Loss of white matter integrity is associated with gait disorders in cerebral small vessel disease

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Gait disturbances are common in the elderly. Cerebral small vessel disease, including white matter lesions and lacunars infarcts, is thought to disrupt white matter tracts that connect important motor regions, hence resulting in gait disturbances. Pathological studies have demonstrated abnormalities in white matter that may appear normal on brain imaging. The loss of integrity in such normal-appearing white matter may partly be due to small vessel disease and may play a role in causing gait disturbances. The white matter regions involved in these gait disturbances, both in white matter lesions and normal-appearing white matter, remain unclear. We, therefore, aimed to investigate the relation between the location of white matter lesions and gait using voxel-based morphometry analysis, as well as between white matter integrity and gait by applying tract-based spatial statistics to diffusion tensor imaging parameters. Magnetic resonance imaging was carried out on 429 individuals in the age range of 50 and 85 years, with cerebral small vessel disease without dementia or parkinsonism. Gait was assessed quantitatively. White matter lesions, especially in the centrum semiovale and periventricular frontal lobe, were related to a lower gait velocity, shorter stride length and broader stride width. Loss of white matter integrity, as indicated by a lower fractional anisotropy and higher mean diffusivity, in numerous regions was related to a lower gait performance. Most of these regions were located in the normal-appearing white matter. The strongest significant association was found in the corpus callosum, particularly the genu. Most of the associations in the normal-appearing white matter disappeared after controlling for white matter lesions and lacunar infarcts, except for some in the corpus callosum. In conclusion, our study showed that using a combination of voxel-based morphometry analysis of the white matter lesions and diffusion tensor imaging is of added value in investigating the pathophysiology of gait disturbances in subjects with small vessel disease. Our data demonstrate that, in elderly subjects with small vessel disease, widespread disruption of white matter integrity, predominantly in the normal-appearing white matter, is involved in gait disturbances. In particular, loss of fibres interconnecting bilateral cortical regions, especially the prefrontal cortex that is
involved in cognitive control on motor performance, may be important. The most important mechanisms underlying affected normal-appearing white matter are probably a direct effect of small vessel disease or, indirectly, remote effects of white matter lesions and lacunar infarcts.

**Keywords:** cerebral small vessel disease; magnetic resonance imaging; diffusion tensor imaging; gait

**Abbreviations:** DTI = diffusion tensor imaging; FLAIR = Fluid-Attenuated Inversion Recovery; TBSS = tract-based spatial statistics

**Introduction**

Gait disorders are common in the elderly and are associated with functional impairment, institutionalization, and death (Guralnik et al., 1995; Verghese et al., 2006). Gait is a complex function that depends on multiple factors, including spinal locomotormotor pattern generators under supraspinal control. Multiple brain regions, connected by white matter tracts, are involved in this supraspinal control of gait (la Fougere et al., 2010). These tracts are vulnerable to lesions, for example, caused by cerebral small vessel disease, including white matter lesions and lacunar infarcts (Jones et al., 1999). Previous studies have shown that cerebral small vessel disease is related to gait disturbances (de Laat et al., 2010), which is supposedly due to the disruption of these white matter tracts. To date, the involvement of white matter tracts has generally been studied at the level of white matter regions affected by small vessel disease visible on conventional MRI, showing the frontal (Benson et al., 2002; Onen et al., 2004; Blahak et al., 2009; de Laat et al., 2010; Srikanth et al., 2010), and in several studies also the parieto-occipital and brainstem regions to be important (Benson et al., 2002; Starr et al., 2003; Moscufo et al., 2009; de Laat et al., 2010). However, these studies did not investigate the whole-brain white matter, including the normal-appearing white matter (white matter with exclusion of T2-weighted white matter lesions), although pathological studies have demonstrated that abnormalities in this part of the white matter may, nevertheless, be present (Grafton et al., 1991).

Diffusion tensor imaging (DTI) measures local water diffusion profiles and provides valuable information on the microstructural integrity of the whole-brain white matter. The diffusion tensor has three eigenvalues; the first is referred to as axial diffusivity, and represents the diffusivity parallel to the white matter tracts. The average of the second and third eigenvalues is termed ‘radial diffusivity’ and reflects the diffusivity perpendicular to these tracts. Two different, but complementary, common DTI metrics can be derived from these eigenvalues: the mean diffusivity, which is the average of the three eigenvalues and represents the overall magnitude of water diffusion, and the fractional anisotropy, a normalized ratio of diffusion directionality (Pierpaoli et al., 1996). Loss of microstructural integrity is typically reflected by a reduction in fractional anisotropy and/or increase in mean diffusivity (Sen and Basser, 2005), which can result from different combinations of changes in axial and radial diffusivity. Investigating these four measures conjointly provides more information about the possible changes of white matter microstructure than fractional anisotropy and mean diffusivity alone. DTI studies in patients with cerebral small vessel disease and gait disturbances are scarce. Two studies showed an association between the loss of microstructural integrity of the corpus callosum and gait impairment (Della Nave et al., 2007; Bhadelia et al., 2009). One of these studies used a region-of-interest approach (Bhadelia et al., 2009), which has the disadvantage of revealing changes only in preselected areas that might not necessarily correspond with areas that are affected in gait disturbances. Whole-brain white matter information can be obtained with voxel-based DTI methods as used by Della Nave et al. (2007). However, potentially important limitations of this analysis are the spurious effects of the smoothing (Jones et al., 2005) and the possible misalignment due to the spatial normalization procedure (Smith et al., 2006). The latter is a common problem in the elderly because of atrophy and ventricular enlargement. Tract-based spatial statistics (TBSS) is a relatively new method that mitigates these two problems (Smith et al., 2006). The rationale behind the TBSS method is that the analysis is restricted to those white matter voxels that constitute the skeleton (core) of the brain’s connectional architecture and that this skeleton can be matched more accurately (compared with whole-brain normalization) across subjects.

We hypothesized that gait disturbances in elderly with cerebral small vessel disease would not only be related to loss of microstructural integrity of the white matter within the white matter lesions, but also of the normal-appearing white matter. Moreover, we suggested that this loss of integrity of the normal-appearing white matter would be related to the concurrent small vessel disease, rather than to degenerative processes such as general brain atrophy. First, we examined the association between white matter lesion location and gait disturbances using voxel-based morphometry analysis. Second, we conducted TBSS to investigate the location of white matter with loss of microstructural integrity related to gait disturbances. Finally, we examined whether the associations in the white matter were primarily explained by small vessel disease or general brain atrophy by appropriate adjustment for these two factors.

**Materials and methods**

**Study population**

This study is embedded within the Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Imaging Cohort study, a prospective cohort study that was designed to investigate risk factors and cognitive, motor and mood consequences of functional and structural brain changes as assessed by MRI among elderly with cerebral...
small vessel disease. The primary outcome of the longitudinal part of this study is the development of dementia or parkinsonism.

Cerebral small vessel disease is characterized by neuroimaging as either white matter lesions or lacunar infarcts. Symptoms of small vessel disease include acute symptoms, such as transient ischaemic attacks or lacunar syndromes, or subacute manifestations, such as cognitive, motor (gait) and/or mood disturbances (Roman et al., 2002). As the onset of cerebral small vessel disease is often insidious, clinically heterogeneous and, typically, with mild symptoms, it has been suggested that the selection of subjects with cerebral small vessel disease in clinical studies should be based on these more consistent brain imaging features (Erkinjuntti, 2002). Accordingly, consecutive patients referred to the Department of Neurology between October 2002 and November 2006, were selected for participation.

Inclusion criteria were: (i) age between 50 and 85 years; and (ii) cerebral small vessel disease on neuroimaging (white matter lesions and/or lacunar infarcts). Subsequently, the abovementioned acute or subacute clinical symptoms of small vessel disease were assessed by standardized structured assessments. Patients who were eligible because of a lacunar syndrome were included only >6 months after the event to avoid acute effects on the outcomes.

Exclusion criteria included: (i) dementia (American Psychiatric Association, 2000); (ii) Parkinsonism (Gelb et al., 1999; Louis and Luchsinger, 2006); (iii) intracranial haemorrhage; (iv) life expectancy of <6 months; (v) intracranial space occupying lesion; (vi) (psychiatric) disease interfering with cognitive testing or follow-up; (vii) recent or current use of acetylcholine-esterase inhibitors, neuroleptic agents, -dopa or dopa-antagonists; (viii) white matter lesion mimics; (ix) prominent visual or hearing impairment; (x) language barrier; (xi) MRI contraindications or known claustrophobia; (xii) inability to walk for 6 m unaided; (xiii) conditions not related to small vessel disease that affected gait (e.g. joint fusion, severe arthritis or psychogenic gait disturbance); and (xiv) territorial infarcts, because they were considered as a confounder and because of methodological issues, such as misalignment, during normalization procedure.

Patients were selected for participation in the study by a three-step approach. After reviewing medical records, 1004 individuals were invited by letter. Of these 1004, 727 were eligible after contact by telephone and 525 agreed to participate. During their visit to our research centre, all subjects underwent standardized structured assessments. Patients who were eligible because of a lacunar syndrome were included only >6 months after the event to avoid acute effects on the outcomes.

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The participants walked twice at self-selected normal gait speed in low-heeled shoes. They started 2 m before the carpet and walked until 2 m beyond it in order to measure steady-state walking. We measured velocity (m/s), consisting of both the stride length (m; the distance between the heel points of two consecutive footprints of the same foot) and cadence (number of steps per minute), and the stride width (cm; the distance between one midpoint of a footprint and the line of progression of the opposite foot). For this study, we chose velocity, stride length and width because our previous studies demonstrated good associations with both neuroimaging aspects of small vessel disease (De Laat et al., 2010) and the known association of velocity with risk for falls (Verghese et al., 2009). As velocity is determined by stride length and cadence, this latter gait parameter was also included.

Magnetic resonance imaging techniques

MRI scans of all subjects were acquired on a single 1.5 T scanner (Magnetom Sonata, Siemens Medical Solutions, Erlangen, Germany). The protocol included a 3D T1 magnetization-prepared rapid gradient-echo sequence: time repetition/time echo/time interval 2250/3.68/850 ms; flip angle 15°; voxel size 1.0 x 1.0 x 1.0 mm; a Fluid-Attenuated Inversion Recovery (FLAIR) sequence: time repetition/time echo/time interval 9000/84/2200 ms; voxel size 1.0 x 1.2 x 5.0 mm, plus an interslice gap of 1 mm; and a DTI sequence: time repetition/time echo 10100/93 ms; voxel size 2.5 x 2.5 x 2.5 mm, four unweighted scans, 30 diffusion-weighted scans with b-value of 900 s/mm2.

Conventional magnetic resonance imaging analysis

White matter signal hyperintensities on FLAIR scans, which were not, or only faintly, hypo-intense on T1-weighted images, were considered white matter lesions, except for gliosis surrounding infarcts. We rated white matter lesions visually on FLAIR images using the Age-Related White Matter Changes scale, which grades white matter lesion severity on a 4-point scale (0–3; Wahlund et al., 2001). White matter lesions were also manually segmented on the FLAIR images by two trained raters. Total white matter lesion volume was calculated by summing the segmented areas multiplied by slice thickness. Lacunar infarcts were rated and defined as areas with a diameter >2 mm and <15 mm with low signal intensity on T1 and FLAIR, ruling out
enlarged perivascular spaces and infrapaternal pseudolacunes (Fisher, 1965; Pullicino et al., 1995; Herve et al., 2005). All imaging analyses were performed by raters blinded to clinical information. In a random sample of 10%, inter-rater variability for total white matter lesion volume yielded an intra-class correlation coefficient of 0.99; intra- and inter-rater reliability for number of lacunar infarcts a weighted kappa of 0.80 and 0.88.

We computed grey and white matter tissue and cerebrospinal fluid probability maps using Statistical Parametric Mapping 5 (Wellcome Department of Cognitive Neurology, University College London, UK) unified segmentation routines on the $T_1$ images (Ashburner and Friston, 2005). Binary tissue maps were created by thresholding the probability maps at 0.5. Total grey and white matter volumes were calculated by summing all voxel volumes that belonged to that class. Total brain volume was taken as the sum of total grey and total white matter volume.

Furthermore, we performed voxel-based morphometry style applied to the white matter lesion maps. For this, we modified the optimized Functional MRI of the Brain Software Library voxel-based morphometry protocol (www.fmrib.ox.ac.uk/fsl; Douaud et al., 2007). First, we registered the skull-stripped $T_1$ images of the study subjects ($n = 429$) non-linearly to the Montreal Neurological Institute 152 template using Functional MRI of the Brain non-linear registration tool (http://www.fmrib.ox.ac.uk/fsl/) and averaged them to obtain the group-specific template. Subsequently, these $T_1$ images were non-linearly normalized to the group-specific template. Then we registered the white matter lesion maps to the $T_1$ images using the transformation matrix from the registration parameters of skull-stripped FLAIR images to the $T_1$-images that were obtained using Functional MRI of the Brain linear image registration tool (http://www.fmrib.ox.ac.uk/fsl/). Next, we normalized the white matter lesion maps non-linearly to the group-specific template using the transformation parameters of $T_1$ images to the group-specific template. To correct for the stretching and contraction of the white matter lesions due to the non-linear registration, the normalized white matter lesion images were divided by the Jacobian of the warp field, allowing us to make inferences about the white matter lesion volume within a voxel. Finally, these images were smoothed with an isotropic Gaussian kernel with a sigma of 2 mm to account for the inter-subject variability.

**Diffusion tensor imaging analysis**

Diffusion data were first preprocessed using an in-house developed algorithm named ‘patching artefacts from cardiac and head motion’ (www.ru.nl/neuroimaging/diffusion; Zwiers, 2009). In short, this iteratively reweighted-least-squares algorithm produces robust diffusion tensor estimates and provides weightings that are used to detect and correct head and cardiac motion artefacts in the diffusion-weighted data. Next, affine misalignments from eddy currents and subject motion were corrected simultaneously by minimization of the residual diffusion tensor errors (Andersson and Skare, 2002). Using DTIFIT within the Functional MRI of the Brain diffusion toolbox, we created the fractional anisotropy, mean diffusivity and the axial and radial diffusivity images, which were then fed into the TBSS pipeline (Smith et al., 2006). In short, a fractional anisotropy skeleton was created by thinning the mean fractional anisotropy image based on the fractional anisotropy values. Subsequently, this skeleton was thresholded at 0.3 to include the major white matter tracts and to account for the inter-subject variability. All normalized fractional anisotropy data were then projected onto this skeleton. By applying the projection vectors from each subject’s fractional anisotropy-to-skeleton transformation, we projected the images of mean diffusivity, axial and radial diffusivity onto the mean fractional anisotropy skeleton. During the normalization procedures, the images were not modulated with the Jacobian of the spatial transformations (i.e. not corrected for the brain volume). These data were then fed into voxel-wise cross-subject statistics.

In addition, we obtained the mean fractional anisotropy, mean diffusivity and axial and radial diffusivity for the three parts of the corpus callosum by performing regions-of-interest analyses. We created the masks for genu, body and splenium of the corpus callosum by applying the white matter atlas (Johns Hopkins University white matter labels, provided by Functional MRI of the Brain Software Library) on the mean fractional anisotropy skeleton. The genu contains fibres connecting areas of the prefrontal cortex, the body of the premotor, motor and somatosensory cortex and the splenium of the parietal, temporal and occipital cortex (Chao et al., 2009). The masks were visually inspected and miscellaneous voxels that belonged to other regions, such as the cingulum bundle, were excluded.

**Other measurements**

For assessment of vascular risk factors, structured questionnaires were used together with measurements of blood pressure taken on several separate occasions. The risk factors included presence of hypertension (mean blood pressure $\geq 140/90$ mmHg and/or use of anti-hypertensive medications; Rosendorff et al., 2007), diabetes (treatment with antidiabetic medications), hypercholesterolaemia (treatment with lipid-lowering medications) and smoking status. Body mass index was also recorded. We used the Mini Mental State Examination score (range 0–30) to assess global cognitive status (Folstein et al., 1975). Functional independence was assessed using the Barthel Index (range 0–20; Mahoney and Barthel, 1965). The total score on the motor section of the Unified Parkinson’s Disease Rating Scale (27 items, score 0–4; Fahn and Elton, 1987) was rated by two trained raters.

**Statistical analysis**

The baseline characteristics were presented as mean $\pm$ standard deviation (SD) and, for the skewed distributed parameters, the median and interquartile ranges were calculated. The quantitative GAITRite parameters were averaged over two walks. When one trial was missing ($n = 3$), the remaining measures were used.

We performed inter-subject voxel-wise regression analyses between white matter lesion volume (within a voxel) and the gait parameters (gait velocity, length, cadence and stride width), while adjusting for age, sex and height. Second, for the TBSS analyses, we assessed voxel-wise regression coefficients between the skeletal DTI parameters (fractional anisotropy and mean diffusivity) and gait, while adjusting for the same confounding factors. To test whether the associations between DTI and gait parameters were attributable to the small vessel disease or general atrophy of the brain, we repeated these analyses with additional adjustment for white matter lesion volume and number of lacunar infarcts or total brain volume. We performed the voxel-wise statistical analyses for both white matter lesions and TBSS data using permutation-based statistical interference tool for non-parametric approach as a part of Functional MRI of the Brain Software Library (Nichols and Holmes, 2002; Smith et al., 2006). The number of the permutation tests was set at 5000 and significant associations were determined using the threshold-free cluster-enhancement with a threshold of $P < 0.05$, corrected for the multiple comparisons (Smith and Nichols, 2009). Third, for the regions-of-interest analyses, we computed (SPSS statistical software,
version 16.0) regression coefficients of the mean fractional anisotropy, mean diffusivity and axial and radial diffusivity of the three regions-of-interest in the corpus callosum (genu, body and splenium) with gait, while adjusting for age, sex, height and total brain volume. Subsequently, we adjusted for the same DTI parameter in the other two regions of the corpus callosum to test an independent effect of the DTI parameter in the three areas. Regression coefficients were presented as standardized β-values.

Results

Characteristics

Table 1 shows demographic, clinical, imaging and gait characteristics. Mean age of the study population (n = 429) was 65.2 years (SD 8.9) and 194 (45.2%) were female. Median volume percentage of normal-appearing white matter of the whole-brain white matter was high: 98.6% (interquartile range 96.3–99.3%).

White matter lesions and gait

White matter lesions were predominantly located in the frontal periventricular regions (Fig. 1). Significant associations between white matter lesions and lower gait velocity, shorter stride length and broader stride width were observed in multiple white matter regions, except for the temporal lobe, brainstem and cerebellum (Fig. 2). No significant associations were identified between white matter lesions and cadence. The strongest significance (P < 0.001) with gait velocity was found in the centrum semiovale on the right side and at a slightly lower significance level (P < 0.005) also on the left side. The regions with a significance P < 0.001 for stride length were more widespread than for gait velocity. These regions were located in the periventricular frontal lobes and centrum semiovale on both sides. White matter lesions in the centrum semiovale also showed the highest association with stride width.

Microstructural integrity of the white matter and gait

As shown in Fig. 3A the fractional anisotropy at almost all voxels on the skeleton were positively related to gait velocity and stride length and negatively to stride width, including the supra- and infratentorial regions (but not the hippocampal regions and fornices). By contrast, a significant positive relation between fractional anisotropy and cadence was found in only a few voxels on the skeleton. We found a similar distribution for the inverse relation between the mean diffusivity and these gait parameters, except for the cingulum and infratentorial white matter areas. Of note, these associations were not only within the white matter lesions, but also where the probability of the white matter lesions was low (<5%) or even absent (Fig. 1). The voxels with the strongest significance (P < 0.001) in the association between the fractional anisotropy and gait velocity were located in the corpus callosum along its complete course and in relation to the mean diffusivity also in the posterior limb of the internal capsule. Similar associations were found for the relation between the fractional anisotropy and mean diffusivity and stride length and stride width. In addition, a high significant association (P < 0.001) was identified in the middle portion of the cingulum bundle in the relation between the fractional anisotropy and stride length. No associations with a P < 0.001 were found with regard to cadence.

Additional adjustment for total brain volume did not change this pattern for the velocity, stride length and stride width. However, these relations disappeared after additional control for white matter lesions and lacunar infarcts, except for some associations between the fractional anisotropy and stride length in the genu and splenium of the corpus callosum and between the mean diffusivity and stride length in the body of the corpus callosum (Fig. 3B). With regard to cadence, no significant associations were found after additional adjustment for both total brain volume or white matter lesions and lacunar infarcts.

As DTI parameters in the corpus callosum showed the strongest significant associations with gait, we analysed the corpus callosum in more anatomical detail by segmenting it in three regions. We found a lower fractional anisotropy and a higher mean diffusivity in the genu significantly related to a lower gait velocity, independently of the DTI parameters in the other two segments (fractional anisotropy β = 0.18, P = 0.021; mean diffusivity β = −0.21, P = 0.010; Table 2). This was mainly due to a change in stride length and, to a lesser extent, in cadence. The relation between DTI parameters and stride width was less clear after adjusting for

Table 1 Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n = 429</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>65.2 (8.9)</td>
</tr>
<tr>
<td>Female</td>
<td>194 (45.2)</td>
</tr>
<tr>
<td>Height, mean</td>
<td>1.7 (0.1)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.6 (4.0)</td>
</tr>
<tr>
<td>Subjects with hypertension</td>
<td>310 (72.3)</td>
</tr>
<tr>
<td>Subjects with diabetes mellitus</td>
<td>60 (14.0)</td>
</tr>
<tr>
<td>Subjects with hypercholesterolaemia</td>
<td>190 (44.3)</td>
</tr>
<tr>
<td>Smokers, current</td>
<td>64 (14.9)</td>
</tr>
<tr>
<td>Smokers, former</td>
<td>233 (54.3)</td>
</tr>
<tr>
<td>Mini Mental State Examination</td>
<td>28.2 (1.6)</td>
</tr>
<tr>
<td>Barthel Index</td>
<td>19.7 (0.7)</td>
</tr>
<tr>
<td>UPDRS motor score</td>
<td>0.0 (0.0; 1.0)</td>
</tr>
<tr>
<td>ARWMC scale</td>
<td>1.0 (1.0; 2.0)</td>
</tr>
<tr>
<td>Total brain volume, ml</td>
<td>1098.2 (119.3)</td>
</tr>
<tr>
<td>White matter volume, ml</td>
<td>467.2 (64.8)</td>
</tr>
<tr>
<td>White matter lesion volume, ml</td>
<td>6.5 (3.2; 17.8)</td>
</tr>
<tr>
<td>Lacunar infarcts</td>
<td>133 (31.0)</td>
</tr>
<tr>
<td>Gait characteristics</td>
<td></td>
</tr>
<tr>
<td>Gait velocity, m/s</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td>Stride length, m</td>
<td>1.4 (0.2)</td>
</tr>
<tr>
<td>Cadence, steps/min</td>
<td>112.0 (10.8)</td>
</tr>
<tr>
<td>Stride width, cm</td>
<td>10.8 (3.1)</td>
</tr>
</tbody>
</table>

Data are mean (SD) unless otherwise indicated.

a Median (interquartile range).

b Number (%).

ARWMC = Age-related white matter changes; UPDRS = Unified Parkinson’s Disease Rating Scale.
the same DTI parameter in the other two segments (mean diffusivity genu $\beta = 0.16$, $P = 0.065$; mean diffusivity body $\beta = 0.17$, $P = 0.088$). These associations with the fractional anisotropy and mean diffusivity were mainly driven by changes in the radial diffusivity, but not in axial diffusivity.

**Discussion**

We found that, in subjects with cerebral small vessel disease, a lower fractional anisotropy and higher mean diffusivity in multiple regions of the white matter skeleton were associated with a lower gait performance. These regions were located both in the white matter lesions and the normal-appearing white matter. The regions that showed the highest significant relations to gait were the internal capsule and the corpus callosum, especially the genu.

Some methodological issues need to be considered. First, cerebral small vessel disease is a term used in different contexts (i.e. pathological, neuroimaging and clinical aspects). As mentioned, neuroimaging plays a central role in defining small vessel disease. White matter lesions and lacunar infarcts are widely accepted signs of cerebral small vessel disease. However, lacunar infarcts may also result from non-small vessel disease-related mechanisms, such as embolism from the heart or proximal arteries (Wardlaw, 2005). We, therefore, cannot rule out some misclassification of lacunar infarcts as small vessel disease-related in our study. As the majority of lacunar strokes are small vessel disease-related (Wardlaw, 2005) and the lacunar infarcts in our study were all accompanied by some degree of white matter lesions, favouring an underlying small vessel disease-related mechanism (Boiten et al., 1993), we feel that this misclassification would be rather small and did not greatly influence our results. Second, our results are based on cross-sectional data, which prevents us from making causal inference. Our study has a longitudinal design and follow-up is already planned to evaluate the effect of progression of small vessel disease on (changes in) gait. Third, in moderate to severe stages of small vessel disease, white matter lesions are diffusely distributed...
throughout the white matter that might lead to a diffuse loss of microstructural integrity. Hence, white matter lesions or loss of structural integrity in one region may be related to those in other regions. This prevents us from drawing conclusions about the importance of white matter lesions or loss of microstructural integrity at a specific location in relation to gait disturbances, independent of other affected white matter regions. To overcome this problem, we analysed the corpus callosum more specifically in three segments and with additional adjustment for the microstructural integrity in the other two segments. Finally, vascular risk factors, such as hypertension or diabetes, were intentionally not taken into account, as they were considered a part of the causal chain between small vessel disease and gait. Disturbances of the peripheral neuromuscular or skeletal systems were also not included as confounders, because these factors were considered as independent contributors to gait disturbances. To our knowledge, this is the first study using both voxel-wise MRI and DTI methods to investigate the pathophysiological mechanisms underlying gait disorders in small vessel disease. A further strength is the manual segmentation of the white matter lesions and quantitative assessment of gait. Finally, our study was large, with a high response, and all subjects were examined by only two investigators in a single centre.

We found that white matter lesions as well as loss of white matter microstructural integrity were associated with a lower gait velocity, mainly due to a reduction in stride length, and a broader stride width. By contrast, only a few voxels with loss of integrity (indicated by a low fractional anisotropy and high mean diffusivity) were related to a lower cadence and white matter lesions were not. We found, in our previous study, a similar lack of association between cadence and total white matter lesion volume. Only lacunar infarcts, in the frontal lobe and brainstem, were related to a lower cadence (de Laat et al., 2010). This, together with the observed discrepancy between a reduced stride length and intact cadence control in patients with Parkinson’s disease and normal pressure hydrocephalus (Stolze et al., 2001), suggests that this gait characteristic is less influenced by the white matter than stride length, at least at the supraspinal level. It would be interesting, in future studies, to unravel the underlying mechanisms of cadence control in more detail.

**Figure 3** Voxel-wise analysis of the fractional anisotropy positively associated with gait velocity, stride length and cadence and negatively with stride width. Adjusted for age, sex and height (A), or age, sex, height, white matter lesions and number of lacunar infarcts (B), thresholded at $P < 0.05$ and corrected for multiple comparisons. The statistical maps are superimposed onto the spatially normalized (Montreal Neurological Institute stereotactic space) and averaged ($N = 429$) fractional anisotropy map. R = right.
A novel finding in our study was that this relation was in line with two smaller DTI studies (Della Nave et al., 2009). The observed relation between the microstructural integrity of the genu of the corpus callosum and gait significantly weakened this relation, suggesting that the damage to this normal-appearing white matter might be, in part, small vessel disease-related. Although all participants had some degree of small vessel disease, we considered it not likely that the association between the microstructural integrity loss of the normal-appearing white matter and gait would completely disappear after adjustment for white matter lesions and cognitive control and, subsequently, to gait disturbances at older age.

Using TBSS, we found that disruption of almost all regions on the skeleton, corresponding to multiple association, projection and commissural fibres, was associated with a lower gait performance. The involvement of multiple regions is consistent with a positron-emission-tomography study in healthy individuals that reported activation in multiple cortical and subcortical regions after walking, such as the primary motor and somatosensory cortex, the parahippocampal, fusiform and occipital gyri, cerebellum and ponto-mesencephalic tegmentum and deactivations in the multisensory vestibular cortices (La Fougere et al., 2010). In addition, even more brain regions are involved in motor control in elderly than young adults (Seidler et al., 2010). Interestingly, the majority of voxels on the skeleton with loss of microstructural integrity related to gait disturbances was localized in regions were the white matter lesion probability was low or even absent. The integrity of the commissural fibres in the corpus callosum showed even the strongest significant association with gait. This highlights the importance of microstructural integrity of fibres in the normal-appearing white matter in gait disorders, in addition to white matter lesions visible on conventional MRI.

Both MRI techniques revealed that a shorter stride length was predominantly related to abnormalities in the frontal white matter (the frontal white matter lesions and the microstructural integrity of the genu). Previous quantitative volumetric MRI studies are conflicting. Srikanth et al. (2010) demonstrated that frontal white matter lesions, especially, were related to poorer gait, whereas one other small study reported this for parieto-occipital white matter lesions (Moscufo et al., 2009). The observed relation between the microstructural integrity of the genu and gait is in line with two smaller DTI studies (Della Nave et al., 2007; Bhadelia et al., 2009). A novel finding in our study was that this relation was independent of the integrity of the other corpus callosum segments. The genu is known to contain fibres connecting the bilateral prefrontal cortex (Chao et al., 2009). As the prefrontal cortex receives, among others, information from virtually all sensory systems and has preferential connections with the motor processing structures, it is proposed to play a central role in the cognitive control of motor performance (Miller and Cohen, 2001). This part of the frontal lobe was seen to be additionally involved in motor performance in elderly, by contrast to young adults (Seidler et al., 2010). Moreover, elderly are thought to rely more on bilateral activation of the frontal cortices during motor performance (Seidler et al., 2010), reflecting the importance of commissural fibres at older age. Hence, our data suggest that loss of microstructural integrity of the genu of the corpus callosum could lead to decreased cognitive control and, subsequently, to gait disturbances at older age.

As loss of microstructural integrity of fibres in the normal-appearing white matter played an important role in the gait disturbances in our subjects with small vessel disease, it would be interesting to know more about the mechanisms underlying this loss of integrity. Of note, small vessel disease as defined in our study was not a dichotomous, but rather a continuous variable and we therefore adjusted for the degree of small vessel disease (and not merely the absence or presence thereof). The introduction of white matter lesions and lacunar infarcts in the association between the microstructural integrity of the normal-appearing white matter and gait significantly weakened this relation, suggesting that the damage to this normal-appearing white matter might be, in part, small vessel disease-related. Although all participants had some degree of small vessel disease, we considered it not likely that the association between the microstructural integrity loss of the normal-appearing white matter and gait would completely disappear after adjustment for white matter lesions and cognitive control and, subsequently, to gait disturbances at older age.

### Table 2 Association between the microstructural integrity of the corpus callosum and gait

<table>
<thead>
<tr>
<th>Microstructural integrity of the corpus callosum</th>
<th>Gait velocity (m/s)</th>
<th>Stride length (m)</th>
<th>Cadence (steps/min)</th>
<th>Stride width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 1</td>
<td>Model 2</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>0.18*</td>
<td>0.19**</td>
<td>0.18*</td>
</tr>
<tr>
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<td>-0.22*</td>
<td>-0.17*</td>
</tr>
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<td>-0.06</td>
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<tr>
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<td>-0.23*</td>
<td>-0.23*</td>
<td>-0.20*</td>
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<td><strong>Body</strong></td>
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<td></td>
</tr>
<tr>
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<td>-0.03</td>
<td>0.16**</td>
<td>-0.03</td>
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<td>-0.04</td>
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<td>-0.18*</td>
<td>-0.04</td>
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<tr>
<td><strong>Splenium</strong></td>
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<tr>
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<td>0.03</td>
<td>0.17**</td>
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<td>-0.07</td>
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<td>-0.01</td>
<td>-0.21**</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

Data are standardized β-values.

Model 1 is adjusted for age, sex, height and total brain volume; model 2 is with additional adjustment for the same DTI-parameter in the other regions of the corpus callosum.

*P < 0.05; **P < 0.001.
lacunar infarcts, as it would be very unlikely that the variance in any outcome (therefore also gait disturbances) would completely be explained by the addition of just a single factor to the model. For example, it is known that different pathological mechanisms, such as vascular and degenerative diseases often coexist in one subject (Vermeer et al., 2002). This is in line with our study, as some relations in the corpus callosum still existed after adjustment for white matter lesions and lacunar infarcts, suggesting also a mechanism other than small vessel disease. Injury to the normal-appearing white matter by small vessel disease in our study may have occurred by at least two mechanisms or a combination thereof. Small vessel disease could have a direct effect on the normal-appearing white matter. This hypothesis is indirectly supported by a MRI study reporting on an increased blood–brain permeability, which is thought to play a role in small vessel disease, in the normal-appearing white matter (Topakian et al., 2009) and a pathological study showing reduced myelin staining in the normal-appearing white matter of the lateral corpus callosum (Tomimoto et al., 2004), in subjects with small vessel disease. Another possibility is the remote effect of white matter lesions on the normal-appearing white matter by antero (Wallerian) or retrograde neuronal degeneration of fibres in the surrounding normal-appearing white matter traversing these lesions, as indicated by pathological studies among patients with multiple sclerosis (Evangelou et al., 2000; Dziedzic et al., 2010). Wallerian degeneration after ischaemic stroke is, furthermore, a well-known phenomenon (Thomalla et al., 2004). White matter lesions in the frontal white matter, shown to be a predilection site of white matter lesions and relevant in gait disturbances in our study, may have at least in part, affected the microstructural integrity of the genu of the corpus callosum through this mechanism.

The pattern of changes in fractional anisotropy, mean diffusivity, and axial and radial diffusivity may provide some information on these underlying histopathological mechanisms of loss of callosal integrity in our study, although the exact (combination of) histopathological processes leading to changes in the diffusion tensors are difficult to predict in humans. A low fractional anisotropy, high mean diffusivity, with a predominantly high radial diffusivity and, to a lesser extent, axial diffusivity were associated with an impaired gait performance. This pattern is believed to be mainly determined by an increase in extra-axonal fluid and lower membrane density (Sen and Basser, 2005), e.g. as a result of loss of myelin (Song et al., 2005) or axons or axonal atrophy (Sen and Basser, 2005). Both the direct effect of small vessel disease and chronic Wallerian degeneration secondary to white matter lesions can mirror this DTI pattern. Direct effects of small vessel disease on the normal-appearing white matter can result in an increased blood–brain permeability and hence, in an accumulation of fluid in the extracellular space, leading to a predominant increase in radial diffusivity. Conversely, our results are also in agreement with previous DTI studies in chronic Wallerian degeneration (Concha et al., 2006), although a reduction in axial diffusivity has also been described in this process (Pierpaoli et al., 2001). At present, we therefore can only speculate on the mechanisms responsible for the observed association between the microstructural integrity of the normal-appearing white matter and gait.

In conclusion, our study provides new insight into the pathophysiology of gait disturbances in elderly with small vessel disease. It is likely that they are attributable to loss of microstructural integrity of multiple white matter fibres connecting different cortical and subcortical regions, mediating intra- and, particularly, inter-hemispheric integration of motor and sensory signals. Especially, damage to the callosal fibres of the genu projecting to the prefrontal areas is thought to be important, which might indicate loss of cognitive control on motor processing. DTI, e.g. by using TBSS, complements voxel-based morphometry analysis of the white matter lesions as DTI reveals functionally relevant injury of white matter integrity underlying gait disturbances in these subjects not detected by conventional MRI and is as such a promising method in examining the consequences of cerebral small vessel disease. As cerebral small vessel disease is treatable (Dufouil et al., 2005), and probably direct effects of small vessel disease on the normal-appearing white matter also, future studies are needed to elucidate the underlying mechanism of abnormalities in the normal-appearing white matter in subjects with small vessel disease.

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**References**


