Loss of Venous Integrity in Cerebral Small Vessel Disease
A 7-T MRI Study in Cerebral Autosomal-Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy (CADASIL)

François De Guio, PhD; Alexandre Vignaud, PhD; Stefan Ropele, PhD; Marco Duering, MD; Edouard Duchesnay, PhD; Hugues Chabriat, MD, PhD; Eric Jouvent, MD, PhD

Background and Purpose—Previous pathological studies in humans or in animal models have shown alterations of small arteries and veins within white matter lesions in cerebral small vessel disease. We aimed to evaluate in vivo, the integrity of the cerebral venous network using high-resolution MRI both within and outside white matter hyperintensities in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).

Methods—High-resolution $T_2^*$-weighted images were obtained at 7-T in 13 CADASIL patients with no or only mild symptoms and 13 age- and sex-matched controls. Macroscopic veins were automatically counted in the centrum semiovale and compared between patients and controls. In addition, $T_2^*$ was compared between groups in the normal-appearing white matter.

Results—Vein density was found lower in CADASIL patients compared with that in controls ($−14.6\%$ in patients, $P<0.001$). This was detected both within and outside white matter hyperintensities. Mean $T_2^*$, that is presumably inversely related to the venous density, was also found increased in normal-appearing white matter of patients ($+7.2\%$, $P=0.006$). All results were independent from the extent of white matter hyperintensities.

Conclusions—A significant reduction in the number of visible veins was observed in the centrum semiovale of CADASIL patients both within and outside white matter hyperintensities, together with an increase of $T_2^*$ in the normal-appearing white matter. Additional studies are needed to decipher the exact implication of such vasculature changes in the appearance of white matter lesions. (Stroke. 2014;45:2124-2126.)

Key Words: CADASIL ■ magnetic resonance imaging ■ veins

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a rare hereditary small vessel disease of the brain caused by mutations of the NOTCH3 gene. Animal models and pathological studies have shown that both small arteries and small veins are altered within white matter lesions in cerebral small vessel disease. At ultrahigh field, susceptibility effects cause blooming of the signal void secondary to the presence of deoxygenated blood in veins, so that veins with diameter as small as a few tens of micrometers, far inferior to the voxel size, can actually be detected in vivo.

In the present study, we aimed to investigate whether alterations of the venous vascular network can be detected in CADASIL patients at an early stage of the disorder using 7-T high-resolution MRI. Both venous-related signal changes at the macroscopic level and those possibly related to microvascular changes at lower scale (through $T_2^*$ measurement) were assessed in CADASIL patients with no or only mild symptomatology compared with age- and sex-matched healthy controls.

Materials and Methods

Participants

Patients included were not demented (preserved global cognitive abilities and Mini-Mental State Examination >24) and not disabled (defined by modified Rankin scale ≤1). In the present study, 13 patients with high-quality 7-T MRI of the whole brain were included for analyses, by comparison with 13 age- and sex-matched healthy controls. A local ethics committee validated the protocol, and all subjects gave their written consent for participating in the study.

MRI Protocol and Image Processing

Subjects underwent both 3 and 7-T MRI evaluations on the same day, comprising 3-dimensional (3D) $T_2^*$-weighted images at 3 T with 1-mm isotropic resolution and 2D $T_2^*$ acquisitions with 0.7-mm isotropic resolution covering the whole hemispheres at 7 T. A specific postprocessing pipeline was used to obtain a 3D whole brain...
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high-resolution T2* image from different 2D blocks obtained at 7 T.

Details of the MRI protocol and image processing are included in the online-only Data Supplement.

An axial block of interest with 6-mm thickness was defined on the Montreal Neurological Institute template in the centrum semiovale, parallel to anterior commissure–posterior commissure plane with midplane tangent to the corpus callosum (Figure I, in the online-only Data Supplement). Minimum intensity projection along the direction perpendicular to the block was derived for each subject in its native space resulting in a single 2D image where macroscopic veins appear hypointense because of local susceptibility effects caused by deoxyhemoglobin. Given the length L and width W of the brain on that image, the middle of a 60-mm segment was positioned at L/2 and W/3 or −W/3 for both hemispheres. Gray levels along both segments were then extracted, from which vein density within or outside white matter hyperintensities (WMH) was estimated.

To evaluate differences of T2* between patients and controls in normal-appearing white matter (NAWM), 4 regions of interest (ROI) were manually defined on T2*-reconstructed volumes in the NAWM of each patient. Each patient was matched to a control subject (same sex, age as close as possible [mean absolute difference, 2.8 years]). Mean signal and standard deviation were computed to estimate the mean T2* in each ROI.

To evaluate differences of T2* between patients and controls in normal-appearing white matter (NAWM), 4 regions of interest (ROI) were manually defined on T2*-reconstructed volumes in the NAWM of each patient. Each patient was matched to a control subject (same sex, age as close as possible [mean absolute difference, 2.8 years]). Mean signal and standard deviation were computed to estimate the mean T2* in each ROI.

Table. Characteristics of CADASIL Patients and Control Subjects and Results of Both Analyses: Macroscopic Vessel Counting and ROI T2* Analysis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CADASIL Patients</th>
<th>Healthy Controls</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Age, mean±SD, range</td>
<td>51.7±12.9, 32.1–72.6</td>
<td>50.8±12.2, 30.1–71.4</td>
<td>0.80*</td>
</tr>
<tr>
<td>Right handed, n (%):</td>
<td>13/13 (100%)</td>
<td>13/13 (100%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>53.8</td>
<td>53.8</td>
<td>0.99</td>
</tr>
<tr>
<td>Level of education (years)</td>
<td>11.5±3.3</td>
<td>13.0±3.3</td>
<td>0.32*</td>
</tr>
<tr>
<td>MMSE, mean, median, range</td>
<td>28.2, 29, 24–30</td>
<td>29.1, 29, 27–30</td>
<td>0.27*</td>
</tr>
<tr>
<td>WMH volume, mean, median, interquartile range, range in cm³</td>
<td>77.4, 66.6, 44.0, 7.3–233.8</td>
<td>None had significant lesions</td>
<td></td>
</tr>
<tr>
<td>Lacunar lesion volume, mean, median, range in mm³ (n=6/13, 46%)†</td>
<td>779, 644, 14–1975</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Number of microhemorrhages, mean, median, range (n=5/13, 38%)†</td>
<td>3, 2, 1–6</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Macroscopic vessel counting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) vein density (mm⁻¹)</td>
<td>0.176 (0.019)</td>
<td>0.206 (0.018)</td>
<td>0.0006‡§</td>
</tr>
<tr>
<td>Mean (SD) vein density outside WMH (mm⁻¹)</td>
<td>0.189 (0.028)</td>
<td>0.206 (0.018)</td>
<td>0.04‡§</td>
</tr>
<tr>
<td>Mean (SD) vein density within WMH (mm⁻¹)</td>
<td>0.137 (0.028)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Mean (SD) length L (mm)</td>
<td>149.5 (8.0)</td>
<td>155.8 (10.6)</td>
<td>0.10</td>
</tr>
<tr>
<td>ROI analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) T2* over all 52 ROI (ms)</td>
<td>29.9 (1.2)</td>
<td>27.9 (1.7)</td>
<td>0.006¶</td>
</tr>
<tr>
<td>% of ROI with superior T2* compared with matched subject</td>
<td>76.9%</td>
<td>23.1%</td>
<td></td>
</tr>
</tbody>
</table>

CADASIL indicates cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; MMSE, Mini-Mental State Examination; ROI, regions of interest; and WMH, white matter hyperintensities.

*Wilcoxon rank-sum test.
†In patients with such lesions (number given in parentheses).
‡p<0.05.
§ANOVA adjusted for age, sex, and brain length L.
¶Paired Wilcoxon signed-rank test.
||ANOVA adjusted for age and sex.

Analysis

Statistical analyses were made using the R software (http://www.r-project.org/). For categorical variables, χ² tests were used. As samples were limited, the Wilcoxon rank-sum test was used to compare subject’s characteristics between groups. For the ROI analysis, a pairwise Wilcoxon test was used as each ROI in each patient corresponded to an ROI in a control subject. For the vein density results, ANOVA models adjusted for age, sex, and brain length L were used.

Results

Characteristics of the 13 patients and 13 controls included in the present study are presented in the Table. The 2 groups did not differ in terms of age, sex, or Mini-Mental State Examination.

At the macroscopic level, fewer veins were detected in CADASIL patients compared with control subjects (0.176 versus 0.206 veins/mm [−14.6%], P<0.001; Table). This reduction was visually obvious on minimum intensity projection images (Figure). The effect was stronger within WMH (mean vein density in patients, 0.137 veins/mm). Outside WMH, vein density was still inferior compared with controls (0.189 versus 0.206 veins/mm [−8.3%], P<0.04; Table). Vein density was not correlated with age (r=0.05, P=0.85 in patients; r=−0.008, P=0.98 in controls). No significant correlation was detected between vein density and WMH volume (r=−0.22, P=0.46) or between mean T2* and WMH volume (r=0.15, P=0.63).
A significant $T_2^*$ increase was observed in the NAWM of CADASIL patients compared with age- and sex-matched controls (29.9 versus 27.9 ms, +7.2%, $P=0.006$; Table). When each ROI was compared 2-by-2, mean $T_2^*$ was found higher in CADASIL patient than in controls in 76.9% of regions. No significant correlation was found between vein density and mean $T_2^*$ in NAWM ($r=-0.32; P=0.11$).

**Discussion**

In the present study, we observed a significant reduction in the number of small visible veins detected using 7-T MRI within the centrum semiovale of CADASIL patients compared with age- and sex-matched controls. Although venous density was dramatically reduced within WMH, it was also found significantly decreased in NAWM. In addition, $T_2^*$ was increased in the NAWM of patients, a finding that may be related to the reduction of vascular density although other contributing factors cannot be excluded. Altogether, these results suggest that the loss of integrity in the global microvasculature may precede the appearance of WMH, but the cross-sectional nature of our study did not allow confirming this hypothesis.

In this study, we did not assess the relationships between the reduction of venous structure and clinical data, given the limited sample of patients with no or only mild symptoms. Small sample size and the lack of pathological data are the main limitations. However, the present results were highly significant and visually obvious.

Finally, using 7-T in vivo MRI, these data support a reduction of the density of visible white matter venous vasculature in CADASIL, both within and outside WMH and at the early stage of clinical manifestations. Further investigations are needed to understand whether these alterations reflect causal changes in the development of white matter lesions in this disorder.

**Sources of Funding**

This work was funded by a Network of European Funding for Neuroscience Research grant (01EW1207) under the Seventh Framework Programme and the European Research Area Net, with the support of the French-cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy association, the PLANIOL foundation, the NRJ foundation, and the Leducq foundation.

**Disclosures**

None.

**References**

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Stroke. 2014;45:2124-2126; originally published online May 27, 2014; doi: 10.1161/STROKEAHA.114.005726

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SUPPLEMENTAL MATERIAL

LOSS OF VENOUS INTEGRITY IN CEREBRAL SMALL VESSEL DISEASE: A 7 TESLA MRI STUDY IN CADASIL

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Supplemental Methods

MRI protocol

Subjects underwent the same day both 3 and 7-Tesla MRI examinations at NeuroSpin (CEA, Gif-sur-Yvette, France). 3D T1-weighted images were acquired with a 3-Tesla Tim-Trio MRI scanner (Siemens Healthcare, Erlangen, Germany) equipped with a 12-channel head coil, using a standard sagittal magnetization-prepared rapid acquisition gradient echo sequence (in plane resolution: 1x1 mm\(^2\), slice thickness = 1.1 mm, TR = 2300 ms, TE = 2.98 ms, TI = 900 ms, FA = 9°, BW = 238 Hz/pixel, time of acquisition = 7'45 min). High-resolution MRI acquisitions were obtained on a 7-Tesla MRI scanner (Siemens Healthcare, Erlangen, Germany) equipped with a 1Tx/8Rx head coil (Rapid Biomedical, Wurzburg, Germany). 2D axial gradient-echo T\(_2^*\) sequences were acquired using 7 or 8 blocks depending on subject’ brain size and following AC-PC orientation. The use of 2D blocks was chosen because of the impossibility for most subjects to remain free of movements during the long period of acquisition needed to cover the whole brain. Each block comprised 20 slices with 2 different echo times using the following MR parameters: in plane resolution: 0.7x0.7 mm\(^2\), slice thickness = 0.7 mm, TR = 900 ms, TE\(_1\) = 13.7 ms, TE\(_2\) = 29.9 ms, FA = 65°, BW = 70 Hz/pixel, NA = 2, time of acquisition = 6'7 min. Blocks were acquired parallel to each other with no overlap and this was repeated each time image was of poor quality. Total scan time by subject was about 1 hour on the 7-Tesla scanner.

Image processing

Volume reconstruction

T\(_1\) images obtained at 3-Tesla were processed using FreeSurfer version 5.1.0 (http://surfer.nmr.mgh.harvard.edu), that produced surface-based data for each subject\(^1\). Masks of WMH were obtained from FLAIR sequences as previously reported and registered to 3D T1 images\(^2\). To overcome segmentation difficulties, image gray level inside lesions was set up to an average intensity close to that of NAWM. Reconstructed datasets were then visually checked for accuracy. For intra-subject registration, the 7-Tesla T\(_2^*\) blocks were first concatenated to create a rough whole brain reconstruction. We then used the FSL FLIRT algorithm (http://www.fmrib.ox.ac.uk/fsl) to perform an initial affine registration between whole T\(_2^*\) volume at second echo time and 3D T\(_1\) volume which was over-sampled to an isotropic resolution of 0.7 mm. Next, boundary-based registration (BBR) was initialized with this latter transformation and ran separately for each block\(^3\)\(^4\). Quality of blocks registration was checked. When the results were not satisfactory, manual adjustment was iteratively performed to change the initial conditions preceding BBR registration. Fine registered blocks were finally combined to create the reconstructed T\(_2^*\) volume. Pre-processing includes correction for intensity inhomogeneity and exclusion of null points corresponding to voids between blocks.
Macroscopic vessel counting

An axial block of interest with 6 mm thickness was defined on the MNI template in the centrum semi-ovale, parallel to AC-PC plane with midplane tangent to the corpus callosum (supplementary Figure I). The choice of this location was made because of the high consistency of vein orientation in this area, thus limiting the impact of block positioning on vessel counting. Also, the perpendicular orientation of the veins in this region to the main magnetic field enabled to maximize the local susceptibility effects and thus the detection of small veins. A 6 mm thickness was chosen as the best trade-off for discriminating many vessels from susceptibility artefacts. The block of interest was then projected to the native space of each subject using non-linear registration (FSL FNIRT) in order to maximize between-subject registration accuracy and study a similar anatomical region. Minimum intensity projection along the direction perpendicular to the block was derived for each subject resulting in a single 2D image where macroscopic veins appear hypointense due to local susceptibility effects caused by deoxyhemoglobin.

Given the length L and width W of the brain on that image, the middle of a 60 mm segment was positioned at L/2 and W/3 or –W/3 for both hemispheres as represented in supplementary Figure I. Gray levels along both segments were then extracted. Finally, local minima on the gray level profile were detected based on second derivative and on a 5% local minimum intensity decrease criterion. The vein density was defined as the sum of local minima (number of veins) extracted for both hemispheres divided by the total length of the two segments (120 mm). In patients, based on the minimum intensity of WMH along the segments, vessels were defined inside or outside WMH in order to compute the vein density inside or outside WMH.

As microbleeds also appeared hypointense on T2*-weighted images, it could have confounded the measurement of the number of veins. However, counting microbleeds as veins would have artificially increase number of veins in CADASIL patients compared to controls what is the opposite of what we found.

ROI analysis

To evaluate differences of T₂* between patients and controls in NAWN, 4 ROIs were manually defined on T₂* reconstructed volumes in the NAWM of each patient. Sphere radius was set up for not encompassing the cortical ribbon, CSF or any WMH. Size and positioning of those ROI was thus specific to each subject and dependent of the extent of WMH (minimum: 42 mm³, maximum: 487 mm³, mean: 209 mm³, standard deviation: 117 mm³). Each patient was matched to a control subject (same sex, age as close as possible (mean absolute difference: 2.8 years)). ROIs were then projected from each patient’s space to the corresponding control’s space using non-linear wrapping. Mean signal and standard deviation were computed for both echo times enabling to estimate mean T₂* relaxation times in each ROI. Mean NAWM T₂* was computed by weighting each ROI’s mean by its volume for each subject.
Supplemental Figure I: Macroscopic vessel counting after minimum intensity projection in a block defined in MNI template.

Supplementary Figure I legend:

A 6 mm block parallel to ACPC plane was defined in MNI space in the centrum semi-ovale and projected to each subject’s space using non-linear registration. Minimum intensity projection was then computed perpendicular to the block on the 7-Tesla T2*-weighted reconstructed volume. Two segments (in yellow) were positioned parallel to interhemispheric plane at the middle of brain length L and one third of brain width W measured on that image. Profiles of gray level values were extracted for both hemispheres and macroscopic vessel were automatically counted as local minima defined from second derivative and local signal decrease criteria.
Supplemental References


