Progression of Brain Network Alterations in Cerebral Amyloid Angiopathy

Yael D. Reijmer, PhD; Panagiotis Fotiadis, BSc; Grace A. Riley, BA; Li Xiong, MD; Andreas Charidimou, PhD; Gregoire Boulouis, MD; Alison M. Ayres, MSc; Kristin Schwab, BA; Jonathan Rosand, MD, PhD; M. Edip Gurok, MD; Anand Viswanathan, MD, PhD; Steven M. Greenberg, MD, PhD

Background and Purpose—We recently showed that cerebral amyloid angiopathy (CAA) is associated with functionally relevant brain network impairments, in particular affecting posterior white matter connections. Here we examined how these brain network impairments progress over time.

Methods—Thirty-three patients with probable CAA underwent multimodal brain magnetic resonance imaging at 2 time points (mean follow-up time: 1.3±0.4 years). Brain networks of the hemisphere free of intracerebral hemorrhages were reconstructed using fiber tractography and graph theory. The global efficiency of the network and mean fractional anisotropies of posterior–posterior, frontal–frontal, and posterior–frontal network connections were calculated. Patients with moderate versus severe CAA were defined based on microbleed count, dichotomized at the median (median=35).

Results—Global efficiency of the intracerebral hemorrhage–free hemispheric network declined from baseline to follow-up (−0.008±0.003; P=0.029). The decline in global efficiency was most pronounced for patients with severe CAA (group×time interaction P=0.03). The decline in global network efficiency was associated with worse executive functioning (β=0.46; P=0.03). Examination of subgroups of network connections revealed a decline in fractional anisotropies of posterior–posterior connections at both levels of CAA severity (−0.006±0.002; P=0.017; group×time interaction P=0.16). The fractional anisotropies of posterior–frontal and frontal–frontal connections declined in patients with severe but not moderate CAA (group×time interaction P=0.007 and P=0.005). Associations were independent of change in white matter hyperintensity volume.

Conclusions—Brain network impairment in patients with CAA worsens measurably over just 1.3-year follow-up and seem to progress from posterior to frontal connections with increasing disease severity. (Stroke. 2016;47:2470-2475. DOI: 10.1161/STROKEAHA.116.014337.)

Key Words: amyloid angiopathy • brain • cerebral small vessel disease • cognitive impairment • diffusion-weighted imaging

Cerebral amyloid angiopathy (CAA) is a common form of cerebral small vessel disease (SVD). A recent topic of interest and an unmet need in the field is how to quantify the widespread subcortical tissue injury in CAA in a clinically meaningful way.1 Diffusion tensor imaging (DTI)–based network analysis is in this context a promising method because it can quantify white matter tissue changes at a microscopic scale, as well as the effect of white matter tissue injury on brain connectivity. In a previous study, we have shown that white matter connectivity is impaired in patients with CAA compared with controls, resulting in a lower global efficiency of the brain network.2 Lower network efficiency was associated with worse executive functioning and processing speed in these patients.2 Similar cross-sectional associations between network disturbances and cognition have been observed in other patient populations with SVD, for example, patients with type 2 diabetes mellitus,1 and symptomatic stroke.4,5 However, how SVD-related network disturbances progress over time and how this relates to subsequent cognitive decline is not known. Unlike other forms of SVD, CAA is known to primarily affect posterior brain regions.6,7 Our previous work demonstrated that most patients with CAA showed measurable alterations in posterior white matter connections.2 Nonetheless, we found that a subgroup of patients had impaired connectivity in frontal as well as posterior regions. This subgroup also had higher...
levels of amyloid across the whole brain, as measured with Pittsburgh compound B positron emission tomography imaging. High amyloid deposition in CAA has been linked to advanced CAA-related microvascular damage, such as micro- and macrohemorrhages. Based on these observations, we hypothesized that brain network alterations in CAA progress from posterior to frontal regions with increasing disease severity. The specific aims of this study were (1) to assess whether CAA-related impairments in global network efficiency worsen over time; (2) to relate decline in global network efficiency to cognitive decline; and (3) to examine decline in posterior and frontal network connections in patients with moderate versus severe CAA. We addressed these aims in the first longitudinal cohort of patients with CAA who had both advanced brain imaging and a cognitive assessment at 2 time points.

Methods

Study Participants

Data were obtained from an ongoing single-center longitudinal cohort study on the natural history of CAA. The Institutional Review Board approved the study, and informed consent was obtained from all participants or their surrogates. Forty-eight non-demented patients with probable CAA defined by the Boston criteria were prospectively enrolled through the stroke clinic for a 1.5-Tesla research magnetic resonance imaging (MRI), including a DTI scan, between May 2006 and March 2015. Exclusion criteria were dementia, a diagnosis of cerebrovascular disease other than CAA, and contraindication to MRI. Of this group, 40 patients (83%) underwent a second MRI scan between 1 and 2 years after the baseline scan (follow-up time, 1.2±0.4 years). Reasons for loss to follow-up were subjects who were too impaired (n=3) or declined to further participate for other reasons (n=5). Of these 40 patients, an additional 4 patients were excluded because of an intracerebral hemorrhage (ICH) in both hemispheres and 3 patients because of missing or low-quality diffusion scan, leaving 33 patients for the current analysis. Longitudinal neuropsychological data obtained at the time of the MRI scans were available from 25 patients. Longitudinal neuropsychological data of 8 patients were not available because they declined cognitive testing (n=6), were non-native English speakers (n=1), or had aphasia as a result of ICH (n=1). CAA patients underwent a clinical evaluation, cognitive testing (described below), and research 1.5-Tesla MRI scan (Siemens), including a T1-weighted 3D spoiled gradient recalled-echo, fluid-attenuated inversion recovery, susceptibility-weighted imaging (see for details Gurrol et al2), and diffusion-weighted imaging sequence (details below).

DTI Processing and Network Reconstruction

High angular resolution diffusion imaging scans (60 directions, b value 700, 10 b0 images, voxel size 2x2x2 mm) were analyzed and processed in ExploreDTI (http://www.exploredti.com) in accordance with our baseline study. In brief, high angular resolution diffusion imaging scans were corrected for subject motion and eddy current–induced geometric distortions, and the diffusion tensors were calculated using the RESTORE (robust estimation of tensors by outlier rejection) approach. For each data set, whole-brain white matter tractography was performed using deterministic streamline constrained spherical deconvolution. The whole-brain fiber tract reconstructions were parcellated into 90 cortical and subcortical gray matter regions using the automated anatomic labeling atlas. Two brain regions or nodes were considered to be connected if a fiber bundle was present with 2 end points located in these regions, resulting in a 90×90 binary connectivity matrix. A weighted connectivity matrix was obtained by multiplying each connection by the mean fractional anisotropy (FA) of that connection. To account for the effects of ICH on structural connectivity, we also reconstructed the network of the ICH-free hemisphere, resulting in a 45×45 connectivity matrix for each patient. For patients without ICH, we randomly selected the left or right hemisphere. All the analyses in this study were performed on the ICH-free hemispheric networks. We calculated the global efficiency at baseline and follow-up based on the FA-weighted hemispheric connectivity matrices in accordance to our cross-sectional analysis. The global efficiency is calculated as the inverse of the shortest path lengths (ie, the minimum number of FA-weighted connections between each pair of brain regions) and quantifies how efficiently information is exchanged over the network. In addition, we examined 2 basic network properties: degree (number of connections within the network) and strength (mean FA of the connections). To examine the posterior–frontal progression of network connectivity over time, we specifically examined 3 groups of white matter connections: connections between (1) posterior cortical regions, (2) posterior–frontal cortical regions, and (3) frontal cortical regions (Figure 1). The division of the anatomic labeling atlas template in posterior and frontal regions was done as described in one of our previous studies on CAA. The mean FA of posterior–posterior, posterior–frontal, and frontal–frontal connections were selected as outcome measures before the analysis. Because of the relatively small sample size, we were not able to examine each network connection individually.

Other Neuroimaging Markers of CAA-Related Brain Injury

Microbleeds were identified on axial susceptibility-weighted imaging sequences by an experienced rater as described previously. White matter hyperintensity (WMH) volume was calculated using an automated method based on thresholding the segmented white matter fluid-attenuated inversion recovery intensity maps (see for more detail Reijmer et al2). All the results generated by this algorithm were manually checked to verify their accuracy. For individuals with an ICH, the volume estimates of the ICH-free hemispheres were used and multiplied by 2. To account for between-subject differences in head size, WMH volumes were expressed as percentage of intracranial volume.

Cognitive Testing

Measures of cognitive functioning among the probable CAA subjects were assessed using a standardized test battery. Tests include verbal memory (immediate and delayed memory score of the Hopkins Verbal Learning Test), processing speed (Trail Making Test A, Symbol Substitution Test), and executive functioning (Trail Making Test B, Digit Span Test backwards, Verbal Fluency Test). Each cognitive test score was transformed into Z scores based on the mean and SD of the baseline scores. Z scores of tests belonging to the same cognitive domain were averaged to obtain one average Z score per cognitive domain. Raw cognitive test scores of the baseline sample can be found in Reijmer et al2.

Figure 1. Selection of posterior–posterior (red), frontal–frontal (yellow; both shown in left panel), and posterior–frontal (orange; right panel) white matter network connections. Division of posterior and frontal cortical regions is done according to Johnson et al17.
Table 1. Baseline Characteristics

| Age, y | 70.6±8.0 |
| Sex, % male | 85% |
| Years of education | 16±3 |
| Microbleeds, n | 35 (13–72) |
| ICH, % present | 49% |
| Time to follow-up MRI, y | 1.3±0.4 |
| Time from ICH to MRI, y | 2 (1.3–5.2) |
| WMH volume, % ICV | 1.9 (1.1–2.2) |

Network parameters

- Global efficiency: 0.196±0.020
- FA front–front connections: 0.266±0.019
- FA posterior–posterior connections: 0.291±0.024
- FA front–front connections: 0.266±0.019

Data are given as mean±SD, percentage, or median (interquartile range). Network parameters were calculated from ICH-free hemispheres only. FA indicates fractional anisotropies; front, frontal; ICH, intracerebral hemorrhage; ICV, intracranial volume; MRI, magnetic resonance imaging; post, posterior; and WMH, white matter hyperintensity.

**Statistical Analysis**

Change in global network efficiency, mean FA of white matter connection subgroups, and cognitive Z scores over time were analyzed with a paired t test. To assess the relationship with CAA severity, we chose before the analysis to dichotomize patients based on the median number of lobar microbleeds, which was 35 for our study cohort (Table 1). Patients with n<35 and n≥35 microbleeds are herein referred to as moderate and severe CAA. Baseline characteristics between patients with moderate versus severe CAA were compared with an independent t test for continuous variables, a Mann–Whitney U test for non-normally distributed variables, and a Chi-square test for dichotomous variables. Repeated measures analyses of variance were performed to test for a group (moderate versus severe CAA)×time (baseline versus follow up) interaction effect with global network efficiency or FA of the selected connections as outcome measure. Age was entered as covariate. Because sex was not related to any of the DTI outcome measures, it was not included in these models. The association between change in connectivity (predictor) and change in cognition (outcome measure) was analyzed with linear regression analyses, corrected for age, years of education, and baseline values to minimize the confounding effect of regression to the mean.

To evaluate whether our results were not solely driven by an increase in WMH volume, we assessed the association between change in WMH volume, CAA severity, and cognitive performance. In addition, we added WMH volume as covariate in secondary models. Finally, we reran all significant associations with follow-up time as a covariate. The continuous measures used in the regression models were tested for their normal distribution and log-transformed when necessary (WMH volume). All analyses were performed with SPSS statistical software (version 22; SPSS Inc, Chicago, IL).

**Results**

**Progression of Global Network Connectivity**

Baseline characteristics for the whole study sample are shown in Table 1. Other than microbleed count that was used to dichotomize subjects, the baseline characteristics did not differ between patients with moderate versus severe CAA (all P>0.45; see also Table I in the online-only Data Supplement). Follow-up time was also similar in both groups (median [interquartile range]: 1.1 [1.0–1.4] versus 1.1 [1.0–1.3] years; P=0.86). Global efficiency of the ICH-free hemispheric network declined from baseline to follow-up (mean difference±SEM: −0.008±0.003; P=0.029). A significant group×time interaction effect indicated that the decline in global efficiency was present for patients with severe but not moderate CAA (group×time interaction P=0.030; Figure 2). For network degree and network strength, we also found a decline over time (degree: −0.81±0.35, P=0.026; strength: −0.28±0.12, P=0.024), without group×time interaction effect (P=0.96 and P=0.32, respectively). The decline in global efficiency of the network was related to a decline in executive functioning (standardized beta [95% confidence interval]=0.46 [0.04–0.88]; P=0.03; Figure 3). No relationship was found with decline in processing speed or memory (P>0.42). WMH volume increased over time (0.20±0.21% intracranial volume; P<0.001), but the increase was not related to CAA severity (group×time interaction P=0.76) or decline in cognitive functioning (executive functioning: beta=−0.04 (−0.44 to 0.38), P=0.88; processing speed: beta=−0.03 (−0.43 to 0.38), P=0.91; memory: beta=0.23 (−0.29 to 0.66), P=0.42).

**Progression of Local Network Connectivity**

Examination of subgroups of network connections showed that across the whole study sample, the FA of posterior–posterior white matter connections declined over time (−0.006±0.002, P=0.017; group×time interaction P=0.16). The FA of posterior–frontal and frontal–frontal connections showed a decline in patients with severe but not moderate CAA (group×time interaction posterior–frontal P=0.007; frontal–frontal P=0.005; Table 2). Adjusting the above mentioned repeated measures analyses for WMH volume did not change the results (data not shown). Post hoc analyses showed that decline in executive functioning was most strongly related to a decline in FA of posterior–posterior (beta=0.49 [0.06–0.91]; P=0.03) and posterior–frontal connections (beta=0.58 [0.17–0.99]; P=0.008).
and, to a lesser, nonsignificant extent to the FA of frontal–frontal connections (beta=0.42 [−0.003 to 0.85], P=0.05). The above associations with cognition remained independent after adjusting for follow-up time (all P<0.05).

Discussion

We found that brain network connectivity in patients with CAA worsens measurably over 1.3-year follow-up. Greater progression of global network efficiency was related to a greater decline in executive functioning. In patients with moderate CAA (defined here by baseline microbleed count), a decline in connectivity strength was observed in posterior network connections, whereas in patients with more severe CAA, the progression also involved posterior–frontal and frontal connections. To our knowledge, no longitudinal diffusion-based connectome studies have been performed in older healthy individuals or other patients with SVD. However, longitudinal DTI studies have examined progression of white matter FA in healthy older adults. Results of 2 large studies showed an average decline in FA of 0.004 and 0.005 over 2 years,21,22 smaller than the decline in FA found in our study over just 1.3 years (Table 2). In addition to the increased effect sizes, the pattern of network progression is different from the pattern observed in healthy aging. We found a distinct posterior greater than frontal gradient of network deterioration in CAA, which is well in line with the distribution of CAA-related vasculopathy.6,7 By contrast, aging has been associated with a more diffuse decline in white matter connectivity, with some studies suggesting that the frontal white matter is disproportionately affected.22,23 It is, therefore, unlikely that our findings are solely explained by age. Furthermore, network progression varied as a function of CAA severity: network connectivity declined in patients with severe but not moderate CAA despite similar ages at baseline. We hypothesized that although CAA preferentially affects posterior brain regions, within a group of CAA patients with relatively advanced disease, frontal connections would be increasingly affected as the disease further progresses. Serial examination of imaging markers sensitive to SVD pathol-ogy, such as diffusion-based network analysis, provides one approach to testing this hypothesis. Future studies in larger sample sizes should further evaluate the validity of this model and relate network findings to progression of cerebrovascular amyloid deposition measured with in vivo tools, such as molecular positron emission tomography imaging.9 Our findings support the view that the progression of CAA is not a linear process, but rather follows a faster rate of decline when the disease becomes more severe. The accelerated decline was driven by a change in connectivity strength of posterior–frontal and frontal–frontal connections. Posterior–frontal connections have relatively high network centrality, that is, those connections participate in a large number of shortest paths and, therefore, are expected to carry a large proportion of the overall information flow within the brain.24 Structural injury in those connections may, therefore, have a profound impact on global network efficiency and brain function. As CAA progresses throughout the brain, more of these central connections become affected, suggesting an accelerated decline in global network efficiency and cognitive function. Previous work has indeed demonstrated the relevance of connections with high network centrality to global efficiency and executive functioning in patients with SVD.19 This is the first longitudinal study on network changes and cognitive decline in a well-described cohort of patients with CAA. A previous longitudinal study in the same cohort25 showed that acute microinfarcts in the white matter, as depicted by hyperintense

![Figure 3. Correlation between progression of global network efficiency and decline in executive functioning in patients with cerebral amyloid angiopathy (CAA). Units are standardized residuals adjusted for age, education, and baseline value. Negative values indicate a decline over time.](http://stroke.ahajournals.org/)

<table>
<thead>
<tr>
<th>Network parameters</th>
<th>Whole Sample (n=33)</th>
<th>Moderate CAA (n=16)</th>
<th>Severe CAA (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global efficiency</td>
<td>−0.008±0.003*†</td>
<td>−0.001±0.004</td>
<td>−0.014±0.005</td>
</tr>
<tr>
<td>FA post–post connections</td>
<td>−0.006±0.002*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA post–front connections</td>
<td>−0.007±0.004†</td>
<td>0.005±0.005</td>
<td>−0.017±0.006</td>
</tr>
<tr>
<td>FA front–front connections</td>
<td>−0.002±0.003†</td>
<td>0.006±0.004</td>
<td>−0.009±0.003</td>
</tr>
</tbody>
</table>

Data are given as estimated marginal means±SEM. In case of a significant time×group interaction effect, results are given stratified for group. CAA indicates cerebral amyloid angiopathy; FA, fractional anisotropies; front, frontal; and post, posterior.

*Effect of time P<0.05.
†Group×time interaction effect P<0.05.
lesions on diffusion-weighted imaging, were associated with chronic local reductions in FA. The accumulated injury of multiple microinfarcts forms one of the possible mechanisms for white matter network damage in CAA.26 Strengths of this study include the collection of high-resolution structural MRI data in combination with the detailed assessment of cognitive functioning. Our study is limited, however, by the small sample size and the lack of a control group. As noted earlier, previous longitudinal studies of non-CAA subjects suggest that the rate and location of progressive reductions of connectivity observed in the current study are unlikely to reflect aging alone.21,22 Another limitation is the possible confounding effect of Alzheimer pathology to our results. Alzheimer’s pathology is known to often co-occur with CAA pathology. However, the pattern of local diffusion-based network disturbances observed in Alzheimer’s disease27 is markedly different from the posterior pattern observed in our cross-sectional analyses.2 Furthermore, we did not find an association between decline in connectivity and memory, suggesting that our results are not primarily driven by Alzheimer’s disease. Because of the small sample size, our study was underpowered to test for a decline in individual network connections. Larger future studies should examine the progression at a higher spatial resolution. This may reveal whether the disease follows a so-called nodal-to-nodal spread.28 Finally, we were not able to relate our network findings to the progression of vascular amyloid deposition on positron emission tomography because of insufficient quantity of positron emission tomography data in this longitudinal sample.

Conclusions
We found measurable progression of global network efficiency in CAA that correlated with clinically relevant parameters (disease severity and cognitive decline). In addition to identifying different spatial distributions of progression in subjects with more advanced CAA, these results also raise the possibility that global network efficiency might be useful as an outcome marker for early phase CAA drug trials.

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Disclosures
None.

References


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### Table I. Baseline characteristics for patients with moderate and severe CAA based on number of microbleeds

<table>
<thead>
<tr>
<th></th>
<th>Moderate CAA (n=16)</th>
<th>Severe CAA (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>69.5 ± 7.1</td>
<td>71.7 ± 8.9</td>
<td>0.446</td>
</tr>
<tr>
<td><strong>Sex, % male</strong></td>
<td>88%</td>
<td>82%</td>
<td>0.680</td>
</tr>
<tr>
<td><strong>Microbleeds, nr</strong></td>
<td>13 (5-25)</td>
<td>70 (53-144)</td>
<td>-</td>
</tr>
<tr>
<td><strong>ICH, % present</strong></td>
<td>50%</td>
<td>47%</td>
<td>0.866</td>
</tr>
<tr>
<td><strong>Time to follow up MRI, years</strong></td>
<td>1.1 (1.0-1.4)</td>
<td>1.1 (1.0-1.3)</td>
<td>0.858</td>
</tr>
<tr>
<td><strong>Time from ICH to baseline MRI, years</strong></td>
<td>1.9 (1.3-6.3)</td>
<td>2.6 (1.0-5.1)</td>
<td>0.619</td>
</tr>
<tr>
<td><strong>WMH volume, % ICV</strong></td>
<td>1.9 (1.2-2.2)</td>
<td>1.9 (0.9-2.8)</td>
<td>0.862</td>
</tr>
<tr>
<td><strong>Network parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global efficiency</td>
<td>0.196 ± 0.016</td>
<td>0.196 ± 0.024</td>
<td>0.963</td>
</tr>
<tr>
<td>FA post-post connections</td>
<td>0.272 ± 0.017</td>
<td>0.272 ± 0.017</td>
<td>0.964</td>
</tr>
<tr>
<td>FA post-front connections</td>
<td>0.287 ± 0.017</td>
<td>0.295 ± 0.029</td>
<td>0.388</td>
</tr>
<tr>
<td>FA front-front connections</td>
<td>0.265 ± 0.016</td>
<td>0.268 ± 0.023</td>
<td>0.664</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD, percentage, or median (interquartile range).

Post = posterior; front = frontal.