Original research

Brain age gap in neuromyelitis optica spectrum disorders and multiple sclerosis

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Table 1 Demographic characteristics, baseline status and deep learning-derived brain age of participants

	NMOSD	RRMS	HCs	P value
Baseline				
N	199	200	269	
Age at baseline, year (min–max)	41.0±13.0 (16.9–66.0)	37.1±11.4 (16.6–66.9)	38.5±12.7 (17.0–69.0)	NMOSD versus HC 0.071 RRMS versus HC 0.468 NMOSD versus RRMS 0.005
Female, n (%)	176/199 (88.4)	128/200 (64.0)	152/269 (56.5)	<0.001
Seropositive for AQP4-IgG, n (%)	84/132 (63.6)	–	–	–
First onset to diagnosis, year (min–max)	4.5±5.1 (0.0–35.0)	3.2±4.4 (0.0–21.0)	–	0.006
Baseline use of DMT, n (%)	52 (26.1%)	86 (43.0%)	–	–
EDSS at baseline, median (IQR) (min–max)	2.0 (2.0) (0.0–9.0)	3.5 (3.0) (0.0–9.0)	–	<0.001
Brain segmentation volume without ventricles, mL (min–max)	1058.9±94.4 (798.7–1390.1)	1080.1±121.5 (742.6–1484.5)	1154.6±98.5 (910.7–1434.0)	NMOSD versus HC <0.001 MS versus HC <0.001 NMOSD versus RRMS 0.108
Normalised brain volume (min–max)	0.750±0.038 (0.647–0.891)	0.731±0.045 (0.590–0.858)	0.765±0.030 (0.700–0.894)	<0.001*
Total volume of lesion, mL (min–max)	4.9±8.1 (0.0–43.9)	12.7±17.9 (0.0–134.0)	–	<0.001*
Deep learning-derived brain age				
Predicted brain age, year (min–max)	46.4±16.0 (18.8–77.5)	49.8±17.5 (19.5–77.8)	39.3±13.7 (14.8–73.8)	<0.001*
Brain age gap, year (95% CI)	5.4±8.2 (4.3 to 6.5)	13.0±14.7 (10.9 to 15.0)	0.8±6.2 (0.1 to 1.6)	<0.001*
Predicted brain age SD, year (95% CI)	6.0±3.0 (5.6 to 6.5)	7.2±4.2 (6.6 to 7.8)	4.8±1.1 (4.7 to 4.9)	<0.001*
Follow-up				
N with follow-up data, n (%)	85 (42.7)	124 (62.0)	–	
Mean follow-up time, year (min–max)	5.8±1.9 (1.9–9.9)	5.2±1.7 (1.5–9.2)	–	0.020
EDSS worsening, n (%)	31 (36.5)	42 (33.9)	–	0.764

Continuous variables other than EDSS are reported as the mean±SD. EDSS is reported as the median (IQR).
*For all pairwise comparisons, that is, for NMOSD versus HC, RRMS versus HC and NMOSD versus RRMS.
DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; HC, healthy control; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; RRMS, relapsing-remitting multiple sclerosis.

Performance of the brain age prediction model

Model training (using 9794 HCs) was terminated at epoch 108. The mean absolute error (MAE) before inverse linear bias correction was 2.63 years in the developmental validation set, and this model was used as the final model for further analysis.

The model was then tested using 462 images for internal (across-scanner) validation and 267 images for external validation (across-centre). In the internal validation dataset, the MAE was 2.9 ± 3.1 years, with no significant difference across scanner types ($p = 0.581$, $n = 2$). The Pearson's correlation coefficient (r) between age and brain age was 0.957. In the external validation set, the MAE was 4.5 ± 3.9 years, and the Pearson's r was 0.890. The MAE was not significantly different across different centres ($p = 0.660$, $n = 5$; online supplemental table 2).

Increased BAG in NMOSD and RRMS compared with healthy controls

The difference in BAG among patients with NMOSD, patients with RRMS and HCs was relatively consistent across baseline chronological ages (figure 1A). At baseline, patients with NMOSD had a significantly higher BAG than HCs (NMOSD − HC = 4.6 years, 95% CI 2.4 to 6.9, $p < 0.001$), but patients with RRMS had a markedly higher BAG than HCs (MS − HC = 12.1 years, 95% CI 9.9 to 14.3, $p < 0.001$). BAG was lower in NMOSD than in RRMS (NMOSD − RRMS = −7.5 years, 95% CI 5.2 to 9.9, $p < 0.001$) (table 1, figure 1B).

Furthermore, we performed subgroup analyses of BAG in AQP4 seropositive versus seronegative patients with NMOSD, as well as in patients with NMOSD with versus without brain lesions. We observed that there was no significant difference in BAG between the AQP4 seropositive and seronegative subgroups (5.8 ± 8.8 vs 4.2 ± 6.9 years, $p = 0.256$). However, the BAG in patients with brain lesions was significantly higher than those without (7.1 ± 8.5 vs 3.4 ± 7.2 years, $p = 0.001$) (online supplemental table 5).

A significant difference in BAG across centres ($p < 0.001$) was noted, although post hoc analysis revealed consistent trends in disease effects on BAG in all six centres (figure 1C). Sample images and the corresponding output from both the NMOSD and RRMS groups were provided for better understanding (figure 1D–G).

The correlation between raw and lesion-filled 3D T1WI images was very high ($R^2 = 0.984$, $p < 0.001$, online supplemental figure 3A). A Bland-Altman plot showed that the mean difference between raw and lesion-filled brain age was 0.28 ± 2.11 years with no apparent systematic bias (online supplemental figure 3B), indicating that the lesion filling process did not have a particular impact on the model.

Correlation of BAG with clinical variables

At baseline, univariate linear regression analysis demonstrated that BAG was positively associated with EDSS in both the NMOSD and RRMS groups (NMOSD $r = 0.217$, $\beta = 0.86$, $p = 0.002$; RRMS $r = 0.268$, $\beta = 2.31$, $p < 0.001$; figure 2A). Normalised brain volume was inversely associated with BAG in both NMOSD and RRMS groups (NMOSD $r = -0.202$, $\beta = -48.5$, $p < 0.001$; RRMS $r = -0.384$, $\beta = -126.9$, $p < 0.001$; figure 2B). Multivariable linear regression found that BAG was positively predictive of baseline EDSS independent of normalised brain volume and disease duration (NMOSD $p = 0.030$; RRMS $p = 0.009$; online supplemental table 3).

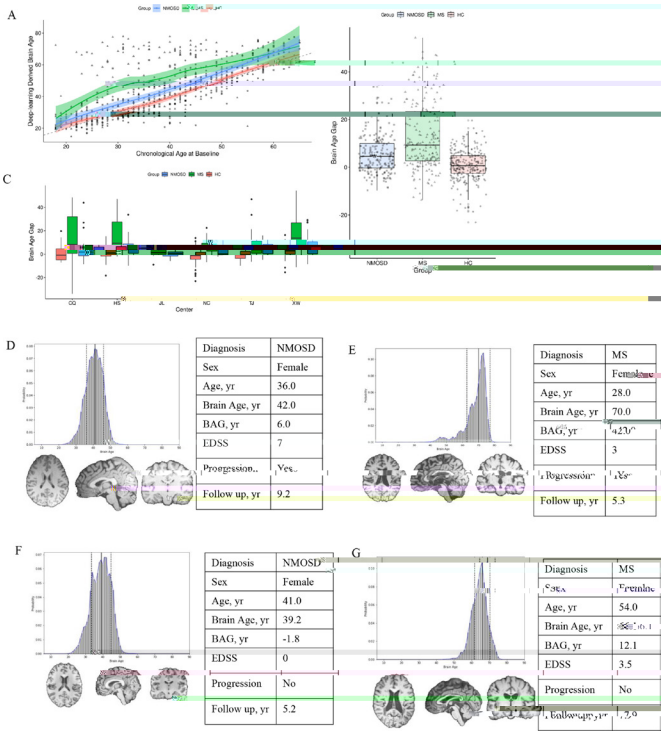

Figure 1 Deep learning-derived brain age versus chronological age in neuromyelitis optica spectrum disease (NMOSD), multiple sclerosis (MS) and healthy control (HC). (A) Deep learning-derived brain age versus chronological age in NMOSD, MS and HC groups. Predicted brain age is consistently higher in NMOSD and MS groups compared with HC group. (B) Patients with NMOSD exhibits lower brain age gap (BAG) over MS and lower BAG over HCs. (C) The difference of BAG across centres in NMOSD, MS and HC groups. The tendency that MS BAG>NMOSD BAG>HC BAG remains consistent even if there are significant differences across centres. (D, E, F, G) A sample input and prediction result of patients with NMOSD and MS. Solid line indicates brain age estimation and dashed lines indicate SD of prediction. The predicted brain age was 42.0±5.1 years for (D) and 70.0±7.2 years for (E), yielding BAG of 6.0 years and 42.0 years namely. Both (D) and (E) experienced disability progression in follow-up sessions. Predicted brain age for (F) and (G) was 39.2±5.6 years and 66.1±4.4 years yielding BAG of −1.8 years and 12.1 years namely. These patients with lower BAG did not experience disability progression within follow-up period. EDSS, Expanded Disability Status Scale.

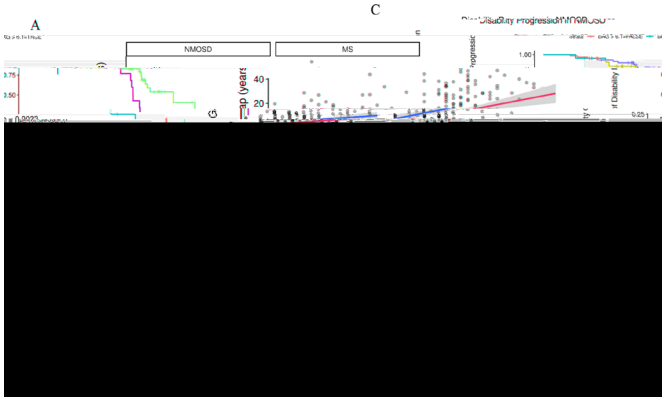

Figure 2 Correlation of brain age gap (BAG) with clinical variables and its prognostic value. (A) Increased BAG was associated with more severe baseline disability status in both neuromyelitis optica spectrum disease (NMOSD), multiple sclerosis (MS), which was more prominent in patients with MS. (B) Normalised brain volume was strongly negatively associated with BAG both in NMOSD and MS indicating possible contribution of atrophy in increased BAG. (C, D) Survival curve of BAG predicting disability progression in patients with NMOSD and MS. Cut-off point was determined by 80% specificity. Operating cut-off point for NMOSD is set to BAG >6.1 (sensitivity 38.7%, specificity 81.5%), MS is set to BAG >24.0 (sensitivity 50.0%, specificity 80.5%).

We performed 1:1 nearest neighbour propensity score matching (PSM)[18] to exclude the possible confounding influence of clinical variables on BAG. This matching yielded adequate balance for all included coefficients. The mean BAG was 5.0±7.1 years in NMOSD and 11.1±12.7 years in RRMS after adjustment for sex, age at diagnosis, baseline EDSS and normalised brain volume, with an estimated difference of −6.1 years (95% CI −8.7 to −3.4) years between NMOSD and RRMS (table 2).

The area under the curve of the receiver operating characteristic for BAG in predicting progression was 0.599 in NMOSD and 0.670 in RRMS. The optimal cut-off of BAG was 6.1 (sensitivity 38.7%, specificity 81.5%) for NMOSD and 24 (sensitivity 50.0%, specificity 80.5%) for RRMS (online supplemental figure 4). Kaplan-Meier survival analysis indicated that BAG was predictive of progression in both groups. For patients with NMOSD, the median time to progression for BAG >6.1 years was 5.79 years vs 7.99 years for BAG ≤6.1 years (p=0.003, figure 2C). The median time to progression for BAG >24.0 years was 5.36 years vs 8.95 years for BAG ≤24.0 years in patients with RRMS (p=0.002, figure 2D).

We used the Cox proportional hazards model to investigate whether BAG could be used to predict time to EDSS worsening independent of age at diagnosis, sex, disease duration, baseline EDSS and normalised brain volume. In univariate models, normalised brain volume and BAG were significantly associated with EDSS worsening in both patients with NMOSD and RRMS (table 3, univariate model). In a multivariable model, BAG was associated with EDSS worsening in patients with NMOSD (HR=1.02 (95% CI 1.00 to 1.04)). p=0.027, table 3), independent of normalised brain volume (p=0.158). However, neither normalised brain volume nor BAG was significant in the RRMS group in multivariable analysis. Interestingly, we found baseline EDSS to be negatively associated with EDSS worsening in NMOSD (multivariable model p=0.001, table 3).

Table 2 Patients with NMOSD exhibit lower brain age gap over RRMS adjusted for sex, age at diagnosis, baseline EDSS and normalised brain volume with propensity score matching

	NMOSD	RRMS	P value
N	119	119	–
Age at diagnosis, years	39.6±13.2	39.9±11.7	0.855
Female, n (%)	96 (80.7)	97 (81.5)	1.000
First onset to diagnosis, year	3.8±4.0	3.5±5.1	0.661
EDSS at baseline, median (IQR)	2.5 (2.0)	2.5 (2.0)	0.300
Normalised brain volume	0.745±0.038	0.742±0.042	0.538
Predicted brain age	44.5±15.5	50.0±16.9	0.008
Brain age gap	5.0±7.1	11.1±12.7	<0.001

Continuous variables other than EDSS are reported as the mean±SD. EDSS is reported as the median (IQR).
EDSS, Expanded Disability Status Scale; NMOSD, neuromyelitis optica spectrum disorder; RRMS, relapsing-remitting multiple sclerosis.

Table 3 Univariable and multivariable Cox proportional hazards model analysis for predicting EDSS worsening by BAG, age at diagnosis, sex, duration between first onset to diagnosis, baseline EDSS and normalised brain volume

	Univariable				Multivariable			
	NMOSD		RRMS		NMOSD		RRMS	
	HR	P value	HR	P value	HR	P value	HR	P value
N	85		124		85		124	
Number of events, n (%)	31 (36.5)		42 (33.9)		31 (36.5)		42 (33.9)	
Age at diagnosis, years	1.04 (1.00 to 1.08)	0.032	0.99 (0.96 to 1.02)	0.540	1.02 (0.98 to 1.06)	0.398	–	
Sex, male	0.52 (0.07 to 3.90)	0.527	1.23 (0.65 to 2.34)	0.523	–		–	
First onset to diagnosis, year	0.97 (0.89 to 1.05)	0.416	1.00 (0.91 to 1.11)	0.890	–		–	
EDSS at baseline								

Analysis of predicted SD in brain age prediction

The predicted SD was positively associated with BAG in all three groups (linear model $p<0.001$ in HC and NMOSD, $p=0.011$ in RRMS, online supplemental figure 5A). The mean SD in NMOSD was higher than HC and lower than RRMS (online supplemental figure 5B), which was consistent with the trend seen in BAG, indicating a higher model uncertainty in those images with greater discrepancy between apparent and chronological age. We examined scans with high model uncertainty and found that some of them could be attributed to low image quality or incomplete anatomical coverage (online supplemental figure 5C), while others were not visually distinguishable from those with lower model uncertainty (online supplemental figure 5D). To analyse whether the difference in BAG was driven by the difference in predicted SD, we performed PSM with predicted SD added as a covariate. The difference in BAG between NMOSD and RRMS, as well as NMOSD and RRMS versus HC, remained statistically significant after PSM adjusted for age, sex, duration to diagnosis, baseline EDSS, normalised brain volume and predicted SD ($p<0.001$, online supplemental table 4).

DISCUSSION

In this study, we developed a deep learning model to accurately predict age from 3D structural MRI scans and demonstrated its robustness in the context of multiple centres and MRI scanners. Using this model, the BAG was estimated to be approximately +5 years in NMOSD and +13 years in RRMS. Baseline BAG was independently predictive of EDSS worsening in both NMOSD and RRMS, suggesting its additional clinical value as a non-invasive biomarker for early triage, stratified follow-up management and clinical trial enrolment.

Previous non-deep learning studies on age prediction tasks reported 2.9-year to 5.0-year MAEs on their validation sets[7 19 20] (some of which included multimodality-derived features, including functional MRI and diffusion tensor imaging), while deep learning studies reported validation MAEs as low as 2.14 years, such as in the original SFCN study.[17] We reached similar performance levels of MAE=2.5 years in the developmental validation set, and the performance was maintained in an internal test set, demonstrating the usefulness of our model and highlighting the versatility and potential of deep learning-based methods. We have also shown that the whole-brain CNN-based model was robust within scanners and centres, supporting the clinical use of the brain age paradigm.

BAG has been investigated extensively as a comprehensive biomarker for accelerated ageing. Increased BAG has been observed in dementia,[21] epilepsy[22] and traumatic brain injury.[23] We report for the first time the meaningfulness of BAG in NMOSD as well as the difference between NMOSD and RRMS. We found a BAG of 5.4 (95% CI 4.3 to 6.5) years in patients with NMOSD, which, although lower than RRMS, is still marked compared with HCs. The degree of BAG increase in NMOSD is similar to what has been reported in epilepsy (4.5 years)[22] and traumatic brain injury (4.7 years).[23]

BAG in NMOSD was positively associated with baseline EDSS score and whole-brain atrophy, with associations comparable to those in RRMS but with a generally less steep slope. BAG was also predictive of EDSS worsening in NMOSD, which is in line with the idea that BAG is a composite marker of abnormal ageing and a disease-related brain. Furthermore, subgroup analysis of patients with NMOSD demonstrated that the BAG of patients with brain lesions was significantly higher than those without. This indicates that lesional brain involvement is associated with older appearing brains in patients with NMOSD. Future longitudinal studies are required to determine the possible causative factors.

In a recent study of brain age using Gaussian processes regression on MS, the authors reported 11.9 (95% CI 10.3 to 13.4) years BAG in patients with MS in the European Magnetic Resonance Imaging in Multiple Sclerosis (MAGNIMS) cohort,[7] which is consistent with our result of 13.0 (95% CI 10.9 to 15.0) years BAG in Chinese patients with MS. Furthermore, increased BAG was predictive of EDSS worsening in MS, also consistent with previous work.[7] Even though we used a fundamentally different methodology and datasets, these results provide additional evidence for the usefulness of BAG in the evaluation of patients with MS. Moreover, using deep learning can substantially shorten the runtime of the analysis pipeline. This acceleration in computation time is potentially of great benefit for widespread application in a clinical setting.

Comparing NMOSD and MS is difficult given the difference in confounding factors that may influence BAG. It has been reported previously that the atrophy patterns in NMOSD and MS are different. NMOSD exhibits more atrophy in the spinal cord but less atrophy in the brain,[24] which can partially explain the lower BAG in NMOSD given the strong association between BAG and brain atrophy. To address the influence of confounding effects such as demographics and brain volume, we used propensity score matching to sample a subset with matched baseline confounding factors. In this matched subset, the difference in BAG between NMOSD and MS was still significantly different even when matched for normalised brain volume. This finding

indicates that the brains of patients with RRMS appear older than those of patients with NMOSD even at the same level of atrophy, implying that BAG can be seen as a global estimation that integrates information beyond simple brain volumetry while being more accessible and informative than tables of volumetric measurements.

The uncertainty and distributional pattern of predicted brain age is an important field of research that has attracted little attention. A recent study modelled brain age uncertainty with a single-layer neural network that addressed aleatoric uncertainty with quantile regression and epistemic uncertainty with the Monte Carlo dropout technique.[25] In contrast to other studies that use quantile regression, the novel method in our study renders aleatoric uncertainty a natural derivative since the model output itself is a distribution instead of the point estimate used in previous studies.[4] Epistemic uncertainty was not derived in this study due to computational cost. Although the uncertainty correlated positively with BAG, the PSM analysis indicated that the BAG difference between NMOSD and RRMS remained statistically significant even after adjustment for predicted SD. We observed that the predicted SD were higher in those scans without enough information for brain age inference (ie, low image quality, etc), and in those with a greater discrepancy between predicted and actual age. This observation suggests a potential use case for the predicted SD. The quantification of individual-level uncertainty in this way could provide an integrated, intuitive metric for image quality control, especially in healthy people, as well as provide a measure of 'confidence' for applications in clinical contexts.

Our study has a few limitations. First, the follow-up duration was relatively short, and the sample size of patients with follow-up was small, which may have introduced selection bias. Second, although previous studies have suggested the longitudinal utility of brain age in healthy cohorts[6] and accelerated ageing measured by BAG has been observed in MS cohorts,[7] our cohort lacked sufficient follow-up assessments for this type of analysis. Finally, the interpretability of the results needs to be further improved; specifically, the anatomical meaning of brain age remains ill-defined. Deep learning-based methods have been cast as 'black boxes'; however, tools such as class activation mapping, guided backpropagation and occlusion analysis are emerging that aim to extract mechanistic information from the network.[26] However, the translation of these methods to 3D data is complex, and they have yet to be validated for use in interpreting medical imaging data. Additionally, our study relied on 3D T1WI MRI, which is not always available in clinical contexts. Future work will take advantage of brain age models developed to work on routine clinical two-dimensional scans.[27]

In conclusion, NMOSD demonstrated a significant BAG compared with HCs, although less marked than RRMS. BAG is a predictive biomarker of EDSS worsening in both NMOSD and RRMS.

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Contributors RW and XX: conception and design of the study, acquisition and analysis of data and drafting the manuscript. Y Liu acts as the guarantor of the study and takes full responsibility for the work. YD, NZ, JS, HL, Y Li, FB, JHC: conception and design of the study, acquisition and analysis of data. Y Li, CZ, XH, FZ, MH, RL, ZZ: acquisition and analysis of data. All authors revised the manuscript and approved the final draft.

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