Clinical and magnetic resonance observations in cerebral small-vessel disease
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Citation for published version (APA):
Kwa, V. I. H. (1999). Clinical and magnetic resonance observations in cerebral small-vessel disease

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Cerebral small-vessel disease compared with large-vessel atherosclerosis: Genetic and vascular risk factors

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Submitted for publication
Abstract

Background and purpose
The association between hypertension and cerebral small-vessel disease (SVD) has been controversial, partly because patients with cerebral SVD often have concomitant large-vessel disease (LVD). Specific risk factors for SVD have not been studied in patients with cerebral SVD only, i.e. without signs or symptoms of LVD.

Methods
We identified signs of cerebral SVD on MRI in a cohort of patients with symptomatic atherosclerotic disease. We compared vascular risk factors and apolipoprotein E (apoE) polymorphism between patients with and without these MRI lesions, and between patients with “pure” cerebral SVD (without signs or symptoms of LVD) and patients with combined SVD and LVD.

Results
Hundred-thirty-two patients fulfilled our MRI-criteria of cerebral SVD. In a multivariate analysis, age (OR 1.1, 95%CI 1.1-1.1), the absence of apoE ε2 (OR 5.4, 95%CI 2.0-14.5) or ε4 alleles (OR 2.1, 95%CI 1.0-4.2) were associated with cerebral SVD. Twenty-seven patients had “pure” cerebral SVD. Hypertension was independently associated with “pure” SVD (OR 2.6, 95%CI 1.1-6.2).

Conclusions
Our findings suggest an interaction between age, apoE polymorphism and hypertension for the development of either cerebral SVD or LVD. Certain patients may develop large-vessel atherosclerosis at a relatively early age, promoted by hypertension and genetic factors. Others may be relatively protected against environmental factors, but develop cerebral small-vessel arteriopathy at a more advanced age, mainly as a consequence of hypertension.
Clinically and pathologically, two main types of ischemic arterial disease in the brain can be distinguished: small-vessel disease (SVD) and large-vessel disease (LVD). SVD is a structural alteration of cerebral arterioles with initial hypertrophy of medial smooth muscle, which is subsequently replaced by extracellular matrix and plasma proteins. SVD is associated with small subcortical infarcts (lacunar infarcts), white matter lesions and intracerebral hemorrhages. Large-vessel atherosclerotic disease is characterized by damage to the intima of the large muscular arteries, which results in the development of atheromas, that may eventually cause stenosis and thrombo-embolic complications. Thrombo-embolic occlusion in the brain typically causes large cerebral infarcts involving the cortex and the adjacent subcortical areas. The major extracerebral manifestations of large-vessel atherosclerosis are myocardial infarction and intermittent claudication.

Why some patients develop SVD and others LVD is not fully understood. Hypertension has been specifically associated with different manifestations of SVD, such as lacunar infarcts, cerebral white matter lesions, and intracerebral hemorrhages. However, hypertension is also a major risk factor for large-vessel atherosclerosis.

One reason why it has been difficult to separate risk factors for SVD and LVD is that both types of arterial disease often occur simultaneously. Genetic factors, such as apolipoprotein E (apoE) polymorphism, may contribute to the development of one type of arterial disease. ApoE e4 is associated with myocardial infarction and large cortico-subcortical cerebral infarcts, both manifestations of LVD. On the other hand, Schmidt et al. found an association between the apolipoprotein e2/e3 genotype and cerebral SVD.

In this study we identified patients with signs of cerebral SVD on MRI in a cohort of patients with symptomatic arterial disease. We studied vascular risk factors and apolipoprotein E polymorphism in these patients and in controls without these MRI lesions. Subsequently, we divided the patients with MRI-signs of cerebral SVD in a group with concomitant
extracerebral large-vessel disease and a group without signs of large-vessel
disease and compared the vascular risk factors in these two subgroups.

**Material and Methods**

**Patients**

Patients with a recent ischemic stroke, myocardial infarction or peripheral
arterial disease were examined prospectively from January 1992 to February
1996. The study was approved by the medical ethics committee. All patients
gave informed consent to participate. Ischemic stroke was defined as an
acute focal neurologic deficit persisting at least one week. Intracerebral
hemorrhage was excluded by an early CT-scan. Myocardial infarction was
defined as angina pectoris for more than 20 minutes and with laboratory- or
ECG-findings consistent with myocardial infarction. Peripheral arterial
disease was defined as intermittent claudication with characteristic leg pain
or a history of surgery for intermittent claudication. Patients with dementia
were excluded. We recorded smoking status, medical history and hyper­tension, defined as a diastolic blood pressure of ≥ 95 mmHg on repeated
prior measurements or a current treatment for hypertension. Plasma levels
of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were
assessed. Hypercholesterolemia was defined as a total plasma cholesterol
level of ≥ 7 mmol/l or a current treatment for hypercholesterolemia.

**Magnetic resonance imaging**

MRI was performed with a 1.5 Tesla Siemens Magnetom 63 SP/4000
(Siemens AG, Erlangen, Germany). Five millimeter transversal images
were obtained with a T1-weighted (T1W-SE), a combined proton density-
weighted (PDW-SE) and a T2-weighted (T2W-SE) spin echo sequence. At
the same levels transversal images were made with a T2W-gradient echo
FLASH 2D sequence. Two investigators (VIHK,BV) independently
assessed the MRI scans without knowledge of clinical or laboratory data.
Lacunes were defined as well-demarcated hyperintense areas on T2W-
SE images of less than 20 mm with corresponding hypointense lesions on
T1W-SE images. Cerebral hemispheric white matter lesions were assessed
on the T2W-SE and PDW-SE images. Hyperintensities limited to small caps and thin linings along the ventricles were considered normal. All other hyperintense lesions in the hemispheric white matter were considered abnormal. Large (non-lacunar) infarcts were defined as cortical and subcortical hyperintense lesions on T2W-SE images and corresponding hypointense areas on T1W-SE. Small old cerebral hemorrhages were defined as local hypointense signal on a T2W-SE image, more pronounced on the T2W-gradient echo images, with corresponding normal or slightly hypointense signals on the T1W-SE image. Flow-void artifacts and symmetrical signal loss in the basal ganglia were ruled out, to avoid inclusion of blood vessels or ferric iron deposits in the basal ganglia. We checked the corresponding areas on the CT-scans and ruled out cerebral calcifications.

B-mode ultrasound examination of common carotid and femoral arteries

An ATL Ultramark IV (Advanced Technology Laboratories, Bothell, Washington) equipped with an HRLA 7.5 MHz linear array transducer was used. The sonographer was blinded to the clinical status of the subjects. The right and left common carotid arterial wall segments were imaged from a lateral transducer angle. Carotid segments were defined by the carotid dilation and carotid flow divider as depicted in the B-mode image of the individual. The right and the left common femoral arterial wall segments were imaged from an anterior transducer angle. Femoral segments were defined by the femoral dilatation and the femoral flow divider. Images of each arterial wall segment were stored on tape. Intima-media thickness (IMT) of the posterior wall segments was measured off-line. The image analysis procedure is described elsewhere.

Apolipoprotein genotyping

Genomic DNA was extracted from peripheral leukocytes in citrated blood using a QIAamp blood kit (QIAGEN, Germany). Apolipoprotein ε2/ε3/ε4 genotyping was performed essentially as described before with minor modifications. In short, the relevant 244 basepairs ApoE gene fragment was amplified with the primers 5'-AGAATTCGCCCCGGCCTGCTACAC-3' and 5'-TAAGCTTTGGCAGCGGTGATCCAAGGA-3'. The reaction mixture was heated for 5' at 95°C and subjected to 30 cycles of 1' 95°C, 1' 60°C and
3' 70°C. Thereafter the mixture was kept at 70°C for 3'. Subsequently 10 µl of the reaction mixture was cut with 5 units Cfo I at 37°C during a three hours incubation. The resulting DNA fragments were visualized on a 4% agarose gel. The ε2 allele results in fragments of 91, 83, 38 and 19 basepairs, the ε3 allele in fragments of 91, 48, 38, 35 and 19 basepairs, and the ε4 allele in fragments of 72, 48, 38, 35, and 19 basepairs. In order to avoid misdiagnosis, each set of reactions included 6 previously typed specimens containing all of the possible genotypes.

Definitions of small-vessel disease and large-vessel disease

Lacunes, white matter lesions and signs of small old intracerebral hemorrhages on MRI were considered to be manifestations of cerebral SVD. Patients with large cortico-subcortical infarcts, myocardial infarction or peripheral arterial disease or a history of one of these diseases, were considered to have LVD.

Statistical analysis

The means of continuous variables were compared with the Student's t-test. Frequency differences were analyzed with the Chi-square test. A p-value of ≤0.05 was considered significant. The independent association between cerebral small-vessel disease and possibly related factors was additionally examined with multivariate logistic regression analysis (with a stepwise forward selection strategy). All univariately identified significant factors (at a level of p ≤0.20) were entered into the model. Effect sizes were expressed as odds ratios (OR), calculated as the antilogarithm of the regression coefficients of the logistic regression model, with 95% confidence intervals (95% CI). Calibration of the model was assessed with Hosmer-Lemeshow goodness-of-fit test. This test compares observed and expected frequencies of the outcome in groups based on the value of the estimated probabilities, using the logistic model.
Results

The cohort consisted of 216 patients with recent ischemic stroke (30%), myocardial infarction (31%) or peripheral arterial disease (39%). Mean age was 62.2 years. Two-thirds of the patients were male and 86% smoked. Forty-two percent had hypertension, about half hypercholesterolemia and 10% diabetes. Forty percent showed white matter lesions and 46% lacunar infarcts on the MRI. Apolipoprotein E allele frequencies were 6.9% for ε2, 76.4% for ε3 and 16.7% for ε4. This distribution is similar to previous findings in comparable populations. The mean common carotid wall thickness was 0.78 (sd 0.20) mm and the mean common femoral wall thickness was 1.40 (sd 0.65) mm. These values are comparable with findings in other studies of atherosclerotic patients.

Hundred-thirty-two (61%) patients fulfilled our MRI-criteria of cerebral SVD. These patients were significantly older than patients without cerebral SVD (mean 66.6 years versus 55.4 years, *p < 0.001*, table 1). They more often had hypertension (48% versus 32%, *p = 0.02*). Diabetes was twice as frequent in patients with SVD (14% versus 7%, *p = 0.14*). There were less smokers among patients with cerebral SVD (83% versus 92%, *p = 0.06*).

<table>
<thead>
<tr>
<th>Characteristics of atherosclerotic patients with and without MRI signs of cerebral small-vessel disease (SVD).</th>
<th>All patients (n = 216)</th>
<th>With cerebral SVD (n = 132)</th>
<th>Without cerebral SVD (n = 84)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mean(SD) age, yr</strong></td>
<td>62.2 (11.7)</td>
<td>66.6 (10.0)</td>
<td>55.4 (11.0)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td><strong>male, %</strong></td>
<td>67</td>
<td>67</td>
<td>65</td>
<td>0.77*</td>
</tr>
<tr>
<td><strong>hypertension, %</strong></td>
<td>42</td>
<td>48</td>
<td>32</td>
<td>0.02*</td>
</tr>
<tr>
<td><strong>smoking, %</strong></td>
<td>86</td>
<td>83</td>
<td>92</td>
<td>0.06*</td>
</tr>
<tr>
<td><strong>hypercholesterolemia, %</strong></td>
<td>47</td>
<td>48</td>
<td>47</td>
<td>0.92*</td>
</tr>
<tr>
<td><strong>diabetes mellitus, %</strong></td>
<td>11</td>
<td>14</td>
<td>7</td>
<td>0.14*</td>
</tr>
<tr>
<td><strong>allele frequency, %:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2</td>
<td>6.9</td>
<td>4.5</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td>ε3</td>
<td>76.4</td>
<td>80.3</td>
<td>70.2</td>
<td>0.02*</td>
</tr>
<tr>
<td>ε4</td>
<td>16.7</td>
<td>15.2</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td><em><em>IMT</em> common carotid art.</em>*</td>
<td>0.78 (0.20)</td>
<td>0.82 (0.20)</td>
<td>0.73 (0.19)</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td><em><em>IMT</em> common femoral art.</em>*</td>
<td>1.40 (0.65)</td>
<td>1.43 (0.62)</td>
<td>1.37 (0.70)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

*chi-square test * Student’s t-test IMT = intima media thickness of the posterior wall in millimeters
The sex ratio and the frequency of hypercholesterolemia did not differ between the two groups. The allele frequency of e3 was higher in patients with cerebral SVD (80.3% versus 70.2%) and correspondingly the frequencies of e2 (4.5% versus 10.7%) and e4 (15.2% versus 19.0%) were higher in patients without cerebral SVD (p = 0.02). The intima-media thickness (IMT) of the common carotid artery (mean 0.82 versus 0.73 mm, p < 0.01) was larger in patients with cerebral SVD. There was no significant difference in the IMT of the common femoral artery (mean 1.43 versus 1.37 mm, p = 0.60). In a multivariate analysis we used the presence of cerebral SVD as a dependent variable and age, sex, hypertension, smoking status, diabetes mellitus and the three apoE alleles as independent variables. Age (OR 1.1, 95% CI 1.1-1.1) was independently associated with the presence of cerebral SVD. The absence of the e2 allele (OR 5.4, 95% CI 2.0-14.5) or the e4 allele (OR 2.1, 95% CI 1.0-4.2) was independently associated with cerebral SVD. The Hosmer-Lemeshow statistic was not significant (p = 0.63), indicating a well calibrated model.

The distribution of MRI signs of cerebral SVD among the patients with different clinical manifestations of arterial ischemic disease is given in table 2. Cerebral SVD was observed in 81% of the patients with stroke, 43% of patients with myocardial infarction and 61% of the patients with peripheral arterial disease.

<table>
<thead>
<tr>
<th></th>
<th>With cerebral SVD (n=132)</th>
<th>Without cerebral SVD (n=84)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral infarct</td>
<td>52 (81%)</td>
<td>12 (19%)</td>
<td>64</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>29 (43%)</td>
<td>39 (57%)</td>
<td>68</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>51 (61%)</td>
<td>33 (39%)</td>
<td>84</td>
</tr>
</tbody>
</table>

Of the 132 patients with cerebral SVD, 27 patients had no signs or a history of myocardial infarction, peripheral arterial disease or large territorial cerebral infarct (“pure” cerebral SVD). We compared these patients with “pure” cerebral SVD with the 105 other patients, who had both SVD and LVD (“combined” SVD and LVD) (table 3). Age, hyper-
cholesterolemia, diabetes mellitus and the frequencies of the apoE alleles did not differ between these two groups. Only hypertension was significantly more frequent in patients with “pure” cerebral SVD (67% versus 44%, p=0.03). There were less males (59% versus 69%, p=0.31) and smokers (74% versus 85%, p=0.19) in the group of “pure” SVD (not statistically significant). The intima-media thickness of the common carotid (mean 0.77 versus 0.83, p=0.21) and common femoral artery (mean 1.37 versus 1.44, p=0.71) was smaller in patients with “pure” SVD (not significant). In a multivariate analysis we used age, sex, hypertension, smoking status and the three apoE alleles as independent variables. Only hypertension (odds ratio 2.6, 95% CI 1.1-6.2) was independently associated with “pure” SVD.

Table 3. Characteristics of patients with MRI signs of cerebral small-vessel disease (SVD) without signs of large-vessel disease (LVD) (“pure” cerebral SVD), compared with those with signs of both SVD and LVD (“combined” SVD and LVD),

<table>
<thead>
<tr>
<th></th>
<th>“Pure” cerebral SVD (n=27)</th>
<th>“Combined” SVD and LVD (n=105)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean(SD) age, yr</td>
<td>64.9 (10.1)</td>
<td>67.0 (10.0)</td>
<td>0.32</td>
</tr>
<tr>
<td>male, %</td>
<td>59</td>
<td>69</td>
<td>0.31</td>
</tr>
<tr>
<td>hypertension, %</td>
<td>67</td>
<td>44</td>
<td>0.03</td>
</tr>
<tr>
<td>smoking, %</td>
<td>74</td>
<td>85</td>
<td>0.19</td>
</tr>
<tr>
<td>hypercholesterolemia, %</td>
<td>48</td>
<td>48</td>
<td>0.95</td>
</tr>
<tr>
<td>diabetes mellitus, %</td>
<td>15</td>
<td>13</td>
<td>0.84</td>
</tr>
<tr>
<td>allele frequency,%:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e2</td>
<td>1.9</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>e3</td>
<td>83.3</td>
<td>79.5</td>
<td>0.56</td>
</tr>
<tr>
<td>e4</td>
<td>14.8</td>
<td>15.2</td>
<td></td>
</tr>
<tr>
<td>IMT* common carotid art.</td>
<td>0.77 (0.14)</td>
<td>0.83 (0.22)</td>
<td>0.21</td>
</tr>
<tr>
<td>IMT* common femoral art.</td>
<td>1.37 (0.30)</td>
<td>1.44 (0.68)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

* chi-square test  Student’s t-test  *IMT = intima media thickness of the posterior wall in millimeters

Discussion

In a cohort of patients with symptomatic arterial ischemic disease we found that age, hypertension and the absence of apoEe2 or e4 alleles were associated with signs of cerebral SVD on MRI. Hypertension was the only independently associated risk factor with “pure” SVD.
From our data hypertension emerges as the most important risk factor for cerebral SVD. Controversies in previous studies about risk factors for SVD and LVD can be partially explained by the frequent co-existence of both types of arteriopathy. Patients with ischemic stroke often have a combination of SVD and LVD. In about 30% of the patients with myocardial infarction asymptomatic cerebral lacunar infarcts have been found. Diabetes mellitus often leads to both small-vessel and large-vessel disease. Likewise, in our study many patients had concomitant SVD and LVD (table 2). Contrary to previous studies we separated patients with cerebral SVD, who did not have symptoms or a history of LVD (“pure” cerebral SVD) from patients with cerebral SVD who also have concomitant LVD (“combined” SVD and LVD). Hypertension was significantly more common in patients with “pure” cerebral SVD than in patients with combined SVD and LVD. After multiple regression analysis hypertension was the only factor independently associated with “pure” cerebral SVD. Also, patients with “pure” cerebral SVD have thinner arterial walls than patients with combined SVD and LVD (table 3), supporting the idea that in the first group hypertension may predominantly have caused changes in the small arteries instead of large artery disease.

We also found, that apoE polymorphism is associated with either cerebral SVD or LVD. Patients with cerebral SVD more frequently carry the allele ε3, and consequently patients without signs of cerebral SVD, who in fact only had manifestations of LVD, more frequently carry alleles ε2 and ε4. Since this is a cross-sectional study, one has to consider a possible selection bias. For example, there may be a selective mortality of patients carrying ε2 or ε4, who had cerebral SVD, leading to an underrepresentation of these patients in our study. However, our results are consistent with previous studies. Alleles ε2 and ε4 have been shown to be associated with LVD, and the apoE ε2/ε3 phenotype was found to be associated with cerebral SVD. In addition, the differences in frequencies of hypertension and apoE alleles are consistent in both comparisons of patients with cerebral SVD to patients without SVD and of patients with “pure” SVD to patients with “combined” SVD and LVD. These findings suggest that ageing, hypertension and genetic risk factors may interact to cause...
different types of arterial alterations. One hypothesis is that certain patients with vascular risk factors, such as smoking and hypercholesterolemia, develop large-vessel atherosclerosis at a relatively early age, promoted by hypertension and possibly the presence of the apoE ε2 and ε4 alleles. Others may be relatively protected against environmental factors, but develop cerebral small-vessel arteriopathy at a more advanced age, mainly as a consequence of hypertension.

In conclusion, in patients with symptomatic arterial ischemic disease we have shown that apoE alleles ε2 and ε4 are associated with LVD without cerebral SVD. Hypertension was significantly more common in patients with “pure” SVD (i.e. without symptoms of LVD) than in patients with combined SVD and LVD. Our findings suggest an interaction between age, apoE genotypes and hypertension for the development of either cerebral SVD or LVD. Future prospective and genetic studies are needed to clarify the exact influences of risk factors to arterial pathology.

Acknowledgements
We thank V.E.A. Gercies, MD, Slotervaart Hospital, Amsterdam, for the DNA-extraction, Mrs. E.W.M. Vogels, Laboratory of Experimental Internal Medicine, for the apolipoprotein-E typing, Prof. R.J. de Haan, PhD, for his comments on the statistical analysis, and Prof. M. Vermeulen, MD, PhD, and WA. van Gool, MD, PhD, neurologists, Academic Medical Center, Amsterdam, for their critical comments on the manuscript.

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References


