Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is a hereditary arteriopathy caused by mutations of the Notch3 gene. The risk factors for cerebral microhaemorrhages (CM), their relationship to other MRI lesions in the disease and their potential clinical impact have not been previously defined. Our purpose was to examine the frequency, number and location of microhaemorrhages in a multicentre cohort study, defining predisposing factors and associated radiographic markers in CADASIL patients. We collected clinical data from 147 consecutive patients enrolled in an ongoing prospective cohort study. Degree of neurological disability and cognitive impairment were assessed by standardized scales. T1-weighted, FLAIR and T2*-weighted gradient-echo (GE) MRI sequences were performed. Volume and location of lacunar infarcts and white matter hyperintensity (WMH) were assessed. Number and location of CM were recorded. CM were present in 35% patients, most commonly occurring in the thalamus, brainstem and basal ganglia. The location of CM qualitatively differed from areas of lacunar infarction and WMH. There was a significant association between the presence of CM and a history of hypertension (P = 0.005), systolic blood pressure (SBP) (P = 0.014), haemoglobin A1c (HbA1c) (P = 0.004) and the volume of lacunar infarcts (P = 0.010) and WMHs (P = 0.046). The number of CM was independently associated with SBP (P = 0.005), the diagnosis of hypertension (P = 0.0004), volume of WMH (P = 0.0005) and lacunar infarcts (P = 0.004). In contrast, no association was found between blood pressure or HbA1c and the load of WMH or lacunar infarcts. The presence of CM was independently associated with increased modified Rankin scores. CM are independently associated with blood pressure and HbA1c as well as with lacunar infarct and WMH volume in CADASIL. Both the vascular risk factors and regional distribution of CM appear distinct from those associated with other MRI markers, suggesting a distinct pathological process. These lesions have a potential clinical impact in CADASIL. These findings further suggest that modulation of blood pressure and glucose levels might influence the course of the disease.

Keywords: CADASIL; cerebral microhaemorrhage; lacunes; white matter damage; haemoglobin A1c

Abbreviations: CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy; CI = confidence interval; CM = cerebral microhaemorrhages; DBP = diastolic blood pressure; FLAIR = fluid-attenuated inversion recovery; GE = gradient-echo; HbA1c = haemoglobin A1c; ICC = intra-cranial cavity; nLV = normalized lacunar volume; SBP = systolic blood pressure; WMH = white matter hyperintensity

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is an arteriopathy caused by mutations of the Notch3 gene (Tournier-Lasserve et al., 1993; Joutel et al., 1996). The main clinical manifestations of the disease include attacks of migraine with aura, mood disturbances, recurrent ischaemic strokes and progressive cognitive decline (Chabrier et al., 1995; Dichgans et al., 1998).

On MRI, there is evidence of widespread white matter hyperintensities (WMHs) on $T_2$-weighted images (Tournier-Lasserve et al., 1991; Chabrier et al., 1998; Auer et al., 2001), lacunar infarctions on $T_1$-weighted images (Chabrier et al., 1995, 1998; Joutel et al., 1996; O’Sullivan et al., 2003; van den Boom et al., 2003) and evidence of old cerebral microhaemorrhages (CM) on $T_2$*-weighted or gradient-echo (GE) images (Lesnik Oberstein et al., 2001; Dichgans et al., 2002; van den Boom et al., 2003). These MRI lesions represent different consequences of the underlying angiopathy in the disease. Although there have been numerous studies investigating the impact of white matter disease in patients with CADASIL (Molko et al., 2002; O’Sullivan et al., 2004, 2005; Holtmannspötter et al., 2005; Buffon et al., 2006), the clinical importance of CM remains unknown.

CM have been reported in several populations including patients with ischaemic and haemorrhagic stroke as well as cerebral amyloid angiopathy (CAA) and represent an important marker of the structural integrity of small blood vessels (Viswanathan and Chabrier, 2006). In CADASIL CM have been reported to occur with frequency ranging between 25 and 69% in several small studies (Lesnik Oberstein et al., 2001; Dichgans et al., 2002; van den Boom et al., 2003) Defined as rounded foci of <5 mm in size that appear hyperintense and distinct from vascular flow voids, leptomeningeal haemosiderosis, or non-haemorrhagic subcortical mineralization (Fazekas et al., 1999; Greenberg et al., 2004). CM can be sensitively imaged by GE MR (Atlas et al., 1988). They are not detected by conventional MR sequences [$T_1$, $T_2$ and fluid-attenuated inversion recovery (FLAIR)]. The reduction of the GE MR signal is caused by haemosiderin, a blood breakdown product that causes magnetic susceptibility-induced relaxation leading to $T_2$* signal loss.

Pathological studies in CADASIL have demonstrated electron-dense osmiophilic granular material within the media of small arteries and capillaries (Ruchoux and Mauring, 1997) in close association with degenerating smooth muscle cells (Ruchoux et al., 1995). These microstructural changes are presumably responsible for wall dysfunction with decreased basal perfusion and haemodynamic reserve (Chabrier et al., 2000; Pfefferkorn et al., 2001; Lacombe et al., 2005) leading to the extensive subcortical ischaemic lesions. Constituting pathological evidence of prior microhaemorrhage, haemosiderin-laden macrophages have been found in the vicinity of 100–300 µm blood vessels whose walls showed the characteristic degenerative changes of the genetic disorder (Dichgans et al., 2002). This suggests that CM in CADASIL also arise from the underlying ultrastructural modifications of the vessel wall and may be reflective of the severity and progression of these changes.

The precise relationship of CM with various clinical risk factors and with the other MRI features of the disease has not been assessed in a large population of patients with the Notch3 gene mutation. In the present study, we sought to determine the risk factors associated with CM and the impact of these lesions on neurological disability in CADASIL patients enrolled in a multicentre cohort study.

Subjects and methods

Subjects

Subjects were drawn from an ongoing multicentre prospective cohort study of patients with CADASIL. Subjects were recruited from consecutive CADASIL patients, at least 18 years of age, evaluated at Lariboisière (Paris) or Ludwig-Maximillians-Universität (Munich) hospitals between October 2003 and July 2005. In all cases, diagnosis was confirmed by identification of a typical mutation in the Notch3 gene (Ekke et al., 1997; Joutel et al., 1997, 2001; Dichgans et al., 2000). Patients who were pregnant or had other contraindications to MRI were excluded. Clinical and demographic data were collected by study investigators at the time of inclusion and included age, sex, history of hypertension [defined as previous diagnosis of hypertension (>140/90) or use of antihypertensive treatment for control of blood pressure (Greenberg et al., 1996)], dementia (defined by DSM IV criteria), diabetes [defined according to the WHO criteria (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003) or as current use of hypoglycaemic agent (Lesnik Oberstein et al., 2001)], hypercholesterolaemia (defined by previous diagnosis), current medication use, smoking history and amount of alcohol use. Blood pressure measurement and laboratory evaluation [which included complete blood count, glucose, haemoglobin A1c (HbA1c), homocysteine and cholesterol panel] was performed in all patients. All enrolled subjects underwent detailed baseline neurological examination during the 2 h preceding MRI examination, including a brief evaluation of cognitive deficits with the Mini-Mental State Examination (MMSE) (MacKenzie et al., 1996; Cockrell and Folstein, 1988) and degree of disability based on the modified Rankin scale (mRS). As in previous studies, we defined poor outcome or functional dependence as modified Rankin score ≥3 (Gebel et al., 2002; Greenberg et al., 2004). Furthermore, detailed neuropsychological testing was performed at each visit.

Informed consent was obtained from each subject or from a close relative if the subject was too severely disabled to give written consent. This study was approved by an independent ethics committee in both participating centres.

MRI

MRI scans were obtained by the use of a 1.5-T system [Vision, Siemens (Munich) or Signa General Electric Medical Systems (Paris)]. $T_1$-weighted axial sequences [Munich: repetition time/echo time (TR/TE) 11.4/4.4 ms, slice thickness 1.2 mm, no interslice gap, 197×256; Paris: TR/TE 9.1/2 ms slice thickness 1.6 mm, interslice gap 0.8 mm, 256×256, FLAIR (Munich: TR/TE = 4284/110, slice
thickness = 5 mm, no interslice gap, 176 × 256; Paris: TR/TE = 840/161 ms, slice thickness = 5.5 mm, no interslice gap, 256 × 160) and T2*-weighted GE planar imaging (Munich: TR/TE = 1056/22 ms, slice thickness = 5 mm, no interslice gap, 256 × 192; Paris: TR/TE = 500/15 ms, slice thickness = 5.5 mm, no interslice gap, 256 × 192) were performed (Fig. 1).

**Image processing**

All MRI sequences were spatially registered by realigning two MRI volumes. For each patient, the T1-weighted images were used as reference images. The registration algorithm used mutual information (MI) in order to assess the quality of match between the two volumes. A rigid 3D transformation was employed. According to MI and derivative values, a gradient descent technique was used to modify the transformation parameters (rotations and translations) iteratively in order to maximize the MI. For this purpose, a multi-resolution, coarse-to-fine strategy was adopted with onset at low resolution and iterations on the basis of the results of the previous step. This strategy was chosen in order to increase the robustness of the registration algorithm by preventing the maximization...

**Fig. 1** MRI sequences in a representative CADASIL patient. (A) GE sequences demonstrate bilateral areas of microhaemorrhage in the thalamus. (B) T1-weighted images show evidence of lacunar infarctions in the thalamus. (C) FLAIR-weighted images demonstrate extensive WMHs in the subcortical white matter.
Image analysis

Image analysis was carried out in two steps. Native and preprocessed data were displayed on identical Unix workstations running image evaluation software developed by Theralitys Inc. (Lyon, France) (Deloire et al., 2005). An automatic computed image processing and preparation phase preceded centralized image review sessions involving study neurologists who were blinded to the clinical data, and during which manual corrections and validation were carried out. Using drawing tools, a trained neurologist or neuroradiologist then validated each discrete lesion (WMH, lacunar infarcts) and corrected its delimitation if necessary.

WMH quantification

WMHs were analysed on FLAIR images. All FLAIR axial slices from the base of the cerebellum to the vertex were analysed. A mask of lesions was generated from FLAIR images after application of the intra-cranial cavity (ICC) mask by applying a threshold on signal intensity derived from the signal intensities histogram. Two trained neurologists using drawing tools then validated each discrete lesion and corrected its delimitation if necessary. The total volume of WMH was normalized to the ICC in each patient \[\text{normalized volume of WMH} = \left(\frac{\text{volume of WMH}}{\text{volume ICC}}\right) \times 100\]. A high interrater reliability was demonstrated for the two readers (intraclass correlation coefficient = 0.995).

Lacunar volume

To assess the number of lacunes and lacunar volume, T1-weighted images were segmented in four tissue classes consisting of white matter, grey matter, CSF/lacunes and non-brain partitions. After segmentation, the class CSF/lacunes was automatically isolated from other tissue classes. Subsequently, two raters (F.B. and R.C.) isolated lacunes from CSF voxels using appropriate 2D and 3D imaging tools. The total volume of lacunes in each patient was normalized to the ICC \[\text{normalized lacunar volume or nLV} = \left(\frac{\text{volume of lacunes}}{\text{volume ICC}}\right) \times 1000\]. Good interrater reliability was demonstrated for both the volume and number of lacunes (intraclass correlation coefficient = 0.830 and 0.824, respectively).

Microhaemorrhages

Microhaemorrhages were defined as rounded foci ≤5 mm in diameter, hypointense on GE sequences and distinct from vascular flow voids, leptomeningeal haemosiderosis or non-haemorrhagic subcortical mineralization. The location and number of microhaemorrhages were recorded. Twenty pairs of images were analysed separately by two raters (A.V. and M.D.). Raters had access to T1- and T2-weighted spin-echo sequences for comparison and validation. It was agreed before the study that only lesions considered to be independent haemorrhagic foci would be counted. Each lesion was marked electronically by the reader on the screen and all lesions were recorded in the corresponding 3D space for each exam. All disagreements were resolved by consensus. The location of lesions was recorded in the corresponding 3D space for each exam.

Results

Cohort demographics

All subjects harboured a characteristic Notch3 gene mutation. The average age of the cohort was 51.8 ± 11.2 years (median: 50.9; range: 24.6–75.9). Vascular risk factors present in the cohort included hypertension (18% of subjects), diabetes (3%), history of smoking (49%) and hypercholesterolaemia (50%). Seventy-one per cent of subjects were on antiplatelet medication, 36% were on anticoagulation, 17% were on ACE inhibitors, 18% were on angiotensin receptor blockers, 17% were on statins and 47% were on other medication. The average age of the cohort was 51.8 ± 11.2 years (median: 50.9; range: 24.6–75.9). Vascular risk factors present in the cohort included hypertension (18% of subjects), diabetes (3%), history of smoking (49%) and hypercholesterolaemia (50%). Seventy-one per cent of subjects were on antiplatelet medication, 36% were on anticoagulation, 17% were on ACE inhibitors, 18% were on angiotensin receptor blockers, 17% were on statins and 47% were on other medication.

Presence of CM and associated factors

Characteristics of patients with and without CM are summarized in Table 1. CM were detected in 35% (52 out of 147) of patients. In those patients with CM, lesions were most commonly multiple (median: 3; mean: 10.9 ± 24.8). In univariate analysis, the presence of CM was strongly correlated with age \((P = 0.0002)\), the diagnosis of hypertension \((P = 0.001)\), decreased HDL cholesterol \((P = 0.02)\), elevated HbA1c \((P = 0.04)\), anticoagulant treatment \((P = 0.008)\) but not with antiplatelet use \((P = 0.28)\). Additionally, individuals with CM had, on average, higher systolic (SBP) and diastolic blood pressure (DBP) \((P = 0.001)\) and \(P = 0.008\), respectively).
Cerebral microhaemorrhage in CADASIL

Table 1 Characteristics of CADASIL cohort with and without CM

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No CM [n %]</th>
<th>&gt;1 CM [n %]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>39 (41)</td>
<td>24 (46)</td>
<td>0.55</td>
</tr>
<tr>
<td>Age (years ± SD)</td>
<td>49.4 ± 11.1</td>
<td>56.3 ± 10.0</td>
<td>0.0002</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>10 (10.6)</td>
<td>17 (32.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Dementia</td>
<td>9 (9.8)</td>
<td>14 (26.9)</td>
<td>0.007</td>
</tr>
<tr>
<td>Past or current smoker</td>
<td>49 (51.6)</td>
<td>23 (44.2)</td>
<td>0.39</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1 (1)</td>
<td>3 (5.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>History of hypercholesterolaemia</td>
<td>45 (48)</td>
<td>28 (54)</td>
<td>0.49</td>
</tr>
<tr>
<td>Anticoagulant use</td>
<td>1 (1)</td>
<td>6 (11.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>Antithrombotic use</td>
<td>65 (68.4)</td>
<td>40 (76.9)</td>
<td>0.28</td>
</tr>
<tr>
<td>Any alcohol consumption</td>
<td>51 (58)</td>
<td>27 (57.5)</td>
<td>0.95</td>
</tr>
<tr>
<td>SBP (mmHg ± SD)</td>
<td>125 ± 15</td>
<td>135 ± 18</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP (mmHg ± SD)</td>
<td>74 ± 10</td>
<td>79 ± 10</td>
<td>0.008</td>
</tr>
<tr>
<td>MMSE</td>
<td>26.8 ± 4.2</td>
<td>24.6 ± 5.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.4 ± 1.1</td>
<td>5.6 ± 1.2</td>
<td>0.30</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.3 ± 0.9</td>
<td>3.6 ± 1.0</td>
<td>0.16</td>
</tr>
<tr>
<td>LDL ≥ 3.1 (mmol/l)</td>
<td>59 (62.8)</td>
<td>38 (76)</td>
<td>0.11</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.52 ± 0.45</td>
<td>1.37 ± 0.33</td>
<td>0.02</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.4 ± 0.39</td>
<td>5.6 ± 0.45</td>
<td>0.04</td>
</tr>
<tr>
<td>HbA1c ≥ 5.6 (%)</td>
<td>25 (26.9)</td>
<td>30 (58.8)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Blood glucose (mmol/l ± SD)</td>
<td>5.24 ± 0.71</td>
<td>5.43 ± 0.71</td>
<td>0.18</td>
</tr>
<tr>
<td>nWMH</td>
<td>6.7 ± 4.7</td>
<td>9.5 ± 4.8</td>
<td>0.0002</td>
</tr>
<tr>
<td>nLV</td>
<td>0.7 ± 1.7</td>
<td>1.1 ± 0.9</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

LDL = low-density lipoprotein; HDL = high-density lipoprotein; HbA1c = haemoglobin A1c; SBP = systolic blood pressure; DBP = diastolic blood pressure; MMSE = Mini-Mental Status Examination; nWMH = normalized white matter hyperintensity volume; nLV = normalized lacunar infarct volume.

The presence of CM was also strongly associated with other MRI markers in the cohort (Table 1). In univariate analysis, those patients with microhaemorrhages had a larger total lacunar infarct volume (nLV) (P = 0.0001) and greater WMH lesion volume (nWMH) (P = 0.0002).

In the final maximally adjusted multivariable logistic regression model, HbA1c (P = 0.004), SBP (P = 0.014), nLV (P = 0.010) and nWMH (P = 0.046) were independently associated with CM. The effect of SBP remained significant (P = 0.04) when only individuals with normal blood pressure (SBP ≤ 140 mmHg) were analysed. In addition, multivariable analysis after removing the four individuals with diabetes did not significantly change the results. These results are summarized in Table 2. Finally, the independent risk factors predisposing subjects to CM (hypertension, SBP, HbA1c) were not found to be associated with white matter lesions or lacunar infarctions after performing similar univariate and multivariate analyses (data not shown).

Number of CM and associated factors

In univariate analysis, the number of CM was associated with age (P = 0.0005), history of hypertension (P = 0.0005), HDL, (P = 0.02), anticoagulant use (P = 0.04), SBP (P = 0.005), DBP (P = 0.01), nWMH (P = 0.0001) and nLV (0.1). Antiplatelet use was not associated with the number of CM. A stepwise ordinal logistic regression model showed that the number of CM was independently associated with SBP (P = 0.005; OR = 1.42 (risk per 10 mmHg increase); 95% confidence interval (CI) = 1.11–1.81), the diagnosis of hypertension (P = 0.0004; OR = 5.19; 95% CI = 2.08–12.95), nWMH (P = 0.0005; OR = 1.16 (risk per cent increase in nWMH); 95% CI = 1.07–1.26) and nLV (P = 0.004; OR = 1.96; 95% CI = 1.24–3.09).

Localization of CM

For the 52 individuals with evidence of microhaemorrhage, the presence or absence of lesions was determined at each cerebral localization. The most common areas of microhaemorrhages were the thalamus (61.5% of patients with microhaemorrhage), brainstem (38.5%), basal ganglia (38.5%) and cortex or cortico-subcortical junction in the temporal (36.5%) and occipital areas (26.9%). CM were detected in the cerebellum in 29% of cases where lacunar infarcts or WMH are typically absent. Thus, this distribution differs from that observed for lacunar infarctions and WMH. The results are illustrated in Fig. 2.

Cerebral microhaemorrhages and neurological outcome

Age (P < 0.0001), presence of dementia (P < 0.0001), anticoagulant use (P = 0.05), alcohol use (P = 0.04), low HDL (P = 0.002), SBP (P = 0.03), HbA1c (P = 0.04), nLV (P < 0.0001), nWMH (P = 0.005) and number of CM (P < 0.0001) were associated with functional dependence (Table 3)

A multivariable stepwise regression analysis that included these variables demonstrated that number of CM (P = 0.034; OR (per one CM) = 1.16, 95% CI = 1.01–1.34) and nLV
$P = 0.004$; OR (per 0.1 per cent increase) = 2.84, 95% CI = 1.40–5.77], as well as SBP ($P = 0.01$; OR = 1.63, 95% CI = 1.13–2.37) and dementia ($P < 0.0001$; OR = 32.97, 95% CI = 6.46–168.34) were independently associated with poor functional outcome.

The presence of CM was also associated with dementia and MMSE scores (Table 1). However, in multivariate analysis examining predictors of dementia, no significant association with CM was found (data not shown).

In all of the above-described analyses, we found no significant centre effect that modified the interpretation of our results.

**Discussion**

The major finding from this large multicentre cohort study is that CM are independently associated with blood pressure levels, HbA1c, as well as lacunar infarct volume and extent of WMH in CADASIL. In addition, the number of CM is an independent predictor of neurological disability.

Blood pressure has not heretofore been thought to play a significant role in the pathophysiology of genetic small vessel diseases (Lesnik Oberstein et al., 2001; Dichgans et al., 2002; Singhal et al., 2004) although very recent evidence in CADASIL patients has demonstrated SBP to be an independent predictor of white matter progression (Holtmannspotter et al., 2005). In CAA, for example, lobar CM are common (Greenberg et al., 1999; Knudsen et al., 2001) but are not associated with hypertension or diabetes (Greenberg et al., 2004). In contrast, in both healthy elderly populations and ischaemic stroke survivors, hypertension has been shown to be a risk factor for CM (Roob et al., 1999; Jeerakathil et al., 2004; Lee et al., 2004a, b; Tsushima et al., 2002). In the present study, the average blood pressure values in CADASIL...
Cerebral microhaemorrhage in CADASIL

Table 3  Analysis of factors of poor outcome in CADASIL patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>mRS &lt; 3 [n (%)]</th>
<th>mRS ≥ 3 [n (%)]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>45 (41)</td>
<td>18 (53)</td>
<td>0.22</td>
</tr>
<tr>
<td>Age (years ± SD)</td>
<td>49.8 ± 11.0</td>
<td>59.5 ± 8.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>18 (16)</td>
<td>9 (26)</td>
<td>0.19</td>
</tr>
<tr>
<td>Dementia</td>
<td>4 (17)</td>
<td>19 (83)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Past or current smoker</td>
<td>56 (51)</td>
<td>14 (41)</td>
<td>0.32</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (3)</td>
<td>1 (3)</td>
<td>1.00</td>
</tr>
<tr>
<td>History of hypercholesterolaemia</td>
<td>52 (47)</td>
<td>21 (62)</td>
<td>0.14</td>
</tr>
<tr>
<td>Anticoagulant use</td>
<td>3 (3)</td>
<td>4 (12)</td>
<td>0.05</td>
</tr>
<tr>
<td>Antithrombotic use</td>
<td>75 (68)</td>
<td>28 (82)</td>
<td>0.11</td>
</tr>
<tr>
<td>Any alcohol consumption</td>
<td>63 (62.4)</td>
<td>14 (42.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.58 ± 1.01</td>
<td>5.24 ± 1.25</td>
<td>0.11</td>
</tr>
<tr>
<td>LDL</td>
<td>3.51 ± 0.90</td>
<td>3.36 ± 0.96</td>
<td>0.42</td>
</tr>
<tr>
<td>HDL</td>
<td>1.52 ± 0.42</td>
<td>1.27 ± 0.32</td>
<td>0.002</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.5 ± 0.44</td>
<td>5.3 ± 0.37</td>
<td>0.04</td>
</tr>
<tr>
<td>Blood glucose (mmol/l ± SD)</td>
<td>5.3 ± 0.75</td>
<td>5.3 ± 0.58</td>
<td>0.49</td>
</tr>
<tr>
<td>SBP (mmHg ± SD)</td>
<td>126 ± 14</td>
<td>136 ± 21</td>
<td>0.03</td>
</tr>
<tr>
<td>DBP (mmHg ± SD)</td>
<td>75 ± 10</td>
<td>77 ± 12</td>
<td>0.28</td>
</tr>
<tr>
<td>nWMH</td>
<td>7.13 ± 4.82</td>
<td>9.61 ± 4.94</td>
<td>0.005</td>
</tr>
<tr>
<td>nLV</td>
<td>0.58 ± 0.72</td>
<td>1.82 ± 2.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of microhaemorrhages</td>
<td>0.97 ± 2.5</td>
<td>13.5 ± 30.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

LDL = low-density lipoprotein; HDL = high-density lipoprotein; HbA1c = haemoglobin A1c; SBP = systolic blood pressure; DBP = diastolic blood pressure; nWMH = normalized white matter hyperintensity volume; nLV = normalized lacunar infarct volume.

subjects with CM and in those without were found to be in the normal range (<140/90) (Table 1). In addition, when hypertensive patients were removed from the analysis, the association remained highly significant (Table 2, Model C). These results suggest that small increases in SBP may contribute to CM through an additive effect on the ultrastructural vessel wall modifications caused by Notch3 mutations (Ruchoux and Maurage, 1997). Other studies are needed to determine which factors (pulsatility, cerebrovascular resistance or vessel wall stiffness) most strongly influence the rupture of the cerebral microvessel wall in the setting of moderate elevations of blood pressure. These results emphasize that acceptable blood pressure values in the setting of an existing cerebral microangiopathy may well differ from the established normal range.

The correlation between HbA1c levels and presence of CM in multivariable modelling suggests that long-term serum glucose levels may also play a role in CADASIL vessel dysfunction. Although a specific association between HbA1c and CM has not been previously reported, there is evidence to suggest that serum glucose levels increase the risk associated with intracerebral haemorrhage. Hyperglycaemia and diabetes have been identified as independent risk factors for fatal outcome (Wong, 1999; Arboix et al., 2000; Passero et al., 2003; Rosand et al., 2004), and other observations suggest that hyperglycaemia predisposes to increased bleeding (de Courten-Myers et al., 1992; Williams et al., 1998; Demchuk et al., 1999; Meigs et al., 2000; Kase et al., 2001; Bruno et al., 2002; Song et al., 2003). There is some evidence in experimental models to suggest that increased HbA1c may alter erythrocyte deformability and increase shear stress on the microvessel wall (Tsukada et al., 2001). Additionally, in the setting of ischaemia, hyperglycaemia leads to increased blood–brain barrier breakdown (Dietrich et al., 1993). Our observations of CM in individuals with higher HbA1c (even in non-diabetic patients) may imply that in the setting of this genetic arteriopathy, chronically increased serum glucose levels may lead to vessel fragility and microhaemorrhage. In contrast with our findings with blood pressure, HbA1c was not found to be related to the number of CM, suggesting that serum glucose levels and blood pressure may differently influence CM pathology.

In the present study, the other MRI markers of CADASIL (lacunar infarctions and WMH) were not associated with these vascular risk factors. Additionally, we found that there was a minimal overlap between regions of CM and regions of lacunar infarction or prominent WMH (Fig. 2). This is consistent with previous findings in a group of 16 CADASIL patients showing no spatial association between lacunar infarctions and microhaemorrhages (Dichgans et al., 2002). The difference in risk factors and location between CM and the other MRI lesions in the disease suggests the presence of distinct pathophysiological pathways that lead to ischaemic or haemorrhagic lesions. It has been suggested that vascular wall abnormalities in CADASIL may affect the vulnerability of certain blood vessels, resulting in rupture and microhaemorrhage (Dichgans et al., 2002). Our results suggest that certain vascular risk factors (elevated blood pressure and HbA1c) may favour the rupture of the vascular wall and that the cerebral angioarchitecture may promote the occurrence of CM in locations different from ischaemic lesions.

CM have been associated with poor functional outcome in CAA (Greenberg et al., 2004) and with higher Rankin scores in previous univariate analysis in CADASIL (Lesnik Oberstein et al., 2001). In the present study we showed that CM were independently associated with increased neurological disability in a large cohort. These results may suggest that CM contribute to the clinical deterioration seen over the course of the disease. Alternatively, CM may not have a direct effect on disability, but rather represent a marker of the severity of other pathologies in CADASIL, such as lacunar infarctions, white matter damage, dementia or other tissue damage not visualized by standard MRI sequences. The observation of a higher risk of poor functional outcome associated with both nLV and dementia could be consistent with this hypothesis.

Our study has limitations. Since the diagnosis of hypertension in our patients was most commonly made on the basis of history, it is possible that bias could have been introduced through misassignment. However, evidence of antihypertensive treatment or elevated blood pressure was
sought in all patients with this diagnosis in order to minimize any bias. Additionally, the association between SBP and CM further reinforces these findings. Because <5% of the cohort had previously received anticoagulation, we were unable to analyse this factor in multivariable models. Although results from univariate analysis indicate that anticoagulation may increase the risk of CM (Table 1), any definitive conclusions regarding the effect of anticoagulant therapy on CM in CADASIL will require larger studies. Because quantification of the number of CM and lacunar infarctions at each cerebral location was not performed in our cohort, regional correlations between these lesions could not be made. However, the semi-quantitative methods employed clearly demonstrate different cerebral localizations for the ischaemic and haemorrhagic lesions.

Finally, because this study demonstrates the clinical importance of CM in CADASIL, it also raises the possibility that interventions to reduce microhaemorrhages could be effective in reducing disease-related disability. Recently, treatment with perindopril and indapamide has been shown to reduce white matter lesion progression in patients with cerebrovascular disease (Dufouil et al., 2005). The significant association between blood pressure and CM in this study as well as recent findings demonstrating the influence of blood pressure on disease progression in CADASIL (Holtmannspötter et al., 2005) suggest that antihypertensive therapy might be of benefit in these patients. Similarly, strict glucose control may also reduce neurological impairment in the disease. Carefully designed trials should help address these questions in the near future.

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References
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