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

FC Moreton[1], M Düring[2], T Phan[3], V Srikanth[3], R Beare[3], X Huang[1], E Jouvent[4], H Chabriat[4], M Dichgans[2], KW Muir[1].

[1]Institute of Neuroscience and Psychology, University of Glasgow, Queen Elizabeth University Hospital Glasgow, Glasgow, Scotland, G51 4TF

[2]Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwig-Maximilians-University (LMU), Munich, Germany

[3]Faculty of Medicine, Nursing & Heath Sciences, Monash University, Australia

[4] Department of Neurology, DHU NeuroVasc, University Paris Diderot and INSERM UMRS 1161, Paris, France

Corresponding author:

Professor Keith Muir, Institute of Neuroscience and Psychology, University of Glasgow, Queen Elizabeth University Hospital, Glasgow, G51 4TF.

Telephone: +44 141 4515892, Email: Keith.Muir@glasgow.ac.uk

For Munich-Paris data: M Düring, Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwig-Maximilians-University, Munich, Germany. Telephone: +49 (0)89 4400 46166, Email: marco.duering@med.uni-muenchen.de

Abstract (234)

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

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 MRI 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 (0.04ml ± 0.04ml) compared to second-order vessels (0.48 ± 0.16ml; $p < 0.001$).

Discussion: In this study of CADASIL patients, most lacunes were small and associated with third-order vessel disease. As CADASIL represents an intrinsic cerebral SVD it suggests that lacunes originating from higher order vessels may indicate an alternative cause, which may assist in mechanistic investigation of lacunes in a general stroke population.

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

Short title

Lacunes in CADASIL

Keywords

Infarction, CADASIL, MRI, lacunes

Word Count (Text, figures, tables, references, declarations): 3828

Number of tables: 2

Number of figures: 2

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,[1, 2] 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[3]. Most subcortical infarcts are proposed to be due to intrinsic abnormalities in cerebral small vessels,[4, 5] although a proportion may be due to embolism or intracranial atherosclerosis at the origin of the perforating arteries.[6, 7] Differentiating these varied potential mechanisms radiologically has proved problematic[8] 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 sometime 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[9]. 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,[10] 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.[11] 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. CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is an inherited vasculopathy proposed as a model for cerebral SVD,[12] 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 lacunes and their relation with the

branching order of the basal ganglia 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 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 MRI and (3) treatment with ACE inhibitors or calcium channel blockers (due to vasoreactivity 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, Wisconsin). The protocol included T1-weighted 3-dimensional (3D) images (3D-T1; slice thickness = 1mm, repetition time = 9.0s, inversion time = 450ms, flip angle = 12^{O}, matrix = 320 x 320, FOV = 24), susceptibility weighted image (SWI; slice thickness 3.6mm, repetition time = 40ms, echo time = 25, flip angle = 15^{O}, matrix = 320 x 224), fluid-attenuated inversion recovery sequence (FLAIR; slice thickness = 5mm, interslice gap = 1.5mm, repetition time = 10s, echo time = 148ms, inversion time = 2250ms , matrix 384 x 256), and sagittal inhance 3D velocity MR angiography (MRA; repetition time =

8.6ms, echo time = 3.54, slice thickness = 1.2mm, flip angle = 8 º, matrix 320 x 224).

Validation study (Paris-Munich cohort): Subjects were prospectively recruited between September 2003 and April 2011 through 2 major referral centers 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[13]. Clinical and demographic data were collected along with vascular risk factors (hypertension, diabetes mellitus, hypercholesteraemia, smoking, and alcohol use). Patients were all scanned on 1.5T systems; details on scanner characteristics and magnetic resonance imaging (MRI) sequences have been reported elsewhere.[13, 14] The protocol included millimetric 3D T1-weighted images, axial slices of 5 mm thickness of FLAIR images, and T2*-weighted gradient-echo planar images. 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 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 FLIRT (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 standardised space (axial width and anterior-posterior (AP) length) were measured using the boundary of their locations on the standardised brain map.

Validation study: Lacunes were delineated using custom 2D 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 Magnetic Resonance Imaging 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: 4 raters (2 neurologists, 1 geriatrician, and 1 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 anonymised 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$1.5 was rounded to1 (first-order branch), 1.5-$\leq$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: 2 raters (1 neurologist, 1 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: 22 subjects with CADASIL (50% male, median age 53 years, range 26 - 67) were included in the pilot study. 10 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, 5 (50%) were smokers and 7 (70%) had hypercholesterolaemia. None had evidence of carotid disease on MR angiography and/or carotid ultrasound. Average systolic blood pressure was 119mmHg (standard deviation (SD) 11mmHg).

Prevalent lacunes: 16 lacunes were initially identified but once maps were overlaid 3 were mainly out-with 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). 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: 315 subjects with CADASIL (42.2% male, median age 50 years, range 23 - 77) were included in the validation study. 95 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: 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.

Incident lacunes: 14 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 2 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 (eg hypertension), and application of arbitrary thresholds to distinguish one mechanism from another. In a large cohort of subjects with inherited small vessel disease 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 35mm in diameter noted. Cortical infarctions co-existed with lacunes in 26% of patients. Rather than the result of intrinsic small vessel disease, 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 intrinsic 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 intrinsic cerebral SVD due to CADASIL. In both cohorts the great majority of lacunes were associated with third-order branch disease.

The STRIVE (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”.[2] 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 15mm cut off, with the median dimensions of lacunes associated with tertiary lacunes being around 4mm in the pilot study. A recent

paper examining lacune shape and 88 incident lacunes in CADASIL showed most lacunes are below 15mm in the axial plane, but even in the basal ganglia lacunes up to 25mm could be found.[21] 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 single 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 x 6mm, but smaller collateral branches supplied much smaller areas.[22] 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.[21]

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.[11] In vitro visualization of relevant vasculature requires invasive angiography, or high field MRI,[23] 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.[14] 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 has shown high repeatability in its identification of incident lacunes.[14] 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 in 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 MR imaging 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.

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.[10]

Our findings may have clinical relevance. The finding of large lacunes within the basal ganglia suggestive of higher branch order disease in sporadic stroke patients may suggest a cause other than cerebral SVD, and provoke investigations for alternative causes. 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 due primarily to disease of third-order branches of the LSA and RAH. If CADASIL represents a pure form of

cerebral SVD, then lacunes that originate from first order vessels should provoke a hunt for alternative aetiology.

Declaration of Conflicting Interests

FM, MDü, XH, VS, HC, EJ, MDi 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.

Funding

The Scottish CADASIL study was funded by a project grant from the Chief scientist Office, Scotland (ETM/244). The Paris-Munich CADASIL cohort is supported by grants from the French Ministry of Health (Regional and National PHRC AOR 02-001), Association de Recherche en Neurologie Vasculaire, and the Vascular Dementia Research Foundation.

Ethical approval and informed consent

The West of Scotland Research ethics committee approved this study (REC number: 12/WOS/0295). Written informed consent was obtained from the patients.

Guarantor

KM has responsibility for the Scottish CADASIL data and MD/MD/HC for the Paris-Munich cohort.

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, KM all scored lacune. MDü and EJ segmented 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.

Acknowledgements

We gratefully acknowledge all patients who have participated in this research and their families.

References

1. Bamford J, Sandercock P, Dennis M, Warlow C, Burn J. Classification and natural history of clinically identifiable subtypes of cerebral infarction. The Lancet 1991;337:1521-1526.
2. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. The Lancet Neurology 2013;12:822-838.
3. Fisher CM. Lacunar strokes and infarcts: a review. Neurology 1982;32:871-876.
4. Fisher CM. Lacunes: Small, deep cerebral infarcts. Neurology 1965;15:774-784.
5. Wardlaw JM. What Is a Lacune? Stroke 2008;39:2921-2922.
6. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. The Lancet Neurology 2013;12:483-497.
7. Futrell N. Lacunar Infarction: Embolism is the Key. Stroke 2004;35:1778-1779.

8. Wardlaw JM. What causes lacunar stroke? Journal of Neurology, Neurosurgery & Psychiatry 2005;76:617-619.
9. Marinković S, Gibo H, Milisavljević M, Ćetković M. Anatomic and clinical correlations of the lenticulostriate arteries. Clinical Anatomy 2001;14:190-195.
10. Feekes JA, Hsu S-W, Chaloupka JC, Cassell MD. Tertiary microvascular territories define lacunar infarcts in the basal ganglia. Annals of Neurology 2005;58:18-30.
11. Phan TG, van der Voort S, Beare R, et al. Dimensions of Subcortical Infarcts Association with First- to Third Order Branches of the Basal Ganglia Arteries. Cerebrovascular Diseases 2013;35:262 - 267.
12. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to the therapeutic challenges. Lancet Neurol 2010;9:689-701.
13. Viswanathan A, Guichard JP, Gschwendtner A, et al. Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study. Brain 2006;129:2375-2383.
14. Duering M, Csanadi E, Gesierich B, et al. Incident lacunes preferentially localize to the edge of white matter hyperintensities: insights into the pathophysiology of cerebral small vessel disease. Brain 2013;136:2717-2726.
15. Hervé D, Mangin J-F, Molko N, Bousser M-G, Chabriat H. Shape and Volume of Lacunar Infarcts: A 3D MRI Study in Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy. Stroke 2005;36:2384-2388.
16. Doubal FN, MacLullich AMJ, Ferguson KJ, Dennis MS, Wardlaw JM. Enlarged Perivascular Spaces on MRI Are a Feature of Cerebral Small Vessel Disease. Stroke 2010;41:450-454.
17. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. Medical Image Analysis 2001;5:143-156.
18. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 2004;23:208-219.
19. Woolrich MW, Jbabdi S, Patenaude B, et al. Bayesian analysis of neuroimaging data in FSL. Neuroimage 2009;45:S173-186.
20. Ay H, Oliveira-Filho J, Buonanno FS, et al. Diffusion-Weighted Imaging Identifies a Subset of Lacunar Infarction Associated With Embolic Source. Stroke 1999;30:2644-2650.
21. Gesierich B, Duchesnay E, Jouvent E, et al. Features and Determinants of Lacune Shape. Relationship With Fiber Tracts and Perforating Arteries 2016;47:1258-1264.
22. Slobodan V. Marinković, Miroslav S. Kovačević, Jelena M. Marinković. Perforating branches of the middle cerebral artery. Journal of Neurosurgery 1985;63:266-271.
23. Seo SW, Kang CK, Kim SH, et al. Measurements of lenticulostriate arteries using 7T MRI: new imaging markers for subcortical vascular dementia. J Neurol Sci 2012;322:200-205.

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

	Second-order **n = 3**	**Third -order** **n = 10**	**p value**
Volume, mL, median (range)	0.48 (0.32 – 0.65)	0.03 (0.01 – 0.14)	0.007
Axial width, mm, median (range)	11 (8 – 11)	4 (3 – 6)	0.007
Coronal height, mm, median (range)	14 (12 – 17)	4 (3 - 9)	0.007
AP length, mm, median (range)	10 (9 – 15)	4 (2 - 7)	0.007

Table 2: Characteristics of Pilot and validation data

	Pilot Data	Validation Study
Number of subjects	10	95
Number of prevalent lacunes scored	13	166
Age in years, median (IQR)	53 (16)	51 (12)
Male:Female	8:2	59:36
Cardiovascular risk factors, n (%)		
Ever smoker	5 (50)	61 (64)
Hypertension	0 (0)	24 (25)
Hyperlipidaemia	7 (70)	45 (51)
Diabetes	0 (0)	3 (3)
Atrial fibrillation	0 (0)	1 (1)

Figure legends

Figure 1: Microangiographic template

(A) Medial and lateral lenticulostriate arteries (arrow) and recurrent artery of Heubner (arrowhead) shown overlaying a MNI-registered T1 MR image of the basal ganglia in a patient with CADASIL. Cd – caudate, Ic = internal capsule, Pu = putamen. Examples of third-order (B), second-order (C) and first-order or main stem (D) arterial branch lacune.

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 second-order branch disease (B) and third-order branch disease; C).

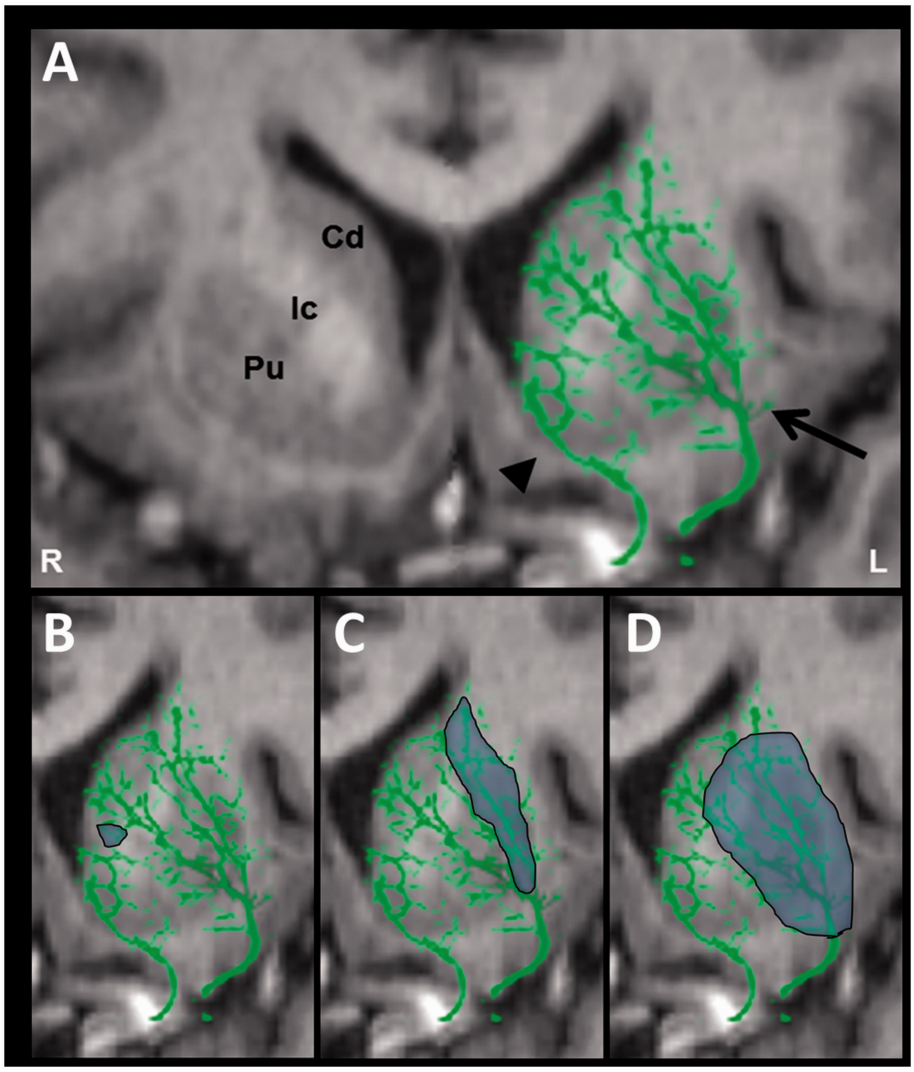

Figure 1. Microangiographic template. (a) Medial and lateral lenticulostriate arteries (arrow) and recurrent artery of Heubner (arrowhead) shown overlaying a MNI-registered T1 MR image of the basal ganglia in a patient with CADASIL. Examples of (b) third-order, (c) second-order and (d) and first-order or main stem arterial branch lacune. Cd: caudate; Ic: internal capsule; Pu: putamen.

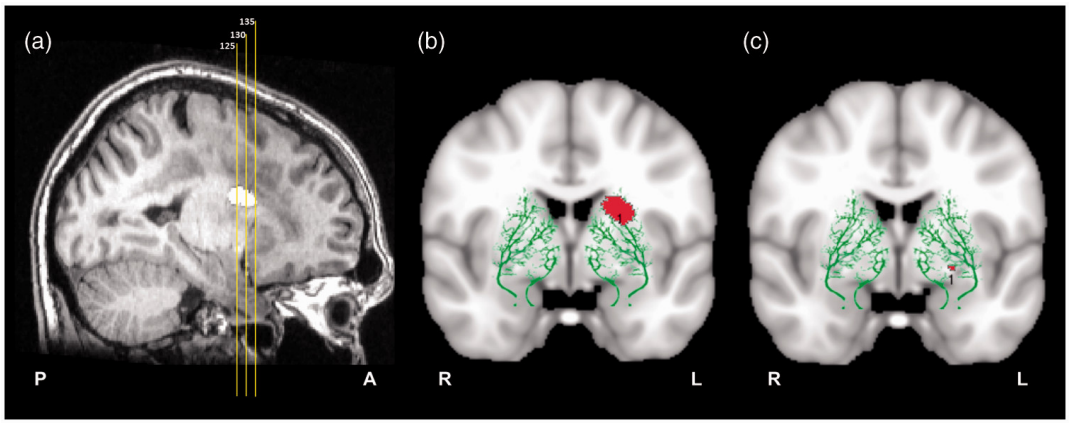

Figure 2. Template overlay and example lacunes. Location of coronal slices 125,130 and 135 on 3D-T1 image transformed into MNI space from pilot study (a). Coronal MNI slices with microangiographic template showing an example of a lacune associated with (b) second-order branch disease and (c) third-order branch disease.