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Longitudinal decrease in blood oxygenation level dependent response in cerebral amyloid angiopathy

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ABSTRACT

Lower blood oxygenation level dependent (BOLD) signal changes in response to a visual stimulus in functional magnetic resonance imaging (fMRI) have been observed in cross-sectional studies of cerebral amyloid angiopathy (CAA), and are presumed to reflect impaired vascular reactivity. We used fMRI to detect a longitudinal change in BOLD responses to a visual stimulus in CAA, and to determine any correlations between these changes and other established biomarkers of CAA progression. Data were acquired from 22 patients diagnosed with probable CAA (using the Boston Criteria) and 16 healthy controls at baseline and one year. BOLD data were generated from the 200 most active voxels of the primary visual cortex during the fMRI visual stimulus (passively viewing an alternating checkerboard pattern). In general, BOLD amplitudes were lower at one year compared to baseline in patients with CAA ($p = 0.01$) but were unchanged in controls ($p = 0.18$). The longitudinal difference in BOLD amplitudes was significantly lower in CAA compared to controls ($p < 0.001$). White matter hyperintensity (WMH) volumes and number of cerebral microbleeds, both presumed to reflect CAA-mediated vascular injury, increased over time in CAA ($p = 0.007$ and $p = 0.001$, respectively). Longitudinal increases in WMH ($r_s = 0.04$, $p = 0.86$) or cerebral microbleeds ($r_s = -0.18$, $p = 0.45$) were not associated with the longitudinal decrease in BOLD amplitudes.

1. Introduction

Cerebral amyloid angiopathy (CAA) is characterized by the accumulation of beta-amyloid in the media and adventitia of the leptomeningeal and cortical vasculature (Vinters, 1987). This pathology causes cerebral arterial bleeding and manifests as lobar intracerebral hemorrhages (ICH), cerebral microbleeds, subarachnoid hemorrhages, and superficial siderosis (Knudsen et al., 2001; Charidimou et al., 2012). CAA is also implicated as a cause of vascular cognitive impairment and dementia (MRC CFAS, 2001), which may be due in part to ischemic lesions caused by subsequent blood flow reduction (Greenberg et al., 2004; Smith et al., 2012).

Vascular beta-amyloid deposition has been shown to cause thickening of the vascular walls and loss of smooth muscle cells, thus impairing the vascular response to functional hyperemia (Davis-Salinas et al., 1995). Animal models of CAA have demonstrated impaired vascular reactivity in response to a vasodilatory challenge in CAA (Shin et al., 2007; Smith et al., 2008; Park et al., 2014). Cross-sectional functional magnetic resonance (fMRI) studies of CAA in humans have demonstrated delayed time-to-peak and reduced amplitude of the blood oxygenation level dependent (BOLD) responses to a visual stimulus (Dumas et al., 2012; Peca et al., 2013) even though clinical tests of visual function and visual evoked potentials were normal. Altered BOLD responses are typically more prominent within the occipital lobe compared to the frontal lobe, consistent with the preferential deposition of vascular beta-amyloid in posterior brain regions, a characteristic feature of CAA (Peca et al., 2013). In addition, the degree of reduction of BOLD response amplitude was strongly correlated with two markers of CAA-related brain injury: volume of white matter hyperintensity (WMH) of presumed vascular origin and the number of cerebral microbleeds

Abbreviations: BOLD, blood oxygenation level dependent; CAA, cerebral amyloid angiopathy; fMRI, functional magnetic resonance imaging; FLAIR, fluid attenuated inversion recovery; ICH, intracerebral hemorrhages; SWI, susceptibility-weighted imaging; WMH, white matter hyperintensity.

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(Dumas et al., 2012; Peca et al., 2013). Thus reduced BOLD response amplitude in response to a visual stimulus may provide a potential in vivo biomarker of CAA severity.

Animal models of CAA show that vascular beta-amyloid accumulates over time, and is associated with progressive loss of vascular reactivity (Dubois et al., 2007); however, the timing and rate of cerebral hemodynamic impairment in patients with CAA is unknown because there are no published longitudinal studies to date.

The present study is designed to determine if BOLD FMRI can track the progression of the characteristic vascular reactivity impairment observed in CAA, using a one-year prospective longitudinal study design. We hypothesized that the BOLD response to a visual stimulus would decrease over one year in CAA, but remain unchanged in healthy similarly-aged controls. Additionally, we hypothesized that the decrease in BOLD response in CAA would be significantly associated with an increase in the volume of WMH of presumed vascular origin and the number of cerebral microbleeds, which are presumed to reflect the severity of CAA-related vascular injury.

2. Methods

2.1. Study population

Study participants included 22 patients with CAA and 16 non-cognitively impaired, stroke-free, healthy controls recruited as a part of a prospective longitudinal study. Patients presented with MR evidence of ICH, microbleeds, or superficial siderosis without other evident cause, consistent with the diagnosis of probable CAA by the validated Boston criteria (Knudsen et al., 2001; Linn et al., 2010). Specifically, the CAA population contained 12 patients that presented with ICH, 3 that presented with headache or cognitive impairment with neuroimaging evidence of CAA-related inflammation (all of whom were studied in a phase of remission without MRI FLAIR evidence of acute vasogenic edema at the time of study), 5 with cognitive symptoms without dementia, and 2 with transient focal neurological episodes. Three patients were recruited from a cognitive clinic and nineteen were recruited from a stroke prevention clinic. Patients were excluded if they resided in a nursing home or long-term care facility, had moderate to severe dementia (defined as a Clinical Dementia Rating (CDR) score > 1.0), had abnormal visual acuity (<20/50 Snellen visual acuity), or were not fluent in English (because English language cognitive testing was part of the study). Patients with recent symptomatic stroke (<90 days) were also excluded to avoid any acute effects of ICH. Patients with MRI evidence of ICH in the occipital pole were excluded if their hemorrhagic lesion extended into the occipital region of interest used to calculate the BOLD response amplitudes. Healthy controls were recruited from the community by advertising in a newsletter or poster, and did not have a history of stroke or dementia as determined by neurologist assessment. Each participant had a repeat study visit and MR imaging at one year. Subjects provided written consent to participate in the study, which was approved by our Institutional Review Board.

2.2. Measurements

All imaging was performed using a 3.0 Tesla MR scanner (either GE Signa VH/*i* or Discovery 750; GE Healthcare, Waukesha, WI) with a 12-channel phased-array neurovascular coil. Because of a MR scanner upgrade, 9 of the 22 patients with CAA and 10 of the 16 control subjects had their baseline scan on a GE Signa VH/*i* scanner and their one-year scan on a GE Discovery 750 scanner, while the remaining patients with CAA and control subjects had both baseline and one-year scans on the same GE Discovery 750 scanner. A T_2-weighted, two-dimensional fluid attenuated inversion recovery (FLAIR) sequence was used to measure the WMH of presumed vascular origin volume (TR/TE/TI = 9000/149/2250 ms, voxel size 0.9 × 0.9 × 3.5 mm, 39 slices, 3.5 mm slice thickness, 256 × 256 matrix size). Susceptibility-weighted imaging (SWI) was used to detect the number of cerebral microbleeds (TR/TE = 30/20 ms, voxel size 0.9 × 0.9 × 5 mm, 120 slices, 2 mm slice thickness, 256 × 256 matrix size). The entire imaging protocol took approximately 1 h and included diffusion tensor imaging and arterial spin labeling acquisitions that were not used in the present study.

All T_2*-weighted fMRI scans were acquired using a gradient-recalled echo, echo planar imaging (GRE-EPI) sequence (TR/TE = 2000/30 ms, voxel size 3.75 × 3.75 × 4 mm, 34 slices, 4 mm slice thickness, field of view = 240 × 240 mm). During fMRI scans, participants viewed four repetitions of 40-second blocks of an 8-Hz contrast-reversing checkerboard visual stimulus followed by 40 s of a grey screen with a central fixation cross.

2.3. Image analysis

All fMRI data were processed using the FMRIB Software Library (FSL version 5.0.1, Oxford, UK). Following brain extraction (Smith, 2002), fMRI data were corrected for interleaved slice timing, corrected for motion using the MCFLIRT tool (Jenkinson et al., 2002), spatially smoothed (using a 5-mm full-width half maximum Gaussian kernel) and temporally filtered using a high-pass temporal filter with a cutoff of 0.01 Hz. A voxel-by-voxel analysis of each participants fMRI data was then performed using a time-series General Linear Model (GLM) as implemented in the fMRI Expert Analysis Tool (FEAT) (Worsley, 2001) of FSL. The regressor of interest was a time-series model consisting of the binary timing of the visual stimulus convolved with a canonical hemodynamic response function. Estimates of brain activity magnitude in response to the stimulus were then computed using FEAT and converted to a *z*-statistic. The 200 most active voxels (11.3 cm^3) exhibiting the greatest *z*-statistic within the primary visual cortex were selected as the region of interest, and the amplitude of the BOLD response (calculated as the percent change in the MR signal between visual fixation and the checkerboard stimulus, as estimated using the *Featquery* tool of FSL) was compared across imaging sessions for each of the CAA and healthy control groups using a paired *t*-test for within-group changes and a two-sample *t*-test for between-group differences.

Due to the difference in prevalence of hypertension between the groups and the variation in MR scanner used between subjects, a mixed-model linear regression was used to determine whether association between CAA and longitudinal BOLD amplitude change over time was independent of age, sex, hypertension, or MR scanner. Because of our modest sample size, we used forward selection to serially enter and retain covariates significantly associated with the outcome, retaining only significant covariates ($p < 0.05$) or where there was evidence of confounding of the CAA group effect (defined as a 20% shift in the model beta-coefficient).

Because analyzing the most active voxels, in which the area of activation might differ between the baseline and one year scans, could minimize changes over time, we also performed a secondary analysis where we analyzed the BOLD amplitude changes in a pre-specified anatomically defined region of interest centered on the primary visual cortex, which is the brain region most heavily affected by vascular amyloid deposition. The anatomically defined visual cortex V1 region of interest (Amunts et al., 2000) was extracted from the Juelich Histological Atlas structures within FSL, and encompassed a 21.7 cm^3 volume of the primary visual cortex in Montreal Neurological Institute (MNI) space (Fig. 1). The anatomically defined ROI was coregistered into the fMRI space of each individual and the BOLD amplitude was calculated as an average of all voxels within the ROI.

In addition, each participant's preprocessed fMRI data set was registered to the standard MNI brain template to permit a voxel-by-voxel statistical analysis of the estimates of brain activity for each group across imaging sessions. This analysis was performed within FEAT using a mixed effects linear model, and the resulting comparisons between imaging sessions were computed as a *z*-statistic, with clusters of significantly activated voxels ($z > 2.3$) corrected for multiple comparisons at

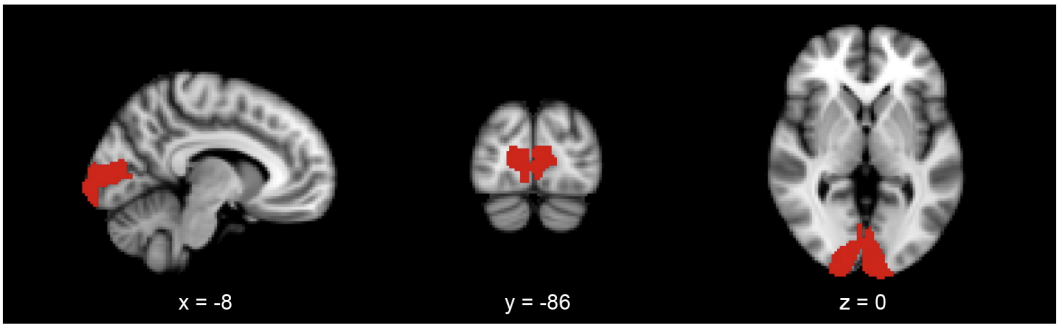

Fig. 1. Anatomically defined region of interest. The region is a modified version of the Visual Cortex V1 structure from the Juelich Histological atlas (Amunts et al., 2000). Mean BOLD response amplitudes were extracted from all voxels within the anatomical region to compare the sampled voxels longitudinally.

a p-value of 0.05 (using Alphasim, part of the AFNI image analysis package, http://afni.nimh.nih.gov/afni/doc/manual/AlphaSim). The change in BOLD response amplitude across imaging sessions were restricted to those voxels that exhibited a z-statistic > 0 for each imaging session. This ensured that differences in BOLD response amplitudes across sessions did not occur spuriously due to sub threshold negative responses during any one session. This analysis was performed to determine whether there were common brain regions within each group that exhibited a change in BOLD response amplitude across imaging sessions.

WMH and microbleeds were classified and reported according to the standards for reporting vascular changes on neuroimaging (STRIVE) (Wardlaw et al., 2013). WMH volume within the T_2-weighted FLAIR images collected at baseline and one year was determined for each participant by a single rater using *Quantomo*, a custom-designed software application (Cybertrials Inc; Calgary, AB, Canada). *Quantomo* software employs a semi-automated threshold-based seed-growing algorithm to detect the volume of hyperintense signal by a trained rater (Kosior et al., 2011). To account for differences in head sizes between subjects, WMH volumes were normalized to the average intracranial volume of participants in a population-based study such that reported WMH volume represented the volume of WMH in a subject with an average intracranial volume (1449 cm^3) (Smith et al., 2015). WMH volumes were measured on baseline and one year FLAIR images to determine WMH progression over time, blinded to fMRI results.

SWI sequences were independently viewed to determine the number of cerebral microbleeds by two raters (ES and SB), with the final number determined by consensus. One patients SWI was not collected at baseline. Raters were blinded to clinical information, time of scan, and fMRI results. We have previously demonstrated good reliability for counting microbleeds using this technique (Cheng et al., 2013).

2.4. Statistical analysis

BOLD response amplitudes were normally distributed and were compared within each group by paired t-test. The absolute difference between BOLD response amplitudes at one year and baseline were compared between CAA and healthy control groups by two-sample t-test. G*Power version 3.1.9.2 (Universitat Kiel, Germany) was used to determine sample sizes for a putative trial of an agent to preserve the BOLD response amplitude in CAA, assuming the placebo-treated group would change by −0.34% (standard deviation 0.71%) as observed in the patients with CAA in our study. These sample size calculations used two-tailed t-tests for independent means with 80% power and alpha 0.05. WMH volumes and number of cerebral microbleeds had right-skewed distributions and were log transformed to allow for longitudinal comparison by paired t-test. For patients with CAA, the associations between change in BOLD response amplitude across sessions and WMH volume and number of cerebral microbleeds was assessed by computing the Spearman correlation coefficient (r_s) using SAS version 9.3 (Cary, NC). For all statistical tests, a p-value < 0.05 was considered as significant.

3. Results

3.1. Demographics

Clinical characteristics of each group are described in Table 1. Patients with CAA were more likely to have hypertension than the healthy controls ($p < 0.001$), but had similar distributions of coronary artery disease, atrial fibrillation, hypercholesterolemia, diabetes mellitus, and tobacco use.

3.2. BOLD response amplitude

Mean BOLD signal change for CAA and healthy controls at both baseline and one year, are plotted in Fig. 2. The amplitude of the BOLD response at baseline and one year are displayed graphically for healthy controls and patients with CAA (Fig. 3). In healthy control subjects, the mean BOLD response amplitude did not change between baseline and one year sessions (Fig. 3A). In patients with CAA, the mean BOLD response amplitude at one year was significantly less than at baseline (Fig. 3B).

The results of mixed-model linear regression are shown in Table 2. There was a significant decrease in BOLD response amplitude over one year in patients with CAA, from 1.98% to 1.64% (difference −0.34%, 95% CI −0.97 to −0.14, $p = 0.01$) but no significant change in healthy controls, from 2.54% to 2.76% (difference +0.21%, 95% CI −0.10 to +0.53, $p = 0.18$). The change over time between patients with CAA and control subjects was also significant ($p < 0.001$).

Table 1
Characteristics of the study populations.

Characteristics	Control ($n = 16$), n	CAA ($n = 22$), n	p-Value
Age in years (mean ± SD)	68.4 ± 5.9	72.6 ± 6.9	0.06
Female	9	7	0.19
Hypertension	1	18	<**0.001**
Coronary artery disease	1	2	0.99
Atrial fibrillation	0	2	0.50
Hypercholesterolemia	12	8	0.99
Diabetes	0	5	0.06
Smoker	1	2	0.99
Baseline WMH volume (mL)	2.34 (1.71–5.27)	24.8 (10.77–44.01)	<**0.001**
WMH volume change (mL)	0.33 (−0.15–1.09)	1.36 (−0.48–7.37)	**0.03**
Baseline microbleeds	0	24 (6–54)	<**0.001**
New microbleeds at one year	0	11	<**0.001**
Number of new microbleeds	0	1 (0−23)	<**0.001**

Values are mean ± standard deviation, median (interquartile range) or number. Significance testing by t-test (age), Wilcoxon rank-sum test (WMH and number of new microbleeds), or Fisher's exact test (categorical variables). WMH volume change defined as WMH volume at one year subtracted by volume at baseline.
For all statistical tests, a p-value < 0.05 was considered to be significant.

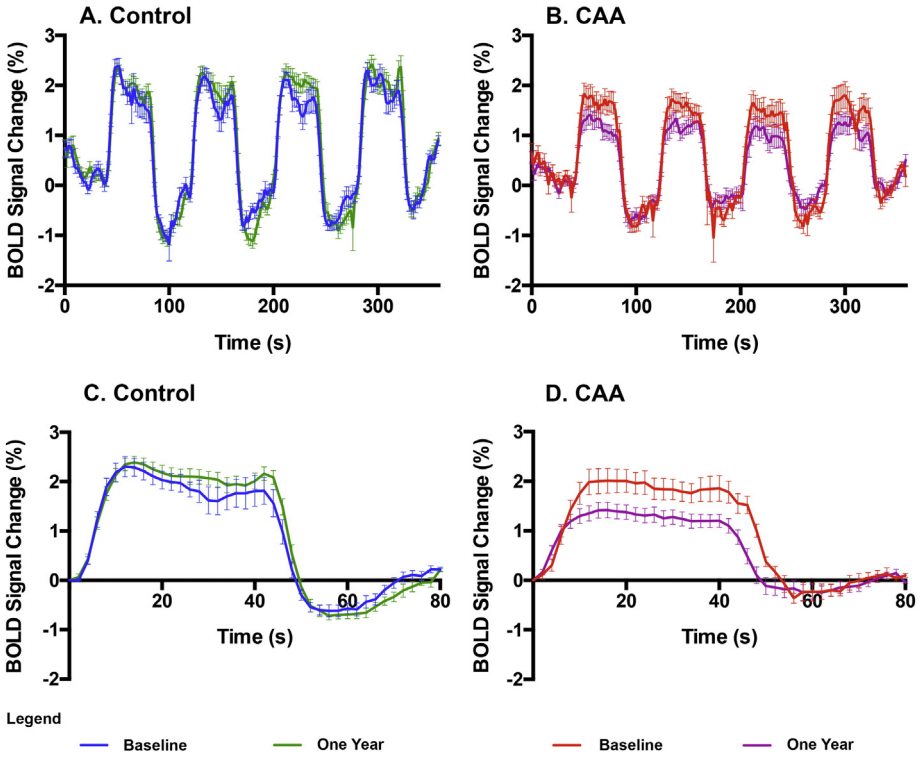

Fig. 2. Mean BOLD signal change in response to a visual stimulus for healthy controls and patients with CAA. Fig. 2A and B show mean BOLD signal change in healthy controls (A) and patients with CAA (B) at baseline and one year, in response to viewing four repetitions of 40-second blocks of an 8-Hz contrast-reversing checkerboard visual stimulus followed by 40 s of a grey screen. Fig. 2C and D show mean BOLD signal change averaged across the four stimulus blocks in healthy controls (C) and patients with CAA (D) at baseline and one year. BOLD signal is expressed as percent change from baseline (defined as 0%). Error bars indicate standard error of the mean.

To assess for potential confounders, the model in Table 2 was repeated, serially adding additional covariates for age, sex, hypertension and scanner effect. In the model controlling for CAA group status, there was no effect of age ($p = 0.15$), sex ($p = 0.28$), hypertension ($p = 0.87$), or scanner model ($p = 0.19$) on change in BOLD response amplitude response across sessions.

The longitudinal decrease in BOLD response amplitudes in patients with CAA remained significant in secondary analyses that employed an anatomically defined ROI including the primary visual cortex (Fig. 1), rather than the 200 most active voxels for extracting BOLD response amplitudes (1.23% ± 0.56% vs. 1.01% ± 0.51%, $p = 0.03$).

The results of the whole-brain group analyses are shown in Fig. 4. The amplitude of the BOLD response significantly decreased across imaging sessions within the primary visual cortex of patients with CAA.

In a hypothetical early phase randomized controlled trial designed to provide 80% power to detect differences between a BOLD amplitude-preserving intervention vs. placebo (expected change in placebo −0.34% at one year), 110 subjects (total of treatment and placebo groups) would be needed to demonstrate no change in BOLD response amplitudes at one year in the treated group (i.e., no change from baseline in the treated group), 432 subjects would be needed to demonstrate a 50% reduction in loss of BOLD amplitude (i.e., absolute −0.17% change

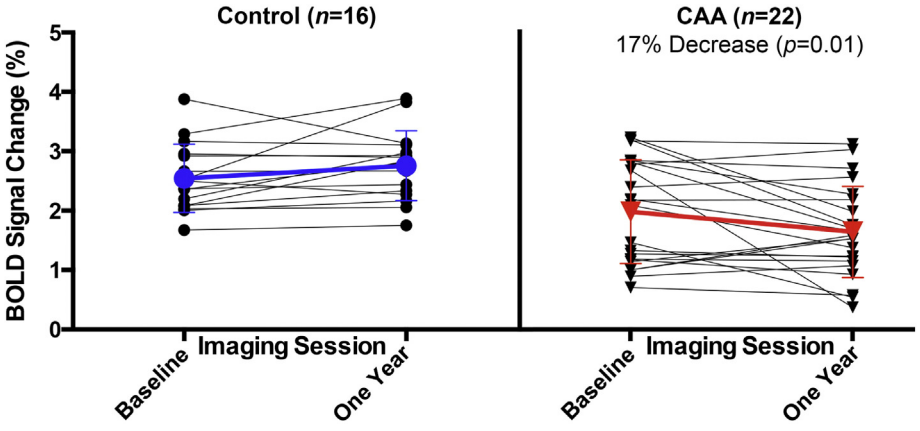

Fig. 3. BOLD response amplitude (expressed as the percentage change in signal between the stimulus and fixation) at baseline and one year for control subjects and patients with CAA. BOLD response amplitude did not change across imaging sessions in controls (2.54 ± 0.58% vs. 2.75 ± 0.61%, $p = 0.18$), but decreased across sessions for patients with CAA (1.92 ± 0.87% vs. 1.64 ± 0.77%, $p = 0.01$). Colored markers denote mean BOLD response amplitude and error bars represent standard deviations. Significance was determined by mixed model linear regression.

Table 2
Mixed model linear regression results.

	Baseline	One year	Difference over time (baseline minus one year)	*p*-Value for difference from baseline to one year	*p*-Value (baseline difference between CAA and control)	*p*-Value (difference between CAA and control in change over time)
CAA	1.98%	1.68%	−0.34%	**0.01**	<**0.001**	**0.01**
Control	2.54%	2.76%	+0.21%	0.18		

The change from baseline to one year was significant for CAA ($p = 0.01$) but not for controls ($p = 0.18$).
For all statistical tests, a *p*-value < 0.05 was considered to be significant.

from baseline in the treated group), and 50 subjects would be needed to demonstrate a 50% increase in BOLD amplitude (i.e., +0.17% increase from baseline in the treated group).

3.3. Longitudinal WMH volume and cerebral microbleed count changes

In patients with CAA, WMH volumes significantly increased over time (median 1.36 mL, interquartile range −0.48 to 7.37 mL, $p = 0.007$) (Fig. 5A). At one year, the median number of new microbleeds was 1 (interquartile range 0 to 23, $p = 0.001$) (Fig. 5B). New microbleeds were seen in 11 of the 21 patients. None of the patients developed new symptomatic hemorrhages over the course of observation.

3.4. Association between BOLD response changes and WMH and microbleeds

Longitudinal decrease in BOLD response amplitude in the visual cortex was not correlated with higher baseline WMH volume ($r_s = 0.17$, $p = 0.46$), WMH volume change over time ($r_s = 0.04$, $p = 0.86$), number of microbleeds at baseline ($r_s = 0.36$, $p = 0.11$) or number of new microbleeds ($r_s = -0.18$, $p = 0.45$) (Fig. 6). A post-hoc analysis also revealed that the change in BOLD response amplitude across sessions was not significantly different between patients with new microbleeds ($n = 11$; −0.42% over one year) and patients without new microbleeds ($n = 10$, −0.23% over one year) ($p = 0.56$).

4. Discussion

A detectable decrease in BOLD response amplitude over a one-year period in patients with probable CAA but not in healthy controls implies that progressive impairment of vascular reactivity is a distinct feature of CAA. Our results also confirm previous literature showing that BOLD responses to visual stimuli do not decrease over one year in older patients without cerebrovascular disease (Stefanova et al., 2013). The present study provides the first evidence that blood flow responses to visual stimuli worsen over time in CAA; however, we did not find correlations between baseline or incident WMH or microbleeds and change in BOLD response amplitude.

The results presented here are consistent with previous cross-sectional studies suggesting impaired visual stimulus-driven vascular reactivity as a novel characteristic of CAA (Dumas et al., 2012; Peca et al., 2013). Previous studies suggest that lower BOLD response amplitudes in response to a visual stimulus are a result of vascular impairment and not neuronal dysfunction in CAA (Peca et al., 2013). Further, another study showed reduced blood flow responses to a visual stimulus in patients with CAA compared to healthy controls, despite constant resting blood flow in both populations (Dumas et al., 2012). These findings suggest that decreased BOLD response amplitudes in CAA result from impaired vascular reactivity rather than lower baseline blood flow delivery due to decreased neuronal activity or abnormal cerebral blood supply. While the two previous cross-sectional studies have provided evidence for impaired vascular reactivity as an important feature of CAA, the present study is the first to detect progression over time. A previous study showed that vascular reactivity was impaired in a patient with pre-symptomatic hereditary CAA (Smith et al., 2008); the present study shows that impaired vascular reactivity does not plateau in the early stages of the disease and instead progressively worsens over time.

In our primary analysis we defined the region of interest based on the most active voxels for each scan, analogous to the approach in our cross-sectional study (Peca et al., 2013). However, because the most activated voxels may not overlap completely across imaging sessions due to progressive disease activity as well as normal physiological variation (Bennett and Miller, 2010; Raemaekers et al., 2012), we performed a secondary analysis where we determined the BOLD response in a larger anatomically defined region, selected independently of voxel activation. The findings of our secondary analysis were concordant with our primary analysis method; with both approaches showing reduced BOLD response amplitudes at one year compared to baseline in patients with CAA.

Longitudinal BOLD response amplitude could be a useful biomarker for early phase trials in CAA; however, more than 100 randomized patients may be needed to elucidate whether the intervention could preserve the BOLD amplitude response. Further reductions in sample sizes could be achieved by allowing a longer duration of follow-up or by implementing repeated MRI measurements to more precisely define individual trajectories over time.

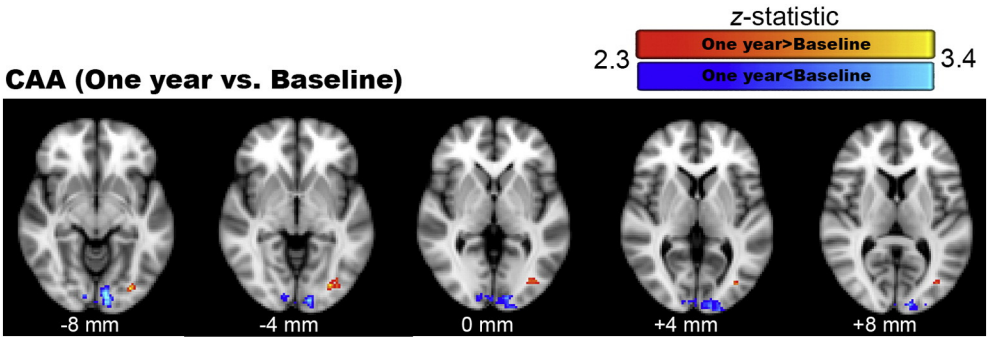

Fig. 4. Change in BOLD response amplitude across imaging session for patients with CAA. BOLD response was significantly lower at one year within the primary visual cortex only (blue). Areas of activation were cluster corrected to a significance level of $p = 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

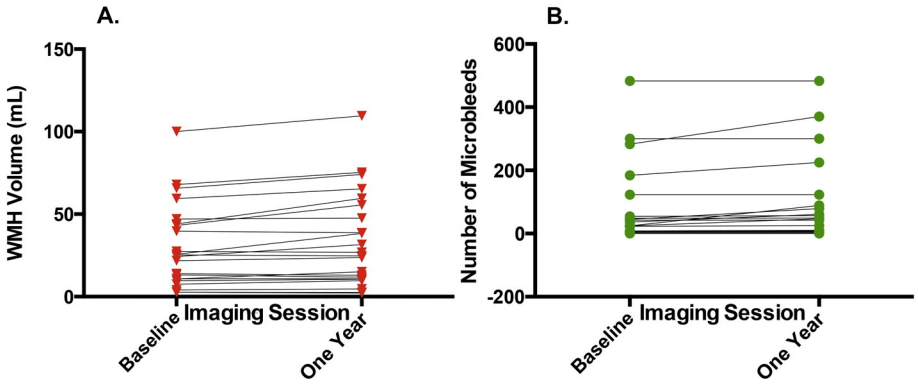

Fig. 5. Progression of white matter hyperintensity (WMH) and microbleeds in patients with CAA. A) WMH volume increased over time (median 1.36 mL, interquartile range −0.48 to 7.37 mL, $p = 0.007$). B) More microbleeds were found at one year than baseline (median 1, interquartile range 0 to 23, $p = 0.001$).

WMH of presumed vascular origin are observed in normal elderly individuals, but volume is much higher in patients with CAA or other vascular diseases (Holland et al., 2008). WMH is considered a marker of CAA severity (Greenberg et al., 2014), and WMH progression has been detected in patients with CAA over a median of 14 months (Chen et al., 2006). Our results confirm that WMH progression can be detected in CAA over a short time interval. However, we did not find a correlation between either baseline WMH or WMH progression and the decrease in BOLD response amplitudes. Similarly, we did not find a correlation between baseline microbleed count, or an increase in microbleed number, and BOLD response amplitude change. The reason for the lack of correlations between these CAA markers is unclear. It could be related to imprecision due to the small study sample size and short period of follow-up (one year). Alternatively, it could indicate that although impaired vascular reactivity is a feature of CAA, it is not directly associated with WMH or microbleeds. It may be that vasoreactivity changes precede the development of CAA-related vascular injury, such as WMH of presumed vascular origin and cerebral microbleeds.

The principal limitation to our study is the small sample size with only one year of follow-up. Although we were able to detect a significant decrease in BOLD response amplitude over time in CAA we had limited power to detect whether BOLD amplitude signal decreases were associated with other markers of CAA-related brain injury and could not look for associations with clinical hemorrhagic events because none occurred during the timeframe of this study. Larger studies will be needed to investigate the relationship between BOLD response amplitude change over time and clinical events. CAA may present as one of several different clinical syndromes, including stroke due to intracerebral hemorrhage, cognitive impairment or transient focal neurological episodes. Future, larger studies will be needed to determine whether BOLD decreases are similar in these different CAA syndromes. Patients were only reassessed at one year and although a significant longitudinal decrease in BOLD response amplitude was detected, a longer follow-up with one year intervals could be helpful in determining the time course of BOLD response changes in CAA, including the identification of stages in CAA severity, and how it can be used to predict brain injury and CAA-related ICH. BOLD response amplitude was chosen as our primary outcome; further studies are needed to determine how other features of the BOLD response (e.g. time to peak) change over time. Finally, measuring vascular reactivity more directly (for example by using carbon dioxide inhalation as a vasodilatory stimulus), could yield measurements that correlate better with brain injury in CAA, although such techniques are more technically challenging than visual stimulus-related fMRI.

This study extends our knowledge of blood flow regulation disturbances in CAA by showing that changes in BOLD response amplitude to a visual stimulus can be detected over one year, with reduced BOLD responses at one year in the CAA group but not in similarly-aged controls. By showing that change over time can be detected during a clinically feasible follow-up period of one year, these findings support the utility of visual stimulus-related BOLD response amplitude as one of a growing number of biomarkers in CAA. Because BOLD response amplitude change is a continuously distributed measure of vascular function, it could potentially be improved in the short term by treatments that restore more normal vascular function. This offers a potential advantage over other biomarkers, such as MRI evidence of microbleeds or WMH, that represent non-modifiable and slowly accruing irreversible structural changes. Therefore, BOLD fMRI may be a useful surrogate outcome marker for early phase clinical trials where it would not be feasible to recruit the large numbers of patients needed to detect outcomes such as symptomatic recurrent ICH. (Greenberg et al., 2014) The present

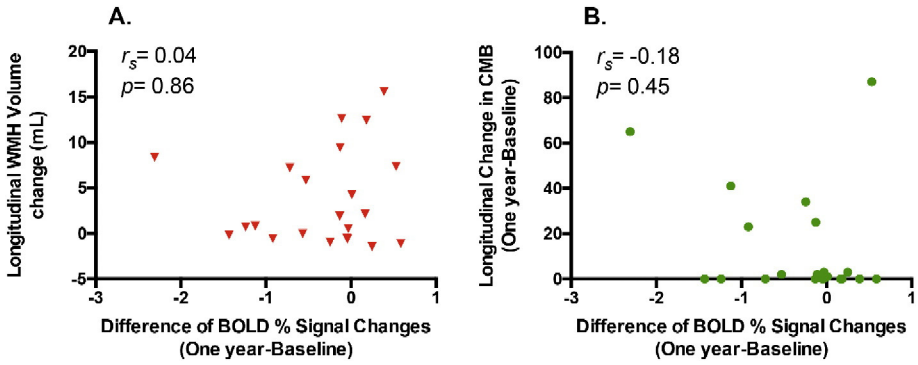

Fig. 6. Longitudinal relationships between absolute longitudinal difference in BOLD amplitudes and WMH volume or microbleed count progression in CAA. A) Longitudinal decrease in BOLD amplitude in the visual cortex was not correlated with WMH volume increase over time ($r_s = 0.04$, $p = 0.86$; $n = 22$). B) Longitudinal decrease in BOLD amplitude in the visual cortex was not correlated with the number of new microbleeds ($r_s = -0.18$, $p = 0.45$; $n = 21$).

study shows progressive decreases in BOLD response, suggesting progressively impaired vascular reactivity, are detectable in CAA; whether modifying vascular reactivity is possible and beneficial to the patient awaits the development of effective treatments for CAA, for which there are currently none. However, a visual fMRI surrogate outcome, similar to our visual fMRI stimulus, has been incorporated as the principal surrogate outcome measure in an early phase clinical trial of the anti-abeta monoclonal antibody ponezumab for treatment of CAA (clinicaltrials.gov NCT01821118). This trial will provide the first evidence for whether CAA-associated vascular dysfunction is potentially modifiable.

5. Conclusions

Reduced BOLD response amplitude was observed at one year compared to baseline in patients with CAA but not in similarly-aged controls. However, we did not find an association between the degree of BOLD response amplitude reduction over time and the progressive increase in WMH of presumed vascular origin or number of cerebral microbleeds. These findings support the utility of visual stimulus BOLD response amplitude as a marker of disease progression in CAA, which may reflect aspects of disease progression independent of WMH or cerebral microbleed progression.

References

Amunts, K., Malikovic, A., Mohlberg, H., Schormann, T., Zilles, K., 2000. Brodmann's areas 17 and 18 brought into stereotaxic space—where and how variable? NeuroImage 11, 66–84. http://dx.doi.org/10.1006/nimg.1999.0516.

Bennett, C.M., Miller, M.B., 2010. How reliable are the results from functional magnetic resonance imaging? Ann. N. Y. Acad. Sci. **1191**, 133–155. http://dx.doi.org/10.1111/j.1749-6632.2010.05446.x.

Charidimou, A., Gang, Q., Werring, D.J., 2012. Sporadic cerebral amyloid angiopathy revisited: recent insights into pathophysiology and clinical spectrum. J. Neurol. Neurosurg. Psychiatry 83, 124–137 (doi:jnnp-2011-301308 [pii]10.1136/jnnp-2011-301308 [doi]).

Chen, Y.W., et al., 2006. Progression of white matter lesions and hemorrhages in cerebral amyloid angiopathy. Neurology **67**, 83–87.

Cheng, A.L., et al., 2013. Susceptibility-weighted imaging is more reliable than T2*-weighted gradient-recalled echo MRI for detecting microbleeds. Stroke 44, 2782–2786. http://dx.doi.org/10.1161/STROKEAHA.113.002267.

Davis-Salinas, J., Saporito-Irwin, S.M., Cotman, C.W., Van Nostrand, W.E., 1995. Amyloid β-protein induces its own production in cultured degenerating cerebrovascular smooth muscle cells. J. Neurochem. **65**, 931–934. http://dx.doi.org/10.1046/j.1471-4159.1995.65020931.x.

Dubois, B., et al., 2007. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS–ADRDA criteria. Lancet Neurol. 6, 734–746. http://dx.doi.org/10.1016/S1474-4422(07)70178–3.

Dumas, A., et al., 2012. Functional magnetic resonance imaging detection of vascular reactivity in cerebral amyloid angiopathy. Ann. Neurol. **72**, 76–81. http://dx.doi.org/10.1002/ana.23566.

Greenberg, S.M., Gurol, M.E., Rosand, J., Smith, E.E., 2004. Amyloid angiopathy-related vascular cognitive impairment. Stroke **35**, 2616–2619.

Greenberg, S.M., et al., 2014. Outcome markers for clinical trials in cerebral amyloid angiopathy. Lancet Neurol. 13, 419–428. http://dx.doi.org/10.1016/S1474-4422(14)70003–1.

Holland, C.M., et al., 2008. Spatial distribution of white-matter hyperintensities in Alzheimer disease, cerebral amyloid angiopathy, and healthy aging. Stroke **39**, 1127–1133.

Jenkinson, M., Bannister, P., Brady, J.M., Smith, S.M., 2002. Improved optimisation for the robust and accurate linear registration and motion correction of brain images. NeuroImage **17**, 825–841.

Knudsen, K.A., Rosand, J., Karluk, D., Greenberg, S.M., 2001. Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. Neurology **56**, 537–539.

Kosior, J.C., et al., 2011. Quantomo: validation of a computer-assisted methodology for the volumetric analysis of intracerebral haemorrhage. Int. J. Stroke **6**, 302–305. http://dx.doi.org/10.1111/j.1747-4949.2010.00579.x.

Linn, J., et al., 2010. Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy. Neurology **74**, 1346–1350.

MRC CFAS, 2001. Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. Lancet 357, 169–175. http://dx.doi.org/10.1016/S0140-6736(00)03589–3.

Park, L., et al., 2014. Age-dependent neurovascular dysfunction and damage in a mouse model of cerebral amyloid angiopathy. Stroke **45**, 1815–1821. http://dx.doi.org/10.1161/strokeaha.114.005179.

Peca, S., et al., 2013. Neurovascular decoupling is associated with severity of cerebral amyloid angiography. Neurology **81**, 1659–1665.

Raemaekers, M., du Plessis, S., Ramsey, N.F., Weusten, J.M.H., Vink, M., 2012. Test-retest variability underlying fMRI measurements. NeuroImage 60, 717–727. http://dx.doi.org/10.1016/j.neuroimage.2011.11.061.

Shin, H.K., et al., 2007. Age-dependent cerebrovascular dysfunction in a transgenic mouse model of cerebral amyloid angiopathy. Brain **130**, 2310–2319. http://dx.doi.org/10.1093/brain/awm156.

Smith, S.M., 2002. Fast robust automated brain extraction. Hum. Brain Mapp. **17**, 143–155.

Smith, E.E., et al., 2008. Impaired visual evoked flow velocity response in cerebral amyloid angiography. Neurology **71**, 1424–1430. http://dx.doi.org/10.1212/01.wnl.0000327887.64299.a4.

Smith, E.E., Schneider, J.A., Wardlaw, J.M., Greenberg, S.M., 2012. Cerebral microinfarcts: the invisible lesions. Lancet Neurol. **11**, 272–282. http://dx.doi.org/10.1016/S1474-4422(11)70307-6.

Smith, E.E., et al., 2015. Early cerebral small vessel disease and brain volume, cognition, and gait. Ann. Neurol. **77**, 251–261. http://dx.doi.org/10.1002/ana.24320.

Stefanova, I., et al., 2013. Age-related changes of blood-oxygen-level-dependent signal dynamics during optokinetic stimulation. Neurobiol. Aging 34, 2277–2286. http://dx.doi.org/10.1016/j.neurobiolaging.2013.03.031.

Vinters, H.V., 1987. Cerebral amyloid angiopathy. A critical review. Stroke **18**, 311–324. http://dx.doi.org/10.1161/01.str.18.2.311.

Wardlaw, J.M., et al., 2013. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 12, 822–838. http://dx.doi.org/10.1016/S1474-4422(13)70124–8.

Worsley, K.J., 2001. In: Jezzard, P., Matthews, P.M., Smith, S.M. (Eds.), Functional MRI: An Introduction to Methods. Oxford University Press Ch. 14.