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## Advanced Brain Age and Chronic Poststroke Aphasia Severity

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## Abstract

**Background and Objectives:** Chronic post-stroke language impairment is typically worse in older individuals or those with large stroke lesions. However, there is unexplained variance that likely depends on intact tissue beyond the lesion. Brain age is an emerging concept which is partially independent from chronological age. Advanced brain age is associated with cognitive decline in healthy older adults, therefore we aimed to investigate the relationship with stroke aphasia. We hypothesized that advanced brain age is a significant factor associated with chronic post-stroke language impairments, above and beyond chronological age and lesion characteristics.

**Methods:** This cohort study retrospectively evaluated participants from the POLAR clinical trial (NCT03416738), recruited through local advertisement in South Carolina (US). Primary inclusion criteria were left-hemisphere stroke and chronic aphasia ( $\geq 12$  months post-stroke). Participants completed baseline behavioral testing including the Western Aphasia Battery-Revised (WAB-R), Philadelphia Naming Test (PNT), Pyramids and Palm Trees Test (PPTT), and the Wechsler Adult Intelligence Scale matrices subtest, before completing 6-weeks of language therapy. The PNT was repeated 1-month post-therapy. We leveraged modern neuroimaging techniques to estimate brain age and computed a proportional difference between chronological age and estimated brain age. Multiple linear regression models were used to evaluate the relationship between proportional brain age difference and behavior.

**Results:** Participants (N=93, 58 males, 35 females, average age=61 years) had estimated brain ages ranging from 14-years younger to 23-years older than chronological age. Advanced brain age predicted performance on semantic tasks (PPTT) and language tasks (WAB-R). For participants with advanced brain aging (n=47), treatment gains (improvement on the PNT) were

independently predicted by proportional brain age difference ( $T=-2.0474$ ,  $p=0.0468$ , 9% of variance explained).

Discussion: Through the application of modern neuroimaging techniques, advanced brain aging was associated with aphasia severity and performance on semantic tasks. Notably, therapy outcome scores were also associated with proportional brain age difference, albeit only among participants with advanced brain aging. These findings corroborate the importance of brain age as a determinant of post-stroke recovery and underscore the importance of personalized health factors in determining recovery trajectories, which should be considered during the planning or implementation of therapeutic interventions.

## Introduction

Stroke-related damage to peri-sylvian language-related regions is often associated with varying types and degrees of aphasia<sup>1-3</sup>. Although some individuals with aphasia spontaneously recover in the first few months after the stroke, many stroke survivors experience persistent long-term disabling language and communication problems. Lesion size and location are important predictors of long-term language disability<sup>2,4</sup>, however, these factors only account for a proportion of the variance in aphasia severity (approximately 20-50%<sup>5,6</sup>). In addition to lesion factors, there is considerable evidence that language impairment following stroke is more severe in older adults<sup>7</sup>. Emerging data also supports the idea that stroke age is associated with greater long-term decline, with older individuals showing poorer trajectories than their younger counterparts<sup>8</sup>. The degree of chronic aphasic impairment is also remarkably variable across individuals, and factors which determine likelihood and severity of long-term deficits remain incompletely understood. No current model of aphasia severity is capable of accurately predicting impairment in this heterogeneous population.

It is well documented that there are declines in multiple cognitive skills in typical aging, but there are substantial differences in the trajectory of age-related decline across individuals<sup>9,10</sup>. This led to the emerging ideas of cognitive reserve as an explanation for why some individuals may be more resilient to the detrimental effects of aging<sup>11</sup>. Stern and colleagues described this

theory but suggest the underlying mechanisms by which brain and cognitive reserve enable individuals to maintain cognitive performance over time are still unclear<sup>11</sup>. Similarly, recent research suggests advanced brain aging is an important factor related to declining cognitive skills among adults in general<sup>12,13</sup>. Brain age can be estimated by comparing neuroimaging measures (e.g., regional tissue volume from gray and white matter) from one individual against a large normative database of healthy individuals. The primary measure of advanced brain aging is the difference between an individual's chronological age and the same individual's predicted brain age based on brain tissue integrity. Advanced brain aging (higher estimated brain age than chronological age) is now being increasingly recognized as an important factor related to lower cognition among older adults, as well as among individuals with dementia or with risk-factors for neurocognitive disorders<sup>14,15</sup>. It may be that advanced brain aging is associated with poor cognitive reserve, and conversely, delayed brain aging (lower estimated brain age than chronological age) may be associated with a resilience to age-related cognitive changes.

Given the known relationship between cognition and MRI-based brain aging metric, we hypothesized that brain age may be related to language impairments after a stroke beyond the effects of chronological age and characteristics of the stroke lesion. By controlling for these known factors, our analysis sought to measure the independent predictive power of brain age in a large sample of adults with chronic stroke aphasia. The brain age measure used quantifies the structural integrity of brain tissue, irrespective of the stroke lesion, i.e., not taking into context the stroke-related tissue loss, but the overall structural integrity of brain tissue that remains after the stroke.

Measuring brain age in the context of stroke can be technically challenging since existing approaches rely on tissue segmentation, which, in turn, depends on the quality of normalization (i.e., warping each individual brain into standard space). Existing approaches to brain normalization are typically designed to work with intact brains. When these methods are applied to brains with large lesion, they can fail catastrophically, providing outputs which dramatically displace relatively proximate voxel locations<sup>16</sup>. One potential solution to this problem, which has been used successfully by multiple groups studying stroke<sup>17,18</sup>, is to apply an enantiomorphic 'healing' algorithm<sup>17</sup> to damaged brains prior to normalization that effectively mimics healthy brains. This healing process relies on the fact that brains are generally left/right symmetrical to

replace damaged left hemisphere tissue with a mirror image of homologous healthy right hemisphere tissue.

In this study, we tested the hypothesis that chronic post-stroke aphasia severity is negatively influenced by advanced brain age. We investigated data from a cohort of stroke survivors with chronic aphasia, leveraging modern neuroimaging techniques (i.e., enantiomorphic healing) to adequately infer brain age from individuals with large stroke lesions and test their relationship with chronic deficits and treated recovery.

## Materials and Methods

### Study design and participants

We retrospectively evaluated an existing longitudinal dataset from participants with a history of one or more chronic (>12 months prior to enrollment) left hemisphere strokes. Participants were part of the POLAR (Predicting Outcomes of Language Rehabilitation in Aphasia)<sup>19,20</sup> clinical trial (NCT03416738). As part of the POLAR protocol, all participants underwent a baseline battery of language and neuropsychological testing at the time of enrollment. The baseline battery included the Western Aphasia Battery-Revised (WAB-R)<sup>21</sup>, Philadelphia Naming Test (PNT)<sup>22</sup>, Pyramids and Palm Trees Test (PPTT)<sup>23</sup>, and the Wechsler Adult Intelligence Scale matrices subtest (WAIS). The battery was administered by American Speech-Language-Hearing Association certified speech and language pathologists with experience working with individuals with aphasia.

Following the baseline behavioral battery, participants underwent 6-weeks of semantic and phonological treatment using the following timeline: 3-weeks of treatment, 4-weeks of rest, then 3-weeks of the alternative treatment. Treatment order was randomized, with half the participants receiving semantic treatment first and the other half receiving phonological treatment first. The PNT was the primary outcome measure and was completed again 1-month following treatment (see Kristinsson and others<sup>20</sup> for a more detailed description of the procedure). The primary goal of POLAR was to provide information about neurobiological and demographic factors associated with aphasia treatment outcomes.

The following inclusion/exclusion criteria applied: Participants were included in the study if they i) incurred a left-hemisphere ischemic or hemorrhagic stroke to the middle cerebral

artery, ii) had chronic aphasia ( $\geq 12$  months post-stroke), iii) were 21-80 years of age, iv) spoken English was their primary language for  $\geq 20$  years, and v) could provide written or verbal consent. Participants were excluded if they had i) severely limited verbal output (measured by a WAB-R Spontaneous Speech score of 0-1), ii) severely impaired auditory comprehension (measured by a WAB-R Auditory Comprehension score of 0-1), iii) bilateral or cerebellar stroke, iv) contra-indications to testing with magnetic resonance imaging (MRI), or v) history of neurological disorders. Individuals with multiple strokes were eligible if all lesions were confined to left supratentorial territory.

### **Standard Protocol Approvals, Registrations, and Patient Consents**

All participants were recruited through local advertisement at the University of South Carolina (Columbia) or at the Medical University of South Carolina (Charleston). The study was approved by the Institutional Review Boards of both institutions. Participants were retrospectively evaluated from an existing longitudinal clinical trial; POLAR (Predicting Outcomes of Language Rehabilitation in Aphasia, clinicaltrials.gov ID: NCT03416738).

### **Neuroimaging acquisition and preprocessing**

All participants underwent research MRI scanning on a Siemens Trio 3T scanner equipped with a 20-channel head coil. In this study, only T1-weighted images were used for brain age estimation, and T2-weighted images were used for stroke lesion identification. These were acquired using the following parameters: T1-weighted imaging (MP-RAGE) sequence with 1mm isotropic voxels, a 256x256 matrix size, a 9° flip angle, and a 92-slice sequence with repetition time (TR)=2250ms, inversion time (TI)=925ms, and echo time (TE)=4.11ms. T2-weighted scans were acquired using a 3-dimensional T2-weighted SPACE sequence covering the whole head with a resolution of 1mm<sup>3</sup> and a field of view=256x256mm, 160 sagittal slices, variable degree flip angle, TR=3200ms, TE=212ms.

Chronic stroke lesions were manually drawn using each participant's T2-weighted image in native space. All lesion tracings were performed by an expert neurologist (author LB) or by a trained study staff member (author RNN) and were blinded to the behavioral data. Enantiomorphic normalization was then conducted using the *nii\_preprocess* pipeline ([https://github.com/neurolabusc/nii\\_preprocess](https://github.com/neurolabusc/nii_preprocess)<sup>24</sup>), a set of Matlab-based (R2017b, The

MathWorks) scripts that leverage multiple best-of-breed programs (SPM12; Functional Imaging Laboratory, Wellcome Trust Centre for Neuroimaging, Institute of Neurology [[www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)], FSL v6.0.3<sup>25</sup>, ASLtbx [<http://www.cnf.upenn.edu/~zewang/ASLtbx.php>], and MRITrix [<https://www.mrtrix.org/>]) in order to normalize and process MRI data acquired from individuals with lesioned brains. These scripts utilized enantiomorphic normalization<sup>17</sup>

To create chimeric images (i.e., ‘healed’ brains) in which the damaged portion of the left hemisphere was temporarily replaced with the mirror image of intact areas from the healthy right hemisphere (using the SPM12’s Clinical Toolbox which leverages SPM12’s unified segmentation-normalization<sup>26</sup> method to warp this chimeric image to standard (MNI) space, and the resulting spatial transform was then applied to the native-space T1 scan as well as the native-space versions of the hand-drawn lesion map. This additional step (enantiomorphic normalization) ensures that segmentation-normalization methods designed for intact brains do not incorrectly warp scans with large lesions to the left hemisphere.

## Brain age estimation

Brain age estimation was performed based solely on T1-weighted images using the BrainAgeR analysis pipeline ([github.com/james-cole/brainager](https://github.com/james-cole/brainager))<sup>27</sup>. However, since the BrainAgeR analysis pipeline involves the iterative segmentation-spatial normalization routines (described below) designed to handle healthy non-lesioned brains, we used the enantiomorphic healing approach described above to remove the stroke lesions from the MRI scans to allow for adequate tissue estimation before the images were input into the BrainAgeR pipeline. By removing the stroke lesion, we ensured that the lesion did not affect the brain age calculation, and that brain age was based on the intact brain tissue outside the lesion.

As such, the lesion maps in native T1-weighted space were smoothed with a 3mm full-width half maximum Gaussian kernel to remove sharp edges and to include the peri-lesional tissue. The enantiomorphic segmentation-normalization was then employed using SPM12 and custom MATLAB scripts (publicly available at: [https://github.com/neurolabusc/nii\\_preprocess](https://github.com/neurolabusc/nii_preprocess)<sup>24</sup>). The enantiomorphic approach creates a mirrored image of the right hemisphere, which is overlaid onto the left hemisphere that contains the stroke lesion. A chimeric image is then created in which the lesioned tissue is fully



replaced by tissue from the contralateral hemisphere. In other words, the stroke-related damaged area is substituted by the homologue tissue in the preserved right hemisphere. This ‘healed’ brain does not include the stroke lesion, and it was this image that was input into the BrainAgeR pipeline described below.

The BrainAgeR pipeline was applied using the default protocol<sup>27,28</sup>. First, T1-weighted images were segmented into gray matter and white matter before being normalized using non-linear spatial registration<sup>27</sup> and SPM12's DARTEL toolbox<sup>26</sup>. The BrainAgeR analysis pipeline uses a customized version of FSL slicesdir to generate a directory of images and corresponding index.html files for quality controlling in a web browser ([github.com/james-cole/brainageR](https://github.com/james-cole/brainageR)). These probabilistic tissue maps were visually inspected by an expert neurologist (author LB) to ensure quality of the segmentation. The cerebrospinal fluid tissue was removed, and the gray and white matter probabilistic tissues were vectorized, concatenated, and subjected to a principal component analysis to reduce dimensionality. The components explaining the top 80% of the variance were used for brain age prediction. A machine-learning algorithm using a pretrained Gaussian regression model implemented in R package Kernlab was used to estimate brain age. This pretrained model was based on scans of 3,377 healthy individuals from 7 publicly available datasets and tested on 611 different scans of healthy individuals aged between 18 and 90 years<sup>27</sup>. See Cole and colleagues for more detail on the BrainAgeR pipeline<sup>27,28</sup>. The image processing steps are shown in Figure 1.

The relative difference between the estimated brain and the participant's chronological brain age was determined using the formula:

$$(\text{estimated brain age} - \text{chronological age}) / \text{chronological age}.$$

As such, for each participant, a proportional brain age difference was calculated representing the proportion of the participant's chronological age accounted for by the difference between brain age and chronological age. More specifically, *proportional brain age difference* (PBAD) is the measure of advanced (or delayed) brain age beyond chronological age, adjusted proportionally for chronological age. Positive values suggest that the predicted brain age is older than the chronological age of the participant (i.e., advanced brain aging), and negative values suggest chronological age is older than predicted brain age (i.e., delayed brain aging). This measure is hereafter referred to as the *proportional brain age difference*.

## Statistical analyses

Normality of primary behavioral variables used for analysis were tested using a one-sample Kolmogorov-Smirnov test. The relationship between continuous demographic variables were assessed using Pearson's or Spearman's correlations for parametrically or non-parametrically distributed variables, respectively (as indicated in the results section).

To assess the relationship between PBAD and language/cognitive measures, we performed two main sets of analyses. First, we evaluated the relationship between the proportional brain age difference and baseline performance (pre-treatment assessments) using multiple linear regression models in which the behavioral variable was set as the dependent variable, and the following variables were used as independent variables: PBAD, chronological age, and lesion volume.

Second, we evaluated the relationship between PBAD and POLAR treatment outcomes, where the relative gain in language function was set as the dependent variable in multiple linear models. More specifically, relative gain in language function was evaluated as the *proportion of maximal gains* according to the formula: (correct items post-therapy – correct items pre-therapy) / (maximum score – correct items pre-therapy).

The same independent variables were used to assess treatment outcomes: chronological age, PBAD, and lesion volume. Additionally, baseline performance (correct items pre-therapy) was also included as an independent variable.

It should be noted that PBAD used in all analyses (concerning baseline behavioral measures or post-treatment recovery) were computed based on the MRI obtained at baseline, i.e., before language treatments.

## Data Availability

The conditions of our ethics approval do not permit sharing of the raw MRI data supporting this study with any individual outside the author team under any circumstances. However, anonymized data not published within this article will be made available by request from any qualified investigator.

## Results

### Participants

We retrospectively evaluated an existing longitudinal dataset from participants (N=109) with a history of one or more chronic (>12 months prior to enrollment) left hemisphere strokes. After confirming participants met our inclusion/exclusion criteria, the results cohort comprised of 93 participants. Demographics information describing this sample are provided in Table 1.

### Brain Age

The estimated brain age difference ranged from 14 years younger to 23 years older than the participant's chronological age. The mean difference between brain age and chronological age was 2.18 $\pm$ 7.6 years. The PBAD was 0.04 $\pm$ 0.14. Figure 2 demonstrates two examples highlighting (A) a participant whose estimated brain age differed substantially from the participant's chronological age, and (B) a participant whose estimated brain age and chronological age were similar.

There was a significant relationship between participants' chronological age and the estimated brain age (Spearman's  $r=0.7190$ ,  $p<0.0001$ ). Older participants had a lower difference between brain age and chronological age (Spearman's  $r=-0.2775$ ,  $p=0.007$ ), as well as a smaller PBAD (Spearman's  $r=-0.3317$ ,  $p=0.001$ ). These results are shown in Figure 3.

There was not a significant relationship between chronological age and lesion volume (Spearman's  $r=-0.1524$ ,  $p=0.15$ ), between brain age and lesion volume (Spearman's  $r=-0.1757$ ,  $p=0.1$ ), or between the PBAD and lesion volumes (Spearman's  $r=-0.006$ ,  $p=0.96$ ).

### Baseline cognitive and language variables

Pyramids and Palms Trees Test (PPTT): Models composed of chronological age, PBAD, and lesion volume significant predicted performance on the PPTT ( $F=7.9079$ ,  $p=0.0001$ ). Chronological age was not significantly associated with the PPTT scores ( $T=-0.6424$ ,  $p=0.5223$ ), but higher PBAD ( $T=-2.5174$ ,  $p=0.0136$ ) and larger lesion volumes ( $T=-4.2830$ ,  $p<0.0001$ ) were associated with worse performance on this test.

WAIS Matrix Reasoning: A similar model showed significant associations between WAIS Matrix Reasoning scores, chronological age, PBAD and lesion volume ( $F=4.1546$ ,

$p=0.0084$ ). Chronological age ( $T=-3.1200$ ,  $p=0.0024$ ) and lesion volumes ( $T=-2.0311$ ,  $p=0.0452$ ) were associated with lower WAIS scores, with older participants having significantly worse behavioral scores. However, PBAD was not an independent predictor ( $T=-1.2832$ ,  $p=0.2027$ ).

Western Aphasia Battery-Revised (WAB): Linear modelling revealed that all WAB scores were significantly associated with chronological age, PBAD, and lesion volume. PBAD was an independent predictor of worse scores for all measures, except for comprehension. All models are summarized in Table 2 and Figure 4. Figure 5 demonstrates the relationship between the WAB scores and PBAD scores after regressing out variance due to chronological age and lesion volume.

Philadelphia Naming Test (PNT): We observed a significant relationship with the prediction model ( $F=8.0514$  Model,  $p=0.0001$ ), with higher chronological age ( $T=-2.4007$ ,  $p=0.0187$ ) and larger lesion volume ( $T=-4.4976$ ,  $p=0.0000$ ) as predictors of worse scores. PBAD was not an independent predictor ( $T=-1.5197$ , PBAD  $p=0.1326$ ). Of note, the PNT was available for only a subset ( $n=82$ ) of all participants.

## Language therapy outcomes

The PNT was available for 78 participants at both baseline testing and testing conducted 1-month after therapy. At baseline testing, participants had an average PNT score of 80.83 ( $SD=61.77$ ,  $\min=0$ ,  $\max=172$ ). At one month follow up, the average PNT score was 86.76 ( $SD=63.73$ ,  $\min=0$ ,  $\max=173$ ). The average change in PNT score between baseline and one month was 5.93 ( $SD=9.20$ ,  $\min=-12$ ,  $\max=43.5$ ). The average proportion of maximal gains score was 0.13 ( $SD=0.17$ ,  $\min=-0.11$ ,  $\max=0.625$ ).

Language improvement as proportion of maximal gains in correctly named items in the PNT was associated with the linear model not including baseline performance ( $F=6.1530$ ,  $p=0.0009$ ), in which the chronological age ( $T=-2.5217$ ,  $p=0.0138$ ) and lesion volumes ( $T=-3.8274$ ,  $p=0.0003$ ) were independent predictors of recovery. PBAD was not a significant predictor ( $T=-1.2824$ ,  $p=0.2037$ ).

In the model with baseline performance included as an independent variable, there was also a significant relationship with improvement ( $F=2197.9094$   $p<0.0001$ ) but only baseline performance was a significant predictor ( $T=83.7965$ ,  $p<0.0001$ ).

Forty-seven participants had an estimated brain age which *was equal or higher than the participant's chronological age*. For these participants the average baseline PNT score was 80.42 ( $SD=61.85$ , min=0, max=169). At one month follow up, the average PNT score was 86.15 ( $SD=63.98$ , min=0, max=171). In these participants, the average change in PNT score between baseline and one month was 5.73 ( $SD=9.19$ , min=-12, max=43.5). The average proportion of maximal gains score was 0.13 ( $SD=0.18$ , min=-0.11, max=0.625). Among participants whose estimated brain age *was equal or higher than the participant's chronological age* ( $n=47$ ), the improvement in the PNT was independently associated with a lower PBAD. Specifically, not including baseline performance in the model, the proportion of maximal gains in correctly named items in the PNT was significantly associated ( $F=3.1104$ ,  $p=0.0361$ ) and independently predicted by a lower PBAD ( $T=-2.0474$ ,  $p=0.0468$ , 9% of variance explained) and lesion volumes ( $T=-2.3682$ ,  $p=0.0224$ ). Chronological age was nearing statistical significance but not an independent predictor ( $T=-1.9985$ ,  $p=0.0520$ , 8% of variance explained).

Importantly, *including baseline performance in the model*, PNT improvement was associated ( $F=1362.0020$ ,  $p<0.0002$ ) and independently predicted by PBAD ( $T=-2.3095$ ,  $p=0.0259$ , 11.2% of variance explained).

## Discussion

In this study, we evaluated the hypothesis that chronic post-stroke aphasia severity is influenced by brain age. We tested this by investigating the contribution of brain age (in addition to typical explanatory variables such as chronological age and lesion volume) using regression models designed to predict behavioral scores and treatment trajectories. This was made possible through the application of modern neuroimaging techniques (i.e., enantiomorphic healing), which enabled us to apply brain age estimation algorithms to non-standard (i.e., lesioned) brains. Specifically, we used output from the brain age algorithm to compute a proportional brain age difference (PBAD) score. Using this metric, we found that the PBAD was associated with performance on both the PPTT and well-known language tasks including WAB subscores (naming, spontaneous speech, repetition), and overall aphasia severity (WAB-AQ). Notably, therapy outcome scores between baseline and 1-month were also associated with PBAD, albeit

only among participants whose estimated brain age was equal to or higher than their chronological age.

## **Challenges of Computing Brain Age in Stroke**

Brain age predictions have been used previously in clinical populations<sup>28</sup>, including schizophrenia<sup>29</sup>, and epilepsy<sup>30</sup>. However, computing brain age within clinical populations with brain lesions or resections poses additional challenges<sup>31,32</sup>. The presence of large lesions is not only methodologically cumbersome, but the large volume of damaged tissue is known to influence behavioral and cognitive outcomes substantially<sup>2-4</sup>. This phenomenon is highlighted by the current study in which lesion volume was found to be significantly related to all behavioral scores. These results may indicate that more subtle effects of advanced brain aging are not as prominent as in otherwise healthy populations with either smaller or perhaps more diffuse brain lesions (e.g., atrophy). Further, the strong relationship between stroke lesion volume and behavioral performance may explain why we did not find associations between PBAD and general cognition (measured by the WAIS), a finding that has been previously described<sup>12,13</sup>. Thus, the current results highlight the importance of considering brain age in stroke aphasia, as it was a predictor of language abilities even when accounting for lesion volume and chronological age.

## **The Role of Brain Age in Language Performance**

The results from this study demonstrate a relationship between PBAD and aphasia severity (measured by the WAB). While chronological age and lesion volume were also predictors of aphasia severity, PBAD was able to explain some of the previously unexplained (additional) variance. Although lesion volume is often reported as a reliable predictor of post-stroke aphasia severity, the contribution of other health and demographic factors is less consistent. One possibility is that specific combinations of health and demographic factors influence brain age, and therefore, brain age mediates the relationship between these factors and overall aphasia severity. The association between PBAD and PPTT scores is particularly interesting, as chronological age was not a significant predictor in this case. This finding suggests the intriguing possibility that advanced aging, as defined in the current study, may preferentially impact participants' ability to access semantic information. As accessing semantic

information is typically associated with the ventral language stream<sup>2,33</sup> and the bilateral temporal lobes<sup>34,35</sup>, it may be that advanced brain aging particularly affects these connections and regions. Future studies could investigate the spatial distribution of advanced age-related changes to identify the brain regions most affected.

## **The Role of Brain Age in Language Recovery**

Our results demonstrating that PBAD independently predicts therapy gains is of clinical relevance and has clear clinical implications. A common view suggests that aphasia recovery is limited in the chronic stage<sup>36</sup>, with outcome largely predicted by initial impairment<sup>36</sup>. However, recent research casts doubt on these conclusions<sup>8,37</sup>. While measures of aggregate performance may appear to plateau, this may reflect different patient trajectories based on factors related to brain health (cognitive reserve), environment, and genetics<sup>38</sup>. This suggests that personalized patterns in aphasia impairments are highly variable, and the factors that influence who shows therapy gains are not completely understood<sup>39</sup>. This view suggests that understanding these factors may prove valuable in providing accurate prognosis, recommending bespoke treatment and compensation regimes, and improving statistical power in clinical trials by matching individuals for predictable gains. Although recently health factors have been considered in models of language recovery<sup>8,40,41</sup>, brain age has not yet been considered in these models. However, the significance of the health and integrity of the spared tissue in populations with cortical damage, such as stroke survivors, is becoming increasingly clear within the literature<sup>41,42</sup>. The results of the current study are consistent with recent reports that the integrity of the spared tissue is related to cognitive reserve<sup>12</sup> and capacity for therapy gains<sup>41,42</sup>, as well as the finding that diffuse neuronal diseases, such as small vessel disease (which can result in leukoaraiosis) is associated with worse stroke outcomes<sup>43</sup>, post-stroke cognitive decline<sup>44</sup>, and poorer language recovery<sup>41,42</sup>. As advanced aging is thought to represent reduced cortical integrity, it is perhaps not surprising that this would affect a complex cognitive skill such as language. Notably, the lack of a relationship between therapy outcome scores when including participants with a younger brain age than chronological age suggests that there are other factors influencing therapy outcomes, particularly in otherwise healthy individuals. These factors might include things like age at the time of stroke, cumulative amount of therapy received since stroke,

and other systemic health measures. There is a critical need to investigate these factors in combination with brain age in future research.

### **Association with Cardiovascular Risk Factors**

In this study, estimated brain age ranged from 14-years younger to 23-years older than chronological age, but no associations were found between lesion volume and chronological age or PBAD. Although brain age and chronological age were positively correlated, we observed considerable variation in brain age within participants with the same chronological age. Previous studies have suggested that brain age in stroke patients is higher than that of chronologically age-matched controls<sup>45</sup> at 6-weeks, 3-months, and 12-months post-stroke. There are several factors which may influence brain age, including education<sup>46</sup> and physical activity<sup>46</sup>, and it is possible that brain age itself is a biomarker for stroke risk<sup>45</sup>. Chronological aging is both a risk factor for stroke<sup>47</sup> and is associated with poorer stroke outcomes<sup>8</sup>, as highlighted by the consistent negative relationship found between age and language scores in the current study. Similarly, since cardiovascular risk factors such as BMI, hypertension, and diabetes are associated with both poorer brain health<sup>48</sup> and increased risk of stroke<sup>49</sup>, these factors may also be related to advanced aging. Future studies could combine measures of brain age with other markers of overall brain health (e.g., small vessel disease) to determine the relative influence of each of these in determining severity of post-stroke aphasia as well as treatment outcomes. Furthermore, future studies could investigate which health or demographic factors may predict who is likely to have delayed/advanced brain aging.

Vascular mechanisms are also one of the major contributing factors to dementia in the elderly, and vascular dementia is common post-stroke. As brain age is a measure of cortical integrity, advanced brain aging may be directly related to mild cognitive impairment or subclinical Alzheimer's disease. Among individuals with aphasia, the ascertainment of cognitive impairment can be limited by language barriers and was not something that was directly tested in this study but is an important avenue for future research.



## Limitations and Future Directions

Although using enantiomorphic ‘healing’ enabled the estimation of brain age in individuals with large stroke lesions, the natural physiological asymmetry in brain hemispheres is therefore lost and this is a limitation of this approach.

Furthermore, this is a retrospective study and ninety-three participants constitutes a relatively small sample size for studies using brain age estimations, however, to our knowledge, this is the first application of the BrainAgeR pipeline to individuals with stroke aphasia and included participants had a broad distribution of chronological age (29-80 years), time post-stroke (12-241 months), education (12-20 years), and aphasia severity (WAB-AQ: 20.1-92.6) across 6 different aphasia subtypes.

It is important to note that it is likely that all regions of the brain do not age at the same rate, and therefore regional distributions of aging in aphasia (and healthy aging) would be an important avenue for future research. The BrainAgeR methodology uses principal components of voxel ensembles that are responsible for most of the explained variance and are not necessarily associated with specific regions (i.e., they may involve more than one region, or parts of regions). Therefore, future research could focus on the development of region based aging methodologies which would allow the identification of region-specific premature brain aging.

## Conclusions

Overall, the current study supports the theory that brain age is an important factor for language deficits post-stroke, highlighting the importance of personalized health factors beyond the characteristics of chronological age and the stroke lesion in determining post-stroke impairment and treatment response. Future studies could focus on integrating measures of brain age with other markers of brain health to better understand potential interactions between various factors affecting language outcomes post-stroke.

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## Tables

**Table 1.** Demographic information of all participants (n=93).

Demographic Variables	Value
Stroke age, mean (SD)	56.81 (11.95)
Test age, mean (SD)	61.00 (11.37)
Education, mean (SD)	15.54 (2.26)
Sex (males:females), count	58:35
Handedness (left:right), count	9:84
Months post-stroke, mean (SD)	49.32 (52.47)
Fluency (fluent:nonfluent), count	45:48
Western Aphasia Battery Aphasia Quotient, mean (SD)	58.87 (22.38)
Aphasia Types (count)	
Anomic	25
Broca's	44
Conduction	13
Global	4
Transcortical Motor	1
Wernicke's	6

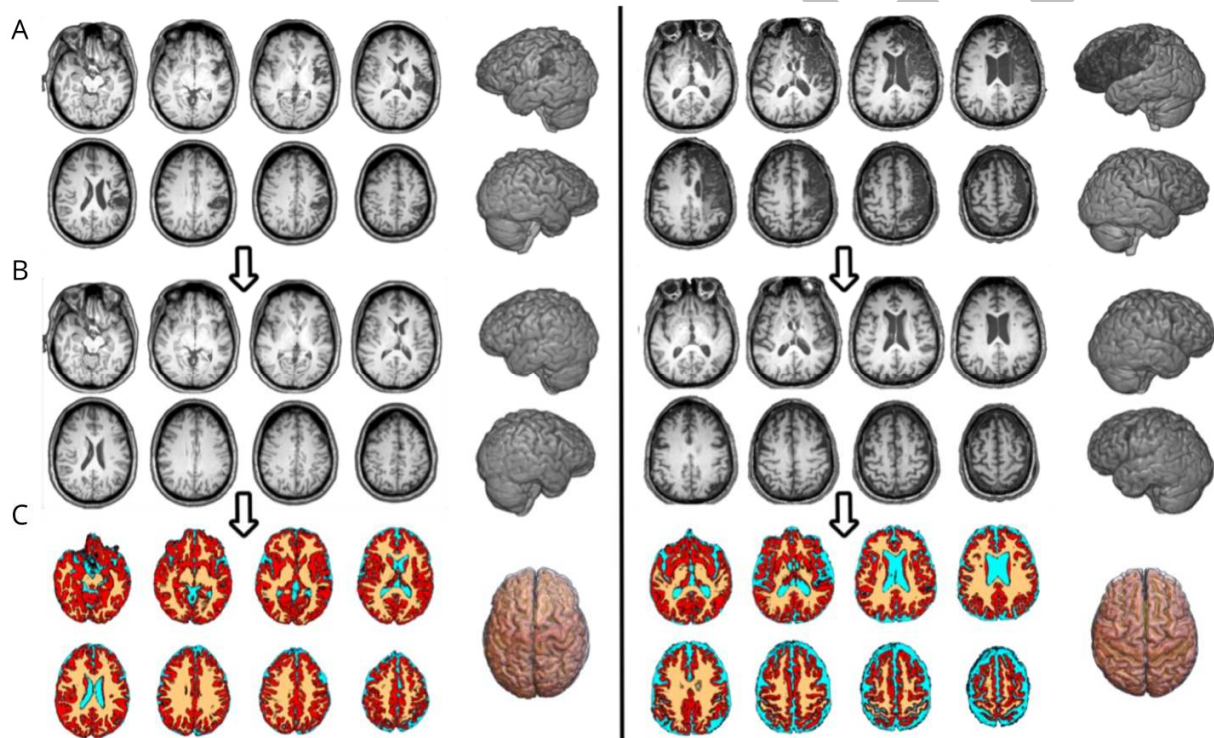
**Table 2.** Summary of prediction models

Test	WAB Naming	WAB Spontaneous Speech	WAB Repetition	WAB Comprehension	WAB-AQ
Model statistics	F=9.8275 $p < 0.0001$	F=17.2809 $p < 0.0001$	F=12.3260 $p < 0.0001$	F=11.6551 $p < 0.0001$	F=16.4073 $p < 0.0001$
Chronological age	T=-3.1649 $p = 0.0021$ Variance explained = 10.1%	T = -3.6159 $p = 0.0005$ Variance explained = 12.8%	T = -2.6299 $p = 0.0101$ Variance explained = 7%	* Not significant T = -0.4878 $p = 0.6269$ Variance explained = 0.2%	T = -3.1828 $p = 0.0020$ Variance explained = 10.2%
Proportional brain age difference	T=-2.0310, $p = 0.0452$ Variance explained = 4.4%	T = -3.5747 $p = 0.0006$ Variance explained = 12.5%	T = -2.3492 $p = 0.0210$ Variance explained = 5.8%	* Not significant T = -1.7117 $p = 0.0904$ Variance explained = 3.2%	T = -2.9847 $p = 0.0037$ Variance explained = 9.1%
Lesion volume	T=-4.6939 $p < 0.0001$ Variance explained = 19.8%	T = -6.1931 $p < 0.0001$ Variance explained = 30.1%	T = -5.5982 $p < 0.0001$ Variance explained = 26%	T = -5.7210 $p < 0.0001$ Variance explained = 26.9%	T = -6.3333 $p < 0.0001$ Variance explained = 31.1%

## Figure Legends

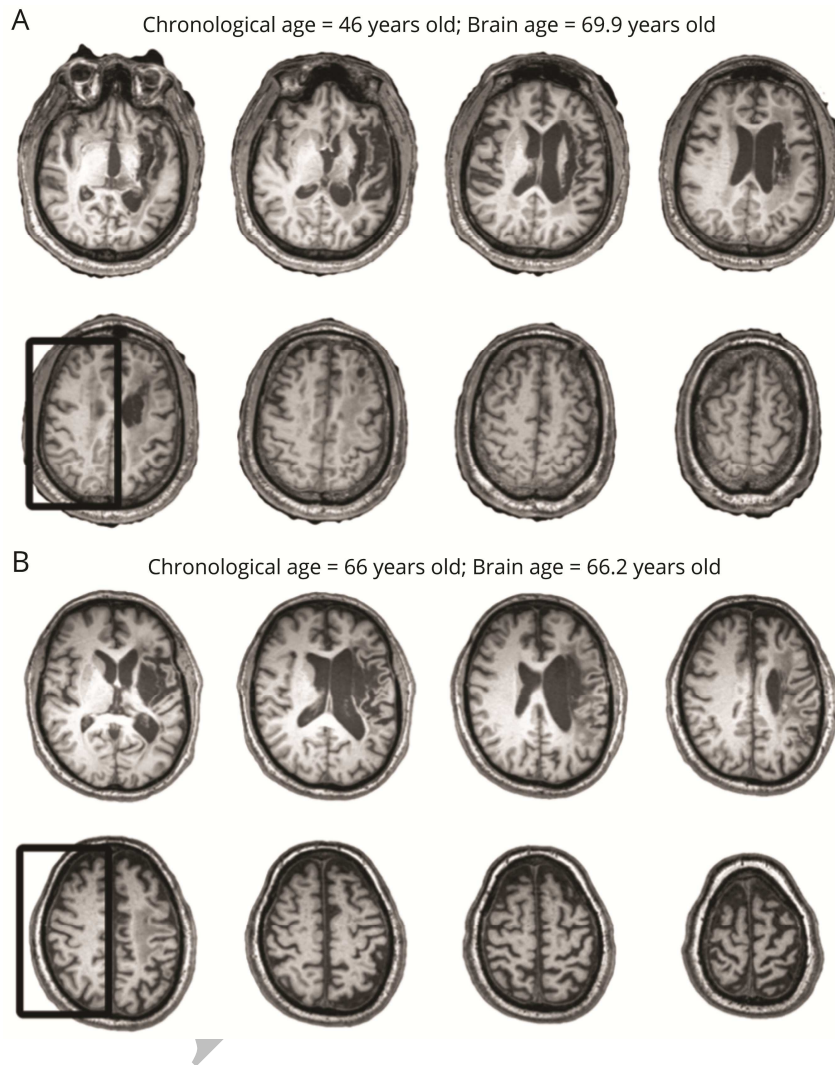
### Figure 1. Steps used to estimate brain age.

This figure illustrates the steps used to estimate brain age. Images from two representative participants are shown. The T1-weighted images with the stroke lesion (A) were segmented using an enantiomorphic process to “heal” the location of the stroke lesion. The resulting images (B) were then processed using BrainAgeR. Probabilistic tissue maps (C), i.e., gray matter (red), white matter (orange) and CSF (teal) were used in machine learning models trained with a large dataset of healthy individuals to estimate each participant’s brain age.



**Figure 2. Example of brain ages.**

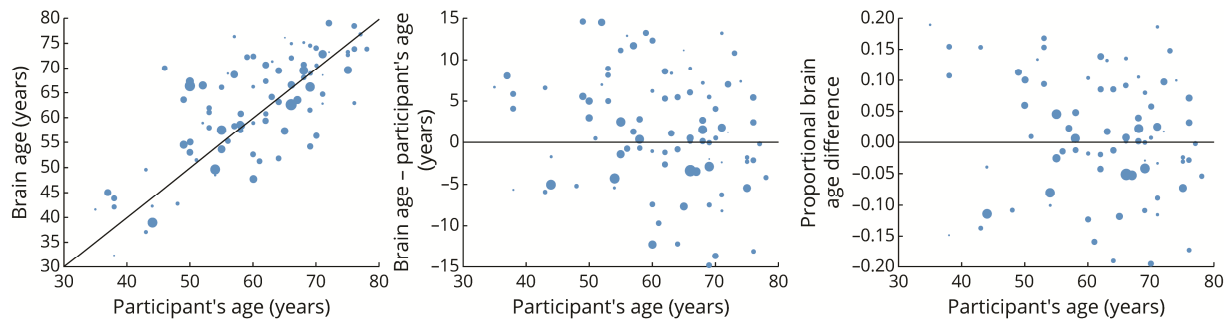
Examples of two participants whose estimated brain ages were substantially higher (A) or similar (B) to the participant's age. The box outlines the noticeable gray and matter atrophy in the participant with a large the brain age difference compared with the other participant. Notice that the size of the lesion pre-enantiomorphic correction was overall similar.





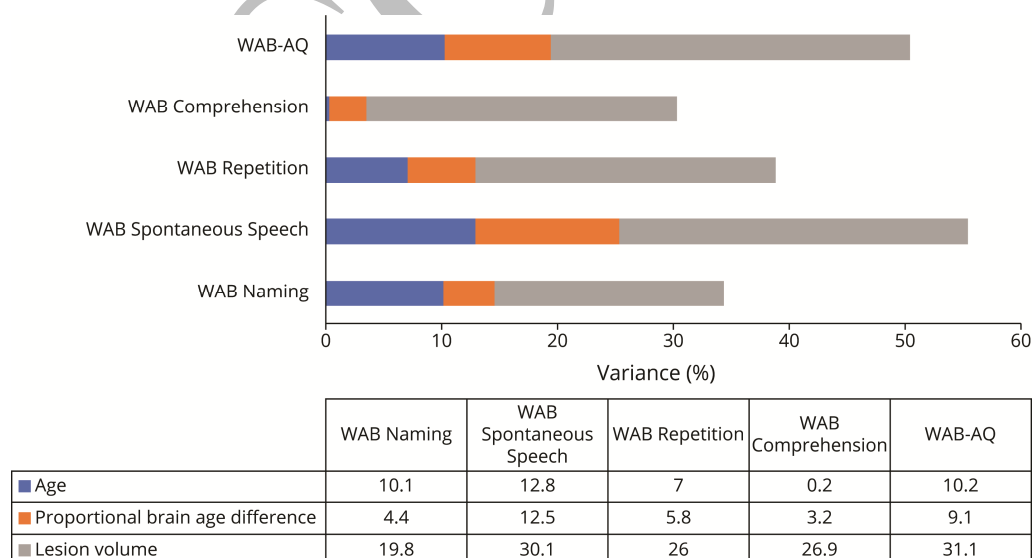
**Figure 3. Scatterplots to show the relationship between chronological age and brain age.**

The size of each data point in the scatterplot is proportional to lesion volume.



**Figure 4. Graphs to show the percentage of variance explained by each factor.**

Percentage of variance explained by chronological age, proportional brain age difference and lesion volume for WAB-AQ and WAB subscores. Note that only lesion volume was statistically associated with WAB comprehension. In all other cases, significant variance in WAB scores was associated with all three predictors.



**Figure 5. Scatterplots to show to the relationship between WAB scores and proportional brain age difference.**

The relationship between WAB scores and the residualized proportional brain age difference from chronological age and lesion volume. WAB comprehension is not shown since it was not significantly associated with proportional brain age difference.

