Incident subcortical infarcts induce focal thinning in connected cortical regions

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SUPPLEMENTARY METHODS

Study cohort

The study sample consisted of 276 CADASIL patients, which were drawn from a longitudinal, two-center study (Medical Center, University of Munich, Germany and Hôpital Lariboisière, Paris, France). Follow-up was scheduled at 18, 36 and 54 months after baseline examination. 45 patients had a single incident subcortical infarct during the follow-up period. After rigid quality control we excluded 36 subjects from the analysis for the following reasons: insufficient quality of T1-weighted scans (n = 22); insufficient quality of DTI scans (n = 9); inability to register DTI and T1-weighted images (n = 3); territorial infarction possibly from another vascular cause (n = 2). Both the quality control steps and the decision on the final sample were completed prior to analyzing focal cortical thinning. The final study sample consisted of 9 patients.

The 9 patients in the final sample did not significantly differ from the excluded 36 patients regarding age (U=187, p=0.49, two-tailed Mann-Whitney-test), sex (chi-square=2.45, df=1, p=0.12, chi-square-test), time since first stroke (U=59, p=0.977), normalized lacunar lesion volume (U=202, p=0.27) and the number of microbleeds (U=134, p=0.43). There was a borderline significant difference for the normalized white matter hyperintensity volume (larger in excluded patients, U=232, p=0.048). Lesions were segmented as described previously.\(^\text{1,2}\)

Identification of incident infarcts using difference images

After intensity normalization, the T1 scan at baseline was subtracted from all follow-up T1 scans, thereby highlighting incident hypointensities as dark intraparenchymal regions (supplementary figure e-1). The reliability for the identification of incident lesions through difference imaging was excellent (Cohen’s kappa coefficient of 0.98). Incident hypointense lesions with a signal identical to cerebrospinal fluid (CSF), sharp delineation, no contact to pre-existing CSF-isointens structures and a diameter greater than 2 mm were segmented as incident infarcts by E.C. and R.R. The Dice coefficient – as a measure for the overlap of segmentations between raters\(^\text{e3}\), calculated by dividing the lesion volume segmented by both
investigators through the average volume of the individual segmentations – was good (0.89). Incident infarcts were distinguished from enlarged perivascular Virchow-Robin spaces by inspecting axial, coronal and sagittal planes and considering size, form, location and the typical orientation of Virchow-Robin spaces along perforating vessels.

**Probabilistic tractography**

Connectivity between brain tissue affected by an incident infarct and cortical regions was assessed on the scan prior to infarction using probabilistic diffusion models and tractography (bedpostx and protrackx \textsuperscript{4,5} from the FMRIB Software library \textsuperscript{6}, version 4.1). Probabilistic diffusion models with multi-fiber approaches are more sensitive to subordinate pathways than single deterministic approaches.\textsuperscript{4} This method has been successfully applied to map connections between subcortical areas (e.g. thalamic nuclei\textsuperscript{5}) and cortical regions. A segmentation mask of the incident infarct, manually drawn on the T1-weighted scan and registered to the DTI scan preceding infarction, was used as a seed in protrackx. Probabilistic tractography (protrackx) was performed using 5000 samples and a curvature threshold of 0.2. Resulting tracts were registered back to the T1 image. Regions where the tracts reached the white matter surface (as determined by Freesurfer, see below) were defined as cortical region-of-interest (ROI) representing the connected area.

Probabilistic tractography was performed using different probability thresholds: Lower thresholds are more susceptible to noise and may lead to false-positive results while higher thresholds are more specific but might remove subordinate pathways. We defined three probability thresholds: Using 5 tractography samples we determined cortical ROIs with low probability of connectivity. The number of samples for medium and high probability were then determined by the ROI surface size: We aimed for 50% (medium threshold) and 25% (high threshold) of the initial (low probability) ROI surface. In addition, we analyzed cortical thinning in ROIs for every probability threshold between 1 and 200 tractography samples (Figure e-2).

**Cortical thickness analysis**

Automated surface reconstruction and measurements of cortical thickness were obtained with the Freesurfer software package using standard procedures and parameters.\textsuperscript{7-9} The validity and reliability of cortical thickness measurements using Freesurfer has been shown by comparisons with manual post-mortem\textsuperscript{10} and test-retest analyses.\textsuperscript{11} Sub-millimeter changes of thickness can be detected in individual subjects.\textsuperscript{8}

Freesurfer results were inspected visually slice by slice. In several subjects white matter hyperintensities located directly beneath the cortex interfered with determination of the white-
matter surface as was common in the temporal pole. Whenever there was a regional segmentation error, the respective brain region was excluded from the analysis on images from both time points (before and after infarct). The surface of excluded brain regions ranged from 1.62% to 6.92% of the whole brain surface. Cortical thickness was determined by the distance between the white-matter surface and the pial surface across the cortical mantle.

**Measurements of focal cortical thinning**

Cortical thickness was measured in the connected ROI and in the non-connected reference region (RR), comprising all cortical areas outside the ROI. The RR was used to control for unspecific global changes in cortical thickness. Absolute thickness values may vary over time because of global atrophy or changes in scanner parameters, image quality and hydration status. Focal cortical thinning (FCT) was thus defined as the change of cortical thickness in the ROI (in %) in excess of the change of cortical thickness in the RR (in %):

\[
FCT = \frac{ROI_{before} - ROI_{after}}{ROI_{before}} - \frac{RR_{before} - RR_{after}}{RR_{before}}
\]

For a calculation example see table e-2.

**Control analysis**

To check the specificity of our analysis, we conducted the exact same analysis using control seeds. Control seeds were picked in every subject according to the location of the incident infarct. In case of an incident infarct predominantly affecting fibers of one hemisphere, we placed the control seed in the contralateral hemisphere, while trying to match the anatomical position as closely as possible. In case of incident infarcts affecting commissural fibers (mainly lesions in the corpus callosum) the projection of the lesion location on the contralateral side would have resulted in the same fiber tracts. We therefore used a control seed in the posterior part of the corpus callosum if the incident infarct was in the anterior part of the corpus callosum and vice versa.
SUPPLEMENTARY REFERENCES

SUPPLEMENTARY TABLES

**Table e-1:** MRI acquisition parameters

<table>
<thead>
<tr>
<th>Subject</th>
<th>Center</th>
<th>T1-weighted, before infarction</th>
<th>T1-weighted, after infarction</th>
<th>DTI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TR [ms]  TE [ms]  Slice [mm] SA</td>
<td>TR [ms]  TE [ms]  Slice [mm] SA</td>
<td>B-value</td>
</tr>
<tr>
<td>1, 3</td>
<td>Paris</td>
<td>8.6   1.9    0.8  axial</td>
<td>1.02 x 1.02  8.6  1.9  0.8  axial</td>
<td>1.02 x 1.02</td>
</tr>
<tr>
<td>2, 5</td>
<td>Paris</td>
<td>9.1   2.0    0.8  axial</td>
<td>1.02 x 1.02  8.6  1.9  0.8  axial</td>
<td>1.02 x 1.02</td>
</tr>
<tr>
<td>4</td>
<td>Paris</td>
<td>11.7  5.2   1.6  axial</td>
<td>0.94 x 0.94  8.6  1.9  0.8  axial</td>
<td>1.02 x 1.02</td>
</tr>
<tr>
<td>6–9</td>
<td>Munich</td>
<td>22    6     1.2  coronal</td>
<td>0.90 x 0.90  22  6    1.2  coronal</td>
<td>0.90 x 0.90</td>
</tr>
</tbody>
</table>

DTI, diffusion tensor imaging. TR, repetition time. TE, echo time. SA, slice acquisition. DD, number of diffusion directions.
Table e-2: Calculation of focal cortical thinning following an incident subcortical infarct in a single subject (example: subject 3).

<table>
<thead>
<tr>
<th>Region</th>
<th>Thickness before infarction</th>
<th>Thickness after infarction</th>
<th>Cortical thinning</th>
<th>Focal cortical thinning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region-of-interest</td>
<td>2.68 mm</td>
<td>2.40 mm</td>
<td>10.45%</td>
<td>7.30%</td>
</tr>
<tr>
<td>Reference region</td>
<td>2.54 mm</td>
<td>2.46 mm</td>
<td>3.150%</td>
<td></td>
</tr>
</tbody>
</table>
Table e-3: Summary statistics across all 9 subjects for cortical morphology at the three thresholds for probability of connectivity.

<table>
<thead>
<tr>
<th>Probability of connectivity</th>
<th>Region</th>
<th>Thickness before infarction mean (SD)</th>
<th>Thickness after infarction mean (SD)</th>
<th>Mean focal cortical thinning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>ROI</td>
<td>2.61 mm (0.34)</td>
<td>2.51 mm (0.39)</td>
<td>2.69%</td>
</tr>
<tr>
<td></td>
<td>RR</td>
<td>2.43 mm (0.14)</td>
<td>2.40 mm (0.14)</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>ROI</td>
<td>2.63 mm (0.37)</td>
<td>2.47 mm (0.44)</td>
<td>5.21%</td>
</tr>
<tr>
<td></td>
<td>RR</td>
<td>2.43 mm (0.14)</td>
<td>2.40 mm (0.15)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>ROI</td>
<td>2.65 mm (0.44)</td>
<td>2.39 mm (0.49)</td>
<td>8.85%</td>
</tr>
<tr>
<td></td>
<td>RR</td>
<td>2.43 mm (0.14)</td>
<td>2.40 mm (0.15)</td>
<td></td>
</tr>
</tbody>
</table>

ROI, region of interest. RR, reference region
SUPPLEMENTARY FIGURES

Figure e-1: Identification of incident infarcts using difference images. Two subjects (numbers correspond to those in the table of the main manuscript) are shown for illustration. Incident infarcts are identified as dark regions on the difference images.
Figure e-2: Relationship between the probability of connectivity and focal cortical thinning. Results are shown for individual subjects (n=9, grey lines) and for the overall group (mean, black line). Depicted are measurements for thresholds from 1 to 200 tractography samples. Graphs were smoothed using a moving average of 5 measurements.
**Figure e-3:** Relationship between the probability of connectivity and focal cortical thinning in the analysis using control seeds. The graphical representation is similar to figure e-2. Results are shown for individual control seeds (n=9, grey lines) and for the overall group (mean, black line).