain atrophy, leading to poorer long-term outcomes.[8] Early prediction of risk for accelerated atrophy could have great clinical benefit for treatment decisions and patient management. However, making such early predictions of future brain atrophy rates is challenging, not least because normal aging also results in brain atrophy.

Neuroimaging, particularly magnetic resonance imaging (MRI), offers a window into the longitudinal evolution of atrophy patterns in MS and aging, allowing them to be compared and contrasted.[9–12] For example, Ghione and colleagues showed similar rates in the percentage of brain volume change between patients with MS and age-matched healthy controls, although patients with MS started from a lower baseline volume. Conversely, this study found scant percentage lateral ventricle volume change in patients with MS, whereas healthy controls volumes increased to reach similar volumes as patients with MS by age 60 years.[9] Meanwhile, Azevedo and colleagues focused on thalamic volume, showing significantly greater reductions in MS (−0.71%/year) compared to healthy aging (−0.28%/year).[11] Similarly, Bishop and colleagues reported "excess" grey matter volume reductions in subcortical regions, including but not limited to the thalamus, in both early and late-onset patients with MS groups, relative to age-matched controls.[12] These studies suggest that, although there is some neuroanatomic overlap between aging and MS-related atrophy, the spatial patterns and rates of change differ. Although whole-brain volume is likely to add value to clinical decision making in MS,[13] potentially more "high-dimensional" approaches that capture patterns across all voxels from a brain scan could add value.

An alternative approach to consider the relationship between disease and healthy aging is the so-called "brain-age" paradigm.[14] Rather than correlate age and brain volume, this approach aims to predict chronological age from neuroimaging data; analogous to efforts to model biological age from biogerontology research. The difference between an individual's chronological age and the age predicted by machine-learning analysis of voxelwise neuroimaging data, the brain-predicted age difference (brain-PAD), has been proposed as an age-adjusted index of structural brain health. Research has shown brain-PAD to be sensitive to neurological and psychiatric diseases,[15,16] including MS.[17,18] Specifically, MS was associated with between 4 and 6 years of added brain-PAD, similar in magnitude to previous work on traumatic brain injury[19] and epilepsy[20] and greater than well-treated HIV[21] or Down's syndrome.[22] Kaufmann and colleagues reported that brain-age gap (equivalent to brain-PAD) was associated with Expanded Disability Status Scale (EDSS) score. Meanwhile, Høgestøl and colleagues found a relationship between change in whole-brain brain-age gap measure and disease-modifying therapy status, but no change in EDSS. This study was relatively small (n = 62), so it is still unclear whether brain-PAD has prognostic value in MS, as has been demonstrated in larger studies of dementia[23,24] and normal aging.[25]

Here, we used unique access to large longitudinal cohort of patients with MS and healthy controls to assess whether MS is associated with a higher apparent brain age and whether a patient's brain-PAD has utility in predicting clinical outcomes. Specifically, we tested the following hypotheses: (1) patients with MS have higher brain-PAD than healthy controls; (2) in patients with MS, there is a relationship between brain-PAD and disability at study entry; (3) brain-PAD increases over time as disabilities worsen; and (4) brain-PAD at baseline predicts future disability progression.

## Methods

### *Participants*

This cohort study used data collected from seven European MS centers (MAGNIMS: www.magnims.eu) and Imperial College London on n = 1,354 participants (Table 1), largely overlapping with our previous work

**TABLE 1. Characteristics of Patients with MS, CIS, and Healthy Controls**

| | Healthy controls | All MS/CIS patients | CIS | RRMS | SPMS | PPMS |
|---|---|---|---|---|---|---|
| N | 150 | 1204 | 296 | 677 | 111 | 120 |
| N with follow-up data | 111 | 1155 | 279 | 653 | 104 | 119 |
| Female, n (%) | 82 (55) | 771 (64) | 199 (67) | 453 (67) | 67 (60) | 52 (43) |
| Number of scans per participant mean ± SD [range] | 2.82 ± 1.90 [1–10] | 2.61 ± 1.01 [1–7] | 2.44 ± 0.98 [1–5] | 2.71 ± 1.05 [1–7] | 2.71 ± 1.05 [1–3] | 2.71 ± 1.05 [1–5] |
| Length of follow-up, yr, mean ± SD [range] | 1.97 ± 1.38 [0.5–6.0] | 3.41 ± 3.15 [0.2–15] | 3.63 ± 4.04 [0.2–15] | 3.57 ± 3.10 [0.45–15] | 2.43 ± 1.12 [0.5–5.5] | 2.86 ± 1.67 [0.8–6] |
| Age at baseline scan, yr, mean ± SD [range] | 37.29 ± 9.96 [23–66] | 39.41 ± 10.76 [15–68] | 33.01 ± 8.08 [15–55] | 38.83 ± 9.68 [18–66] | 50.11 ± 9.39 [30–68] | 48.55 ± 10.04 [19–65] |
| Brain-predicted age at baseline, yr, mean ± SD [range] | 38.43 ± 11.12 [14.5–70] | 50.27 ± 14.90 [7.4–92] | 37.60 ± 10.01 [13.7–82] | 51.33 ± 13.32 [7.4–92] | 67.36 ± 10.42 [40.2–89] | 59.77 ± 10.90 [31–84] |
| Time since clinical diagnosis at baseline, yr | - | 7.26 ± 7.96 [0–48] | 0.52 ± 1.50 [0–18] | 7.67 ± 7.31 [0–42] | 17.44 ± 9.04 [2.5–48] | 6.65 ± 5.63 [1–27] |
| EDSS at baseline mean ± SD [range] | - | 2.60 ± 1.95 [0–9] | 1.36 ± 1.02 [0–4.5] | 2.12 ± 1.40 [0–6.5] | 5.83 ± 1.20 [3–9] | 5.10 ± 1.32 [2–8] |
| Disease-modifying treatment at baseline, n (%): yes/no/unknown | - | 475 (39) / 675 (56) / 54 (5) | 60 (20) / 234 (79) / 2 (1) | 356 (53) / 285 (42) / 36 (5) | 51 (46) / 52 (47) / 8 (7) | 8 (7) / 104 (87) / 8 (7) |

CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; PPMS = primary-progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis.

(Table S1).[10] Patients had all received a diagnosis of MS according to 2010 McDonald Criteria or clinically isolated syndrome (CIS).[26,27] Patients with MS and CIS were scored on the EDSS.[28] Healthy controls without history of neurological or psychiatric disorders were also included (n = 150). For longitudinal imaging analysis, participants were required to have undergone at least 2 high-resolution T1-weighted MRI acquired with the same protocol with an interval of ≥1 month.

The final protocol for this study was reviewed and approved by the European MAGNIMS collaboration for analysis of pseudo-anonymized scans and the Imperial NHS Trust (London Riverside Research Ethics Committee: 14/LO/0343). All participants provided written, informed consent to take part in the research.

### *Expanded Disability Status Scale Progression*

Time-to-event, where a progression event was an individual's progression on the EDSS, was defined as per our previous work.[10] That is, if a patient showed a longitudinal change of: a 1.5-point increase in EDSS if the baseline EDSS was 0; a 1-point increase if baseline EDSS was 1 to 6 inclusive; and a 0.5-point increase if EDSS was greater than 6.

### *Neuroimaging Acquisition*

Overall, 3,565 T1-weighted MRI scans were used in the study according to local MRI protocols, which used similar acquisition parameters. Thirteen different scanners (Siemens, GE, and Philips) were used in patients recruited from 1998 onward (Supplmentary Table S2).

### *Machine-Learning Brain-Age Analysis*

Brain-predicted age calculation followed our previously established protocol, which has high test–retest reliability (intraclass correlation coefficient = 0.97).[21,29] In brief, all structural images were preprocessed, using the SPM12 software package (www.fil.ion.ucl.ac.uk/spm/software/spm12/), to generate grey matter and white matter segmentations. Visual quality control was then conducted to verify segmentation accuracy; all images were included. However, n = 13 participants were excluded from a single site, due to labeling errors. Segmented grey matter and white matter images were then nonlinearly registered to a custom template (based on the training dataset). Finally, images were affine registered to Montreal Neurological Institute (MNI) 152 space (voxel size = 1.5 $mm^3$), modulated and smoothed (4 mm). Summary volumetric measures of grey matter, white matter, cerebrospinal fluid (CSF), and intracranial volume were also generated.

Brain-predicted ages were generated using Pattern Recognition for Neuroimaging Toolbox (PRoNTo version 2.0, www.mlnl.cs.ucl.ac.uk/pronto) software.[30] First, a model of healthy brain aging was defined. Brain volumetric data (from in a separate training dataset,[21] n = 2001 healthy people screened to exclude comorbidities, aged

**TABLE 2. Comparisons of Estimated Marginal Means in Brain-PAD Between MS subtypes**

| Group comparison | Estimated difference | Standard error | DF | *t*-ratio | *p* |
|---|---|---|---|---|---|
| CIS-healthy controls | −2.15 | 0.916 | 724 | −2.35 | 0.13 |
| RRMS-healthy controls | −7.30 | 0.811 | 868 | −9.00 | <0.0001 |
| SPMS-healthy controls | −8.74 | 1.013 | 1,227 | −8.63 | <0.0001 |
| PPMS-healthy controls | −6.16 | 0.952 | 1,296 | −6.47 | <0.0001 |
| RRMS-CIS | −5.15 | 0.575 | 1,100 | −8.95 | <0.0001 |
| SPMS-CIS | −6.59 | 0.916 | 1,295 | −7.19 | <0.0001 |
| PPMS-CIS | −4.01 | 1.011 | 659 | −3.97 | <0.0001 |
| SPMS-RRMS | −1.44 | 0.764 | 1,342 | −1.89 | 0.32 |
| PPMS-RRMS | 1.14 | 0.871 | 700 | 1.30 | 0.69 |
| PPMS-SPMS | 2.58 | 0.986 | 1,110 | 2.62 | 0.06 |

Brain-PAD = brain-predicted age difference; CIS =clinically isolated syndrome; DF = degrees of freedom; MS = multiple sclerosis; PPMS = primary-progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis.

18 to 90; see Table S2 for data sources) were used as the independent variables in a Gaussian Processes regression, with age as the dependent variable. This regression model predicted chronological age with a mean absolute error of 5.02 years, assessed using 10-fold cross-validation, which explained 88% of the variance in chronological age.

Next, the coefficients from the full training model were applied to the current test data (ie, patients with MS and CIS and healthy controls) to generate brain-predicted ages. These values were adjusted to remove age-related variance, as discussed by Le and colleagues,[31] by subtracting 3.33 and then dividing by 0.91; the intercept and slope calculated from a linear regression of brain-predicted age (outcome) on chronological age (predictor) in the training dataset. Finally, brain-PAD scores were calculated by subtracting chronological age from brain-predicted age and used for subsequent analysis. A positive brain-PAD score indicates that the individual's brain is predicted to be "older" than their chronological age.

### *Statistical Analysis*

Using brain-PAD values, further statistical analysis was carried out to test our hypotheses, using R version 3.5.2. A full list of R packages and versions and analysis code is included in the accompanying R Notebook (https://github.com/james-cole/UCL-MAGNIMS-Brain-age). We used linear mixed effects models, enabling incorporation of fixed and random effect predictors to model each given outcome measure. In these models, brain-PAD was used as the outcome variable. Each model included fixed effects of group (eg, patients with MS and CIS vs. healthy controls; MS subtype [CIS, relapsing-remitting, secondary-progressive, and primary-progressive]), age, age$^2$, sex, normalized brain volumes (ie, whole-brain to intracranial volume ratio), and MRI scanner field strength (1.5 T or 3 T) and a random effect of original study cohort (modeling intercept). Estimated marginal means or trends (for interactions) and confidence intervals (CIs) from linear models were calculated, using asymptotic CIs where appropriate. This analysis was repeated using data from a single cohort from a single center (UCL, London, UK), where all MS clinical subtypes were present (not CIS). To test the influence of disease-modifying treatments on the analysis, we repeated the mixed-effects modeling with the addition of baseline treatment status as an additional covariate, before calculating estimated marginal means for those patients with MS receiving treatment compared to those not.

A random effects meta-analysis was conducted to explore the heterogeneity of the group effects on brain-PAD across different study cohorts. Only cohorts that included healthy controls and patients with MS or CIS were included in this analysis.

To establish whether brain volume measurements were driving the variability in brain-PAD, we performed a linear regression with hierarchical partitioning of variance, with brain-PAD as the outcome variable and age, sex, normalized brain volume, cohort, and field strength as predictors.

Subsequent analyses were conducted to test for fixed-effect influences of EDSS score (patients with MS

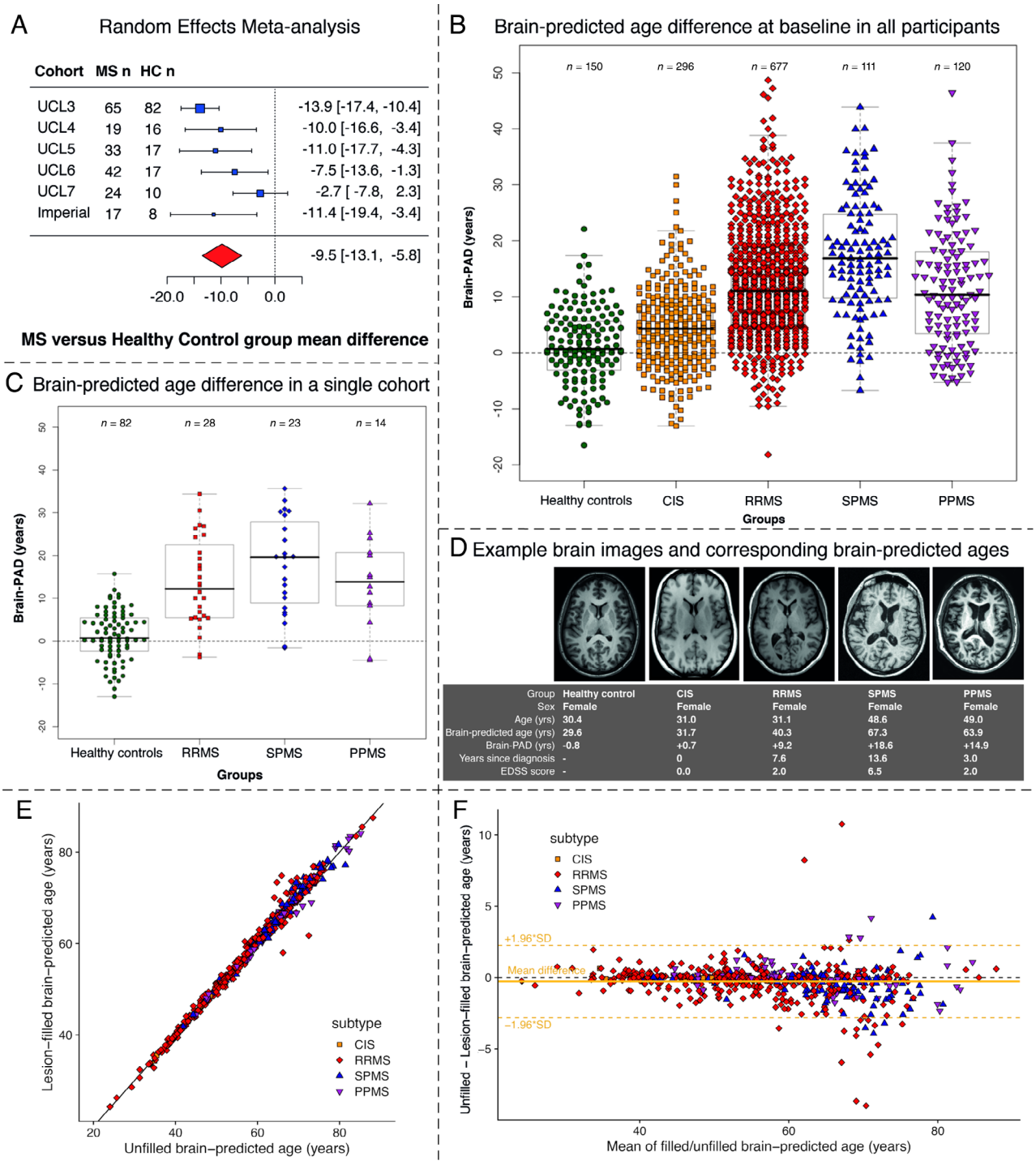


**FIGURE 1: Brain-predicted age difference (brain-PAD) for patients with multiple sclerosis (MS) and clinically isolated syndrome (CIS) and healthy controls (HCs) at baseline. (A) A random-effects meta-analysis of the 6 cohorts that included both patients with MS and CIS and healthy controls found the pooled effect of multiple sclerosis and CIS on brain-PAD compared to healthy controls was 9.45 years (95% confidence interval [CI] = 13.11–5.80), across a total of n = 200 patients with MS and CIS and n = 150 healthy controls. Heterogeneity was estimated at $I^2$ = 59% [95% CI = 3–91%]. (B) Grouped scatterplot depicting the distributions of brain-PAD at baseline in years. Solid lines represent the group median, boxes show the interquartile range, and whiskers 1.5 times the interquartile range from the median. (C) Data from cohort "UCL3," where all MS subtypes were present, confirms a result similar to that of the total cohort. (D) Examples of how brain structure relates to brain-PAD, with axial slice from T1-weighted magnetic resonance imaging (MRI) from 1 healthy control and 4 individuals with CIS or MS. A control brain from a 30-year-old woman with a brain-PAD of −0.8 years can be compared to a 31-year-old woman with CIS, Expanded Disability Status Scale (EDSS) of 0.0 and a brain-PAD of +0.7 years, and 31-year-old with relapsing-remitting multiple sclerosis (RRMS), EDSS of 2.0, and a brain-PAD of +9.2 years. In addition, we illustrate a 48-year-old with secondary-progressive multiple sclerosis**
*(Figure legend continues on next page.)*

**TABLE 3. Standardized Beta Coefficients from Linear Mixed Effects Models of Clinical Measures**

| Baseline clinical measure | Brain-PAD | Normalized brain volume | Age | Age$^2$ | Sex | Field strength |
|---|---|---|---|---|---|---|
| EDSS | 0.141 | −0.143 | 0.265 | 0.096 | 0.000 | −0.068 |
| Time since diagnosis | 0.245 | −0.102 | 0.407 | 0.074 | −0.041 | −0.062 |
| Age at onset | −0.202 | 0.086 | 0.774 | −0.065 | 0.028 | 0.044 |

Brain-PAD = brain-predicted age difference; EDSS = Expanded Disability Status Scale.

and CIS), and time since clinical diagnosis and age at clinical diagnosis (patients with MS only). Model fits were considered using F-tests and post hoc pairwise comparisons using *t*-tests or Tukey tests where appropriate.

We explored how longitudinal changes in brain-PAD related to changes in disability over time in 2 ways: (1) by correlating annualized change in brain-PAD (ie, the difference between first measured brain-PAD and last brain-PAD, divided by the interval in years) with the annualized change in EDSS score; and (2) by using linear mixed effects models to investigate group (patients with MS and CIS vs. healthy controls; patient subtype) by time interactions. These analyses included a random effect of participant (modeling slope and intercept), alongside age, age$^2$, sex, field strength, and cohort effects.

Survival analysis, using a Cox proportional hazards regression, was used to test whether baseline brain-PAD predicted time-to-EDSS progression, including age at baseline MRI and sex as covariates. An additional survival analysis was conducted with normalized brain volume as a further covariate.

We investigated the impact of MS lesions on brain-PAD in MS. Using cross-sectional data from a subset of n = 575 patients with MS and CIS, for which manually annotated lesion maps were available, we explored the relationship between MS lesions and measurements of brain-PAD using the FSL lesion-filling algorithm[32] by artificially removing lesions from T1-weighted MRI scans. Both "lesion-filled" and "unfilled" scans were run through the brain-age prediction procedure, then resulting brain-PAD scores were compared.

## *Results*

### *Multiple Sclerosis is Associated with Older Appearing Brains*

The MAGNIMS sample forms part of a well-characterized population (see Table 1). The combined cohort involves patients from 6 countries with a mean follow-up of 3.41 years in patients. At baseline, age was correlated with age at diagnosis (r = 0.69), time since diagnosis (r = 0.49), and EDSS score (r = 0.51). As would be expected, age at diagnosis was negatively correlated with time since diagnosis (r = −0.27). EDSS score was correlated with age at diagnosis (r = 0.21) and time since diagnosis (r = 0.40).

Patients with MS and CIS had markedly greater brain-PAD scores at the time of the initial MRI scan compared with healthy controls (estimated marginal means 10.3 years [95% CI = 8.5–12.05] vs. 4.3 [2.1–6.4]; $p < 0.001$). The linear mixed-effects model was adjusted for the age, age$^2$, sex, field strength normalized brain volume, and cohort.

Despite heterogeneity between study cohorts, due to clinical characteristics and technical factors (eg, MRI scanner system), the difference between patients with MS and CIS and healthy controls was robust in a random-effects meta-analysis of the 6 cohorts that included both patients with MS and CIS and healthy controls (Fig 1A). The heterogeneity in the group differences was substantial ($I^2$ = 59% [95% CI = 3–91%]).

MS subtype (CIS, relapsing-remitting, secondary-progressive, and primary-progressive) significantly influenced brain-PAD ($F_{3,773}$ = 28.1; $p < 0.001$; Fig 1B). Estimated marginal mean brain-PAD per subtype were: CIS 6.7 years (95% CI = 5.0–8.4), relapsing-remitting 11.9 years [95% CI = 10.3–13.4], secondary-progressive 13.3 years [95% CI =

**(SPMS), EDSS of 6.5, and a brain-PAD of +18.6 years and a 49-year-old with primary-progressive multiple sclerosis (PPMS), EDSS of 2.0, and a brain-PAD of +14.9 years. (E) Scatterplot showing brain-predicted age derived from original, "unfilled" T1-weighted MRI scans (x-axis), plotted against brain-predicted ages generated from T1-weighted MRIs that had undergone the automated lesion "filling" procedure. (F) Bland–Altman plot of brain-predicted age from unfilled T1-weighted MRI scans and brain-predicted ages generated from "filled" T1-weighted MRIs. The plot shows the mean value from the 2 measures for each participant (x-axis) and the difference between the 2 measures (y-axis). The mean difference line is solid (mean difference = −0.28 years), and the corresponding limits of agreement (±1.96 * standard deviation of difference) are dashed lines. [Color figure can be viewed at www.annalsofneurology.org]**

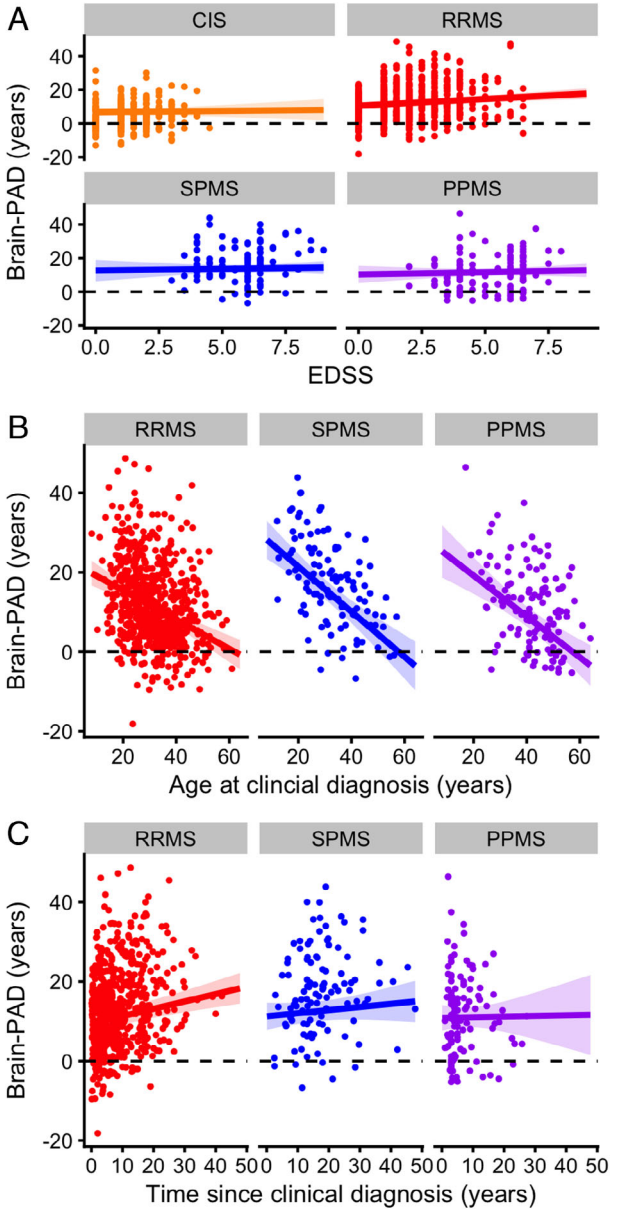


**FIGURE 2: Scatterplot of brain-predicted age difference by age at diagnosis, time since diagnosis, and Expanded Disability Status Scale (EDSS) score. (A) Baseline EDSS score (x-axis) and concurrent brain-predicted age difference (brain-PAD; y-axis). (B) Age at clinical diagnosis at first scan (x-axis) and concurrent brain-PAD (y-axis). (C) Time since diagnosis at baseline (x-axis) and concurrent brain-PAD (y-axis). Panels show patients with clinically isolated syndrome (CIS), relapsing-remitting multiple sclerosis (RRMS), secondary-progressive multiple sclerosis (SPMS), and primary-progressive multiple sclerosis (PPMS) separately. Lines represented the linear regression lines calculated per group, and shaded areas are the 95% confidence intervals. [Color figure can be viewed at www.annalsofneurology.org]**

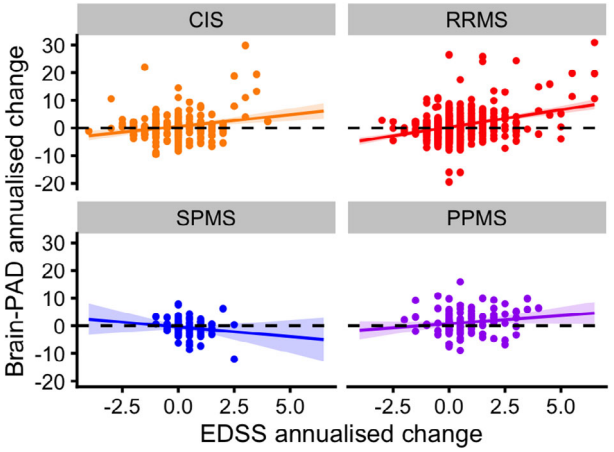


**FIGURE 3: Scatterplot of annualized changed in Expanded Disability Status Scale (EDSS) score and brain-predicted age difference. Panels show patients with clinically isolated syndrome (CIS), relapsing-remitting multiple sclerosis (RRMS), secondary-progressive multiple sclerosis (SPMS), and primary-progressive multiple sclerosis (PPMS) separately, with annualized change in EDSS score between baseline and final follow-up (x-axis) and annualized change in brain-predicted age difference (brain-PAD) between baseline and final follow-up (y-axis). Lines represented the linear regression lines calculated per group, and shaded areas are the 95% confidence intervals. [Color figure can be viewed at www.annalsofneurology.org]**

11.3–15.3], and primary-progressive 11.2 years [95% CI = 9.2–13.3]. Post hoc pairwise group comparison based on the estimated marginal means (Table 2) showed statistically significant differences ($p < 0.05$) in brain-PAD between each subtype and healthy controls (excluding CIS), and between patients with CIS and each of the 3 MS groups (relapsing-remitting, secondary-progressive, and primary-progressive). There were no differences among the 3 clinical MS groups. The findings of differences in brain-PAD among each MS subtypes and healthy controls ($p < 0.001$) can also be seen in a single cohort from a single center (cohort UCL3; Fig 1C). Again, there were no pairwise differences among subtypes (relapsing-remitting multiple sclerosis [RRMS] and secondary-progressive multiple sclerosis [SPMS] $p = 0.74$; RRMS and primary-progressive multiple sclerosis [PPMS] $p = 0.96$; and SPMS-PPMS $p = 0.98$).

Brain-PAD scores and corresponding T1-weighted MRI scans of individual female participants with different subtypes of MS are illustrated in Fig 1D to demonstrate the atrophy associated with higher brain-PAD.

### *The Relationship Among Lesions, Brain Volume, Scanner, and brain-PAD*

We considered the impact of lesions of brain-PAD, by comparing brain-PAD values on a single MRI scan from n = 575 patients with both a lesion-filled and unfilled version of the same image. The correlation between brain-predicted age using filled and unfilled scans was r = 0.99, $p < 0.001$ (Fig 1E) suggesting that the presence of lesions did not overly influence the brain-PAD values used throughout the study (which were unfilled). A Bland–Altman plot showed a mean difference between filled and unfilled scans

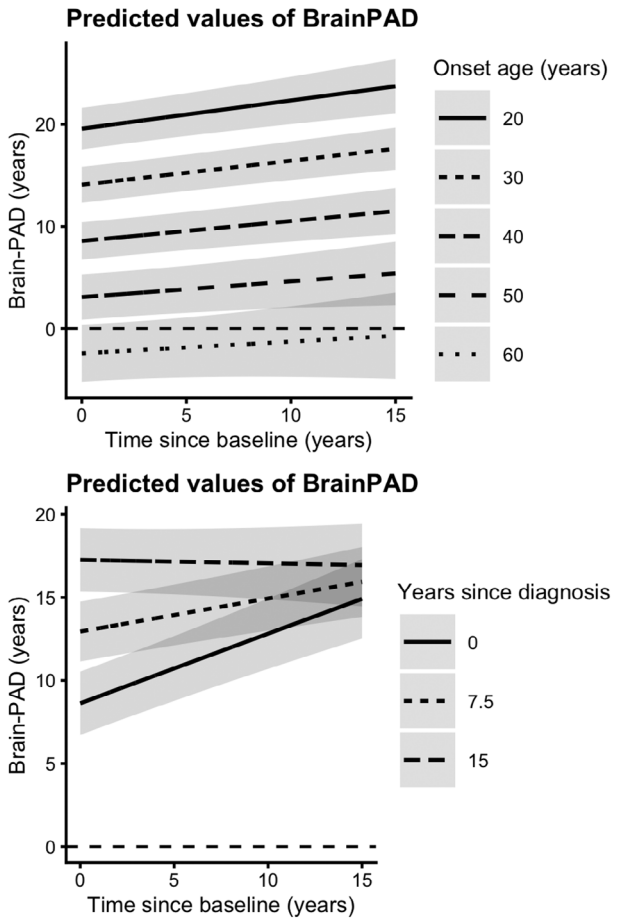


**FIGURE 4: How baseline age at onset and time since diagnosis interact with changes in brain-predicted age difference (brain-PAD). (A) Predicted slopes from estimate marginal means analysis of 3-way interaction between interval since baseline scan (x-axis), brain-PAD (y-axis), and age at disease diagnosis (ie, onset age). Predicted values for the estimated model were made for 5 exemplar onset ages, ranging from 20 years old to 60 years old. The predicted changes (ie, slopes) in brain-PAD over time from baseline scan are plotted for each, along with their 95% confidence intervals. No interaction is evident. (B) Predicted slopes from estimate marginal means analysis of 3-way interaction among intervals since baseline scan (x-axis), brain-PAD (y-axis), and time since disease diagnosis. Predicted values for the estimated model were made for 3 exemplar times since diagnosis, 0 years, 7.5 years, and 15 years since diagnosis at study baseline. The predicted changes (ie, slopes) in brain-PAD over time from baseline scan are plotted for each, along with their 95% confidence intervals. A strong interaction is evident.**

was −0.28 ± 1.29 years with no systemic bias caused by lesion filling evident, although there was increased variability between ages 60 and 70 years (Fig 1F).

We examined whether normalized brain volume, scanner field strength, and study cohort were driving the variability in brain-PAD using hierarchical partitioning of variance. The combination of chronological age, sex, grey matter, white matter, and CSF volume, field strength, and cohort explained an adjusted $R^2 = 0.56$ of the variance in brain-PAD. Independent contributions were: age (5.6% variance explained), sex (0.004%), normalized brain volume (38.9%), and field strength (0.006%). Cohort (ie, scanner) explained a further 10.5% of the variance in brain-PAD, highlighting the importance of statistically accounting for scanner or study site.

### *Brain-Predicted Age Difference at Baseline is Associated with Disability, Age at Clinical Diagnosis, Time since Diagnosis, and Disease-Modifying Treatments*

At baseline, a higher brain-PAD was associated with higher disability, as measured by the EDSS, when adjusting for age, age$^2$, sex, normalized brain volume, field strength, and cohort: for every 0.64 years increase in brain-PAD, EDSS increased by one (95% CI = 0.36–0.91; $p < 0.001$). There was no statistically significant interaction between MS subtype and EDSS score ($F_{3,1159} = 2.43$; $p = 0.06$; Fig 2A). With the same adjustments, a higher brain-PAD was associated with both younger age at diagnosis and longer time since diagnosis: for every year increase in brain-PAD, the age at diagnosis was reduced by 0.16 years (95% CI = -0.23 to -0.09; $p < 0.001$); for every year increase in brain-PAD, the time since diagnosis increased by 0.16 years (95% CI = 0.08–0.23; $p < 0.001$). There was no statistically significant interaction between subtype and age at diagnosis ($F_{2,877} = 0.60$; $p = 0.55$; Fig 2B). For time since diagnosis, the interaction was also not significant ($F_{2,797} = 0.97$; $p = 0.38$; Fig 2C). When predicting baseline clinical measures, brain-PAD was a significant predictor, alongside normalized brain volume, and age (see Table 3). Patients receiving a disease-modifying treatment had a higher estimated marginal mean brain-PAD (12.1 [95% CI = 10.6–13.7]) compared to those not receiving treatment (10.2 [95% CI = 8.8–11.6). This estimated difference of 1.91 years brain-PAD was significantly different (t = 3.9; $p < 0.001$).

### *Longitudinal Brain-Predicted Age Difference Increase Correlates with EDSS Worsening and Time since Diagnosis*

In patients who had 2 or more scans (n = 1155), annualized change in brain-PAD was a significant predictor of annualized change in EDSS (r = 0.26; $p < 0.001$). This relationship remained significant when accounting for change in normalized brain volume using partial correlations. There was a significant interaction between EDSS change and disease subtype, when predicting brain-PAD change in linear model ($F_{3,1089} = 3.90$; $p = 0.009$; Fig 3). This interaction remained significant when covarying for

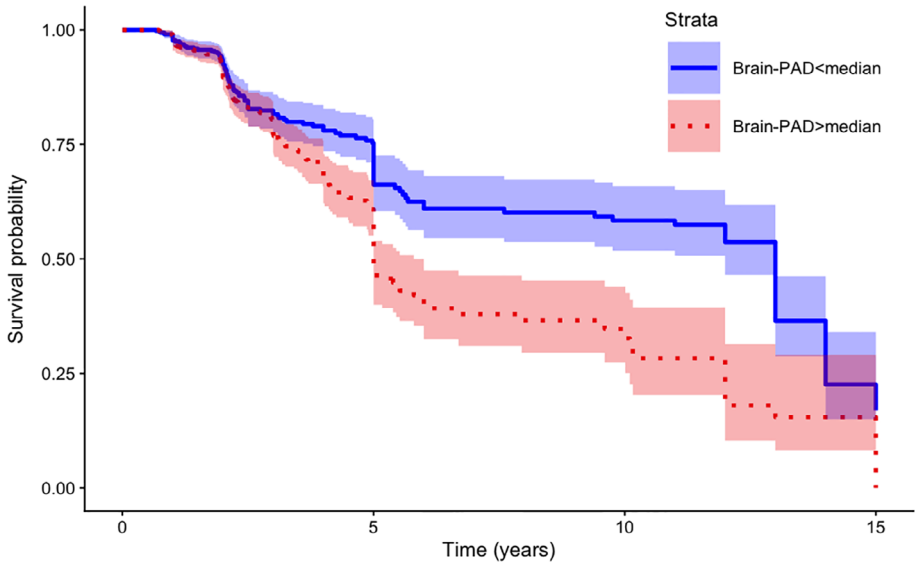


**FIGURE 5: Time-to-Expanded Disability Status Scale (EDSS) progression survival curves based on baseline brain-predicted age difference (brain-PAD). Kaplan–Meier plot illustrating the relationship between brain-PAD at first scan and survival prior to an EDSS progression "event." Based on a median split of brain-PAD within patients with multiple sclerosis (MS) and clinically isolated syndrome (CIS; median brain-PAD = +9.68 years). The solid line is the survival curve for patients > median brain-PAD, the dashed line for patients < median brain-PAD. Shaded areas represent the 95% confidence intervals for the survival curves. [Color figure can be viewed at www.annalsofneurology.org]**

change in normalized brain volumes. The slopes were positive in CIS (beta = 0.84; $p$ = 0.0001) and relapsing-remitting (beta = 1.25; $p$ < 0.001), although flatter in primary-progressive (beta = 0.59; $p$ = 0.090) and negative (although not significant) in secondary-progressive (beta = −0.70; $p$ = 0.29). To explore the latter finding post hoc, we correlated baseline brain-PAD with the number of follow-up scans completed. This showed a significant inverse correlation (n = 104; Spearman's rho = −0.29; $p$ = 0.0028). We also explored whether baseline age at disease onset and time since diagnosis predicted rates of change in brain-PAD, using linear mixed effects models, accounting for fixed effects of age, age$^2$, sex, field strength, and random effects of cohort and participant. When predicting brain-PAD, the interaction between interval and age at onset was not significant ($F_{1,693}$ = 1.06; $p$ = 0.30), but the interaction between time since diagnosis was ($F_{1,745}$ = 33.27; $p$ < 0.0001). The direction of this effect was negative, whereby those with greater time since diagnosis has less change in brain-PAD across the study duration (Fig 4).

### *Initial Brain-Predicted Age Difference Predicts EDSS Worsening*

In patients who had EDSS assessed at ≥2 timepoints (n = 1,143), baseline brain-PAD significantly predicted EDSS worsening. Of these patients, 303 (26.5%) experienced EDSS worsening during the follow-up period. Using a Cox proportional-hazards regression model, adjusted for age, age$^2$, sex, and field strength, the hazard ratio for brain-PAD was 1.023 (95% CI = 1.012–1.038; $p$ < 0.001; Fig 5). In other words, for every 5 years of additional brain-PAD, there was a 12.2% increased chance of EDSS progression during follow-up. The assumptions of proportional hazards were met ($p$ > 0.05). However, when including baseline normalized brain volume as a covariate, the relationship between brain-PAD and EDSS worsening was no longer significant ($p$ = 0.54), whereas the relationship between normalized brain volume and EDSS worsening was (hazard ratio = $8.14e^{-5}$; 95% CI = $2.75e^{-6}$ to $2.41e^{-3}$; $p$ < 0.001).

### *Brain-Predicted Age Difference Increases over Time in Patients with Multiple Sclerosis*

A total of 1,266 participants had 2 or more MRI scans (patients with MS and CIS = 1,155 and healthy controls = 111). This included 573 with 3 or more scans (patients with MS and CIS = 509 and healthy controls = 64). When using these data, we found a significant interaction between group and time ($F_{1,1325}$ = 5.85; $p$ = 0.016) and between MS subtypes and time ($F_{4,845}$ = 4.99; $p$ = 0.002), when adjusting for age, age$^2$, sex, field strength, and cohort. This indicated that the annual rate of increase in brain-PAD over time was faster in patients with MS and CIS than in healthy controls and was significantly different among MS subtypes. The estimate marginal trends (ie, slopes) of change in brain-PAD per group was as follows: healthy controls −0.17 = [95% CI = -0.46

to 0.13]; CIS = 0.15 [95% CI = 0.01–0.30]; relapsing-remitting patients = 0.24 [95% CI = 0.14–0.34], secondary-progressive patients = −0.26 [95% CI = -0.54 to 0.02]; primary-progressive patients = 0.42 [95% CI = 0.19–0.65]; and all patients combined (MS and CIS) = 0.61 [95% CI = -0.03 to 1.27].

## Discussion

By applying the brain-age paradigm in a large longitudinal cohort of patients with MS, we found that indeed MS has a pronounced effect on the brain-PAD metric, indicative of poor structural brain health. As the disease develops from a clinically isolated episode to clinically diagnosed MS, brain-PAD increases, reaching similar levels to patients with dementia.[33,34] A single baseline brain-PAD was independently associated with higher disability (measured by EDSS), younger age at diagnosis and longer time since diagnosis, irrespective of disease phenotype. Using scans performed at multiple sites in different scanners we observed that longitudinal brain-PAD increases correlate with worsening disability. Baseline brain-PAD also predicted future disability accumulation, although only when not accounting for normalized brain volume. In the whole cohort, we show that measures of brain-PAD increase more rapidly than normal chronological aging in relapsing-remitting and primary-progressive MS, implying that the brain-age approach is sensitive to accelerations in brain atrophy in MS.

In a life-long disease, the accumulation of neurological disability is the main clinical and societal burden,[35] estimated to cost $10.6 billion/year in the United States.[36] Tracking disease evolution is hampered by the lack of a simple and powerful outcome measure. MRI-assessed brain atrophy is a surrogate outcome for this process, but the requirement for precise longitudinal assessments, usually over at least a 12-month interval, reduces the feasibility of use. Here, we demonstrate that with a single T1-weighted MRI, brain-PAD values can index elements of MS disease progression. First, we show that a single point estimate can place a patient's disease and disability in context of their age. Our results suggest that the "brain-age" framework can provide informative data without the need for longitudinal scans.[37] Second, we demonstrate that a single measure can give prognostic value for disability accumulation. However, this effect seemed to be explained by normalized brain volumes, suggesting that brain-age would not be suitable to replace more conventional measures for prognostic purposes. The ability to make prognostic predictions from cross-sectional data should prove highly valuable to facilitate early use of therapy to prevent future disability accumulation,[38] however, it remains to be seen if the brain-age paradigm can add significant value over more commonly used volumetric measures in this context.

The brain-age paradigm has been applied widely in neuropsychiatric diseases,[14] although only recently in MS.[17,18] Cross-sectional comparisons showed a strong effect of MS on brain-age (mean increases of 4.4 and 5.6 years), whereas preliminary longitudinal evidence (n = 62) reported an additional 0.41 years of brain aging per year in relapsing-remitting patients, although no controls were included in this longitudinal analysis.[17] Here, we utilize serial MRI scans acquired over 15 years in a wide range of settings (eg, different countries, institutions, and scanners), analyzing n = 1,155 patients with MS and CIS. The mean magnitude of the apparent brain aging we observed in patients with MS (10.3 years) is greater than has been reported in dementia (9 years),[33] epilepsy (4.5 years),[20] or after a traumatic brain injury (4.7 years),[19] as well as being greater than the 2 previous, related brain-age studies in MS.[17,18] We show that brain-PAD increases faster than chronological age in patients with MS and CIS, with an additional 0.61 (95% CI = -0.03 to 1.27) years of brain aging per year across all MS subtypes. This is not dissimilar to the rate reported in Høgestøl and colleagues' study of n = 62 patients,[17] although it is important to be mindful of the precision of these estimates, hence, the CIs are important to consider. In our case, brain-PAD did not increase longitudinally in secondary-progressive patients, which widened the CIs (across zero) when considering the patients with MS and CIS together. The most rapid change in brain-PAD over time was in PPMS (0.42 [95% CI = 0.19–0.65]), and whereas the CIs robustly indicate that the brain-PAD changes are greater than expected in aging, the exact rate of change cannot be stated precisely. The decreasing brain-PAD in the SPMS group is potentially due to a survivor bias or a floor effect in this group, whereby those patients with rapidly deteriorating disease did not return for longitudinal follow-up. Evidence for this comes from the inverse correlation between brain-PAD at baseline and the number of follow-up scans acquired in secondary-progressive patients. Interestingly, brain-PAD relates to baseline EDSS as well as tracking changes in EDSS over time. The relationship is moderate, and of a similar magnitude to previous reports,[17,18] suggesting that it is robust. These changes in disability status co-occurred with changes in apparent "brain-age" even when adjusting for normalized brain volume.

We addressed some potential issues with the use of a nonspecific aging biomarker like brain age for the assessment of MS. Brain lesions, the overt MRI marker of MS disease activity, had minimal impact of the brain-PAD

measurement in MS. Brain volume is strongly similar to brain-age, however, statistically these measures are partially independent. In other words, brain volume correlated with brain-PAD, although >50% of the variance in brain-PAD is independent from brain volumes or age. Brain-age incorporates voxelwise MRI data in the statistical model, thereby capturing more information than using tissue-volume summary statistics. This means that more widespread and distributed patterns of features (ie, voxelwise grey matter and white matter volumes) can contribute to the age-prediction model, capturing elements of cortical thinning, sulcal widening, and ventricular enlargement, alongside more macroscopic loss of tissue volume. This study complements our recent work in MAGNIMS that assessed brain-regional volumes,[10] providing spatial information on brain atrophy during MS progression; the current work offers an alternative perspective on MS progression, using a global biomarker of age-related brain structural patterns. A key advantage of the brain-age paradigm over brain volume or longitudinal atrophy measures is that it automatically places an individual's brain health in context for their age, summarizing complex information in an intuitive and accessible manner. Brain-age also has the potential to go beyond merely brain structure, as recent work has shown the potential of incorporating multiple neuroimaging modalities into brain-age prediction models,[39,40] which could provide greater sensitivity to changes in brain health.

Our study has some strengths and weaknesses. The sample size for both training and test sets is relatively large but one potential limitation is the multiple sources of training data, although previous work has shown high between-scanner reliability.[29] Thus, application to a single individual needs to be in the context of individual scanner performance. Comprehensive biomedical data were not available on all training dataset participants, meaning some may have had undetected health conditions. However, individuals in this sample were screened according to various criteria to ensure the absence of manifest neurological, psychiatric, or major medical health issues. For longitudinal analysis, the follow-up time was short relative to the duration of MS (mean = 3.41 years), which means that the brain-PAD trajectories are only a restricted window onto the underlying disease dynamics. For controls, follow-up time was shorter still (mean <2 years), nevertheless, we were still able to estimate the temporal progression of brain-PAD in this healthy group (which was stable), which is informative given that the only previous longitudinal study of brain-age in MS did not include longitudinal controls. The MS and control cohort included data from multiple sites and scanners, which does impact brain-PAD values, and could potentially add a bias given that not every site included healthy controls. Nevertheless, we were able to statistically remove variance associated with each site, and by using a meta-analysis, able to demonstrate that the effects were robust in separate study sites, suggesting that scanner-related variance did not influence the study findings. Regarding lesions, we used unfilled images here, as our analysis indicated that lesion filling had minimal impact on brain-PAD values. Potentially, this means that lesion-severity, a key characteristic of MS, is not accounted for in our analysis. However, this may also be a strength, indicating the invariance of brain-age to pathological (ie, not healthy-aging related) changes to brain structure. Finally, our brain-age analysis pipeline only generates a whole-brain age prediction, thus does not provide any spatial local information about differential patterns in different brain regions, unlike some alternative methods,[18] meaning that neuroanatomic insights from this method are limited. However, we justify the use of the current approach as previous testing shows that whole-brain models result in higher correlations and lower errors than regional ones, and the aim of this study was to provide a robust and reliable biomarker that relates to clinical measures and outcomes, rather than investigate local neuroanatomy, which we have previously addressed using regional brain volumes in other related work.[10] Finally, we saw some effect of disease-modifying treatments on brain-age, whereby those receiving treatments had a higher brain-PAD than those who did not. This intriguing finding would benefit from follow-up in a clinical trial, where exposure to treatments is controlled experimentally, as studying treatment in a cohort study has several limitations, including the variable adherence and dosages, patients changing regimens, and, moreover, the confound that patients who are clinically worse at baseline are more likely to receive treatment. Potentially the observed higher brain-PAD in those receiving treatment is an artifact of the clinical requirements, rather than relating to brain-PAD directly.

This work supports the use of the "brain-age" paradigm in MS. We propose that brain-PAD has potential value for: (1) MS disease monitoring; potentially capturing the progressive processes that start early on in all disease phenotypes including CIS. (2) Integrating MRI measures of brain injury in MS in a wide range of centers and different scanners. (3) Conveying complex neuroanatomic information in a conceptually simple and intuitive manner. (4) Assessing both current brain health and prognosis. (5) Aiding clinical trial design, by stratifying enrolment based on high brain-PAD, or using brain-PAD as a surrogate outcome measure, reflecting age-associated neurodegeneration. Further work is needed to determine its utility, over and above common brain volumetric measures, in larger clinical cohorts, but its ease of use makes it an exciting candidate for such cohorts. Extensions of

brain-PAD could also incorporate multiple neuroimaging modalities and improve the anatomic interpretability of brain-age, both in general and specifically to MS. Ultimately, this may offer insight into an individual's disease course, in line with the move toward precision medicine in the treatment of MS.

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## Author Contributions

Concept and design of the study: J.C., J.R., T.F., A.E., W.B., D.C., N.D.S., C.E., L.P., M.F., C.G., M.A.R., A.R., S.R., J.S.-G., M.L.S., B.U., H.V., F.B., R.N., and O.C. Data analysis: J.C., J.R., T.F., and R.N. Manuscript drafting: J.C., J.R., T.F., R.N., and O.C.

## Potential Conflicts of Interests

The authors report no potential conflicts of interest.

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