Bone Marrow-Derived Progenitor Cells in Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy

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Background and Purpose—Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited disease due to cerebral microangiopathy presenting with variable pictures, including stroke, progressive cognitive impairment, and disability. Mechanisms leading from vessel structural changes to parenchymal damage and eventually to clinical expression are not fully understood. Among pathogenic processes, endothelial dysfunction has been hypothesized. Endothelial progenitor cells and circulating progenitor cells (CPCs) derived from bone marrow participate in endothelium structure and function maintenance and contribute to ischemic area revascularization. No data are available about these cells in CADASIL. Our objective in this study was to evaluate endothelial progenitor cells and CPCs role in CADASIL.

Methods—Twenty-nine patients with CADASIL and 29 sex- and age-matched control subjects were enrolled. Cells were measured in peripheral blood using flow cytometry. Endothelial progenitor cells were defined as positive for CD34/KDR, CD133/KDR, and CD34/CD133/KDR; and CPCs as positive for CD34, CD133, and CD34/CD133.

Results—Endothelial progenitor cells were significantly lower in patients with CADASIL than in control subjects (CD34/KDR: 0.05 versus 0.1 cells/µL, P=0.005; CD133/KDR: 0.07 versus 0.1 cells/µL, P=0.006; CD34/CD133/KDR: 0.05 versus 0.1 cells/µL, P=0.001). The difference remained significant after adjusting for age, sex, and statin use. CPCs were not significantly lower in CADASIL, but patients with stroke or dementia had significantly reduced CPC levels than patients without (CD34: 1.68 versus 2.95 cells/µL, P=0.007; CD133: 1.40 versus 2.82 cells/µL, P=0.004; CD34/CD133: 1.44 versus 2.75 cells/µL, P=0.004). CPC levels significantly correlated with cognitive and motor performance measures.

Conclusions—We have documented an association between endothelial progenitor cells and CPCs and CADASIL, extending previous data about the presence of endothelial dysfunction in this disease and its potential role in modulating phenotype. (Stroke. 2010;41:218-223.)

Key Words: CADASIL • endothelial dysfunction • phenotype • progenitor cells • small vessel

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a rare inherited disease linked with a nonarteriosclerotic nonamyloid microangiopathy. It is caused by missense point mutations of the NOTCH3 gene (chromosome 19p13.1).1,2 The disease is clinically characterized by migraine, early-onset transient ischemic attack/stroke, cognitive disturbances, mood disorders, and more rarely seizures.3 It has a progressive course leading to disability and dementia in three fourths of patients.4 The neuroimaging hallmarks are severe and diffuse leukoencephalopathy, typically involving the anterior temporal pole and the external capsule, and multiple subcortical lacunar infarcts. The disease phenotype may be variable ranging from minimal symptoms at late-age to full-blown dementia and disability in middle-aged patients.3,5,6 Variability is only partially explained by the effect possibly exerted by genetic or acquired cofactors.7,8 It has been hypothesized that the structural microvessel changes lead to impaired vasoreactivity with consequent reduced cerebral perfusion and tissue damage. Cerebral
hypo-perfusion has been documented in CADASIL using blood flow imaging studies,9,10 and, although not univocally, vasoreactivity changes have been reported by transcranial Doppler sonography studies.11,12 Altered skin microvessel reactivity13 and endothelial-dependent vasodilatation in cerebral and forearm arteries studies have been observed.14,15 Over the last years, increasing evidence has accumulated showing that bone marrow-derived cells, named endothelial progenitor cells (EPCs), possess the capacity to home to sites of vascular injury contributing to the maintenance of the homeostasis of vascular endothelium and to in vivo neoangiogenesis.16 To date, studies that have investigated EPCs in extracerebral vascular diseases have shown that increased levels of EPCs are associated with a reduced risk of vascular events and with better outcomes, including death from cardiovascular causes.17 Only few studies have addressed EPCs in cerebrovascular diseases reporting a lower EPC number in patients with both stable and acute stroke compared with control subjects18 and a better outcome in patients with increased EPC number after stroke.19,20 Experimental studies suggest a role of the more undifferentiated bone marrow-derived circulating progenitors cells (CPCs) in neovascularization of cerebral ischemic areas.21 Furthermore, in one human study, CPC levels were correlated inversely with the number of cerebral infarctions and directly with preserved cerebral perfusion, suggesting that they could play a role in the maintenance of cerebral circulation after stroke.22 In the framework of a prospective study (Microvascular LEukoencephalopathy prospective multicenter Study [MILES], conducted in Italy) aimed at evaluating clinical, biochemical, and genetic factors and mechanisms possibly modulating the phenotypic expression of genetic and sporadic leukoencephalopathies, we have evaluated the possible role of both EPCs and CPCs in CADASIL.

Materials and Methods
This was a case–control study: consecutive patients with CADASIL were matched 1:1 for age (±3 years) and sex with healthy control subjects.

Patients with CADASIL were selected from case series already existing in 3 of the 4 centers (Florence, Siena, and Genoa) participating in MILES. Inclusion criteria were to be ≥18 years old, free from stroke and myocardial infarction in the previous 6 months and from severe comorbidities with impact on clinical status and on short-term prognosis; the Mini Mental State Examination23 score had to be ≥18. Genetic diagnosis had been established by the detection of a NOTCH3 mutation as previously reported.5 The patients were divided into 2 groups according to clinical picture severity; the severe group was composed of patients with stroke or dementia and the mild group of patients without stroke and dementia.

For each patient with CADASIL, a control subject matched according to the previously mentioned criteria was recruited among the hospital employers and their relatives in one center. Control subjects were excluded if they had a history of cerebro- and cardiovascular diseases, peripheral arteriopathy, cancer, or dementia (a standard questionnaire was used for the screening).

Both patients and control subjects were assessed for the presence of vascular risk factors and drug use. In the CADASIL group, a standardized protocol was also applied to assess: (1) detailed medical history mainly focused on the typical disease disturbances; (2) clinical and functional status by means of neurological examination and documentation of the Disability Assessment for Dementia scale24; (3) cognitive performance, applying the neuropsychological battery used in the European multicenter “Leukoaraisosis and Disability in the Elderly Study” (LADIS)25; (4) mood disturbances using the Geriatric Depression Scale26; and (5) motor performance using a slightly modified Short Physical Performance Battery.27 Duplex and transcranial Doppler sonography was also performed in all patients with CADASIL to exclude extracranial and intracranial artery stenosis.

The study complies with the Declaration of Helsinki and was approved by the local ethic committee of each center. All subjects gave informed consent.

Definition of Associated Diseases and Risk Factors
Hypertension was defined according to the National High Blood Pressure Education Program Coordinating Committee28 or to the use of antihypertensive medications as verified by the physician. Diabetest mellitus was defined in agreement with the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus29 or by the use of specific medications. Dyslipidemia was defined according to the National Cholesterol Education Program (Adult Treatment Panel III).30 Smoking was considered as present in case of current or previous history. Dementia was defined according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.31

Blood Collection
Blood samples were obtained from an antecubital vein in the morning after an overnight fasting and were collected into evacuated plastic tubes (BD Vacutainer Systems, Plymouth, UK) containing ethylenediaminetetraacetate 0.17 mol/L for EPC and CPC evaluation.

Because inflammatory events are known to influence EPC number,32 blood was withdrawn after excluding the occurrence of infectious events (defined according to previously published criteria33) in the previous 15 days.

Flow Cytometric Analysis of EPCs and CPCs
EPC and CPC number was assessed contemporarily using flow cytometry as previously described with minor modifications.32,34 Briefly, 200 μL of peripheral venous blood was incubated for 20 minutes in the dark with the appropriated monoclonal antibodies and 300 000 cells within the leukocyte gate were acquired using a FACSCanto analyzer (Becton Dickinson, San Jose, Calif). Data were processed using BD FacsDiv software. Cells positive for CD34/KDR, CD133/KDR, and CD34/CD133/KDR were considered EPCs. By using a modification of the International Society of Hemotherapy and Graft Engineering guidelines,35 CPCs were defined as cells forming a cluster with low side scatter and low-to-intermediate CD45 staining and positive for CD34, CD133, and CD34/CD133.

Statistical Analysis
To assess the statistical significance of differences in clinical data and progenitor cell numbers between patients with CADASIL and control subjects, the χ2 test for categorical variables and Mann–Whitney test for numeric variables were used. Logistic regression analysis, including age, drug use, and sex as variables possibly influencing the cells number, was performed to test the independence of associations. In this analysis, the logarithm of the cell number was used for a better evaluation of the OR.

To study possible associations between cell numbers and the clinical manifestations, patients with CADASIL were divided into 2 groups (severe and mild) as previously defined. χ2 test and Mann–Whitney tests were applied for comparisons between these groups. Correlation analysis with nonparametric test (Spearman) was performed to establish the possible relationship between CPC and EPC numbers and patient performances on cognitive, functional, and motor tests.

All analyses were performed using the SPSS (Statistical Package for Social Sciences, Chicago, Ill) software for Windows (Version 15.0).
Results

Twenty-nine patients with CADASIL belonging to 24 unrelated families and 29 control subjects were enrolled. Each group was composed of 12 males and 17 females; the mean age was 54.5 ± 14.6 years (range, 29 to 81 years) and 54.1 ± 14.6 years (range, 29 to 84 years), respectively, for patients and control subjects. Demographic data, risk factors, and drug use of patients (all and the 2 severity groups) and control subjects are reported in Table 1. Comparing patients with control subjects, there was a similar distribution of vascular risk factors, whereas patients were more frequently on antiaggregants. Patients who were more severely affected were older and used more frequently antiaggregants than patients with a milder picture. Table 2 reports the distribution of CADASIL typical features and the mean values obtained in patients on neurocognitive tests and on functional, motor,

Table 1. Demographic Data, Vascular Risk Factors and Therapies of Patients With CADASIL (Mild and Severe Groups and All Patients) and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>CADASIL Patients, Mild Group (N=13)</th>
<th>CADASIL Patients, Severe Group (N=16)</th>
<th>CADASIL Patients, Mild Versus Severe, P</th>
<th>CADASIL Patients, All (N=29)</th>
<th>Control Subjects (N=29)</th>
<th>CADASIL Patients Versus Control Subjects, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (± SD)</td>
<td>47.7 (± 14.6)</td>
<td>60.0 (± 12.6)</td>
<td>0.03†</td>
<td>54.5 (± 14.6)</td>
<td>54.1 (± 14.6)</td>
<td>0.87†</td>
</tr>
<tr>
<td>Males, no. (%)</td>
<td>5 (38%)</td>
<td>7 (44%)</td>
<td>0.77*</td>
<td>12 (41%)</td>
<td>12 (41%)</td>
<td>1.00*</td>
</tr>
<tr>
<td>Education, years (mean ± SD)</td>
<td>10.4 (± 3.7)</td>
<td>8.3 (± 3.4)</td>
<td>0.10†</td>
<td>9.2 (± 3.6)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>3 (23%)</td>
<td>8 (50%)</td>
<td>0.14*</td>
<td>11 (38%)</td>
<td>10 (35%)</td>
<td>1.00*</td>
</tr>
<tr>
<td>Hypercholesterolemia, no. (%)</td>
<td>5 (38%)</td>
<td>6 (40%)</td>
<td>0.93*</td>
<td>11/28 (39%)</td>
<td>10 (34%)</td>
<td>0.79*</td>
</tr>
<tr>
<td>Hypertriglyceridemia, no. (%)</td>
<td>3 (23%)</td>
<td>2 (13%)</td>
<td>0.50*</td>
<td>5/28 (18%)</td>
<td>3 (10%)</td>
<td>0.47*</td>
</tr>
<tr>
<td>Diabetes mellitus, no. (%)</td>
<td>0</td>
<td>1 (6%)</td>
<td>0.36*</td>
<td>1 (3%)</td>
<td>0</td>
<td>1.00*</td>
</tr>
<tr>
<td>Smoking, no. (%)</td>
<td>4 (31%)</td>
<td>3 (19%)</td>
<td>0.45*</td>
<td>7 (24%)</td>
<td>7 (24%)</td>
<td>1.00*</td>
</tr>
<tr>
<td>Drug use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiaggregants</td>
<td>4 (31%)</td>
<td>14 (87%)</td>
<td>0.002*</td>
<td>18 (62%)</td>
<td>1 (3%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Statins</td>
<td>3 (23%)</td>
<td>4 (25%)</td>
<td>0.90*</td>
<td>7 (24%)</td>
<td>3 (10%)</td>
<td>0.30*</td>
</tr>
<tr>
<td>Angiotensin converting enzyme inhibitors</td>
<td>1 (8%)</td>
<td>5 (31%)</td>
<td>0.12*</td>
<td>6 (21%)</td>
<td>4 (14%)</td>
<td>0.73*</td>
</tr>
</tbody>
</table>

*χ².
†Mann–Whitney test.

Table 2. Clinical Features and Mean Scores of the Cognitive, Motor, Functional, and Mood Tests/Scales Performed in All Patients With CADASIL and in the 2 Severity Subgroups (Severe Versus Mild)

<table>
<thead>
<tr>
<th></th>
<th>All CADASIL Patients (N=29)</th>
<th>Severe (N=16)</th>
<th>Mild (N=13)</th>
<th>Severe Versus Mild, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke, no. (%)</td>
<td>14 (48%)</td>
<td>14 (87%)</td>
<td>0</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>TIA, no. (%)</td>
<td>8 (28%)</td>
<td>8 (50%)</td>
<td>0</td>
<td>0.004†</td>
</tr>
<tr>
<td>Dementia, no. (%)</td>
<td>3 (10%)</td>
<td>3 (19%)</td>
<td>0</td>
<td>0.099†</td>
</tr>
<tr>
<td>Mild cognitive impairment, no. (%)</td>
<td>13/26 (50%)</td>
<td>6/14 (43%)</td>
<td>7/12 (58%)</td>
<td>0.431†</td>
</tr>
<tr>
<td>Psychiatric disturbances, no. (%)</td>
<td>21 (72%)</td>
<td>13 (82%)</td>
<td>8 (61%)</td>
<td>0.238†</td>
</tr>
<tr>
<td>Migraine, no. (%)</td>
<td>15 (52%)</td>
<td>9 (56%)</td>
<td>6 (50%)</td>
<td>0.457†</td>
</tr>
<tr>
<td>Seizures, no. (%)</td>
<td>2/27 (7%)</td>
<td>2/14 (14%)</td>
<td>0</td>
<td>0.173†</td>
</tr>
<tr>
<td>Gait disturbances, no. (%)</td>
<td>11/28 (39%)</td>
<td>9/15 (60%)</td>
<td>2 (15%)</td>
<td>0.023†</td>
</tr>
<tr>
<td>Dysphagia, no. (%)</td>
<td>8/28 (29%)</td>
<td>7/15 (47%)</td>
<td>1 (8%)</td>
<td>0.023†</td>
</tr>
<tr>
<td>MMSE, mean (± SD)</td>
<td>26.5 (± 4.4)</td>
<td>25.3 (± 4.7)</td>
<td>27.9 (± 3.2)</td>
<td>0.038*</td>
</tr>
<tr>
<td>Trial Making Test-A, mean time (± SD)</td>
<td>77.4 (± 64.0)</td>
<td>101.6 (± 74.0)</td>
<td>47.2 (± 30.5)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Trial Making Test-B, mean time (± SD)</td>
<td>168.0 (± 96.1)</td>
<td>211.0 (± 91.9)</td>
<td>114.2 (± 73.6)</td>
<td>0.009*</td>
</tr>
<tr>
<td>Stroop test (interference trial) mean time (± SD)</td>
<td>46.7 (± 31.2)</td>
<td>61.5 (± 35.4)</td>
<td>32.8 (± 16.5)</td>
<td>0.007*</td>
</tr>
<tr>
<td>Verbal fluency, mean words, no. (± SD)</td>
<td>17.7 (± 9.4)</td>
<td>13.6 (± 8.0)</td>
<td>21.5 (± 9.0)</td>
<td>0.020*</td>
</tr>
<tr>
<td>Delayed word recall, mean (± SD)</td>
<td>4.4 (± 3.0)</td>
<td>5.2 (± 3.3)</td>
<td>3.8 (± 2.4)</td>
<td>0.202*</td>
</tr>
<tr>
<td>Short Physical Performance Battery, mean (± SD)</td>
<td>9.2 (± 3.1)</td>
<td>7.8 (± 3.6)</td>
<td>10.6 (± 1.7)</td>
<td>0.022*</td>
</tr>
<tr>
<td>Disability Assessment for Dementia, mean (± SD)</td>
<td>89.8% (± 19.1)</td>
<td>83.0% (± 24.0)</td>
<td>98.0% (± 4.0)</td>
<td>0.128*</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>4.4 (± 3.2)</td>
<td>4.4 (± 3.2)</td>
<td>4.5 (± 3.2)</td>
<td>0.912*</td>
</tr>
</tbody>
</table>

*Mann–Whitney test.
†χ².
TIA indicates transient ischemic attack; MMSE, Mini Mental State Examination.
EPCs

CD34+/kdr+
0.06 (0.001–0.27)
0.04 (0.001–0.15)
0.211
0.05 (0.001–0.27)
0.10 (0.02–0.34)
0.005

CD133+/kdr+
0.09 (0.001–0.31)
0.06 (0.001–0.13)
0.062
0.07 (0.001–0.31)
0.10 (0.02–0.34)
0.006

CD34+/CD133+/kdr+
0.06 (0.001–0.31)
0.04 (0.001–0.13)
0.124
0.05 (0.001–0.31)
0.10 (0.02–0.34)
0.001

CPCs

CD34+
2.95 (1.75–4.79)
1.68 (0.72–3.69)
0.007
2.29 (0.72–4.79)
2.61 (0.80–5.19)
0.499

CD133+
2.82 (1.55–4.77)
1.40 (0.66–3.42)
0.004
2.26 (0.66–4.77)
2.60 (0.80–5.19)
0.316

CD34+/CD133+
2.75 (1.61–4.77)
1.44 (0.66–3.40)
0.004
2.29 (0.66–4.77)
2.55 (0.80–5.19)
0.266

*Mann–Whitney test.

and depression scales. Except for 3 demented patients, global
cognitive performances and functional abilities were relatively
well preserved. However, as expected, the more severe
group had worse performance on neuropsychological and
motor tests. In the mild group, 3 patients were asymptomatic
and one had migraine only. Considering bone marrow-
derived cells, median EPC count was significantly lower in
patients with CADASIL with respect to control subjects
(Table 3). The difference remained significant after adjusting
for age, sex, and statin use in multivariate logistic regression
[78, 79]. The strength of the association did not change. Also, CPC levels were lower in
patients with CADASIL than control subjects, but the differ-
ce did not reach statistical significance (Table 3).

Taking into account disease severity, patients with a more
severe clinical picture, compared with those with a milder
phenotype, had a statistically significant lower number of
CPCs (Table 3). After adjusting for age, this difference
remained significant (P values and regression coefficient
[Wald statistic], respectively, for CD34, CD133, and CD34/
133 were 0.044 and −2.56 [5.75], 0.041 and −2.50 [4.20],
and 0.034 and −2.87 [5.65]). The differences in EPC values
between patients with a mild or severe picture narrowly failed
to reach the statistical significance.

Some correlations between CPC levels and cognitive or
motor performances proved significant (Table 4).

**Discussion**

To the best of our knowledge, this is the first study to evaluate
EPCs and CPCs in CADASIL and their possible association
with measures of clinical severity. There was an association
between low bone marrow-derived circulating cells levels
and CADASIL, particularly in patients with the most severe
manifestations of the disease. The strength of the association
was corroborated by the fact that the number of CPCs was
correlated with cognitive and motor performances.

EPCs contribute to the maintenance of the endothelium by
replacing injured mature endothelial cells and by serving as a
cellular reservoir for the replacement of dysfunctional endo-
thelium. Our finding that patients with CADASIL have a
reduced number of circulating EPCs is consistent with previous
data, obtained using different markers, pointing to the

**Table 3. Median Values of the EPC and CPC Numbers (Cells/Microliter) in the 2 Severity Groups (Mild, 13 and Severe, 16) and All
Patients With CADASIL (29) and Control Subjects (29)**

<table>
<thead>
<tr>
<th>EPCs</th>
<th>CADASIL Patients, Median (Range)</th>
<th>CADASIL Patients, Severe Group, Median (Range)</th>
<th>Mild Versus Severe, P*</th>
<th>All CADASIL Patients, Median (Range)</th>
<th>Control Subjects, Median (Range)</th>
<th>CADASIL Patients, Versus Control Subjects, P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+/kdr+</td>
<td>0.06 (0.001–0.27)</td>
<td>0.04 (0.001–0.15)</td>
<td>0.211</td>
<td>0.05 (0.001–0.27)</td>
<td>0.10 (0.02–0.34)</td>
<td>0.005</td>
</tr>
<tr>
<td>CD133+/kdr+</td>
<td>0.09 (0.001–0.31)</td>
<td>0.06 (0.001–0.13)</td>
<td>0.062</td>
<td>0.07 (0.001–0.31)</td>
<td>0.10 (0.02–0.34)</td>
<td>0.006</td>
</tr>
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<td>CD34+/CD133+/kdr+</td>
<td>0.06 (0.001–0.31)</td>
<td>0.04 (0.001–0.13)</td>
<td>0.124</td>
<td>0.05 (0.001–0.31)</td>
<td>0.10 (0.02–0.34)</td>
<td>0.001</td>
</tr>
<tr>
<td>CPCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+</td>
<td>2.95 (1.75–4.79)</td>
<td>1.68 (0.72–3.69)</td>
<td>0.007</td>
<td>2.29 (0.72–4.79)</td>
<td>2.61 (0.80–5.19)</td>
<td>0.499</td>
</tr>
<tr>
<td>CD133+</td>
<td>2.82 (1.55–4.77)</td>
<td>1.40 (0.66–3.42)</td>
<td>0.004</td>
<td>2.26 (0.66–4.77)</td>
<td>2.60 (0.80–5.19)</td>
<td>0.316</td>
</tr>
<tr>
<td>CD34+/CD133+</td>
<td>2.75 (1.61–4.77)</td>
<td>1.44 (0.66–3.40)</td>
<td>0.004</td>
<td>2.29 (0.66–4.77)</td>
<td>2.55 (0.80–5.19)</td>
<td>0.266</td>
</tr>
</tbody>
</table>

*Values represent the Spearman rho; P values shown in parentheses.

MMSE indicates Mini Mental State Examination; TMT, Trial Making Test; SPPB, Short Physical Performance Battery; DAD, Disability Assessment for Dementia; GDS, Geriatric Depression Scale.
presence of endothelial dysfunction in CADASIL. Although the disease pathology affects mainly the tunica media, endothelial changes, including cytoplasmic swelling, disruption of tight junctions, and appearance of bundles of microfilaments, have been also reported.36 Altered endothelial-dependent vasodilatation in cerebral and forearm arteries and higher levels of asymmetrical dimethylarginine, a nitric oxide endogenous inhibitor, have been found.14,15,37 Even if our findings support the involvement of the endothelium in the pathogenesis of the disease, differently from the experimental setting, in vivo studies do not allow to establish whether this is associated with a failure of angiogenesis. Moreover, it is not possible to determine whether the endothelium is damaged and cells are not being replaced or there might be endothelium degeneration not compensated by increase in EPCs. Further studies are warranted to better elucidate the relationship between EPCs and CADASIL pathogenesis.

Regarding the different behavior of EPCs and CPCs, there are a few possible explanations: one is technical and is related to the fact that EPCs are rare in the circulation; from a biological viewpoint, EPCs and CPCs likely represent different progenitor cell phenotypes with different biological properties. The EPC circulating pool represents a population of more mature cells that are just “committed” to differentiate into endothelial cells able to participate mainly in the processes of the re-endothelialization and revascularization. On the other hand, CPCs are a more heterogeneous and undifferentiated cell population that can evolve in different cells types. They are known for being involved in sustaining the homeostasis of damaged brain. In animal models, it has been shown that within the central nervous system, these cells can give rise to neurons,38 astrocytes,39 and oligodendrocytes40 as well as endothelial cells.41 In vitro studies are able to produce several growth factors.42 In a few human studies, their role in angiogenesis33,44 and cerebral circulation22 has been shown.

The main limitation of our study is the small number of patients examined. This implies that potential confounders, both those involved in the relation between cell numbers and the disease (for example, age, sex, or drugs55) and those involved in the relation between cell numbers and the disease (for example, age, sex, or drugs55) and those influencing the severity of phenotype (for example, hypertension and smoking7,8), cannot be adequately accounted for. Moreover, we cannot exclude completely a chance effect. However, CADASIL remains a rare disease and published series are usually not large. This remains a preliminary observation to be expanded in future studies. Being a cross-sectional study, we are not able to show any data about variations in progenitor cells occurring over time possibly related to aging or to disease progression.

**Conclusions**

We documented, for the first time, an association between EPCs and CPCs and CADASIL. These data corroborate the hypothesis that endothelial dysfunction in CADASIL plays an active role and may contribute to its phenotypic expression. Further and more robust data are needed to prove this hypothesis. If this will be the case, pharmacological strategies aimed at favoring stem cell mobilization could be considered to limit the clinical and functional consequences of CADASIL.

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**Disclosures**

None.

**References**


9. Chabrier H, Pappata S, Ostergaard L, Clark CA, Pachot-Clouard M, Chabriat H, Mouton P, Bousser MG, Chabriat H. Characteristic features of in vivo skin microvascular changes, including cytoplasmic swelling, disruption of tight junctions, and appearance of bundles of microfilaments, have been also reported.36 Altered endothelial-dependent vasodilatation in cerebral and forearm arteries and higher levels of asymmetrical dimethylarginine, a nitric oxide endogenous inhibitor, have been found.14,15,37 Even if our findings support the involvement of the endothelium in the pathogenesis of the disease, differently from the experimental setting, in vivo studies do not allow to establish whether this is associated with a failure of angiogenesis.


11. Pfefferkorn T, von Stuckrad-Barre S, Herzog J, Gasser T, Hamann GF, Pfefferkorn T, von Stuckrad-Barre S, Herzog J, Gasser T, Hamann GF. *Characteristic features of in vivo skin microvascular changes, including cytoplasmic swelling, disruption of tight junctions, and appearance of bundles of microfilaments, have been also reported.36 Altered endothelial-dependent vasodilatation in cerebral and forearm arteries and higher levels of asymmetrical dimethylarginine, a nitric oxide endogenous inhibitor, have been found.14,15,37 Even if our findings support the involvement of the endothelium in the pathogenesis of the disease, differently from the experimental setting, in vivo studies do not allow to establish whether this is associated with a failure of angiogenesis.*


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