Arterial branching and basal ganglia lacunes: A study in pure small vessel disease

Fiona C Moreton, Marco Düring, Thanh Phan, Velandai Srikanth, Richard Beare, Xuya Huang, Eric Jouvent, Hugues Chabrier, Martin Dichgans and Keith W Muir

Abstract

Introduction: Lacunes are defined morphologically by size and location, but radiological characteristics alone may be unable to distinguish small vessel disease aetiology from alternative mechanisms. We investigated the branching order of arterial vessels associated with basal ganglia lacunes in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), in order to improve the understanding of their pathogenesis in pure cerebral small vessel disease.

Patients and methods: Adults with a confirmed diagnosis of CADASIL were included. A pilot study was conducted in a Scottish CADASIL cohort. The Paris–Munich CADASIL cohort was used for independent validation. Lacunes identified on T1-weighted magnetic resonance imaging scans were registered to a standard brain template. A microangiographic template of the basal ganglia vasculature was automatically overlaid onto coronal slices, and raters estimated the vessel branching order related to each lacune.

Results: Of 179 lacunes, 150 (84%) were associated with third-order vessels. In 14 incident lacunes, 11 (79%) were associated with third-order vessels. In the pilot study, lacune volume was significantly lower in lacunes associated with third-order vessels (0.04 ml ± 0.04 ml) compared to second-order vessels (0.48 ± 0.16 ml; p < 0.001).

Discussion: In this study of CADASIL patients, most lacunes were small and associated with third-order vessel disease. This suggests that these are the vessels primarily affected in cerebral small vessel disease. Microangiographic template techniques could be used to further investigate in a general stroke population whether finding large lacunes originating from higher order vessels indicates an alternative cause of stroke.

Conclusion: Lacunes in pure small vessel disease are associated with the smallest vessels in the basal ganglia.

Keywords

Infarction, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, magnetic resonance imaging, lacunes

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Introduction

Regarded as a classic radiological manifestation of cerebral small vessel disease (SVD), lacunes are proposed to be due to subcortical infarct or haemorrhage in the territory of a single perforating artery and are commonly found in the basal ganglia, subcortical white matter and brainstem.

They may be associated with characteristic neurological syndromes but can occur without concurrent clinical manifestations. Most subcortical infarcts are proposed to be due to intrinsic abnormalities in cerebral small vessels, although a proportion may be due to...
embolism or intracranial atherosclerosis at the origin of the perforating arteries. Differentiating these varied potential mechanisms radiologically has proved problematic due in part to limited resolution of in vivo vascular imaging. Post-mortem studies require complex techniques to study small vessels and are usually only available some time after the development of the lacune.

The vascular anatomy of the basal ganglia, a common site for lacunes, has been the focus of anatomical studies. The medial and lateral lenticulostriate arteries (LSA), which originate from the middle cerebral artery, supply portions of the caudate, putamen, internal capsule and external globus pallidus. The recurrent artery of Heubner (RAH) usually arises from the anterior cerebral artery and supplies anterior parts of the basal ganglia. All these vessels branch into smaller vessels which terminate in defined territories.

The principal anatomy of these vessels has been shown by contrast injection of 40 cerebral hemispheres to be highly consistent across individuals, with little overlap of arterial territories, allowing the production of a microangiographic template (Figure 1). This template was used previously to estimate the branching order of arteries related to basal ganglia subcortical infarcts seen in a general stroke population. The investigators concluded that the dimensions of the subcortical infarct were dependent on the branching order of the vessel involved. In a heterogeneous stroke population, with multiple risk factors, it is not possible to determine whether these infarcts were
caused by cerebral SVD or whether other mechanisms might be relevant.

We aimed to assess a population with pure cerebral SVD. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited vasculopathy proposed as a model for cerebral SVD, due to similar radiological findings but with a typically younger age of onset and hence a lower prevalence of confounding pathologies. We investigated the size of basal ganglia lacunes and their relationship with the branching order of the arteries using a microangiographic template in a pilot study of CADASIL patients with limited co-existent cardiovascular pathology, and subsequently in a larger cohort with both prevalent and incident lacunes, as representative of a pure cerebral SVD model. The hypothesis was that in CADASIL disease of third-order branches would lead to the development of basal ganglia lacunes.

**Methods**

**Samples**

**Pilot study (Scottish sample).** All subjects were drawn from a study of cerebral and peripheral haemodynamics in CADASIL (UK CRN 13794). Competent adults with a genetic diagnosis of CADASIL were included. Exclusion criteria were (1) other non-CADASIL-related degenerative neurological disease, (2) contraindications to magnetic resonance imaging (MRI) and (3) treatment with angiotensin-converting enzyme inhibitors or calcium channel blockers (due to vaso-reactivity tests in the main study). Clinical data including medical history, cardiovascular risk factors and medication were obtained.

Patients were scanned on a 3.0T GE Signa HDxt (GE Medical Systems, Milwaukee, WI, USA). The protocol included T1-weighted three-dimensional (3D) images (3D-T1; slice thickness = 1 mm, repetition time = 9.0 s, inversion time = 450 ms, flip angle = 12°, matrix = 320 × 320, FOV = 24), susceptibility-weighted image (SWI; slice thickness 3.6 mm, repetition time = 40 ms, echo time = 25, flip angle = 15°, matrix = 320 × 224), fluid-attenuated inversion recovery sequence (FLAIR; slice thickness = 5 mm, interslice gap = 1.5 mm, repetition time = 10 s, echo time = 148 ms, inversion time = 2250 ms, matrix 384 × 256) and sagittal enhance 3D velocity MR angiography (MRA; repetition time = 8.6 ms, echo time = 3.54, slice thickness = 1.2 mm, flip angle = 8°, matrix 320 × 224).

**Validation study (Paris–Munich cohort).** Subjects were prospectively recruited between September 2003 and April 2011 through two major referral centres for CADASIL (University Hospital Lariboisière, Paris (n = 198) and Institute for Stroke and Dementia Research, Munich (n = 117)). Adults with a documented mutation in the NOTCH3 gene or with the presence of granular osmiophilic material in skin biopsy were included, and written informed consent was obtained either from a patient or a legal guardian. Details of the study protocol have been reported elsewhere. Clinical and demographic data were collected along with vascular risk factors (hypertension, diabetes mellitus, hypercholesterolaemia, smoking and alcohol use). Patients were all scanned on 1.5 T systems; details on scanner characteristics and MRI sequences have been reported elsewhere. The protocol included millimetric 3D T1-weighted images, axial slices of 5 mm thickness of FLAIR images and T2*-weighted gradient-echo planar images.

![Figure 2. Template overlay and example lacunes. Location of coronal slices 125,130 and 135 on 3D-T1 image transformed into MNI space from pilot study (a). Coronal MNI slices with microangiographic template showing an example of a lacune associated with (b) second-order branch disease and (c) third-order branch disease.](image-url)
Follow-up examinations were scheduled at 18 and 36 months.

**MRI analysis**

Prevalent lacunes were identified on 3D T1 images as cystic lesions with a cerebrospinal fluid (CSF) signal identical on T1 and T2 (or FLAIR) images. Well-established criteria were used to distinguish lacunes from perivascular spaces including size (>2 mm diameter for lacunes), shape, location and the typical orientation of perivascular spaces along the course of perforating vessels.\(^{15,16}\) Incident lacunes in the longitudinal dataset were identified using difference imaging and Jacobian maps as described elsewhere.\(^{14}\)

**Pilot study.** A seed placed within each lacune using Analyze v 11.0 (AnalyzeDirect Inc, United States). SWI sequences were examined to assess if there was any evidence of haemosiderin around the lacune. Seed-based thresholding was used to outline the lacune. Segmented lacunes were fused with the 3D-T1 scan, which was transformed into Montreal Neurological Institute (MNI) standard 1mm space using FMRIB's Linear Image Registration Tool\(^{17}\) from the FMRIB software library (FSL v 5.0, Oxford University, UK). Coronal slices 125, 130 and 135 were extracted from the transformed T1 (FSL slicer). A microangiographic template of the basal ganglia (LSA and RAH) created as part of a recent study\(^{11}\) was overlaid onto coronal images using an in-house programme. This template displays first-order (proximal) to third-order (distal) branching of these arteries in the basal ganglia.

Individual lacunes were transformed into MNI standard space using the matrix derived above. The transformed lacune volume was measured using a voxel-counting method. Lacune dimensions in standardized space (axial width and anterior–posterior (AP) length) were measured using the boundary of their locations on the standardized brain map.

**Validation study.** Lacunes were delineated using custom two-dimensional and 3D editing tools from BioClinica SAS using a semi-automated procedure with intensity thresholding and manual corrections. The normalization procedure to MNI 152 space involved tools from the functional MRI of the Brain Software Library.\(^{18,19}\) Once lacunes were in standard space the microangiographic template was overlaid as detailed above.

**Visual rating**

Raters who were not involved in scan processing were provided with overlaid coronal images where a lacune was visible along with a standardized rating form, which included examples from a previous paper.\(^{11}\) Raters were asked to visually estimate the branching order of the artery related to the lacune, and whether the vessel that was likely to have been diseased was a first-, second- or third-order branch, or out with the LSA/RAH territory.

**Pilot study.** Four raters (two neurologists, one geriatrician and one neurology trainee) who were blinded to clinical information were asked to rate lacunes. Lacunes were excluded if the bulk of the lacune was clearly outside the LSA/RAH territory. Where a lacune was present on more than one image, all images were presented to the rater anonymized but only the scan with the largest lacune included in analysis. Individual ratings were averaged to provide a summary score for each lacune. A summary score of \(\leq 1.5\) was rounded to 1 (first-order branch), \(1.5 – 2.5\) was rounded to 2 (second-order branch) and \(> 2.5\) rounded to 3 (third-order branch), as branching order in a realistic setting is an integer.

**Validation study.** Two raters (one neurologist and one geriatrician) blinded to clinical information rated prevalent and incident lacunes using the above standardized rating form. In the case of disagreement, a third rater (a neurologist) rated the lacune using the same form.

**Statistical analysis**

Statistical analysis was performed with IBM SPSS version 21 (IBM Corp, Armonk, NY, USA). Lacune dimensions and volumes in relation to vessel branch order were compared with independent \(t\)-test, and their relationship to each other with a Spearman’s correlation. Kappa was calculated with an online calculator (http://vassarstats.net/kappa.html). Differences were considered significant at \(p < 0.05.\)

**Results**

**Pilot study**

**Patient characteristics.** Twenty-two subjects with CADASIL (50% male, median age 53 years, range 26–67) were included in the pilot study. Ten subjects had one or more discrete lacunes in the LSA/RAH territory (none of which was associated with blood on SWI). Of those with lacunes, no subject had hypertension, diabetes or atrial fibrillation, five (50%) were
smokers and seven (70%) had hypercholesterolaemia. None had evidence of carotid disease on MRA and/or carotid ultrasound. Average systolic blood pressure was 119 mmHg (standard deviation, 11 mmHg).

Prevalent lacunes. Sixteen lacunes were initially identified, but once maps were overlaid, three were mainly outwith the vessel territory. Of the 13 remaining lacunes, none was associated with first-order, 3 with second-order and 10 with third-order vessels. Lacunes associated with second-order vessels had significantly greater dimensions than third-order vessel lacunes (see Table 1). All dimensions correlated with lacune volume (n = 13; axial width, r_s = 0.630, p = 0.021; coronal height, r_s = 0.9, p < 0.001; AP diameter, r_s = 0.899, p < 0.001).

Validation study

Patient characteristics. A total of 315 subjects with CADASIL (42.2% male, median age 50 years, range 23–77) were included in the validation study. Ninety-five subjects had one or more prevalent lacunes in the LSA/RAH territory with a total of 166 lacunes. Cardiovascular risk factors are shown in Table 2. Prevalent lacunes. In sum, 166 lacunes were identified within the LSA/RAH territory. There was agreement on branching order between the two primary raters in 151/166 lacunes; 149 were associated third-order vessels and 17 with second-order vessels. Incident lacunes. Fourteen incident lacunes occurred within the LSA/RAH territory during the follow-up period. There was agreement in 12/14 lacunes between the two primary raters; 12 were associated with third-order and two with second-order vessels.

Discussion

Mechanistic classification of stroke carries therapeutic implications but is confounded by the prevalence of risk factors common to multiple mechanisms (e.g. hypertension), and application of arbitrary thresholds to distinguish one mechanism from another. In a large cohort of subjects with inherited SVD due to CADASIL, we have found that lacunes within the basal ganglia are small^{15} and associated with third-order arterial branch disease. This was the case for both prevalent and incident lacunes and was validated in two cohorts. If these lacunes reflect intrinsic vasculopathy due to third-order branch arterial disease, then refinement of the arbitrary upper limit for lacune size in cerebral SVD may allow better characterization of stroke mechanism.

Fisher’s seminal work on lacunes was based on autopsy data well after an acute ischaemic event. A range of lacune sizes was observed in his studies, with ‘giant lacunes’ up to 35 mm in diameter noted. Cortical infarctions co-existed with lacunes in 26% of patients. Rather than the result of SVD, giant lacunes have been proposed to be due to embolism^{20}.

In our pilot study, patients with CADASIL and without hypertension, diabetes, atrial fibrillation or evidence of carotid disease were investigated as representative of cerebral SVD as the likely sole mechanism. The validation cohort was more heterogeneous and had greater prevalence of vascular risk factors, but the biggest contributing factor to their lacunes was likely to have been cerebral SVD due to CADASIL. In both cohorts, the great majority of lacunes was associated with third-order branch disease.

Table 1. Dimensions of basal ganglia lacunes by branching order in pilot study.

<table>
<thead>
<tr>
<th></th>
<th>Second order n = 3</th>
<th>Third order n = 10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume, ml, median (range)</td>
<td>0.48 (0.32–0.65)</td>
<td>0.03 (0.01–0.14)</td>
<td>0.007</td>
</tr>
<tr>
<td>Axial width, mm, median (range)</td>
<td>11 (8–11)</td>
<td>4 (3–6)</td>
<td>0.007</td>
</tr>
<tr>
<td>Coronal height, mm, median (range)</td>
<td>14 (12–17)</td>
<td>4 (3–9)</td>
<td>0.007</td>
</tr>
<tr>
<td>AP length, mm, median (range)</td>
<td>10 (9–15)</td>
<td>4 (2–7)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of pilot and validation data.

<table>
<thead>
<tr>
<th></th>
<th>Pilot data</th>
<th>Validation study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>Number of prevalent lacunes scored</td>
<td>13</td>
<td>166</td>
</tr>
<tr>
<td>Age in years, median (IQR)</td>
<td>53 (16)</td>
<td>51 (12)</td>
</tr>
<tr>
<td>Male:Female</td>
<td>8.2</td>
<td>59:36</td>
</tr>
<tr>
<td>Cardiovascular risk factors, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smoker</td>
<td>5 (50)</td>
<td>61 (64)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0 (0)</td>
<td>24 (25)</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>7 (70)</td>
<td>45 (51)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0 (0)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>0 (0)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>
The Standards for Reporting Vascular changes in Neuroimaging criteria for a lacune of presumed vascular origin is a round or ovoid, subcortical, fluid-filled (similar signal as CSF) cavity, of between 3 mm and 15 mm in diameter, consistent with a previous acute small deep brain infarct or haemorrhage in the territory of one perforating arteriole. The lower boundary was chosen to distinguish lacunes from perivascular spaces, but the upper boundary is arbitrary, and there remains a lack of consensus as to lacune size in cerebral SVD. This study, examining basal ganglia lacunes in pure cerebral SVD suggests the vast majority of lacunes due to this pathology are well below this 15 mm cut off, with the median dimensions of lacunes associated with tertiary LSA branches being around 4 mm in the pilot study. A recent paper examining lacune shape and 88 incident lacunes in CADASIL showed most lacunes are below 15 mm in the axial plane, but even in the basal ganglia lacunes up to 25 mm could be found. However, whether these larger lacunes are due to cerebral SVD, or alternative pathology, cannot be determined with certainty.

CADASIL may represent a model of sporadic cerebral SVD since radiological features and key histopathological findings are similar. Despite molecular pathophysiology that is likely to differ, a common pathway of lacune formation in third-order vessel branch disease (regardless of the exact pathology) is likely to be common to both and is consistent with autopsy studies. One study reported the dimensions supplied by collateral branches (likely representing third-order or below) as a maximum of 18 × 6 mm, but smaller collateral branches supplied much smaller areas. In CADASIL, either haemodynamic failure due to impaired vasoreactivity or occlusion of these vessels would result in ischaemia and give rise to lacunes of the size identified in this study. Studies in CADASIL patients have shown that lacunes tend to align with the orientation of these perforating vessels.

Our study has several methodological strengths. The initial pilot study was conducted in a small number of patients but used 3T isotropic T1 scans with high resolution in all planes. The larger cohort allowed validation of the study, in a well-phenotyped group, with imaging analysed by experienced researchers. The use of an arterial template represents a novel, if indirect method of examining small vessels. In vitro visualization of relevant vasculature requires invasive angiography, or high-field MRI, but small vessels of relevant size are beyond the spatial resolution of current routine non-invasive vascular imaging. Co-registration of the map and the brain is likely to be preferable to methods requiring side-to-side inspection and a reliance on anatomical skills. Co-registration did require the transformation of scans to an MNI template, thus changing lacune dimensions to an extent. There are some limitations to our study. Only the LSA and RAH territories were included, since only these areas have a defined microangiographic template. In the pilot study, a single rater identified lacunes, which may have resulted in lacune misclassification; however, the Paris–Munich data have shown high repeatability in its identification of incident lacunes.

As discussed, a lacune may be related to ischaemia or haemorrhage. In the larger study, no attempt was made to differentiate between the two with reference to SWI or T2* scans. However, damage from either mechanism may well relate to common vessel pathology (and thus vessel size) given that these are end-artery territories. We have not included size analysis of lacunes in the validation cohort, but this analysis (on lacunes throughout the brain) has been published elsewhere. One potential criticism could be that lacune size alone could be a surrogate for vessel branch order such that additional reference to vascular templates could have been avoided. However, this idea assumes that one knows beforehand what the relationship between branch vessel order and lacune size. Had this been known, then the axial diameter of 15 mm on conventional MRI would not have been used to define the lacune. Variation in brain size and the volume of tissue subtended by vessels may have led to misclassification and the template reference was felt to represent the optimal methodological approach. In some lacunes, the bulk of the lacunes was outside the LSA/RAH territory but a small proportion of the lacune crossed into the RAH/LSA territory covered by the templates. When the bulk of the lacune was mainly outside the territory, they were excluded as they were not likely to be due to RAH/LSA arterial disease. In the periphery of the LSA/RAH territory, there may be some minimal crossover of supply from different blood vessels. We did not exclude any lacunes from the second study, so we cannot exclude that this was not the case in the second study.

Even when using agreed criteria, the distinction between lacunes and perivascular spaces, particularly when small and in the basal ganglia, is difficult. Enlarged and numerous perivascular spaces may be seen in CADASIL, and dividing CSF-filled spaces visualized on MRI into ‘non-pathological’ perivascular spaces and ‘pathological’ ischaemic lacunes is likely to be subject to error. There are no controls in this study, since healthy subjects would be unlikely to have lacunes. Patients with sporadic cerebral SVD could be used as an alternative control group, and this could be explored in the future, but defining aetiology in individual patients remains difficult for the reasons described. We cannot be sure that CADASIL patients have the same vascular anatomy as subjects used to derive the
microangiographic templates; however, the principal anatomy is thought to be uniform across individuals. Our findings may have clinical relevance. Identifying small lacunes from third- and second-order vessels on scans from stroke patients would suggest cerebral SVD as the likely aetiology. Further work in sporadic stroke patients would be needed to confirm whether the finding of large lacunes within the basal ganglia, associated with first-order vessel disease, suggests a cause other than cerebral SVD. Applying this microangiographic template to acute infarcts within this territory may add further mechanistic insights, which could be performed on already available clinical imaging cohorts.

In conclusion, our data suggest the dimensions on MRI of basal ganglia lacunes in CADASIL patients are small and likely to be primarily due to disease of third-order branches of the LSA and RAH.

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Declaration of Conflicting Interests
The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: FM, MD, XH, VS, HC, EJ, MDü, and KM declare no competing interests. TP has an Honorarium from Bayer/Genzyme/Boehringer Ingelheim/Pfizer/BMS and is on the Advisory Board for Genzyme on Fabry Disease.

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Ethical approval
The West of Scotland Research Ethics Committee reviewed and approved the final version of the manuscript. XH, TP, VS, and KM scored lacunes. MDü and EJ delineated lacunes in the Paris–Munich cohort. RB designed study image processing software. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Contributorship
TP, KM and MDü conceived the study. FM researched literature, analysed the results and wrote the first draft of the manuscript. XH, TP, VS and KM scored lacunes. MDü and EJ delineated lacunes in the Paris–Munich cohort. RB designed study image processing software. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Trial registration
UK CRN 13794 (Scottish study).

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