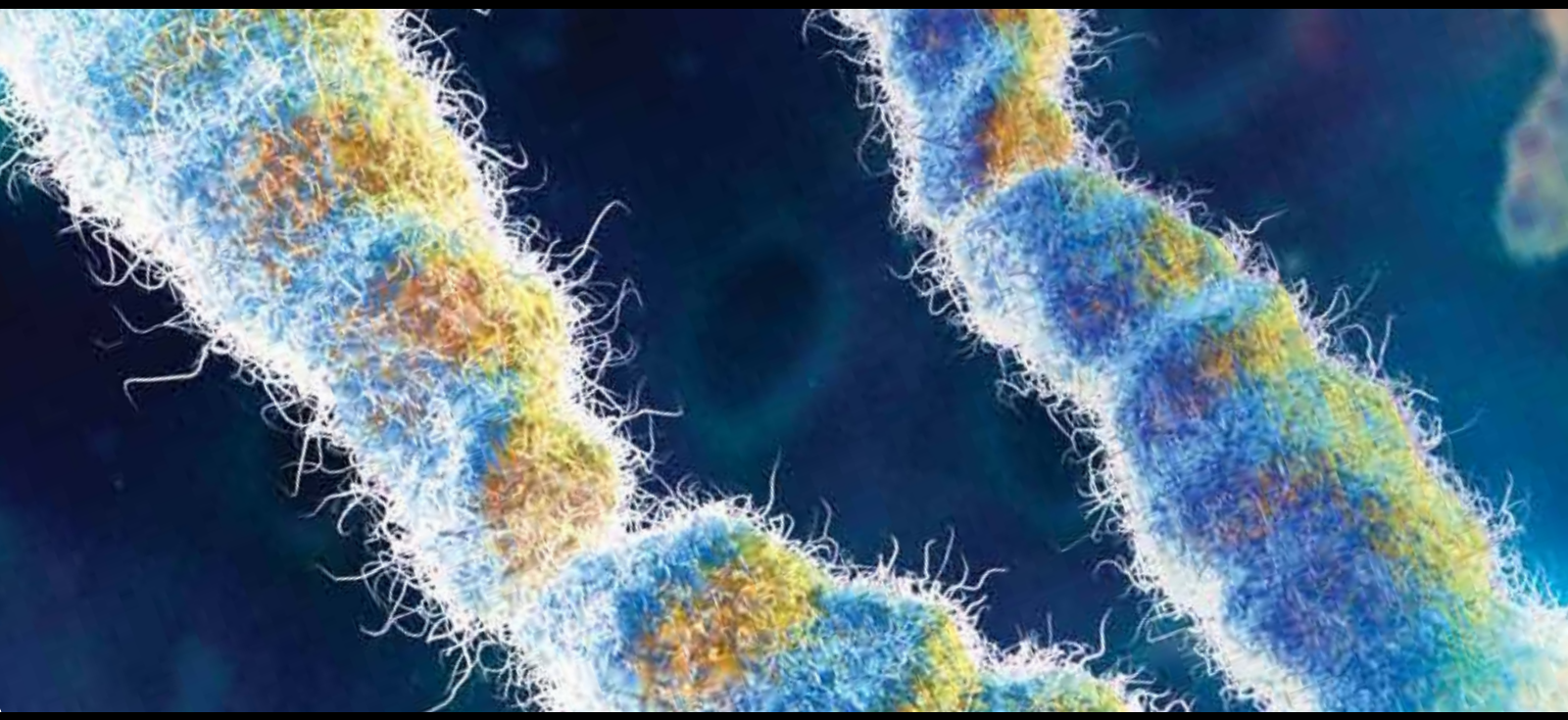
Journal of Aging Research

White Matter Changes: New Perspectives on Imaging, Clinical Aspects, and Intervention

Guest Editors: Sofia Madureira, Ana Verdelho, Leonardo Pantoni, and Philip Scheltens



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Editorial Board

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 841913, 2 pages
doi:10.4061/2011/841913

Editorial

White Matter Changes: New Perspectives on Imaging, Clinical Aspects, and Intervention

Sofia Madureira,[1] Ana Verdelho,[1] Leonardo Pantoni,[2] and Philip Scheltens[3]

[1] *Department of Neurosciences and Mental Health, Hospital de Santa Maria, University of Lisbon, 1649-004 Lisbon, Portugal*
[2] *Department of Neurological and Psychiatric Sciences, University of Florence, 50121 Florence, Italy*
[3] *Department of Neurology and Alzheimer's Center, VU University Medical Center, 1007 MB Amsterdam, The Netherlands*

Correspondence should be addressed to Sofia Madureira, pmadureira@fm.ul.pt

Received 4 October 2011; Accepted 4 October 2011

In the last three decades, the development of more sensitive techniques used in the assessment of *in vivo* brain functioning, such as computed tomography (CT) scan and magnetic resonance imaging (MRI), has contributed to identify cerebral changes that are either associated with clinical features (dementia and cognitive impairment) or described in normal subjects [1].

The presence of age-related white matter changes (ARWMCs) is described with the increasing age and the presence of vascular risk factors as the major determinants for these findings [2–4], but also in subjects over 50 years old [5].

The research of the clinical significance of ARWMC has clearly demonstrated the association between the presence of white matter changes and cognitive impairment, behavioural changes, urinary disturbances, and gait difficulties. However, and despite the increasing interest in this field, the interpretation of the data and explanation of these mechanisms are still under discussion.

The aim of the current special issue was to explore the heterogeneity of the areas of impact of the ARWMC, by including different and less frequent approaches that allow for a more comprehensive picture.

Focused in this general goal, we included a variety of papers ranging from an overview of the pathophysiology, assessment, and clinical manifestations of ARWMC, as well as a complete revision of proposed treatments—illustrated by the review of Y. Y. Xiong and V. Mok—to the proposal of a mouse model of chronic cerebral hypoperfusion as a possible tool on the explanation of the molecular pathology of white matter lesions—presented by M. Ihara and H. Tomimoto. With the same perspective, we included the paper of C. Sierra et al. that presents a nice review about the relation between hypertension and long-standing effect in the cerebral white matter. L. Yang reviewed issues related to brain plasticity and its possible relation to age-related white matter changes, a topic of novel interest. Also of interest is the work of A. Xekardaki et al. that explores the use of diffusion tensor imaging in bipolar disorder.

Besides the review papers, 8 original articles were included in this special issue and are grouped in three blocks. The first one tried to focus on the anatomical correlations and clinical features that are related with the presence and progression of white matter changes—cognitive changes (memory), motor dysfunction, late-onset depression, and health-related quality of life. E. T. Schulze et al. provided us with an innovative approach by using multifaceted imaging techniques on the evaluation of brain functional changes during working memory tasks. In a less addressed field of research, M. Viana-Baptista et al. reflected on the importance of frontal white matter lesions for the compromise of motor performance. J. A. Brommelhoff et al. also pointed out that late-onset depression could be a process different from neurodegenerative changes and describe no differences in frontal lobe deep or subcortical white matter between Alzheimer's patients with or without late-onset depression. A. M. Grool et al. discussed the relation between progression of WMC volume and health-related quality as part of the

large SMART Study adding data to the existing body of literature on the role of progression of white matter lesions on decline in mental functioning in patients with symptomatic vascular disease. A very interesting paper by V. Rajagopalan et al. reports innovative imaging data in amyotrophic lateral sclerosis (ALS) patients with predominant upper motor neuron (UMN) signs.

A second section, mainly addressed to MRI analysis and measurement of white matter changes, is represented in the paper by S. D. Smart et al., who present the validation of automated WMH segmentation. These authors showed that automated segmentation of the brain provides good to very good estimates of the volume of the affected white matter, which will enable further research into the role of ARWMC in various diseases.

Finally, the last block of papers refers to different basic sciences, reflected both on the interesting paper of H. Chen et al. that evidences reductions in fiber tract volumes and loss of axonal neurofilaments, and on the paper by B. D. James et al., that brought surprising findings on genetic precursors of dementia and led us into an open discussion for possible future directions.

Sofia Madureira
Ana Verdelho
Leonardo Pantoni
Philip Scheltens

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 286564, 10 pages
doi:10.4061/2011/286564

Review Article

White Matter Changes in Bipolar Disorder, Alzheimer Disease, and Mild Cognitive Impairment: New Insights from DTI

Aikaterini Xekardaki,[1] Panteleimon Giannakopoulos,[1,2] and Sven Haller[3]

[1] *Division of Mental Health and Psychiatry, Department of General Psychiatry, University Hospitals of Geneva, Chemin du Petit-Bel-Air, Geneva, Switzerland*
[2] *Division of Old Age Psychiatry (PG), University of Lausanne School of Medicine, Lausanne, Switzerland*
[3] *Service Neuro-Diagnostique et Neuro-Interventionnel DISIM, University Hospitals of Geneva, Switzerland*

Correspondence should be addressed to Aikaterini Xekardaki, aikaterini.xekardaki@hcuge.ch

Received 15 April 2011; Revised 10 August 2011; Accepted 5 September 2011

Academic Editor: Sofia Madureira

Neuropathological and neuroimaging studies have reported significant changes in white matter in psychiatric and neurodegenerative diseases. Diffusion tensor imaging (DTI), a recently developed technique, enables the detection of microstructural changes in white matter. It is a noninvasive *in vivo* technique that assesses water molecules' diffusion in brain tissues. The most commonly used parameters are axial and radial diffusivity reflecting diffusion along and perpendicular to the axons, as well as mean diffusivity and fractional anisotropy representing global diffusion. Although the combination of these parameters provides valuable information about the integrity of brain circuits, their physiological meaning still remains controversial. After reviewing the basic principles of DTI, we report on recent contributions that used this technique to explore subtle structural changes in white matter occurring in elderly patients with bipolar disorder and Alzheimer disease.

1. Introduction

White matter (WM) comprises 40–50% of the adult human brain. At a macroscopical level, it consists of collections of tightly wrapped axons that connect different brain regions. At a biochemical level, WM is mainly formed by myelin, a multilayer sheath of proteins (30%) and lipids (70%) around the axons. Small unwrapped regions called Ranvier nodes serve to mediate the salutatory conduction of the electrical impulse and enhance the velocity of conduction [1]. Neuropathological studies [2] indicate that myelination continues until at least the third decade of life. Other scientists found that WM increases in a roughly linear way until at least the age of 20 [3]. *In vivo* studies using volumetric imaging showed that WM volume increases in frontal and temporal lobes by the fourth decade of life [4, 5] and then steadily decreases. A neuropathological study found WM volume reduction by 28% as a function of age [6]. Interestingly, an assessment of WM volume in piano players suggests that practicing induces plasticity in early age when fiber tracts are still under maturation [7]. The development of modern MR techniques allowed for documenting WM changes in several neurological and psychiatric entities. In this paper, we describe a recent *in vivo* noninvasive technique for WM imaging referred to as diffusion tensor imaging (DTI) and explore its relevance in bipolar disorder and Alzheimer disease. The goal of this paper is to develop the different parameters obtained with DTI, the principal analysis methods, as well as the correlation of DTI-derived parameters with clinical and neuropathological findings in human brains and animal models. We will refer to the application of DTI on Alzheimer disease (a neurodegenerative disease with well-established associated morphological abnormalities in particular in the hippocampal region) and bipolar disorder (a psychiatric disease with currently disputed associated morphological abnormalities) for detection of white matter changes. The choice of these two entities is related to our previous and actual work in these fields of research and our recent work using DTI in bipolar disorder [8]. We included articles analyzing the basic principles of

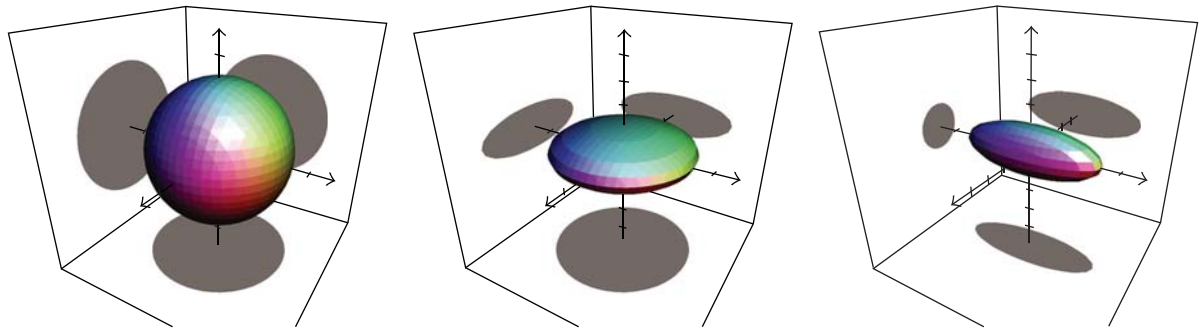

Figure 1: This illustrates the basic tensor shapes of diffusion tensor imaging (DTI). If the diffusion is not restricted, the resulting tensor is a sphere (a). If the diffusion is restricted in only one direction, the resulting tensor is lens-shaped (b). If the diffusion is restricted in two directions, the resulting tensor is cigar-shaped (c).

the technique, its correlation to neuropathology as exposed from animal models and human brain banks. DTI, white matter changes, bipolar disorder, AD, and mild cognitive impairment were the key words used to search for articles of DTI applications on these medical entities in Pubmed. In the discussion part, our aim is to provide authors with a critical review of advantages and disadvantages of the DTI technique and its different ways of data analysis, as well as future directions of improvement.

2. Diffusion Tensor Imaging

Diffusion tensor imaging is a promising technique that evaluates *in vivo* brain structure, especially white matter integrity. DTI was originally presented in 1994 [9, 10] and takes advantage of the fact that MR images are essentially sensitive to water protons. Molecular diffusion refers to the Brownian random motion resulting from the thermal energy carried by these molecules [11]. Diffusion is a three-dimensional process. Water is the most convenient molecule to study with MRI. Water proton displacement depends on surrounding tissue microstructure. In cerebrospinal fluid (CSF), water molecules move equally in all directions in space and the resulting diffusion tensor is isotropic. In contrast, diffusion is anisotropic in white matter. Water follows a path along the white matter fiber that is constrained by barriers such as the myelin sheath causing movement to be greater along the long axis of the fiber than perpendicular. Thus, axial diffusion along the fiber is greater than radial diffusion across the fiber [12, 13]. The degree of anisotropy can be expressed by the fractional anisotropy (FA). This is an absolute value that ranges from zero (diffusion equal in all directions resulting into a spherical diffusion tensor, see Figure 1) to 1 (diffusion only in one direction yet zero in the other orthogonal directions resulting in a cigar-shaped tensor of unlimited length). To quantify anisotropic diffusion, the computation of a tensor is required based on data from at least six or more noncollinear gradient directions. The diffusion tensor is a three-dimensional ellipsoid depicting the magnitude and orientation of diffusion in an individual voxel (Figure 1). The ellipsoid has three axes called eigenvectors corresponding to the three orientations of the tensor, and their lengths are called eigenvalues. The longest eigenvalue pointing along the axon direction is called $\lambda 1$ or axial diffusivity, and the two small axes orthogonal to the long one are called $\lambda 2$ and $\lambda 3$. By using the diffusion tensor imaging model we assess the following parameters.

2.1. Axial and Radial Diffusivities. The diffusivity parallel to the principal axis of the axon within a voxel of interest is called longitudinal or axial diffusivity or $\lambda 1$ [10]. Radial diffusivity represents diffusivity perpendicular to the first eigenvector: $\lambda\tau$: $(\lambda 2 + \lambda 3)/2$. Song et al. used a mammalian model (shiverer mouse) that has incomplete myelin formation without any signs of axonal damage or inflammation to find direct associations of directional diffusivity changes with pathological findings [13]. Radial diffusivity increased significantly in these otherwise intact axons reflecting the freer movement of water molecules related to the reduction of myelin. No change of axial diffusivity was reported, suggesting that radial diffusivity could serve as a biomarker of myelin loss or damage. The same group of scientists used a mouse model of retinal ischemia that provokes axonal degeneration in optic nerve and correlated DTI and pathological findings [14]. They found a significant decrease of axial diffusivity, but not radial diffusivity, 3 days after ischemia coinciding with detectable axonal degeneration but with no demyelination. An increase of both axonal and radial diffusivity was reported on the fifth day associated with myelin degeneration at this time. Thus, axial diffusivity is thought to correspond to axonal damage and radial diffusivity to myelin damage. These findings were further confirmed by more recent studies [15, 16], yet basic research is still needed in this domain.

2.2. Mean Diffusivity. Mean diffusivity represents the average magnitude of a tensor's water diffusion and is equal to the average of the three eigenvalues $(\lambda 1 + \lambda 2 + \lambda 3)/3$. Mean diffusivity is the mean molecular motion in a certain voxel, but it provides no elements regarding diffusion directionality. To date, the physiological correlates of this DTI parameter are not well understood.

2.3. Fractional Anisotropy. The most commonly used DTI parameter assessed in brain research is fractional anisotropy (FA) [11]. Fractional anisotropy represents the normalized standard deviation of the three diffusivities and is thought to be a marker of WM integrity. In CSF, where diffusivity is equal in all directions, the FA index is zero. In WM, FA increases, showing fast diffusivity along the fibers and a slow diffusivity perpendicular to them. The microstructural changes corresponding to anisotropy changes in WM tissues still remain quite unclear. Decreased FA has been described in tissues with demyelination, edema, gliosis, and inflammation. Myelin is a characteristic anatomical feature of white matter and is thought to play a crucial role in DTI signal. Beaulieu and Allen reported that anisotropy is observed in nonmyelinated fibers as well. The water diffusion of nonmyelinated olfactory nerve was similar to myelinated trigeminal one in garfish [17, 18]. Wimberger et al. revealed anisotropic water diffusion in pup rats in not myelinated tissues [19]. Gulani et al. compared diffusion anisotropy of myelin deficient rats and age-matched controls. They concluded that myelin is not a required factor for the presence of diffusion anisotropy (consistent with the above studies), but its presence plays a major role in diffusion anisotropy generation in WM [20].

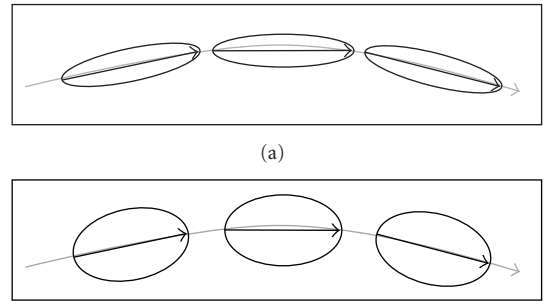

Figure 2: Schematic illustration of deterministic tractography, in a normal subject (a), three adjacent voxels have a clearly directed primary diffusion direction (longitudinal diffusion) indicated as ellipsoidal tensor. A deterministic tractography analysis would result in the indicated tract. Another subject, for example, a patient with a neurodegenerative disease (b) might have a reduction of the directivity of diffusion, evident as less ellipsoidal and more spherical tensors. The direction of the principal direction is however unchanged. This explains why a deterministic tractography analysis may result in the same reconstructed tract (primary direction unchanged) although the diffusion tensor is less ellipsoidal (reduced FA value).

3. Types of DTI Data Analyses

We will summarize most commonly used methods of DTI data analysis.

3.1. ROI Analysis of Diffusivity Values (e.g., FA or MD). Region of interest analysis consisting of manually designed or template-based comparison of specific regions among different subjects. They are used in order to calculate and compare DTI parameters in a specific region implicated in a disease. The advantage of this method is the simplicity and the high sensitivity because multiple voxels within a given ROI are averaged thereby enhancing the signal to noise ratio. The disadvantages of this method are that it is time consuming and operator-dependent when regions of interest are drawn manually. Furthermore, as the ROI is designed manually, it does not necessarily correspond to the anatomic borders of a given area. Voxels with significant differences can be averaged with other voxels without differences, masking their effect.

3.2. White Matter Tractography

3.2.1. Deterministic Tractography. White matter tractography is an alternative method to describe 3D patterns of WM connections [9, 21, 22]. The most commonly implemented methods are deterministic tractography approaches using the first eigenvector to estimate the trajectory of a white matter fiber. The analysis starts from a certain point or ROI and progresses voxel by voxel along the first eigenvector along the white matter fiber in order to estimate the anatomical trajectory of a bundle within the limits predefined parameters. The most important parameters are the minimum FA value (directivity of a voxel) and acceptable angle between the two first eigenvectors of two adjacent voxels. Alternatively, fibre tracts can be calculated that connect two or more predefined ROIs. The advantage of this method is the very high illustrative value of the resulting tracts. The disadvantages of this method include definition of one or multiple ROI and the resulting operator-dependency and dependence of the reconstructed "tracts" on tractography parameters. The sensitivity of tractography analysis may be lower compared to direct assessment of FA or other diffusion parameters, for example, in the domain of Alzheimer disease [23]. The authors found significant differences in FA and diffusivity in Alzheimer disease compared to controls yet no significant differences in deterministic tractography in the same regions. This can be explained by the fact that deterministic tractography uses the principal direction of the first eigenvector to calculate fiber tracts. Note that FA may decrease in a given disease, yet as long as the primary direction of the diffusion tensor remains unchanged, the resulting deterministic tractography remains unchanged (see Figure 2).

An additional problem of deterministic tractography is the difficulty to reconstruct trajectories across regions with crossing fibres. Possibilities to overcome this limitation include assessment of higher-order diffusion images such as Q-ball imaging [24] or diffusion spectrum imaging (DSI) [25]. The basic principle of these "second generation" diffusion techniques is the acquisition of higher resolution imaging with several diffusion directions within each voxels, thereby trying to overcome the crossing fiber problem. The resulting data acquisition time is however considerably longer than conventional DTI sequences with the resulting motion artifacts mainly in elder patients.

3.2.2. Probabilistic Tractography. Other possibilities to overcome the crossing fiber problem are probabilistic tractography approaches [26]. In contrast to the deterministic

tractography described above, probabilistic approaches do not follow a trajectory along the first eigenvector from voxel to voxel in a deterministic approach, yet calculate the probability with which two voxels or regions are connected. While such analyses may successfully overcome the crossing fiber problem, yet the resulting images are clearly less intuitive than those of the deterministic tractography.

3.3. TBSS: Tract-Based Spatial Statistics. A recent method was developed by Smith et al. [27] that projects all individual DTI parameters onto a group average white matter skeleton. The advantages of this method is the alignment of all subjects' FA images in order to create a "mean FA skeleton," that is, a group average brain skeleton of the major WM tracts. This technique provides us with data of the whole brain and is operator-independent. The disadvantages include the multitude of required processing steps. Additionally, the resulting images are less intuitive than the deterministic tractography described above. Moreover, this technique is aimed for group level analysis but not analysis of individual patients.

4. Applications of DTI Analyses

In order to illustrate the relevance of this new technique in the exploration of the structural correlates of major psychiatric illnesses, we will focus the following chapters on DTI application in bipolar disorder and Alzheimer disease.

4.1. Bipolar Disorder. Bipolar disorder is a psychiatric illness affecting 1–3% of the population. It is characterized by alternating depressive and maniac phases. Several structural studies support the hypothesis of neurodevelopmental disruptions in early life implicated in the pathophysiology of bipolar disorder [28]. Different neurodevelopmental models suggest that deficits of maturational processes in adolescence in combination with early developmental changes result in psychiatric illness [29]. MRI imaging has provided us with valuable information concerning neuroanatomical abnormalities in bipolar patients during the past decades. Findings from structural and functional neuroimaging suggest that the basis of mood dysregulation results from disruptions along the frontocortical-striatal-thalamic circuits [30, 31]. Consistent with this hypothesis, a neuropathological study revealed reduced volumes of the left nucleus accumbens, bilateral external pallidum, and right putamen [32]. At the microscopic level, neuropathology revealed changes in glial density and neuronal abnormalities in the prefrontal and anterior cingulate cortex of patients with bipolar disorder [33, 34]. The majority of the structural brain research of bipolar disorder concerned gray matter. The role of fiber tracts interconnecting cortical and subcortical regions remains to be elucidated because their potential lesions could be implicated in brain dysfunctioning. White matter hyperintensities (WMH) on T2-weighted and fluid attenuated inversion recovery (FLAIR) images have been repeatedly reported during the last years in bipolar disorder. MRI signal hyperintensities in deep white matter were first described by Dupont et al., and their presence was associated with an increased number of hospitalizations [35]. Since then several scientists examined the prevalence of WMH in subjects with bipolar disorder. Most results show an increased prevalence in subjects with bipolar disorder [35–38]. White matter hyperintensities have been associated with cardiovascular risk factors such as hypertension [39] as well as advanced age [37, 40]. An MRI study of children and adolescents revealed a higher risk and severity of WM lesions in children with bipolar disorder located predominantly in the frontal lobes [41]. Lesions described in bipolar disorder were mostly located in the deep white matter [36, 37, 42], recent meta-analysis showed that patients with bipolar disorder had 2.5 times more deep white matter hyperintensities compared to controls [43]. WMH lack specificity having been associated with other illnesses and, thus, cannot be a specific marker for bipolar disorder.

There have been 19 published DTI studies to identify WM changes in subjects with bipolar disorder. Results are highly heterogeneous reporting decreased FA in frontal and prefrontal regions in adolescents, children [44, 45], and adults [46]. Other studies revealed increase in MD [47] and ADC [48] in prefrontal and frontal regions of adults. Regenold et al. was the first to report higher ADC [49] in 8 different ROIs of WM of a bipolar disorder population suggesting microstructural alterations in WM. A recent study by Versace et al. [50] demonstrated abnormal right versus left asymmetry in FA in BD subjects in the orbitomedial prefrontal white matter. DTI research of projection fibers showed reduced FA in the posterior [51] and anterior [52] limbs of the internal capsule in bipolar patients. Mahon et al. [53] first performed a voxel-wise analysis of FA to detect group level differences in FA between BD and control subjects, and then used the identified regions as seed regions for deterministic tractography. The reconstructed tracts included the pontine-crossing tract, corticospinal/corticopontine tracts, and thalamic radiation fibers, consistent with the concept that bipolar disorder implicates dysregulation of cortico-subcortical and cerebellar regions. This study revealed equally reduced FA in left cerebellum consistent with previous studies implicating cerebellar abnormalities in the model of bipolar disorder [28, 31, 54]. Increased FA of thalamic radiation was also described by Versace et al. [50], though a ROI, analysis by Sussmann et al. [52], showed decreased FA in the superior thalamic radiation fibers of patients. A small number of studies examined association fibers and found decreased FA in the uncinate fasciculus [50] connecting the frontal and temporal lobes. Higher or lower FAs were equally described in the superior longitudinal fasciculus [55, 56]. The corpus callosum which connects the two hemispheres was found to have a decreased FA in the rostrum and body of the corpus callosum [44, 57]. Under the assumption of increased cerebral structural abnormality during disease progression and ageing, we recently examined 19 euthymic elderly bipolar patients and 47 controls in a combined analysis of VBM and TBSS. This study found a significant decrease of FA in the ventral part of corpus callosum in patients with bipolar disorder. The VBM analysis of grey matter demonstrated a reduction of grey matter density in bipolar patients in the right anterior insula, the head of the caudate nucleus, nucleus accumbens,

ventral putamen, and frontal orbital cortex as compared to controls. There was no significant group difference in TBSS data of bilateral uncinate fasciculus, anterior, and posterior cingulum. The WM alterations assessed using DTI in this study were more sensitive than changes in grey matter assessed using voxel-based morphometry (VBM) in bipolar disorder [8].

In summary, white matter alterations in BD are very heterogeneous, relating probably to different patient populations and data acquisition and analysis. Nevertheless, there is a trend towards impaired white matter integrity in BD in particular in frontal regions.

4.2. MCI and Alzheimer Disease. Alzheimer disease is the most common form of dementia. Mild cognitive impairment is characterized by memory complaints reported by the patient, preserved cognition, and autonomy in daily activities in life [58, 59]. Mild cognitive impairment has been classified to two subtypes: amnestic (memory deficits) and nonamnestic (other cognitive deficits) [58]. Patients with amnestic MCI are thought to present prodromal lesions of Alzheimer disease [60] and convert to Alzheimer over the years when compared to elderly population without cognitive decline [61, 62].

Alzheimer disease is characterized by the formation of extracellular neurofibrillary tangles and senile plaques. These lesions are found in the cognitively intact subjects as well [63–69], and it still remains unclear if their presence is part of the disease process or the ageing process. Most MR imaging studies have focused their interest on grey matter changes related with the AD. Additional to grey matter abnormalities such as diffuse cortical and hippocampal atrophy [70], white matter damage has been described in several neuropathological [71–73] and neuroimaging [74] studies. Several hypotheses have been proposed concerning the pathophysiology of white matter damage. The first hypothesis is the one of Wallerian degeneration occurring after neuronal loss meaning that white matter damage follows grey matter damage in the same regions [75]. A second hypothesis called retrogenesis suggests that the latest myelinated regions are the more vulnerable ones and that degeneration occurs in the reverse pattern of myelogenesis [76]. The third hypothesis involves vascular damage contribution to white matter pathology [77]. With respect to this last hypothesis, MRI white matter hyperintensities reflecting small vessel disease have been associated with AD. The cognitive impact of these lesions located in periventricular regions and deep white matter remains controversial [78, 79]. It has been suggested that their location is the key element of their implication in cognition [80].

DTI has been used to describe and understand white matter lesions in patients with Alzheimer disease and MCI, as well as normal aging. We will not review in detail DTI findings in normal elderly subjects [46, 81]. We will focus our interest on DTI findings of MCI and AD subjects and their correlation to underlying pathophysiological mechanisms. Numerous studies showed a heterogeneous pattern of changes in mean diffusivity and fractional anisotropy. Increased MD was reported in regions including frontal [82, 83] and temporal lobes [82–87], parahippocampal white matter [84, 88, 89], and the posterior cingulum [88–91]. Decreased FA was reported in the same regions by most scientists [84, 87, 89, 91–93] as well as in white matter tracts such as the superior longitudinal fasciculus [90]. FA differences between MCI and AD have been reported in temporal [92] and posterior cingulum regions [88] reflecting a more widespread WM pathology in AD. Interestingly, a recent study revealed that the decrease of FA in the posterior cingulum tract was associated with all four cognitive domains (memory, language, attention, and visual-spatial processing). This is in agreement with functional MRI studies proposing that posterior cingulate cortex functions as the main connectivity network during resting-state fMRI, with the posterior cingulate being a key structure in the default mode network [94].

The underlying pathophysiology of WM damage still remains under debate. Huang et al. [92] showed that patients with AD presented a pattern of reduced axial diffusivity and increased radial diffusivity in temporal lobe consistent with axonal loss damage and Wallerian degeneration. Similar observations were described by other research teams [82]. These findings are consistent with neuropathological loss of myelinated axons at histopathological studies of postmortem brains [95]. A DTI study of a mouse model revealed demyelination in corpus callosum and axonal loss in the other white matter tracts [96]. Retrogenesis has been supported by several authors as a possible degeneration pattern reflecting a vulnerability of late-myelinated regions in AD evolution [97, 98]. Naggara et al. investigated white matter damage with DTI, and their findings correspond to the retrogenesis hypothesis model with a decreased FA in the WM of temporal, frontal lobe, and the splenium [99]. Other published papers supported this hypothesis as well [100–102].

A study by Lee et al. tested the hypothesis of vascular factors' contribution to degeneration by focusing both on normal-appearing white matter and white matter hyperintensities in controls, MCI and AD subjects and by integrating etiologic contribution of vascular risk and degenerative processes in FA changes [103]. They found that AD subjects had a significantly lower FA in highly organized fibers. On the opposite, vascular risk factors had an impact on less organized fibers in both normal appearing WM and WMH. Decreased FA in WMH regions was not associated with vascular risk or diagnosis, implying that these lesions represent an extreme, diffuse lesional consequence of white matter. Similar to bipolar disorder discussed above, assessment of white matter changes using DTI is more sensitive than grey matter changes assessed by using VBM [97].

In summary, white matter changes can be readily assessed using DTI in MCI and Alzheimer disease, showing a widespread deterioration of multiple DTI bases parameters in widely distributed networks clearly exceeding hippocampus and parahippocampal regions. Recent investigations found evidence supporting both, the secondary Wallerian degeneration hypothesis following neuronal loss and retrogenesis hypothesis, suggesting that a combination of both mechanisms may be present in MCI and AD.

5. Discussion and Future Directions

Findings of DTI parameters remain rather heterogeneous and contradictory both in bipolar illness and Alzheimer disease. White matter changes assessed by DTI affect various regions in the brain, indicating that these diseases affect several cortical circuits consistent with the idea that neurons in a given cortical region are connected with axons in distributed networks. A number of recent investigations that simultaneously assessed grey matter (VBM) and white matter (DTI) in various neurodegenerative diseases consistently reported higher level of significance of group differences of DTI as compared to VBM analysis [8, 97, 104]. This suggests that the DTI assessment of white matter changes is more sensitive than VBM assessment of grey matter changes in neurodegenerative diseases. Future studies are needed to determine whether this is due to more pronounced disease-related changes in white matter or due to a higher sensitivity of the different methods which might more readily detect white matter changes due to, for example, higher signal to noise ratio of the data measurement.

Despite *in vivo* and *ex vivo* animal and human studies, the correlation of the different DTI parameters and pathological lesions of white matter diseases still needs further clarification. Human studies with DTI differ in sample characteristics, sample size, and techniques of data analysis. For example, brain regions including crossing fiber tracts, such as the rostral pons, revealed no (or marginal) changes in diffusion anisotropy, yet an important change in fiber orientation [105]. The need for MR correlations with neuropathology is imperative in the future to better understand and interpret changes in FA, MD, AD, and RD. *Ex vivo* images have a high quality resolution to detect structural changes. DTI use in postmortem brains is complicated by the fact that water diffusion features changes dramatically postmortem, especially after brain fixation. Sun et al. compared calculated axial and radial diffusivity in a retinal ischemia mouse model *in vivo* and *ex vivo* [106]. They found that *ex vivo* radial diffusivity is comparable to *in vivo* in the detection of myelin changes. Axial diffusivity changes were not present in *ex vivo* samples. The same group showed that changes of DTI parameters' sensitivity is due to the fixation of brain tissues rather than the delay between death and their fixation [107].

The use of prefixed brain tissues is an alternative to neuropathology. Schmierer et al. compared histological changes such as myelin content, axonal count, and gliosis with DTI measurements of MD and FA in unfixed postmortem multiple sclerosis brains. They found a decrease of FA and MD that correlated with myelin content and to a lesser degree with axonal count [108]. FA remains the most commonly used parameter representing white matter integrity. Klawiter et al. investigated axial and radial diffusivity correlation with histopathological findings in multiple sclerosis (MS) postmortem brains [109]. They revealed a sensitivity of radial diffusivity in the detection of demyelination, but no correlation between axial diffusivity and axonal loss. Despite these discrepancies, the use of axial and radial diffusivity can be an essential aid in the interpretation of FA changes in the future.

5.1. Group-Level versus Individual Classification Analyses. Most neuroimaging studies use group comparisons to explore the biological substrates of a disease. However, this type of comparison does not provide individual markers of clinical evolution. In the case of MCI, an individual prediction of conversion to AD would be of great interest since not all MCI patients evolve to AD.

In a very recent study, Haller et al. [110] reported significant DTI differences between stable MCI versus progressive MCI subjects. They assessed neuropsychologically 35 controls and 67 MCI subjects among whom 40 were stable and 27 progressive. FA, MD, RD, and LD were measured using TBSS. FA was significantly higher in controls compared to MCI in a network involving the corpus callosum, right temporal and frontal pathways. No significant difference was found between stable versus progressive MCI. Support vector machines (SVMs) [111] have been recently used to provide us with individual risk scores concerning MCI conversion to AD. Haller et al. used TBSS preprocessed DTI FA data and subsequent individual SVM classification in MCI and controls. The accuracy of the individual classification of controls versus MCI was up to 91.4% and stable versus progressive MCI was over 97%. Their results suggest that one SVM classifier may be sufficient to discriminate stable versus progressive cases even if the neuropsychological profile of MCI subgroups is unknown at the time of SVM analysis.

6. Conclusions

DTI is an interesting noninvasive *in vivo* neuroimaging method to assess white matter. Despite the heterogeneity of the experimental data, the increasing application and development of DTI in central nervous system pathologies (such as bipolar disorder and Alzheimer disease) including the combination analysis of the tensor's parameters (axial, radial, mean diffusivity, and fractional anisotropy) may provide in the near future potential biomarkers for early and differential diagnosis of these conditions. Further basic research with animal models and postmortem brain tissues is required to establish a better comprehension of the correlations between DTI findings and microscopic changes in white matter.

Abbreviations

AD: Alzheimer disease
ADC: Apparent diffusion coefficient
DSI: Diffusion spectrum imaging
DTI: Diffusion tensor imaging
CSF: Cerebrospinal fluid
FA: Fractional anisotropy
MCI: Mild cognitive impairment
MD: Mean diffusivity
MRI: Magnetic resonance imaging
RD: Radial diffusivity
SVM: Support vector machines
VBM: Voxel-based morphometry
WMH: White matter hyperintensities.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 606871, 9 pages
doi:10.4061/2011/606871

Research Article

Anatomical Correlates of Age-Related Working Memory Declines

Evan T. Schulze,[1] Elizabeth K. Geary,[1] Teresa M. Susmaras,[1] James T. Paliga,[1,2] Pauline M. Maki,[3,4] and Deborah M. Little[1,3,5,6,7,8]

[1] *Department of Neurology and Rehabilitation, University of Illinois at Chicago, Chicago, IL 60612, USA*
[2] *Duke University College of Medicine, Durham, NC 27704, USA*
[3] *Department of Psychology, University of Illinois at Chicago, Chicago, IL 60612, USA*
[4] *Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612, USA*
[5] *Department of Anatomy & Cellular Biology, University of Illinois at Chicago, Chicago, IL 60612, USA*
[6] *Department of Ophthalmology & Visual Sciences, University of Illinois at Chicago, Chicago, IL 60612, USA*
[7] *Department of Veterans Affairs, VISN 17 Center of Excellence for Research on Returning War Veterans, Waco, TX 76711, USA*
[8] *Texas A&M Health Sciences, College of Medicine, Bryan, TX 77807, USA*

Correspondence should be addressed to Deborah M. Little, deborah.little2@va.gov

Received 16 March 2011; Revised 26 August 2011; Accepted 29 August 2011

Academic Editor: Sofia Madureira

Aging studies consistently show a relationship between decreased gray matter volume and decreased performance on working memory tasks. Few aging studies have investigated white matter changes in relation to functional brain changes during working memory tasks. Twenty-five younger and 25 older adults underwent anatomical magnetic resonance imaging (MRI) scans to measure gray matter volume, diffusion tensor imaging (DTI) to measure fractional anisotropy (FA) as a measure of white matter integrity, and functional magnetic resonance imaging (fMRI) while performing a working memory task. Significant increases in activation (fMRI) were seen in the left dorsal and ventral lateral prefrontal cortex with increased working memory load and with increased age (older showing greater bilateral activation). Partial correlational analyses revealed that even after controlling for age, frontal FA correlated significantly with fMRI activation during performance on the working memory task. These findings highlight the importance of white matter integrity in working memory performance associated with normal aging.

1. Introduction

Although there is some debate about the magnitude of age-related effects on gray matter (GM) and white matter (WM), it is generally accepted that both GM and WM volumes decline with advanced age [1–3]. Furthermore, declines in certain cognitive skills are also anticipated with advanced age [4]. Examinations of cortical volume and behavior suggest a relationship between volume loss and declines in cognitive skills. In particular, fluid-intelligence skills such as working memory, believed largely mediated by frontal-subcortical structures [4–8], appear particularly susceptible to age-related changes [9–12]. Multiple neuroimaging techniques have been utilized in isolation to examine the diffuse neural networks supporting complex behaviors, but only recently have multimodal imaging techniques such as standard structural imaging, functional magnetic resonance imaging (fMRI), and diffusion tensor imaging (DTI) been utilized concurrently to elucidate the relationship between volume loss, white matter integrity, and declines in cognitive functions associated with healthy aging.

Standard magnetic resonance imaging (MRI) structural studies have historically examined structure-function relationships in aging with an emphasis on volumetric alterations. While debate exists regarding the relationship between volume and function, these prior studies have reported correlations between frontal gray matter (GM) density and behavioral measures of executive function [13, 14], frontal white matter (WM) integrity and executive function, and overall prefrontal cortex volume and problem solving, working memory, and processing speed [15].

Diffusion tensor imaging (DTI) is a relatively newer neuroimaging modality, which allows for the examination of the integrity and directionality of white matter tracts. WM integrity is measured using factional anisotropy (FA) values, which are calculated based on the directionality of the diffusion of water. Increased isotropic diffusion and low directionality indicate demyelination or axonal loss and are correlated with a low FA value (near 0). With increased age, a loss of integrity as indicated by a decreased FA value is observed in WM fiber tracts and throughout the whole brain [16–19]. As with studies of GM and WM volumetric changes, differential decreases in FA are often observed within the frontal lobe WM tracts, including the superior frontal gyrus [20–24].

Unlike standard structural MRI, fMRI uses the properties of blood flow and oxygen concentration (Blood oxygenation level-dependent or BOLD contrast) as a measure of a hemodynamic response associated with neuronal firing. Utilizing the BOLD signal allows for investigation of region-specific brain activation during performance of a behavioral task. In terms of frontal lobe function, a great degree of variability exists in the aging literature specific to changes in either patterns of or amounts of BOLD activation. This variability is likely due in part to the specific paradigms used, health of the sample, age of the sample, and differences in magnet hardware [25, 26]. One common debate is whether aging is accompanied by increased recruitment of neurons to complete a task relative to younger adults. Some argue that the recruitment of contralateral brain tissue is a sign of compensation to counteract volume loss [27], while others argue that such recruitment is inefficient and therefore detrimental. While this debate is outside the scope of this paper, of most interest to the present examination is the relationship between alterations in fMRI patterns of activation and structural measures of brain volumes.

The purpose of the current study is to use multimodal imaging techniques to examine working memory performance in healthy older adults. Specifically, the investigation examines structural brain GM and WM volume and WM integrity (FA) in relation to functional activation and behavior during performance on a working memory (*N*-Back) task between healthy younger and older adults. Our primary hypothesis is that reduced integrity of white matter and not alterations in cerebral volumes is associated with poorer working memory performance in normal aging.

2. Methods

2.1. Participants. All participants provided written informed consent, and experimental procedures complied with the code of ethics of the World Medical Association, the University of Illinois Institutional Review Board, and Declaration of Helsinki. Twenty-five healthy younger adults (mean age = 26 years, SEM = 2.04 years; mean education = 16.3 years; SEM = 0.65) and 25 healthy older adults (mean age = 65.9 years, SEM = 2.38 years; mean education = 14.6 years, SEM = 0.73) participated in the study. Although the older adults had a trend for less formal education than the younger adults, this comparison did not reach significance ($P = 0.08$). All were right handed, and none reported cognitive, visual, or motor deficiencies other than the need for corrective lenses. All were native speakers of English. Subjects reported no history of diagnosed neurologic disorder (including, but not limited to Alzheimer's disease, mild cognitive impairment, stroke, Parkinson's disease, traumatic brain injury, or attention deficit disorder), psychiatric disorder (including, but not limited to, major depressive disorder, schizophrenia, or bipolar disorder), or history of substance abuse and/or dependence as defined by the DSM-IV (APA, 1994). Additionally, subjects were excluded if they had any history or current use of medication for either hypertension or hyperlipidemia or had a positive history of cardiac illness.

2.2. Procedure. All subjects received practice on each version of the *N*-back working memory task prior to imaging. Following this brief practice, subjects were placed in the MRI and completed four versions of a working memory task described below followed by a high-resolution anatomical study and DTI.

2.2.1. Functional Imaging Methods: N-Back. During the fMRI assessment, subjects performed four versions of a verbal *N*-Back task [28]. Each version involved two conditions—0-back and "*N*"-back (where *N* is either 1, 2, 3, or 4 back). The *N*-Back is a well validated test of working memory (Lezak, 1995) and requires subjects to monitor a series of letters presented on a computer screen and to respond if the letter is identical to the one that immediate proceeded it (1-Back), the one presented two trials back (2-back), three trials back (3-back), or four trials back in the list (4-back). Instructions were first presented for 10 sec and included information on the nature of the task (e.g., whether the task was 0-, 1-, 2-, 3-, or 4-back). For the 0-back conditions, the participants needed to retain in memory a reference target that had been presented in the instructions. Following instructions, a series of 20 letters (trials) were presented. Each letter was presented for 2 sec and was followed by 500 msec of fixation. Participants indicated whether the currently presented letter matched the identity of the trial (reference, 1, 2, 3, and 4 back) target by pressing an MR compatible response switch (MRIχ Technologies, Bannockburn, Ill, USA). The 0-back task provides an activation map for short-term memory, eye movements, motor planning, and motor execution. Intermixed with blocks of 0-back were the 1-, 2-, 3-, and 4-back conditions. A forced two-choice speeded response was required on each trial (same and different). An example of the materials, appropriate stimulus comparisons relative to condition and with correct button responses is presented in Figure 1.

2.2.2. Functional Imaging Parameters: N-Back. All imaging took place on a 3T MRI (GE, 3T EXCITE 2.0). Functional MRI was performed using a blocked design to optimize spatial localization of function (epiRT, plane = axial, TR = 2500 ms, TE = 25 ms, flip angle 60°, NEX = 1, Bandwidth = 62 kHz, acquisition matrix = 64 × 64, FOV = 20 × 20 cm^2, slice thickness/ISI = 4/1 mm/mm, slices = 30, volumes = 144).

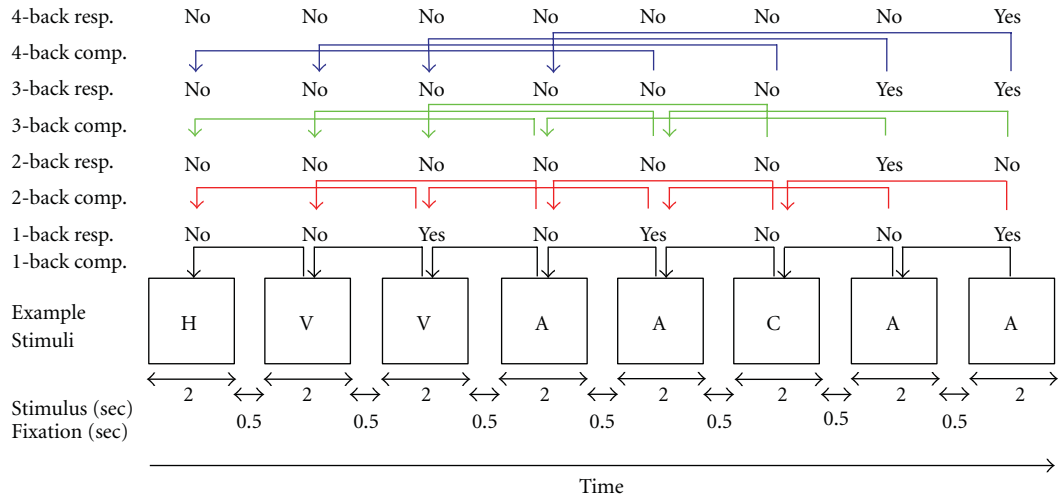


FIGURE 1: Diagram of sample series of *n*-back stimuli, timing of stimuli (bottom), expected correct responses, and appropriate stimuli to match to under different experimental (*n*-back) conditions.

2.2.3. Structural Imaging Parameters: T1 SPGR. Following functional imaging, anatomical imaging for visualization of full brain anatomy was performed with a three-dimensional inversion recovery prepared spoiled gradient recalled echo (3D IRPrepSPGR) acquisition (reconstructed in three planes = axial, coronal and sagittal, TR = 13.8 ms, TE = 2.7 ms, flip angle = 25 degrees, acquisition matrix = 512 × 192, FOV = 22 × 16 cm^2, slices = 120, slice thickness/separation = 1.5/0 mm/mm, NEX = 1, Bandwidth = 15.6 kHz, total acquisition time = 5 : 33 minutes).

2.2.4. Diffusion Tensor Imaging Parameters. To characterize white matter integrity, we collected DTI on each subject. The sequence is based on a single-shot EPI pulse sequence with the capability of compensating eddy currents induced by the diffusion gradients via dynamically modifying the imaging gradient waveforms. The diffusion-weighting orientations are designed based on the electrostatic repulsion model proposed by Jones et al. [29] (TR = 5000–6000 ms, TE = minimum (81 ms), *B*-values = 0, 750 s/mm^2, diffusion gradient directions = 27, FOV = 22 cm, Matrix = 128 × 128, slice thickness = 5 mm, gap = 1.5 mm, ramp-sampling = on, NEX = 2, total acquisition time = 5 : 46).

2.2.5. Functional Imaging Analysis. Analysis on the fMRI data was carried out using Statistical Parametric Mapping (SPM2, Wellcome Department of Imaging Neuroscience, London, UK). Data from each individual subject was initially screened and corrected for head motion. Data with greater than 4 mm of motion (across all tasks) was excluded from further analysis. The functional data (EPI images) were first coregistered with the other functional paradigms and then with the corresponding anatomical images. These coregistered images were then normalized to the Montreal Neurological Institute (MNI) template and spatially smoothed the functional data (FWHM = 8 mm). The preprocessed functional data for each individual was then entered into a general linear model in which the experimental variables (memory load; 1-back, 2-back, 3-back, and 4-back) were evaluated and convolved with the hemodynamic response function (HDF). One significant potential issue in the analysis of functional data for the interpretation of age-related differences is the concurrent finding that normal aging also affects the cerebrovascular system which could affect the neurovascular system [30]. These changes are not believed to affect the shape of the BOLD response or the integrity of this response but do affect the ability to detect a response (signal-to-noise ratio). For this reason, contrasts were calculated between conditions for each subject allowing each subject to serve as their own control. Random effects analyses were conducted on these individual difference maps followed by between-group comparisons.

2.2.6. Structural Imaging Analysis for Quantification of Whole Brain and Regional Volumetric Loss. Manual tracings of prefrontal cortex for each subject were accomplished with the Analyze software package (Mayo Clinic Foundation, Rochester, Minn, USA). Two independent raters produced an inter-rater reliability of 0.92. We broadly defined the prefrontal cortex using Brodmann's definitions to include the area between the superior rostral sulcus, and inferior rostral sulcus, and dorsally by the anterior cingulate. Given the small subject numbers, we will only present these gross volumes in the present paper. Once drawn, the images were segmented for gray matter, white matter, and cerebrospinal fluid. Total prefrontal volume was extracted for both white and gray matter.

2.2.7. Diffusion Tensor Imaging Analysis. The 28 diffusion directions, along with the B0 image, were used to calculate FA as the primary indicator of white matter integrity. The images were reconstructed, and FA was calculated, using the program from Johns Hopkins, DTI Studio [31]. The 28 diffusion-weighted image sets were examined for image quality and head movement. Head movement was required to be

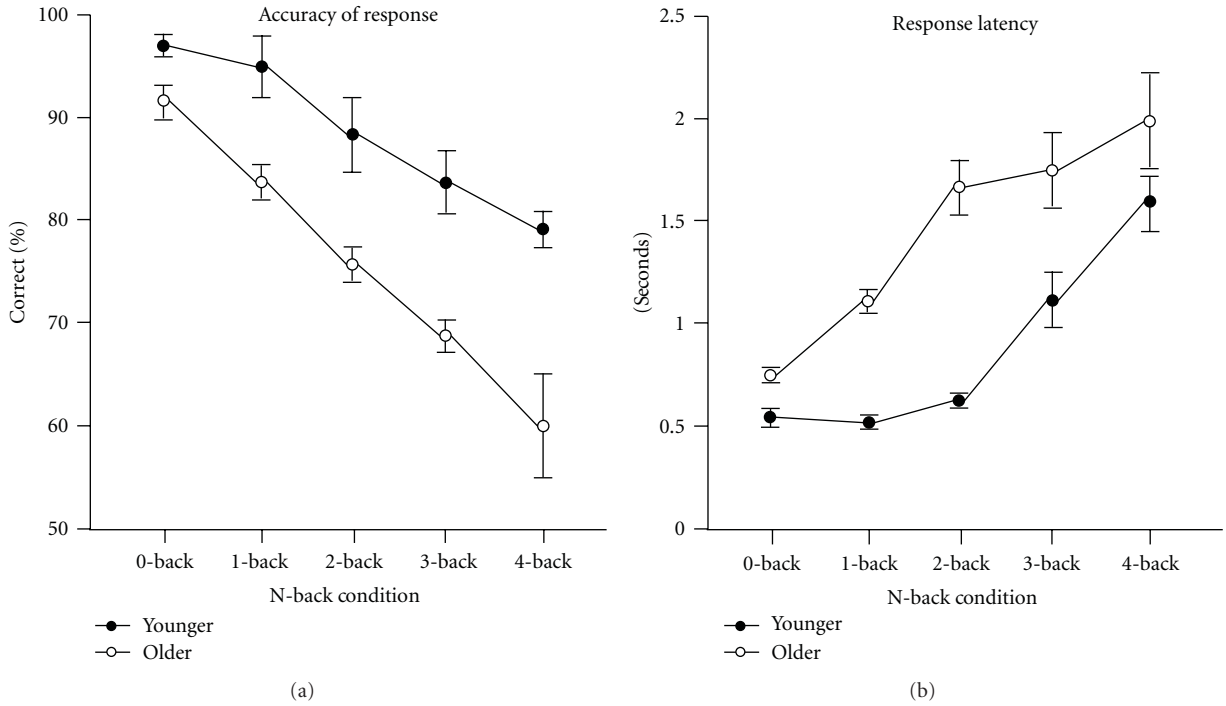


Figure 2: Accuracy (a) and latency (b) for older (open circles) and younger (closed circles) adults collected during fMR data acquisition. Error bars represent 1 SEM.

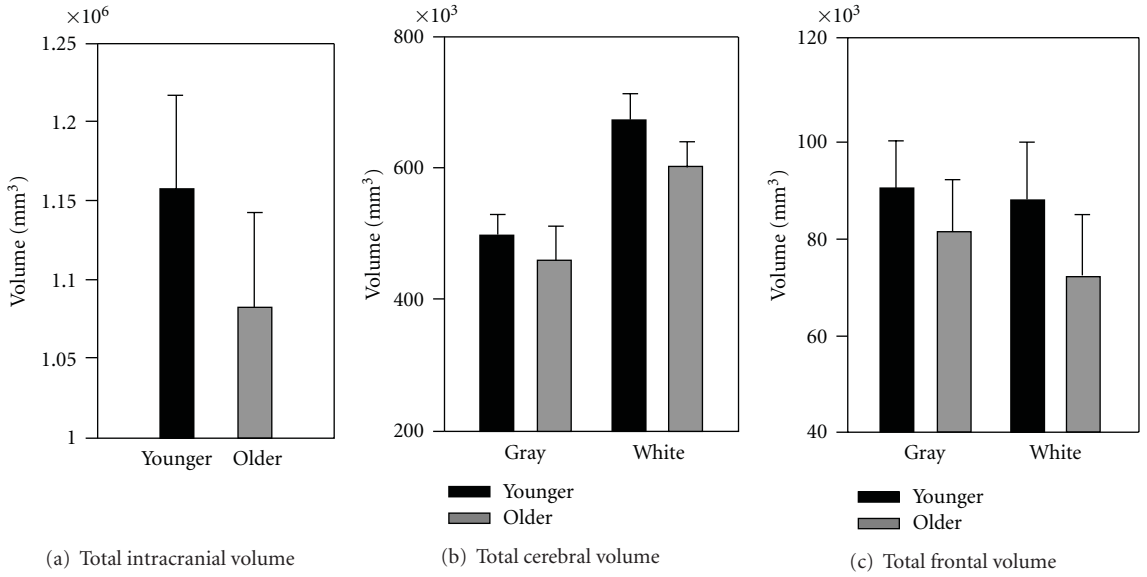


Figure 3: Volumetric data for older relative to younger adults for (a) total intracranial volume, (b) whole brain gray and white cerebral volumes (black bars, young; white bars, older), (c) prefrontal gray and white (black bars, young; white bars, older). Error bars represent 1 SEM.

within one voxel across the image acquisition. Because noise can introduce bias in estimates of the eigenvalues and because noise decreases the signal-to-noise ratio, we applied a background noise level to all subjects prior to calculation of pixelwise FA (background noise = 150 scanner units). It is important to note that the application of this criterion and the noise itself can influence calculation of anisotropy. However, because the analyses focus on differences between groups the bias introduced by this noise floor should not influence group differences. The FA maps were then converted to ANALYSE format and read into SPM2 software for analysis (Wellcome Department of Imaging Neuroscience, London, UK). DTI data from each subject was coregistered with their corresponding T1-weighted anatomic image set

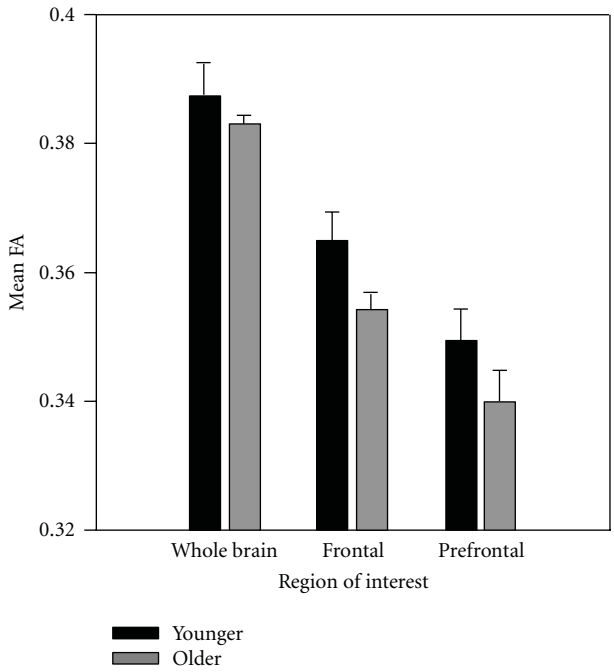


Figure 4: FA for older and younger adults for both frontal and whole brain regions of interest. Error bars represent 1 SEM.

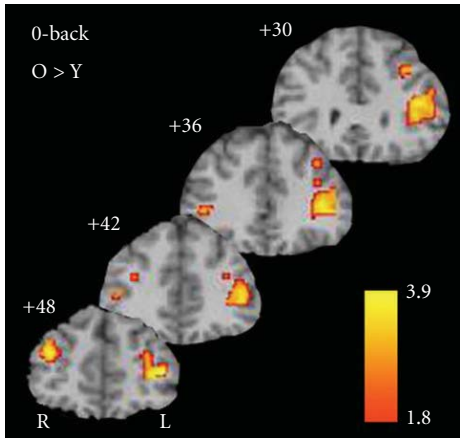


Figure 5: Regions of greater activity for older compared to younger adults on the 0-back. Areas exceeding $P_{corr} < 0.05$ are included.

(after skull stripping) using a normalized mutual information cost function and trilinear interpolation. Normalization parameters were determined based upon the high-resolution T1 image relative to the MNI template. These normalization parameters were then applied to the FA and eigenvalue images. Each image was visually checked for accuracy after both the coregistration and normalization steps.

2.2.8. Statistical Analysis. In additional to the imaging analyses described above the dependent measures extracted from the imaging data (volumes and fractional anisotropy) were submitted to a mixed design analysis of variance with subject group (younger and older) as the between subjects factor with (df = 1,49). For the *n*-back, percent correct, response

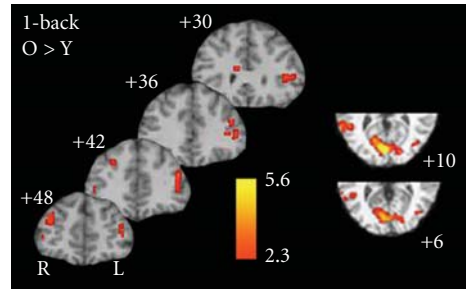


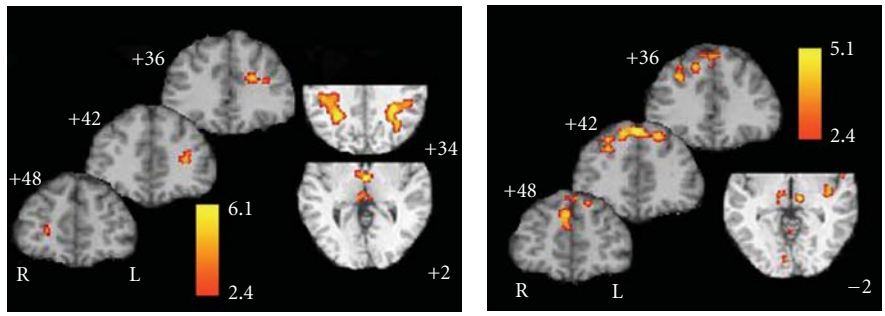
Figure 6: Regions of activation that were differentially greater for older adults on the 1-back (relative to 0-back) compared to younger adults.

latencies, percent of hits and false alarms, and the signal-detection parameter *d*-prime (d') were calculated. The mixed design analysis of variance with participant group as the between subjects factor was performed for each of these measures derived from the behavioral. Because of the sample size, bivariate Pearson correlations were used to examine the relationship between behavioral responses and cerebral tissue volume and integrity.

3. Results

3.1. Behavioral Data. Behavioral data (i.e., accuracy and reaction time) collected during the fMRI sequences are presented in Figure 2 for both the older and younger adults. First, there was an interaction between age and accuracy ($P = 0.04$) and latency ($P = 0.02$) with older adults showing a greater decrease in accuracy and an increase in latency with increased task difficulty relative to the younger adults. Post-hoc tests of independent means of accuracy within each level of the *N*-Back comparing the young to the older adults showed significant differences at the 0-back ($P = 0.041$), 1-back ($P = 0.028$), 2-back ($P = 0.048$), 3-back ($P = 0.046$), and 4-back ($P = 0.032$). Post hoc comparisons on latency data demonstrated significantly increased latencies for the older adults only at the 0-back ($P = 0.043$) and 1-back ($P = 0.018$) conditions. Because the older adults did not perform better than chance on the 4-back ($P = 0.15$), these imaging data have been excluded from the next set of analyses and results.

Because of the nature of the *n*-back task and relatively low numbers of trials for which a "match" between target and reference occur signal detection theory was applied to these data. For this, the percent of hits and false alarms were calculated as was d'. These data are presented in Table 1. When comparing the performance between younger and older adults using these methods, it becomes clear that the older adults had significantly fewer hits (identifying the target as a target; the only significant ($P < 0.05$) comparison was during the 3- and 4-back) and more false alarms (identifying a nontarget as a target) than the younger adults (all comparisons between groups within each condition $P < 0.05$ with the exception of the 1-back). They also differed on d' on all conditions ($P < 0.05$).



(a) Y > O (b) O > Y

Figure 7: Regions of activation that were differentially greater for younger (a) relative to older adults and those that were differentially greater for older compared to younger adults (b) on the 2-back task.

Table 1: Percent hits, false alarms, and d' for each condition of the n-back within each participant group.

	Condition	Hit rate (%)	False alarm (%)	d'
Younger	0-back	96	2	2.69
	1-back	95	10	2.07
	2-back	91	13	1.74
	3-back	85	16	1.44
	4-back	83	15	1.41
Older	0-back	95	10	2.07
	1-back	94	14	1.86
	2-back	88	18	1.48
	3-back	76	21	1.07
	4-back	52	22	0.58

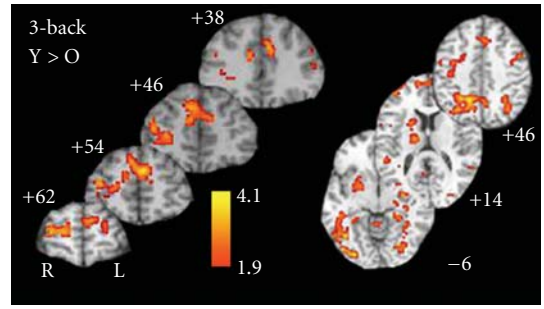


Figure 8: Regions of activation that were differentially greater for younger adults on the 3-back relative to 0-back activation compared to the same contrast in older adults.

3.2. Volumetric Measurements. Volumetric measurements of total intracranial volume, whole brain volumes, and prefrontal volumes for both GM and WM are presented in Figure 3. Differences between younger and older adults on total cranial volume ($P = 0.09$), whole brain GM volume ($P = 0.071$) and prefrontal GM volume ($P = 0.082$) approached, but did not reach, statistical significance. There were, however, significant differences in total WM volume and total prefrontal WM volume, with the older showing smaller tissue volumes than the younger adults (whole brain: $P = 0.048$; prefrontal: $P = 0.038$).

3.3. WM Integrity. Figure 4 shows whole brain and frontal lobe measurements of FA for the younger and older adults. Compared to younger adults, older adults showed lower whole brain FA values ($P = 0.018$), lower frontal lobe FA values ($P = 0.038$), and significantly lower prefrontal values ($P = 0.32$) indicating lower WM integrity in the older group.

3.4. fMRI Activation Patterns. Comparisons between older and younger adults on the 0-back conditions yielded no significant clusters of activation ($P_{corr} < 0.05$, k = 30) for which the younger adults showed greater activity compared to the older adults. Older adults showed differentially greater activity bilaterally in both dorsolateral prefrontal cortex (DLPFC) and ventrolateral prefrontal cortex (VLPFC) compared to the younger adults (Figure 5).

In light of the group differences in baseline prefrontal cortex activity during the 0-back, the next set of analyses examined age differences as a function of condition (e.g., n-back relative to 0-back) in a two-way ANOVA (group × condition (0-back versus 1-, 2-, and 3-back). These analyses address the question of relative increases in activation during parametrically greater memory loads.

As shown in Figure 6, when controlling for baseline activation (0-back), the older adults showed differentially greater activity on the 1 back relative to younger adults especially in prefrontal and ventral-lateral prefrontal regions. The only exceptions to this finding was in primary and higher older visual cortical areas (Figure 6(b)), where the young showed greater activity than the older adults. Additionally, two distinct patterns emerge from the n-back data. In the 2-back, the younger adults showed greater activity in deeper frontal structures, the thalamus, and bilaterally in the intraparietal sulci (Figure 7(a)). In contrast, the older adults showed increased activation superior and medially in the frontal lobe. Interestingly, they also showed increase activation in the dorsomedial and pulvinar regions of the thalamus (Figure 7(b)). During the 3-back (Figure 8), the young showed increase activation in a widespread network including the frontal regions, thalamus, basal ganglia, motor regions, intraparietal sulci, and anterior cingulate compared to the older adults.

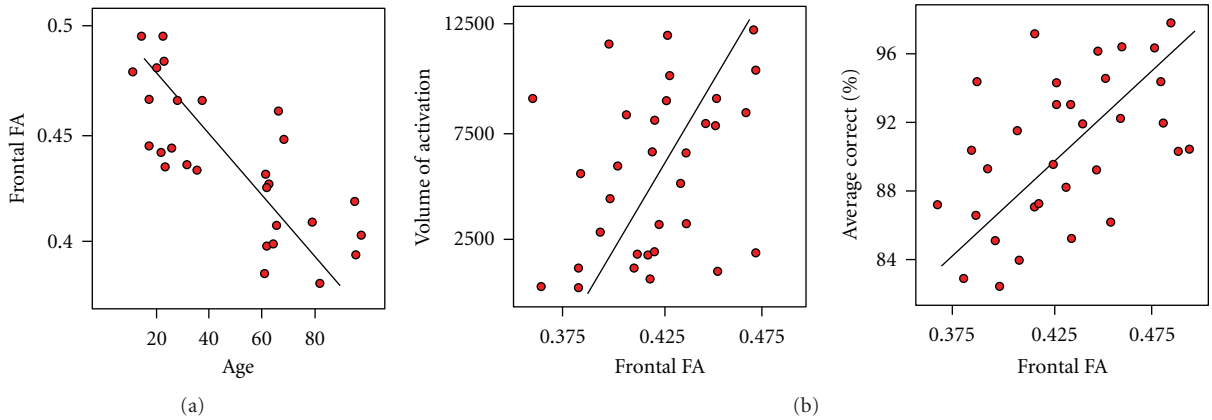


Figure 9: Scatterplots with best-fit lines indicating the relationship between age and FA (a), and FA with volume of activation and behavioral performance (b).

There were no regions which showed increased activation on the 3-back relative to the 0-back for older adults compared to younger adults.

3.5. Structure-Function Correlations. We next conducted a series of correlational analyses to examine structural brain outcomes (e.g., GM volume, WM volume, and WM integrity) in relation to functional activation and behavior during performance of the *n*-back task. For simplicity, accuracy data were collapsed across the 0-, 1-, 2- and 3-back conditions (the 4-back was excluded due to near chance data for the older adults). The results are presented in Figure 9.

Age was negatively correlated with frontal FA ($r = -0.69$, $P = 0.001$) and frontal lobe FA is positively correlated with the volume of frontal lobe activation ($r = 0.807$, $P < 0.001$) and accuracy ($r = 0.511$, $P = 0.036$). When age was included as a covariate, frontal FA still correlated with fMRI activation ($P = 0.006$) but no longer correlated significantly with performance (although the trend still suggests the same relationship). When the relationship with GM volume was investigated after controlling for age, the only significant relationship was between frontal GM and volume of activation, and this relationship appears to be driven by the 2- and 3- back condition. Interestingly, WM volume did not correlate with performance or volume of activation.

4. Discussion

The overall objective of this research was to use multimodal imaging techniques to examine working memory performance in healthy older adults. Two groups of healthy adults (young, 20–30 years of age and older, 60+ years of age) completed four versions of a verbal *N*-Back task (1-back, 2-back, 3-back, and 4-back) while fMRI data were acquired. Additional data was also acquired to characterize integrity of the frontal lobe white matter (diffusion tensor imaging, DTI) and gray matter (inversion recovery spoiled gradient recalled, IRSPGR). With increased age, longer latencies (1-back and 2-back) and decreased accuracy (2-back only) was observed behaviorally. Contrasts between the 1-back and 2-back with a 0-back control task showed the older sample demonstrated increased activation in both dorsal (left) and ventral (left) lateral prefrontal cortical regions for both the 1- and 2-back tasks. Additionally, older adults showed more bilateral activation in prefrontal regions than the younger group. Similar to previous reports, gray matter volumes were negatively correlated with decreased performance (accuracy) on the working memory tasks for the older adults. Findings from Mattay et al. [32] and Cappell et al. [33] show similar patterns of activation in their group of 10 young and 12 older adults. Specifically, Mattay and colleagues showed greater activity in the easier condition in prefrontal cortex for older adults and greater activity in prefrontal cortex during the higher load conditions for younger adults.

Evidence from other functional neuroimaging studies shows altered or disrupted patterns of neuronal function in the prefrontal cortex in elderly adults compared to their younger counterparts [27, 34–37]. Consistent with our findings of recruitment under conditions of lower working memory, memory demand, and reductions in activation under high working memory demand, others have found that the presence or magnitude of recruitment in older adults varies as a function of performance success although these studies are based primarily upon episodic and not working memory.

Recent neuroimaging findings show modest volumetric loss of both GM and WM during aging. Age-related GM loss is conservatively estimated at somewhere between 3%–5% per decade [2, 38]. However, unlike the linear losses of GM, WM remains largely intact until late-middle-adulthood when volumetric loss occurs in an exponential manner [1, 17] and may decline at a greater rate in frontal regions [39–41]. In the present study, fractional anisotropy was calculated from the DTI data to characterize white matter integrity in the frontal lobes. Our data demonstrate that white matter integrity was stable in younger adults but was reduced for the older adults. Observations of increased neuronal activation both locally (greater volume but in the same region) and bilaterally in older adults in response to increased task difficulty has been interpreted as evidence of neuronal recruitment and support the conclusion that active compensatory processes are observed [27, 42, 43]. In the present

study, decreased FA was related to increased baseline activation in the prefrontal cortex offering further evidence of potential compensatory mechanisms.

In summary, the data presented here demonstrate age-related effects on behavior, white and gray matter volume, white matter integrity, and fMRI activation as a function of working memory task difficulty. Although preliminary and limited by power, the present results begin to integrate previously reported, yet distinct, findings of behavioral, functional, and anatomical correlates of working memory declines in normal aging. Although this study represents only a preliminary attempt at describing these relationships, this hypothesis appears to be supported. However, more discrete analyses of specific white matter tracts and the relationship between these tracts and functional activation are needed to fully describe this relationship and are planned for a larger sample of subjects.

Acknowledgments

This work was supported by NIH Grant no. R21 AG028662 (PI: D. M. Little) from the National Institute on Aging, the University of Illinois College of Medicine (D. M. Little), and the Kravis Leadership Institute at Claremont McKenna College (J. T. Paliga). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Aging, Department of Veterans Affairs, United States Government, University of Illinois at Chicago, Texas A&M, or Duke University.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 978761, 11 pages
doi:10.4061/2011/978761

Review Article

Lessons from a Mouse Model Characterizing Features of Vascular Cognitive Impairment with White Matter Changes

Masafumi Ihara[1] and Hidekazu Tomimoto[2]

[1] *Department of Neurology, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo, Kyoto 606-8507, Japan*
[2] *Department of Neurology, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan*

Correspondence should be addressed to Masafumi Ihara, ihara@kuhp.kyoto-u.ac.jp

Received 26 March 2011; Accepted 26 July 2011

Academic Editor: Sofia Madureira

With the demographic shift in age in advanced countries inexorably set to progress in the 21st century, dementia will become one of the most important health problems worldwide. Vascular cognitive impairment is the second most common type of dementia after Alzheimer's disease and is frequently responsible for the cognitive decline of the elderly. It is characterized by cerebrovascular white matter changes; thus, in order to investigate the underlying mechanisms involved in white matter changes, a mouse model of chronic cerebral hypoperfusion has been developed, which involves the narrowing of the bilateral common carotid arteries with newly designed microcoils. The purpose of this paper is to provide a comprehensive summary of the achievements made with the model that shows good reproducibility of the white matter changes characterized by blood-brain barrier disruption, glial activation, oxidative stress, and oligodendrocyte loss following chronic cerebral hypoperfusion. Detailed characterization of this model may help to decipher the substrates associated with impaired memory and move toward a more integrated therapy of vascular cognitive impairment.

1. Introduction

Subcortical ischemic vascular dementia (SIVD) is characterized by white matter (WM) changes and lacunar infarctions, which occur as a result of a reduction in cerebral blood flow (CBF) over an extended period of time, causing small vessel changes [1–3]. Cerebrovascular WM lesions, neurodegenerative manifestations characterized by hyperintense signals on magnetic resonance images, are frequently associated with aging and are responsible for the cognitive decline in the elderly population [1–7]. Chronic cerebral hypoperfusion is likely to cause such WM lesions as CBF is decreased in these patients [2, 8]; indeed, similar WM lesions can be induced in rats, gerbils, and mice after chronic cerebral hypoperfusion, with experimental conditions mimicking chronic cerebral ischemia in humans [9–11]. These model animals can be generated by bilateral common carotid artery (CCA) occlusion in rats (2-vessel occlusion (2VO)) [9, 12, 13] or in mice [14], bilateral CCA stenosis in mice (BCAS) [10] or in gerbils [11], and unilateral CCA occlusion in mice [15]. Nonhuman primates appear to represent the best model for the study of WM lesions, because they have well-developed WM and vascular architectures which closely resemble those in human brains [16]. Nevertheless, most experiments studying chronic cerebral hypoperfusion have been performed in rodents because of the ease of handling and higher acceptability from an ethical viewpoint.

The rat model of chronic cerebral hypoperfusion is accompanied by cognitive impairment and cholinergic deficits [9, 13, 17] and is most widely used [12, 18]. These animals develop WM rarefaction [9, 19], which appears very similar to that found in human cerebrovascular WM lesions. However, this model has some drawbacks. For example, the visual pathway is injured by the occlusion of the ophthalmic arteries and thus may compromise behavioral assessment. Furthermore, genetic studies may be hampered because

of limited accessibility to molecular technologies when using knockout or transgenic animals. To circumvent such limitations, we have established a mouse model of chronic cerebral hypoperfusion, which is subjected to various degrees of CBF reduction by the narrowing of the bilateral CCAs with newly designed microcoils. The model demonstrates good reproducibility in terms of WM lesion appearance and glial activation. The cerebral WM is selectively damaged, while gray matter (including hippocampal) integrity remains intact, if the degree of stenosis is appropriately controlled by internal diameter regulation of the microcoils [10].

The aims of the current paper are to provide a comprehensive survey of the experimental evidence that has accumulated since establishment of this mouse BCAS model, in order to extrapolate the results into human neurological conditions, and to consider the particular strengths and pitfalls of the method.

2. The Procedures for BCAS

Ten-week-old male C57BL/6J mice (24–29 g) are conventionally used to induce chronic cerebral hypoperfusion by BCAS [10, 20, 21]. This model should be applied exclusively to C57BL/6J strain, because the CBF in the other strains may have a greater variability after BCAS. In this paper, unless stated otherwise, the "BCAS mouse" indicates a male C57BL/6J mouse that is subjected to BCAS for 30 days from 10 weeks of age using microcoils of 0.18 mm in diameter.

Mice are anesthetized with 2% halothane or 25–50 mg/Kg sodium pentobarbital and, through a midline cervical incision, both CCAs are exposed and freed from their sheaths. Two 4–0 silk sutures are placed around the distal and proximal parts of the right CCA. The artery is then gently lifted by the sutures and placed between the loops of the microcoil just proximal to the carotid bifurcation (Figures 1(a) and 1(b)). The microcoil is twined by rotating it around the CCA, and another microcoil of the same size is twined around the left CCA after 30 minutes (Figures 1(b) and 1(c)). Four types of microcoils made from piano wire with varying inner diameters from 0.16 mm to 0.22 mm (Figure 1(c)) have been designed in collaboration with Sawane Spring Co., Ltd. (Hamamatsu, Japan). Microcoils with the same diameter are conventionally placed on the bilateral CCA, though a modified model has also been devised where the 0.16 mm microcoil is placed on the left CCA and the 0.18 mm microcoil on the right CCA [22]. The rectal temperature should be maintained between 36.5°C and 37.5°C, and the cessation of CBF for >1 minute should be avoided. All procedures for BCAS are usually accomplished within 15 minutes except an interval for 30 minutes.

2.1. Blood Pressure. The blood pressure of the surviving mice does not change significantly at any postoperative intervals until 30 days, compared with the sham-operated controls [10].

2.2. Mortality Rates. The mortality rates are reported to range from 10% to 20%: 13% in mice with microcoils of 0.22 mm in diameter, 17% in those of 0.20 mm, and 15–19% in those of 0.18 mm [10, 23]. In contrast, 75% (15/20) of mice with microcoils of 0.16 mm placed died within 14 days after the surgery, most of whom were found to have cerebral infarctions [10]. In another study of a modified model with the 0.16 mm microcoil on the left CCA and the 0.18 mm microcoil on the right CCA, the mortality rate is reported to be 18.8% [22].

2.3. Body Weight. Body weight has been shown to decrease after the surgery, but recover to baseline by day 7, in mice with 0.22, 0.20, and 0.18 mm diameter microcoils. Although the mice with the 0.22, 0.20, and 0.18 mm diameter microcoils placement tended to have a lower body weight than those with sham operation, no significant difference is noted at any postoperative interval. In contrast, the mice with the 0.16 mm diameter microcoil placement showed significantly lower body weight at all postoperative intervals, compared with the sham-operated mice [10].

2.4. Neurological Deficits. After placement of the 0.22, 0.20, and 0.18 mm diameter microcoils, the animals regained consciousness within a few hours and occasionally showed transient ptosis but no apparent motor weakness. In contrast, some of the mice with 0.16 mm diameter microcoils placed (∼35%) did not regain consciousness, showing rolling or circling movements lasting 2 to 6 hours after awakening, and severe akinesia with a squatting posture [10].

2.5. Anesthetics. Although anesthetics such as sodium pentobarbital and halothane are known to provide varying degrees of neuroprotection against ischemic injury [24], the selection of anesthesia did not appreciably affect the mortality rates, temporal profile of CBF, and ischemic WM changes after BCAS.

3. The Spatial and Temporal Profiles of Cerebral Blood Flow and Metabolism after BCAS

Although 2VO rats develop specific WM changes without any apparent gray matter changes [9, 12], 2VO in mice will inevitably lead to a severe drop in CBF due to underdeveloped posterior communicating arteries [25]. Therefore, in mice, carotid stenosis, but not occlusion, is required to achieve cerebral hypoperfusion and resultant WM changes. CBF and cerebral metabolism change dynamically following the BCAS operation, with CBF dropping immediately after carotid stenosis and recovering over a period of months via compensatory and adaptive mechanism (i.e., collateral anastomosis).

Figure 2 shows mean CBF values evaluated with laser Doppler flowmetry in surviving mice of 2.5 months of age after application of four types of piano wire with varying inner diameters, varying from 0.16 mm to 0.22 mm, to the bilateral CCAs [10]. The CBF values decreased significantly from the preoperative baseline after the surgery with the 0.20, 0.18, and 0.16 mm diameter microcoils. At 2 hours, there was a significant reduction in CBF values in mice with

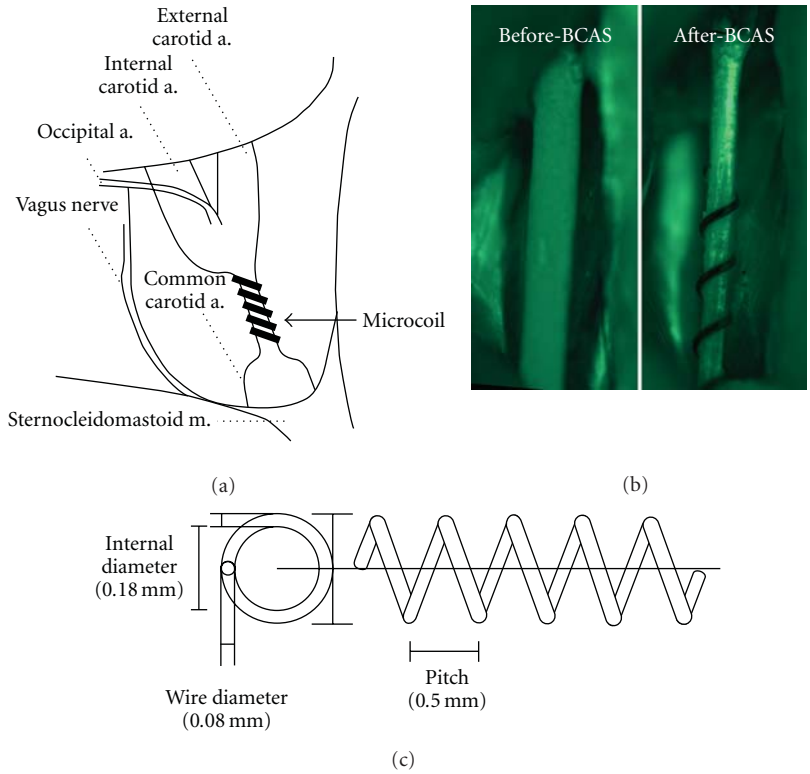

Figure 1: The procedure for BCAS and the microcoil. The microcoil is twined by rotating it around the CCA just proximal to the carotid bifurcation of a C57BL/6J mouse (a). Representative photographs of a FITC-perfused common carotid artery before (left) and after (right) placement of a microcoil (b). The microcoil is made from piano wire (wire diameter of 0.08 mm) with an inner diameter of 0.18 mm, pitch 0.50 mm, and total length 2.5 mm (c).

the 0.20 mm microcoils to 77.3 ± 13.4% (mean ± SD), 67.3 ± 18.5% in those with 0.18 mm, and 51.4 ± 11.5% in those with 0.16 mm. On day 1, the CBF values started to recover but remained significantly lower until 14 days after placement of the microcoils, compared with the control group. At 30 days, CBF values were still decreased in mice with 0.16 mm microcoils placed. Intergroup differences in CBF values were detected between mice with 0.16 mm microcoils but there were no differences among the mice with 0.22, 0.20, and 0.18 mm microcoils placed.

Older 4-month-old mice showed a similar profile of CBF changes after the BCAS operation with the 0.18 mm coils; the CBF values temporarily decreased to 62.9 ± 18.5% (mean ± SE) at 2 hours after BCAS, compared to the sham group but gradually recovered to 81.7% ± 4.0% at 1 month, 83.2% ± 1.8% at 2 months, and 85.0% ± 8.7% at 3 months [26]. Interestingly, this temporal profile of CBF is similar to that of the first 5-minute ^{18}F-fluorodeoxyglucose (FDG) uptake in the cerebral cortex, suggesting that the early ^{18}F-FDG uptake scan can serve as an estimate of CBF. The early ^{18}F-FDG uptake scan in the striatum showed a similar temporal profile to that of the cerebral cortex, suggesting that the CBF values in the cerebral cortex and the striatum change in parallel after BCAS. By contrast, the CBF in the hippocampus did not decrease at 2 hours or 2 months after BCAS but finally decreased at 6 months. The late ^{18}F-FDG scans show that the glucose uptake in the hippocampus did not decrease by 2 months after BCAS but decreased by 20% at 6 months after BCAS. The lack of reductions in hippocampal CBF and metabolism in the early phase after BCAS is probably due to the hippocampus being supplied by both anterior and posterior circulations [27]. However, mild cerebral ischemia of an insufficient magnitude for 6 months has been shown to induce subacute pathologies, which may lead to subsequent changes in the gray matter, including the cerebral cortex and hippocampus [26].

4. Blood-Brain Barrier Disruption after BCAS

A previous study on the rat 2VO system and human material implicated a dysfunction of the blood-brain barrier (BBB), perivascular edema, and microglial activation as the mechanisms underlying the WM lesions [9, 28, 29]. During this process, microglia may play a pivotal role; both microglia activation and WM lesions have been shown to occur concurrently, and both are suppressed by the administration of immunosuppressants, such as cyclosporin A or FK 506 [30, 31]. Proteases derived from the microglia may contribute to the reduction in the basement membrane components and BBB breakdown [32, 33]. The resulting perivascular edema may further exacerbate the degradation of the WM myelin through the actions of extravasated serum factors [28]. The BBB breakdown also leads to leukocyte

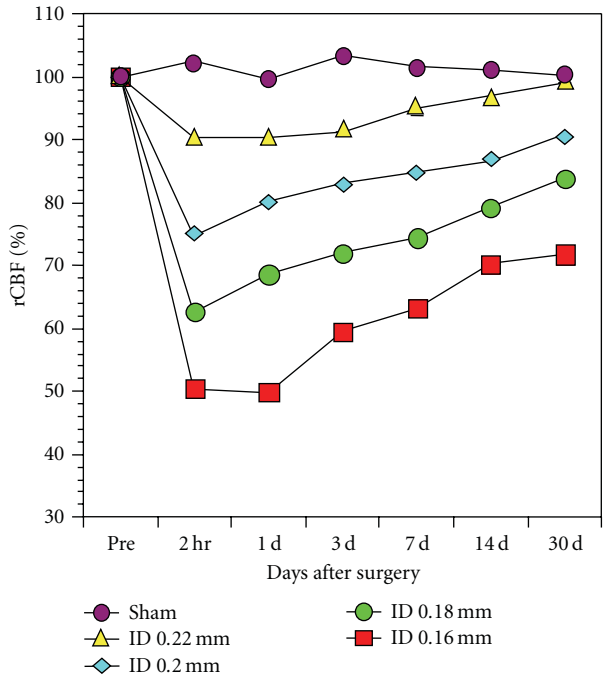

Figure 2: Cerebral blood flow after BCAS. This figure shows cerebral blood flow evaluated with laser Doppler flowmetry in mice at 2.5 months of age after the surgery using microcoils with diameter of 0.16 mm, 0.18 mm, 0.20 mm, and 0.22 mm. The data represent mean values expressed as a percentage of the preoperative value.

diapedesis [34], and the infiltrating leukocytes may cause inflammatory demyelination. Matrix metalloproteinase-2 (MMP-2), through its activity as a type IV collagenase, is activated and degrades components of the basement membrane. In addition, MMP-2 has been shown to degrade myelin basic protein at approximately 100x more potency than MMP-9 [35]. Thus, the MMP-2 released from glial cells may be directly involved in the remodeling of WM myelin [36].

Consistent with the aforementioned notion, BBB disruption has been shown to be accompanied by an upregulation of MMP-2, but not MMP-9, suggesting the specific involvement of MMP-2 in WM lesion manifestation in the 2VO rat model [37]. In rats treated with a relatively selective MMP-2 inhibitor, AG3340, the WM lesions become significantly less severe after chronic cerebral hypoperfusion, and the density of activated astroglia and microglia significantly reduced, compared with the vehicle-treated rats [21]. Gene knockout of MMP-2 also reduced the severity of WM lesions and the density of activated astroglia and microglia in a mice BCAS system. In both rodents, disruption of BBB function, as assessed by IgM staining and the Evans blue extravasation test, was less severe when MMP-2 activity was attenuated. The most marked extravasation in Evans blue test, in the paramedian portion of the corpus callosum facing the lateral ventricle in the BCAS mouse, is consistent with a previous report on a rat model of chronic cerebral hypoperfusion [38] and further indicates a vulnerability of the BBB in this area. Rosenberg et al. showed that the activated astroglia and microglia/macrophages present around arterioles express MMP-2 and MMP-3, but not MMP-9, in the brains of patients with vascular dementia [39]. The major pathologic features of WM lesions, such as demyelination and gliosis, may result from a BBB dysfunction, which may result in the leakage of proteins and fluid through the compromised barrier of the penetrating arteries [40].

5. White Matter Injury after BCAS

In BCAS mice with 0.18 mm microcoils placed, the temporal profile of the WM lesions was examined (Figure 3) [10, 20, 21, 41–43]. WM lesions were not detected in any region of the brain 3 and 7 days after BCAS. After 14 days, the WM lesions were evaluated as grade 0 or 1 in the medial part of the corpus callosum, caudoputamen, and the internal capsule; however, after 30 days, severe rarefaction occurred in these regions. WM lesions were most densely distributed in the medial part of the corpus callosum adjacent to the lateral ventricles; the lesions were moderately distributed in the paramedian part of the corpus callosum, fiber bundles of the caudoputamen, and the internal capsule; lesion distribution was, however, less severe in the anterior commissure and the optic tract. The staining intensity of the myelinated fibers was reduced and the integrity of the myelin compromised in the WM regions. The remaining fibers were disorganized and vacuoles frequently observed in the neuropil. There were relatively few TUNEL positive cells in the corpus callosum [20]. In contrast, the WM lesions in the optic tract did not emerge until 30 days. Atrophy was not found in the optic nerve, though there was evidence of slight rarefaction. In each region of the WM, the numerical densities of the microglia/macrophages immunolabeled for MHC class II antigen increased significantly from 7 to 30 days after BCAS whereas astroglia immunolabeled for GFAP increased in the period from 14 to 30 days and the regions with intense glial activation corresponded to those with a greater loss of WM myelin. There was a significant negative correlation between the CBF at any time point after BCAS and the grading scores of WM lesions at 30 days [10]. Thus, lower CBF appears to be present in the more severe WM injuries. This notion is further strengthened by the finding that mice with 0.16 mm microcoil on the left CCA and 0.18 mm microcoil on the right CCA exhibited more severe WM injury in the left hemisphere [22].

In accordance with the histological findings, *in vivo* MRI showed reductions in fractional anisotropy in the corpus callosum and internal capsule and a significant decrease in the magnetization transfer ratio in the corpus callosum, fimbria, internal capsule, and optic tract following hypoperfusion [44]. Hypoperfused mice demonstrated diffuse axonal and myelin pathology, which was essentially absent in control mice. Both fractional anisotropy and magnetization transfer ratio correlated with markers of myelin integrity/degradation and not axonal pathology. Furthermore, in a rat 2VO model, an increase in apparent diffusion coefficients on MRI was reported to be linked with

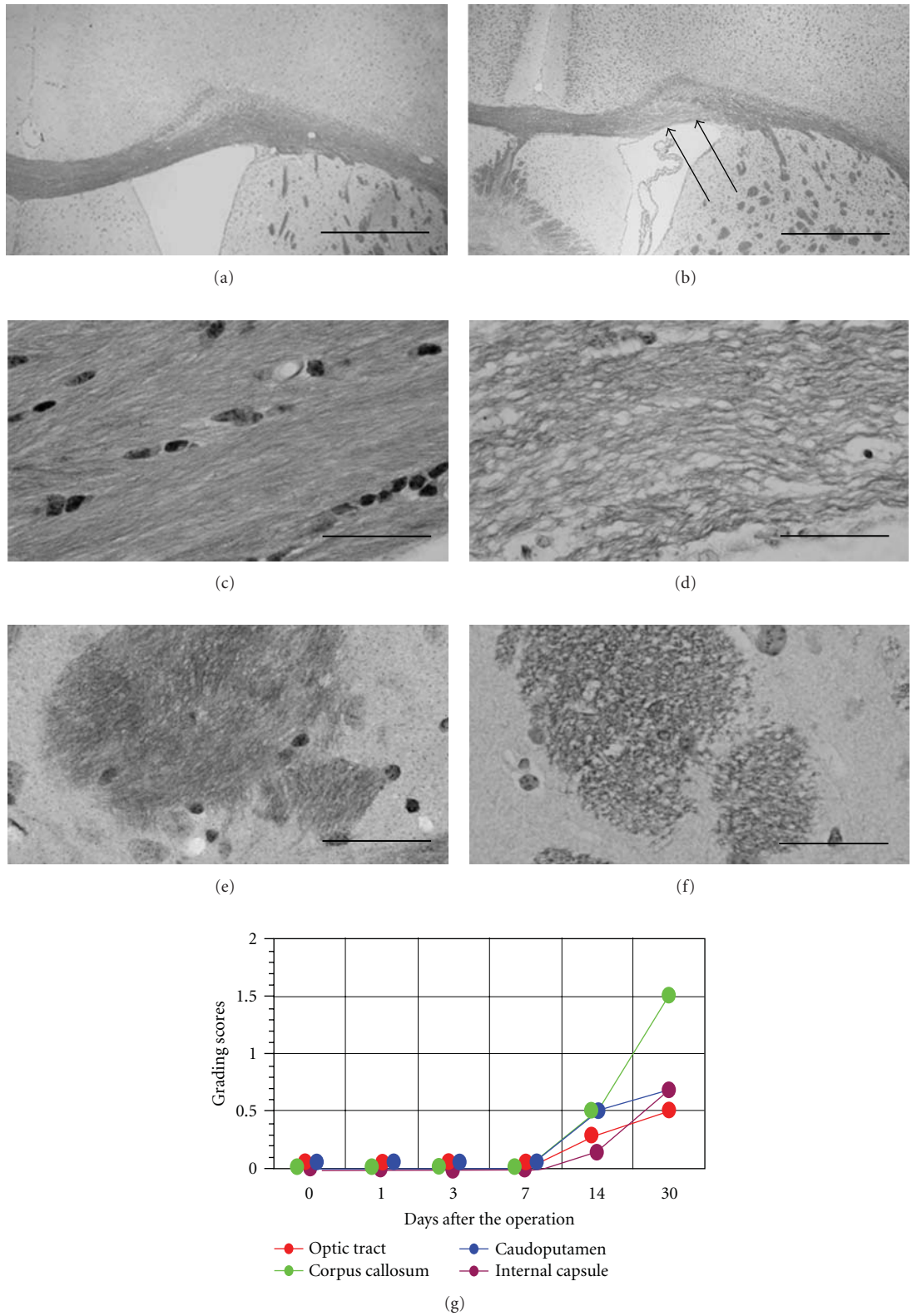

Figure 3: White matter changes after BCAS. Photomicrographs of Klüver-Barrera staining in the cerebral cortex (a, b), corpus callosum (c, d), and caudoputamen (e, f). The left column (a, c, e) indicates the brain from a sham-operated mouse, and the right column (b, d, f) indicates a brain after BCAS-operated mouse using microcoils with 0.18 mm diameter for 30 days. Note that the WM changes are the most intense in the medial part of the corpus callosum adjacent to the lateral ventricle (arrows). The histogram shows temporal profiles of the WM changes, the severity of which is semiquantitatively graded into four levels (g). Scale bar, 500 μm (a, b), 50 μm (c, d), and 25 μm (e, f).

MMP-2 or -9 activity and edema in WM [45]. These data therefore suggest that *in vivo* MRI is a sensitive measure of vasogenic edema and WM changes in the murine brain [44, 45].

6. Impairment of Learning and Memory after BCAS

In working memory tasks, tested with the 8-arm radial maze, BCAS mice made significantly more errors than the control mice following one month of hypoperfusion, although they did show normal spatial reference memory in the 8-arm radial maze test [20]. Spatial reference memory task is related to cognitive domains likely to rely on the integrity of the hippocampus, and therefore preserved reference memory is in agreement with lack of histological damage in the hippocampus [10]. In contrast, working memory impairment may be attributable to either the frontal WM lesions observed and/or hippocampal damage, which is undetectable by the conventional histological methods. In previous studies, working memory deficits have been related either to the hippocampus or frontal subcortical circuits in the rodent [13, 46] and likely primates [47, 48]. Therefore, disruption of WM tracts, especially within the prefrontal cortex, may be another mechanism behind age-related changes in working memory function [49]. A previous study has also shown that there is a selective impairment in spatial working memory, with all other measures of spatial memory remaining intact, in the BCAS mice with selective WM damage [42].

In contrast, in the longer-term BCAS model, in addition to WM changes, there were also significant hippocampal changes (atrophy and cell death) documented 8 months after BCAS (see Section 7). Consistent with these histological changes, a series of behavioral batteries demonstrate deficits in both working and reference memory. Thus, longer-term hypoperfusion more accurately replicates the advanced stages of SIVD and possibly provides evidence linking chronic hypoperfusion and aging [26].

7. Neuropathologic Changes Induced by BCAS

No infarctions or hemorrhage develops in any gray matter regions in mice with the 0.22, 0.20, and 0.18 mm microcoils after 1 month of chronic cerebral hypoperfusion [10]. There are no TUNEL positive neurons in the hippocampus [20]. In contrast, more than half of the BCAS mice with 0.16 mm microcoils placed exhibited microinfarcts in the parietal cortices, neuronal loss in the CA1 subfield of the hippocampus, and patchy necrotic lesions in the caudoputamen [10].

In contrast, at 8 months after BCAS, pyknotic neurons have been frequently observed in the cerebral cortex and the hippocampus. Furthermore, significant atrophy has been noted in the hippocampus but not in the cerebral cortex or the corpus callosum. The number of fragmented or shrunken nuclei stained for single-stranded DNA increased in the CA1 and CA3 sectors of the hippocampus but not in the dentate gyrus.

Given that the shorter-term (conventional) BCAS mice demonstrate WM damage without any apparent hippocampal damage at 1 month after BCAS, hippocampal degeneration in the longer-term BCAS mice may be secondary to the preceding WM damage. This may then subsequently contribute to the dementia syndrome, partly overlapping with Alzheimer's disease (AD) in their cognitive profiles and histological changes. In probable AD patients, a linear relation is found between WM lesions and hippocampal atrophy on MRI, especially for WM lesions in the frontal and parietooccipital regions [50]. A disconnection of the hippocampus by cerebrovascular WM lesions in the white matter tracts subserving the cortical association areas may lead to shrinkage of the hippocampus due to Wallerian degeneration as the hippocampus receives most of the input from the neocortical association cortices [51]. These findings are intriguing given the widely accepted fact that vascular dementia and AD both increase in prevalence with age, frequently occur concomitantly, and overlap considerably in their symptomatology, pathophysiology, and comorbidity [52]. WM damage may thus be one of the pathological substrates that mediates such a linkage between neurodegenerative and cerebrovascular disorders.

8. BCAS-Mediated Acceleration of Neurodegeneration: Linkage between Hypoperfusion and Neurodegeneration

WM attenuation has also been frequently observed in neurodegenerative disorders, such as AD and dementia with Lewy bodies [53]. MR imaging has revealed that such changes manifest as WM lesions, which increase with older age [54]; this is particularly apparent in AD and dementia with Lewy bodies, compared to ageing controls, though to a lesser extent than in vascular dementia. WM lesions in AD progress relatively slowly if a multicomponent intervention is given to reduce vascular risk factors [55], suggesting that ischemic changes underlie the WM lesions in AD. However, different mechanisms have been, at least to a certain extent, associated with myelin degeneration as it has been shown that myelin loss evolves in parallel with shrunken oligodendrocytes in vascular dementia but with their increased density in AD [56]. Further investigation is thus warranted to clarify the wider question of whether vascular brain injury has additive effects on AD pathogenesis [57–59]. To tackle this question, AD model mice have been subjected to chronic cerebral hypoperfusion by BCAS.

Biochemical analyses have indicated that BCAS increases the level of conformationally changed Aβ in soluble extracellular-enriched brain fractions in a relatively low-(J9) [60] and high-expressor line (J20) of the $APP_{Sw/Ind}$ mouse [61]. The latter study also demonstrated that BCAS significantly reduced the density of cored plaques and neurons of the hippocampus [61]. Notably, chronic cerebral hypoperfusion and $APP_{Sw/Ind}$ overexpression interdependently disrupted reference memory [61]. Therefore, soluble, but not insoluble, Aβ species may play a direct role in neurotoxicity and resultant behavioral abnormalities in the hypoperfused

$APP_{Sw/Ind}$ mice. Since the vascular-type lesions reproduced in the BCAS model are oligemic (e.g., noninfarctional) chronic hypoperfusion may accelerate AD neuropathology in a latent manner over an extended period of time via enhanced neuronal loss and altered Aβ metabolism. Given that oligodendrocytes are highly susceptible to Aβ toxicity [62], the results may further provide evidence linking chronic hypoperfusion with neurodegeneration.

9. Treatment: Future Directions on Intervention

9.1. MMP Inhibitor. The MMP inhibitor AG3340 has been shown to possess protective effects against WM lesions after chronic cerebral hypoperfusion in rats [21]. AG3340 administration has been shown to decrease IgM-immunoreactive glial cell density in the vicinity of the microvessels in the corpus callosum, suggesting it helps restore BBB integrity [21, 37]. Furthermore, genetic deletion of MMP-2 has been shown to attenuate the WM lesions after BCAS in mice. These data suggest the potential value of MMP inhibitors in preventing SIVD resulting from BBB dysfunction and chronic cerebral ischemia in humans [39]. An elucidation of the exact roles of MMP-2 in BBB disruption may also provide information useful in developing strategies for controlling WM damage.

9.2. Adenosine A_{2A} Ligand. As an endogenous neuromodulator in the brain, the extracellular levels of adenosine markedly increase under hypoxic/ischemic conditions. Adenosine exerts its physiological actions through activation of four G-protein-coupled membrane receptors, the A_1, A_{2A}, A_{2B}, and A_3 receptors [63]. The A_{2A} receptor has drawn attention for its ability to modify a variety of brain insults; for instance, mice deficient in the A_{2A} receptor have been shown to possess substantially smaller infarct volumes and better neurological behavioral deficit scores after transient focal ischemia [64]. A_{2A} receptor antagonists have also been shown to attenuate ischemic brain injury [65], suggesting a neuroprotective role of A_{2A} in acute ischemic injury. However, adenosine's action is likely to be diverse in the setting of brain injury as brain damage aggravates after hypoxic ischemia in immature A_{2A} knockout mice [66]. A recent study has further indicated that, following the BCAS operation, A_{2A} receptor knockout mice display more extensive demyelination-related damage together with proliferation of astrocytes and microglia in the WM, compared with wild-type mice [23]. Working memory, evaluated by means of an 8-arm radial maze test, is also more seriously impaired in A_{2A} receptor knockout mice relative to wild-type mice. Such effects have been associated with increased expression of proinflammatory cytokines, including tumor necrosis factor-α, interleukin-1β, and interleukin-6 in the WM. Therefore, activation of the A_{2A} receptor by its ligand may ameliorate the WM damage and cognitive deficits induced by BCAS through suppression of proinflammatory cytokines. Although the A_{2A} receptor may be a potential therapeutic target for the treatment of ischemic WM damage, a potential pitfall in their use may be their apparent opposing effects on different cell types such as neurons, inflammatory cells, and glial cells.

9.3. Angiotensin II Type 1 Receptor Blocker. Drugs that target the rennin-angiotensin system seem to have particular potential for prevention of dementias, including AD and vascular dementia. The Perindopril Protection Against Recurrent Stroke Study (PROGRESS) has suggested a protective effect of angiotensin-converting enzyme inhibitors on cognitive function in patients with stroke [67]. Moreover, the Study on Cognition and Prognosis in the Elderly (SCOPE) trial demonstrated a positive effect of the angiotensin II type 1 receptor blocker (ARB), candesartan, in a subgroup of elderly hypertensive patients with mild cognitive impairment [68]. Notably, a prospective cohort analysis of 819 491 participants has suggested that ARBs are associated with a significant reduction in the incidence and progression of dementia, even compared with angiotensin-converting enzyme inhibitors [69].

In accordance with the above clinical findings, telmisartan, an ARB with unique "delta lock" structure that strongly binds to angiotensin II type 1 receptor [70], and possesses a high degree of lipophilicity and thus the ability to cross the blood-brain barrier [71], has been shown to exert protective effects against WM damage and cognitive impairment in the BCAS mice [41]; it is thought to achieve this by alleviating microglial/astroglial activation, endothelial oxidative stress, and oligodendrocyte loss [41]. Notably, such protective effects are observed with a nonhypotensive dose, but not with a hypotensive dose of telmisartan, suggesting that such protective effects against WM lesions are independent of blood pressure, and are at least partially mediated by anti-inflammatory and antioxidative effects that are exerted in part by the pleiotropic effects of telmisartan such as PPAR-γ activation [41, 71]. Thus, telmisartan may be considered as a putative treatment for SIVD, though caution should be exercised when lowering blood pressure if cerebrovascular autoregulation is damaged. In clinical practice, appropriate timing and dose of telmisartan should be considered.

9.4. Adrenomedullin. Adrenomedullin (AM) has a variety of effects on the vasculature that include vasodilation, regulation of permeability, inhibition of endothelial cell apoptosis and oxidative stress, regulation of smooth muscle cell proliferation, and promotion of angiogenesis [72, 73]. AM heterozygosity in mice resulted in increased infarct volume with significant accumulation of inducible nitric oxide, oxidative DNA damage, and lipid peroxidation after transient focal ischemia [74] whilst prophylactic administration of AM alleviated cerebral edema in the striatum and cerebral cortex in a rat stroke model [75]. In BCAS mice, increased levels of circulating AM have been shown to restore cerebral hemodynamics, promote arteriogenesis, as well as angiogenesis, alleviate oxidative damage in cerebral microvessels, and preserve WM integrity; importantly, this subsequently attenuates working memory deficits in an 8-arm radial maze test [43]. In addition, AM selectively upregulates brain levels of cyclic AMP, vascular endothelial growth

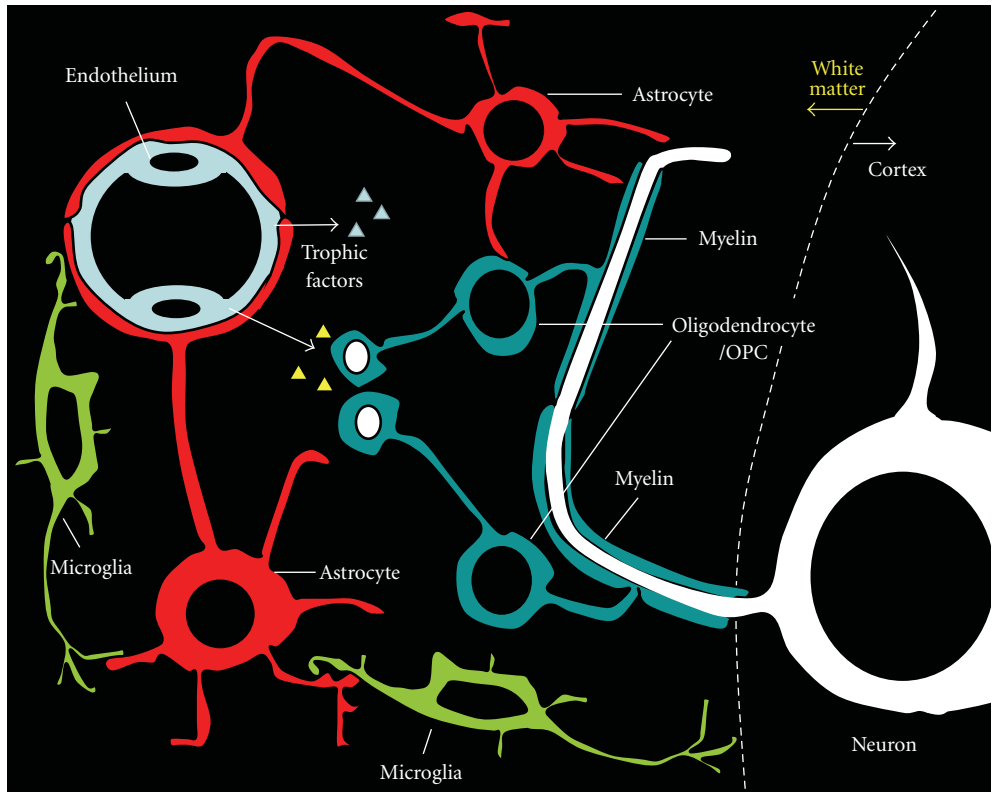

Figure 4: A schematic illustration of oligovascular niche. In the "oligovascular niche," crosstalk between endothelial cells and oligodendrocytes mediated by an exchange of soluble signals (e.g., trophic factors or chemical messengers) might play an important role in sustaining oligodendrocyte homeostasis and WM integrity. Since oxidative stress and inflammation caused by cerebral hypoperfusion would be detrimental for this niche, maintenance of white matter integrity or oligovascular protection could be achieved by proangiogenic, antioxidative, and anti-inflammatory interventions. OPC: oligodendrocyte precursor cell.

factor, and basic fibroblast growth factor in the hypoperfused, but not the normoperfused, brain. Furthermore, proangiogenic/arteriogenic changes did not occur in sham-operated AM-overexpressing mice where the expression of AM receptor component RAMP2 is significantly suppressed, possibly through feedback inhibition. Such tissue selectivity could be an advantage for clinical application of AM in patients with SIVD; AM-induced arteriogenesis and angiogenesis could be induced only in hypoperfused tissue.

9.5. Bone Marrow Transplantation. Therapeutic use of bone-marrow-derived cells has been shown to ameliorate functional deficits after stroke and is accompanied by augmentation of angiogenic and regenerative responses [76]. Although early functional improvement has been noted within days of treatment, its precise mechanism remains to be elucidated. A recent study has demonstrated that administration of bone marrow mononuclear cells (BMMNCs) induces immediate endothelial nitric oxide synthase-dependent vasodilation in ischemic femoral arteries [77].

In BCAS mice, BMMNC treatment has been shown to provide strong protection against WM damage, dependent primarily on CBF recovery beginning from the early phase, and the subsequent endogenous restorative response, including angiogenesis, in a later phase [78]. Both of these responses involve nitric oxide synthase activation. Despite marked protection against WM damage, no direct structural incorporation of donor BMMNCs to oligodendrogenesis was found, although a fraction of donor cells were found to wrap around the microvessels with features suggestive of pericytes [78, 79]. While a direct antiapoptotic effect on oligodendrocytes may be involved in the WM protection [80], it is plausible that CBF recovery after BMMNC treatment is sufficient to maintain WM integrity. Additional investigation is therefore required to assess whether other mechanisms such as direct structural incorporation or direct antiapoptotic effect of BMMNCs play a role in the WM protection. The above findings suggest clinical applicability of BMMNC treatment for SIVD management.

10. A New Concept of "Oligovascular Niche"

Recently, the concept of "oligovascular niche" has been proposed, where crosstalk between the endothelial cells and oligodendrocytes mediated by an exchange of soluble signals (such as brain-derived neurotrophic factor and fibroblast growth factor) might play an important role in sustaining oligodendrocyte homeostasis and WM integrity (Figure 4) [81, 82]. In the oligovascular unit, endothelial cells release trophic factors that promote oligodendrocyte precursor

cell proliferation. Noncytotoxic levels of oxidative stress or blockade of Src and Akt signaling prevents endothelial trophic factors from supporting oligodendrocyte precursor cells. Therefore, to treat or prevent WM damage in SIVD, endothelial cells and oligodendrocytes should be protected from being damaged due to hypoperfusion.

Since cerebral endothelial cells contribute to numerous signaling cascades that help regulate brain homeostasis and function [83], angio-/arteriogenesis and inhibition of oxidative damage in the cerebral endothelial cells might lead to oligovascular protection—namely, successful vascular growth and vasoprotection and preservation of WM/oligodendrocyte integrity—and prevention of cognitive decline after chronic cerebral hypoperfusion. Therefore, the application of proangiogenic, antioxidative, and anti-inflammatory factors, including the aforementioned drug- and cell-based therapy, may offer potential for the treatment of WM changes and SIVD.

11. Summary and Conclusions

The BCAS model characterizing features of vascular cognitive impairment with WM changes may serve as a powerful tool for investigation of the molecular pathology of WM lesions and in the design of therapeutic measures for WM changes induced by chronic cerebral hypoperfusion. Although these models do not (and no other current models can) describe all features of SIVD [84], the BCAS model may help further elucidate the mechanism by which WM pathology and dementia progress in the elderly. Such knowledge may significantly enhance strategies to tackle these disorders.

Acknowledgments

The authors would like to thank Dr. Khundakar for his editorial assistance and helpful discussion, Dr. Maki and Dr. Shibata for providing some of the images used in this paper, and the Ph.D. students involved for their commitment and enthusiasm in pursuit of excellence.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 407074, 8 pages
doi:10.4061/2011/407074

Review Article

Practice-Oriented Retest Learning as the Basic Form of Cognitive Plasticity of the Aging Brain

Lixia Yang

Department of Psychology, Ryerson University, JOR823A, 350 Victoria Street, Toronto, ON, Canada M5B 2K3

Correspondence should be addressed to Lixia Yang, lixiay@ryerson.ca

Received 13 April 2011; Revised 23 August 2011; Accepted 24 August 2011

Academic Editor: Leonardo Pantoni

It has been well documented that aging is associated with declines in a variety of cognitive functions. A growing body of research shows that the age-related cognitive declines are reversible through cognitive training programs, suggesting maintained cognitive plasticity of the aging brain. Retest learning represents a basic form of cognitive plasticity. It has been consistently demonstrated for adults in young-old and old-old ages. Accumulated research indicates that retest learning is effective, robust, endurable and could occur at a more conceptual level beyond item-specific memorization. Recent studies also demonstrate promisingly broader transfer effects from retest practice of activities involving complex executive functioning to other untrained tasks. The results shed light on the development of self-guided mental exercise programs to improve cognitive performance and efficiency of the aging brain. The relevant studies were reviewed, and the findings were discussed in light of their limitations, implications, and future directions.

1. Introduction

In research on cognition and aging, cognitive declines among older adults have been commonly documented in both cross-sectional (e.g., [1]) and longitudinal studies (e.g., [2]). The declines tend to accelerate in the advanced ages [3–6]. The cognitive declines, in combination with physical deterioration, in senior adults make them critically vulnerable in their everyday activities and could eventually deprive them of their independence and thus diminish their quality of life. This will in turn exert burdens on their families and society. This fact has spurred a growing interest in research on cognitive plasticity (i.e., the ability to improve performance through training) in older adults. The accumulated research evidence suggests that cognitive training is effective in reducing or even reversing age-related declines in target abilities that are vulnerable to aging-associated declines (for comprehensive reviews, see [7–10]).

A large body of cognitive training studies has focused on directly teaching older adults strategies, including mnemonic techniques (for meta-analysis, see [11]), cognitive strategies facilitating performance on psychometric tests such as those for reasoning or spatial orientation (e.g., [12]). Direct strategy instruction often effectively lead to performance increments in the target cognitive tests for older adults (e.g., [12–16]; for reviews, see [17]), but the performance improvement is typically specific to the tasks directly corresponding to the taught strategies [11, 18]. In addition, learning, consolidating, and applying a new cognitive strategy are usually effortful and time consuming, thus it is unclear whether older adults would continue to use the learned strategies in their life after training. It has been shown that strategy-based training benefits tend to decrease with age [19]. Oldest-old adults (i.e., primarily in their 80s) were unable to effectively apply and optimize the newly taught mnemonic technique [20].

Alternatively, an emerging body of studies adopted a process-specific training approach to examine the benefits of retest practice of psychometric tests of fluid ability (e.g., reasoning, speed), executive functions (e.g., updating, switching, dual-task coordination), cognitively stimulating activities (e.g., problem solving, brain teasers) on older adults' performance on the trained or other untrained cognitive tasks ([21–23]; for reviews, see [7, 17]). The process-specific training produced sizable retest learning, namely, performance improvement on the target process through

retest practice on taking the test, without instructions on task-specific strategies or adaptive feedback [9, 23]. It has been postulated that retest learning is mainly driven by reactivating or refreshing old skills that decline with age but are still available in repertoires of older adults and thus represents the basic form of cognitive plasticity [23]. Retest learning has been demonstrated in both young-old and oldest-old adults [23]. Most promisingly, some recent studies reported that older adults were able to transfer these practice-oriented training gains, primarily in the domain of executive functions, to other untrained tasks (e.g., [24]), although the majority cognitive training studies reported limited and ability-specific transfer effects.

In the past two decades, there have been a growing number of reviews on cognitive plasticity in old age. Baltes and Linderberger [17] summarized findings of 15 years of cognitive intervention research conducted by Baltes and colleagues, primarily from two projects: ADEPT (Penn State Adult Development and Enrichment Project) and PRO-ALT (Projekt Altersintellingenz). Willis [25] identified some methodological issues in behavioral intervention studies with older adults; Kramer and Willis [26] reviewed research evidence for enhancing older adults' cognitive vitality through domain-relevant experience, laboratory-based cognitive training, and aerobic fitness training; Thompson and Foth [10] reviewed various cognitive intervention programs and their effects in enhancing mental fitness in older adults; Greenwood [27] proposed a hypothesis to view functional plasticity from the perspective of adaption of the aging brains to cognitive declines. Similarly, Goh and Park [28] proposed a "scaffolding theory" to understand neuroplasticity of the aging brain; Hertzog and colleagues [7] provided a comprehensive review of research on cognitive enrichment effects, primarily in older adults, through cognitive intervention, skill development, mentally stimulating activity, physical aerobic exercise, social engagement, as well as positive attitudes and beliefs. Lövdén and colleagues [8] proposed a theoretical framework that views cognitive plasticity as sluggish and limited intrinsic capacity for reacting to a mismatch between supply and demand. Finally, Stine-Morrow and Basak [9] reviewed different variables and approaches adopted in cognitive interventions across life-span, with a focus on aging.

Different from all these aforementioned comprehensive reviews that focus on a general coverage of various types of experience or interventions that promote cognitive functioning of older adults, the current paper zooms in and provides an in-depth and specific review on studies that examined retest learning in aging population. Different from strategy-based training paradigms, retest learning represents a basic form of plasticity because it involves minimal externally-exerted intervention, without a need for any external guidance on task-performing strategies or adaptive feedback. It is most useful to reactivate or refresh old skills that decline with age. Relative to strategy-based training that requires a systematic guidance from an external trainer, retest learning may represent a promising and cost-effective self-guided intervention practice for older adults.

Specifically, this paper focuses on the following core questions which have been addressed in retest learning literature. (1) Does retest learning benefit older adults? (2) Is retest learning endurable in old age? (3) Is retest learning item-specific? (4) Can older adults transfer retest learning to untrained tasks? At the end, the results would be discussed in light of their limitations and implications, together with some proposed future research directions.

2. Does Retest Learning Benefit Older Adults?

Data from longitudinal studies revealed substantial retest effects in cognitive abilities among adults aged 18–70 (e.g., [29–32]). Echoing with these findings, a growing body of research on cognitive plasticity indicates that older adults, including those in advanced ages, also show robust retest learning effects, mostly in fluid intelligence and executive functioning.

2.1. Retest Learning in Fluid Intelligence. Earlier studies on retest learning mainly focused on improving performance on tasks measuring fluid intelligence or cognitive mechanics, such as speed and reasoning (e.g., [33, 34]). Hoyer et al. [34] trained 32 elderly females (mean age = 70 years) on test-related response speed through two sessions of retest practice. The results showed sizable retest learning effects (i.e., increased response speed on the trained tests), suggesting that the age-related intellectual declines in speed may mainly reflect performance declines rather than competence deficits. Hofland et al. [33] conducted a study in which 30 young-old adults (mean age = 69 years) participated in eight sessions of retest practice on two reasoning tests: figure relations and induction. The same tests with identical items were administered across sessions and participants did not receive feedback on individual performance. The results demonstrated remarkable retest learning effects, with a total performance improvement by slightly over 1 Standard Deviation (SD).

To investigate whether the aforementioned retest learning could be extended into oldest-old adults in their 80s and over as well as whether the magnitude of retest learning is moderated by age and cognitive functioning status, Yang et al. [23] compared 34 young olds (mean age = 74, rage = 70–79) and 34 oldest olds (mean age = 84, range = 80–91) in retest learning effects on three psychometric ability domains: reasoning, speed, and attention. Each age group was evenly divided into two groups, with high or low levels of cognitive functions relative to their age norms provided in the Berlin Aging Study (BASE, [4]). Participants completed six 1-hour retest sessions, spreading over a 3-week period, that focus on self-guided practice of 6 tasks measuring reasoning, speed, and attention. The results revealed substantial retest learning effects in both age groups across all the tasks and thus provided evidence for continued cognitive plasticity in the oldest-old age. The magnitude of retest learning was moderated by both age and cognitive functioning status in most complex reasoning tasks, with differentially lower learning rates for the oldest-old and low-status groups. For the speed task (i.e., Digit Symbol), the retest learning effect

was moderated by age, but not by functioning status. For the least demanding visual attention task (i.e., D2 task), neither age nor cognitive functioning moderated the retest learning magnitude. These results suggest that age- and ability-graded declines in the basic form of cognitive plasticity, indexed by retest learning rate, only occur when the training tasks or the trained skills are complex and/or challenging. Taken together, the fluid intelligence retest training studies suggest that older adults, including those in advanced ages, are able to improve their performance on fluid ability tests through retest practice on the standard psychometric tasks.

2.2. Retest Learning in Executive Functioning. Retest practice paradigm has also been employed in some recent studies that aimed to improve older adults' working memory and executive functions ([21, 22, 24, 35–37]). A considerable number of studies indicate that young and older adults are capable of improving effective attentional control skills through practice on dual-task performance across a couple of sessions, with a larger training benefit demonstrated in the variable-priority training condition (i.e., shifting processing priorities between tasks in the dual-task paradigm) relative to the fixed-priority training condition (i.e., always prioritizing one task over the other in the dual-task paradigm) [21, 36, 38]. In another study, Dahlin and colleagues [35] trained young and older adults on a computer-based working memory updating task (i.e., Letter Memory) through five sessions of retest practice. Both age groups showed reliable retest learning in working memory updating performance. Furthermore, Karbach and Kray [24] administered four sessions of computerized task-switching training to children, younger and older adults. All age groups showed reliable retest learning effects on performing the trained task-switching task. In another study, Li and colleagues [22] extensively trained young (ages 20–30) and older adults (ages 70–80) on a spatial n-back working memory task across 45 days for about 15 minutes each day. Both age groups showed substantial retest learning effect as indexed by the significant performance gain on the practiced task. Similarly, Buschkuehl and colleagues [39] trained a sample of 80-year-old adults (mean age = 80.0, SD = 3.3) twice weekly for 12 weeks to practice on a variety of computerized working memory tasks that requires reproducing sequences of stimuli in an adaptive way (i.e., the sequence length was continuously adjusted to the individual's working memory capacity). The results revealed significant improvement in the performance on all the trained working memory tasks. In a recent study, Wilkinson and Yang [37] examined the retest learning in inhibition with older adults (mean age = 71.05, range = 60–84 years) using a 6-session Stroop retest training paradigm. Participants practiced on the classical color word Stroop task for about 30 minutes on each session across six retest sessions. The results showed significant linear decrease in Stroop interference effect, suggesting improvement in inhibition. The learning induced from these executive functioning or working memory training studies is considered as retest learning because the training provided is practice-oriented retest aiming to improve general process efficiency without teaching any task-specific strategies. Taken together, the executive functioning training studies suggest that deliberate retest practice is effective to improve older adults' cognitive performance on working memory or executive functioning performance.

In sum, training studies with a practice-oriented retest paradigm on fluid psychometric intelligence (such as reasoning and speed) and executive functioning (such as updating, switching, inhibition, and working memory) consistently revealed substantial improvement in older adults, suggesting that older adults reserve the basic form of cognitive plasticity.

3. Is Retest Learning Item Specific or General?

In most studies involving retest learning, particularly those longitudinal or training studies on fluid intelligence, participants are repeatedly tested with identical items/tasks across the sessions. It is possible that the retest learning effects may be primarily driven by item-specific effects through memorization/familiarization with specific items and/or solutions.

It has been argued that age effects observed in longitudinal studies are composed of positive effects associated with repeated retest practice (i.e., retest effects) and negative effects associated with age (e.g., [31, 32]). However, in most conventional longitudinal studies, identical versions of tests are administered across testing occasions. It is not very clear whether the retest effects could be minimized or even eliminated by adopting alternate versions of tests on different test occasions. In an article by Salthouse and Tucker-Drob [40], three sets of short-term retest (ranging from 1 day to a few weeks) data were analyzed and compared to evaluate the contribution of short-term retest effects in the interpretation of longitudinal change. The three studies involved in this analysis included moderately large samples of adults ranging from 18 to over 80 years of age who were tested on a cognitive battery of 16 tests at two or three occasions, with either identical or alternate versions of tests administered across two successive occasions. The results indicated that although the retest effects were greatly reduced in most tasks of memory and reasoning when the alternate versions of tests were administered, retest effects remained substantial and almost equivalent in spatial visualization and perceptual speed between the alternate-version condition (i.e., different items) and the identical-version condition (i.e., same items). These findings suggest item-specific effects may differ across ability tests, with some tests (e.g., memory) being more vulnerable to item-specific memorization effects than others (e.g., speed). Nevertheless, the results indicated that retest effects could occur even in the absence of item-specific effects in adults aged between 18 to over 80 years.

To investigate whether older adults show retest learning in the absence of item-specific effects, Yang and colleagues [41] specifically examined the non-item-specific retest learning in 31 older adults (mean age = 71.10, range = 60–82 years). The item-specific effects were eliminated through administering parallel versions of the psychometric tests of reasoning, speed, and visual attention across eight retest sessions so that participants completed a brand new set of items

at each single session. The analysis on the number of correct solutions showed substantial retest improvement across all the three cognitive ability domains. However, the analysis on accuracy (i.e., the proportion of correct solutions out of all attempted items) showed a linear improvement only in the reasoning domain. Echoing with the findings of a strategy-based training study [42], this result suggested increased self-discovered strategy use in completing the reasoning tasks with retest practice. The comparison of retest learning effect size between this study and a comparable study that was vulnerable to item-specific effects (i.e., with identical tasks administered across sessions) [23] suggested that only retest learning in the reasoning domain could potentially take advantage of item-specific effects. Nevertheless, it should be noted that retest learning in reasoning was still reliable even after controlling for the contribution of item-specific effects and anxiety [41], suggesting that retest learning could occur at a more conceptual level in old age.

4. Long-Term Maintenance of Retest Learning

In studies with strategy-based cognitive training, it has been shown that older adults not only improve their cognitive performance through cognitive training but also maintain the training-induced improvement for a long period of time, such as up to 7 years [43, 44]. Echoing with this finding, longitudinal data also estimate that it takes 7 or more years before the positive retest effects become undetectable in longitudinal changes in cognitive functioning of adults aged 18–60 years [31].

However, to our knowledge, only a few studies have examined the long-term maintenance of retest learning effects in cognitive plasticity studies with older adults. Li et al. [22] investigated the practice-oriented retest training for young and older adults with a spatial n-back working memory paradigm and found substantial retest effects and transfer effects to tasks requiring similar processes. Most impressively, both age groups maintained the retest improvements and transfer effects over a period of 3 months, although the maintenance of retest learning was reduced with age. In another study, Yang and Krampe [45] compared long-term maintenance of retest learning in fluid intelligence over a period of 8 months between young-old (in their 70s) and oldest-old adults (in their 80s and onwards). The results suggested that both age groups maintained about 50% of original retest learning gains over six sessions of retest practice on psychometric tests of reasoning, speed, and attention. Together with the original study [23], this study suggests that the retest learning effects in fluid intelligence are robust and can endure for at least 8 months, even in oldest-old age. Strikingly, the maintenance effect size in reasoning over 8 months (0.45 SD) roughly corresponds to the amount of age-related decline naturally occurring over 14 years (0.42 SD) and is comparable to the trainer-guided training gain (0.48 SD) across 10 sessions [13, 45]. The maintenance effect size in speed over the 8-month period (0.66 SD) is more than 4 times the amount of age-related decline naturally occurring over 2 years (0.16 SD) [13]. Recently, some research data collected in my lab suggest that old adults are able to maintain retest learning in inhibitory control, as measured with the reduced interference effects using a Stroop retest training paradigm, for up to 1 year [46]. Taken together, the limited yet accumulating empirical findings suggest that retest learning is endurable and can be maintained for a substantial period of time in old age, even in the oldest-old age.

5. Can Older Adults Transfer Retest Learning to Other Untrained Tasks?

In the domain of fluid ability (e.g., reasoning and speed), transfer effects from either strategy-based training or practice-oriented retest training are very limited and highly ability specific [13, 19]. Transfer, if any, usually occurs to the similar tasks that share surface features and strategies with the practiced tasks. In a study by Baltes et al. [19], a sample of 72 elderly participants (mean age = 72 years) were randomly assigned into three conditions: a tutor-guided training group received five sessions of training that focused on teaching and identifying task-relevant problem-solving strategies and providing performance feedback on two figure relations reasoning tasks; a self-guided retest training group who went through five sessions of retest practice on the same reasoning tasks but did not receive any problem-solving strategies or feedback; a no-contact control group who did not receive any types of training. All participants completed a large set of cognitive tests at the pretest and posttest sessions. In comparison to the no-contact control group, the tutor-guided training group and the self-guided retest training group demonstrated comparable transfer effects, but the transfer was largely limited to the similar figure relations reasoning tasks. The results suggest that elderly adults are capable of achieving the same degree of performance improvement on their own through retest practice as that induced through tutor-guided strategy-based training, thus strongly supported the effectiveness of retest practice as a basic form of cognition training paradigm. Nevertheless, transfer from retest practice of fluid ability tests was largely ability specific and primarily limited to trained tasks. This limited transfer effect seems to be not due to old age of participants. In a recent large-scale study with participants from a wider age range (18–60 year), a 6-week web-based online practice of tasks measuring fluid abilities, such as reasoning, planning, problem solving skills, attention, memory, and visuospatial processing, improved performance on the trained tasks but did not produce any transfer effect to other untrained tasks, despite they measure the same abilities (e.g., reasoning and memory) [47].

Retest learning studies on executive functioning produced promising yet mixed evidence in terms of transfer effects. Similar to the training studies in fluid ability, a number of studies have demonstrated near transfer effects (i.e., transfer to similar but untrained tasks) of practice with the core process, such as executive functioning or working memory performance. In the study of Dahlin et al. [35], both young and older age groups showed substantial retest learning in working memory updating performance across five sessions of retest practice. However,

only young adults transferred the retest benefits to a 3-back working memory task that also required continuous updating. Most importantly, young adults showed a training-induced activity increase in the striatum for both the trained task (i.e., Letter Memory) and the transfer task (i.e., 3 back), suggesting the transfer effect was mainly mediated by the striatum. In contrast, older adults did not show transfer effects, and their striatum activation changes were restricted to the trained task. These results suggested that older adults reserve sizable retest learning in working memory updating but this learning does not help them improve performance on other tasks requiring updating. In Li et al. [22], both young and older age groups transferred the retest learning effect in performance on a spatial working memory n-back task to another more difficult spatial n-back task and to numerical n-back tasks. The practice gains and transfer effects were maintained for 3 months, though older adults showed decrements in performance relative to postpractice performance. It should be noted that these studies focused on practicing with a single component executive functioning task (e.g., updating or n-back). Transfer effects were limited and specific to the tasks that share surface structure or measure similar or the same abilities.

Promisingly, a growing number of studies revealed broader far transfer effects (i.e., transfer to untrained tasks measuring different abilities) from the intervention programs that target at practicing/exercising fundamental core executive functioning using multiple tasks (or multiple versions of the same task) and with complex video games. In the task-switching training study by Karbach and Kray [24], all age groups, including children, younger and older adults, demonstrated sizable near transfer effects from practicing computerized task-switching tasks across four retest sessions to structurally similar tasks and significant far transfer effects to structurally dissimilar "executive" tasks and fluid intelligence. In a related light, another recent study also suggested that practicing on a complex real-time strategy video game that requires frequent shifts in component task priority for fifteen 1.5-hour sessions over a period of 4-5 weeks could improve older adults' executive control performance [48]. In addition, another study trained old-old adults with an average age of 80 years with a 3-month (twice weekly) intervention program with a focus on practicing on a set of working memory tasks requiring reproducing sequences of colored blocks or animals on a computer screen, as well as some Reaction Time (RT) tasks. In comparison to an active control group who received physical training with an eccentric bicycle ergometer, the cognitive training group showed a sizable transfer effect to other untrained working memory and even episodic memory tasks [39]. Taken together, the significant far transfer effects appear to be evident in such training programs that focus on practicing of multiple or different versions of executive functioning tasks or engaging in activities involving complex cognitive control.

Recently, an emerging body of studies suggested that participation in cognitively stimulating activities or immersion in an engaged life style (e.g., practice with brain teasers or puzzles, spontaneous problem solving, and helping in elementary schools), even for a short period of time, could significantly enhance fluid ability, flexible thinking, executive functions, and memory performance [49–51]. For example, in the Senior Odyssey Project conducted by Stine-Morrow and colleagues [50], older adults were randomly assigned to a 20-week program designed to operationalize an engaged lifestyle through active participation in a team-based competition in creative and spontaneous problem solving. Compared to a wait-list control group, the intervention group showed modest improvement in a composite measure of general fluid ability from pretest to posttest. This far transfer effect occurred even in the absence of explicit trainer-guided training, suggesting a possibility that the improvement may be driven by enhanced self-regulation and sustained practice of multiple abilities involved in the engaged activities. Overall, these studies suggest that older adults are able to show a broad benefit on their general cognitive ability from a short-term engagement in cognitively stimulating activities.

Taken together, relative to retest practice of fluid ability, retest practice of executive functioning or activities involving executive control produced encouraging far transfer effects. This is probably because the executive functioning training focuses on nonspecific fundamental processes underlying performance on a broad range of cognitive tasks. In support to this assumption, a large-scale study by Smith and colleagues [52] with community-dwelling older adults suggested that 8-week practice on a brain plasticity-based computerized cognitive training program designed to improve basic cognitive efficiency (i.e., speed and accuracy) of central auditory systems could be transferred to untrained standardized measures of auditory-based memory and attention.

In addition, there has been empirical evidence suggesting that executive functioning is largely regulated by prefrontal cortex [53], a brain region that underlies general cognitive control and shows greater and earlier signs of age-related decline than most other areas of the brain [54]. Extensive practice of executive functioning or related activities may induce neural efficiency of frontal lobe and thus improve other cognitive functions sharing the overlapped brain regions. Support for this argument could be found in Dahlin et al. [35] that suggested transfer effect of working memory updating to a 3-back task in younger adults was mainly mediated by the striatum, commonly shared between the two tasks.

Inspired by promisingly broad transfer effects emerged from retest practice of executive functions or cognitively engaged life style, the following factors/features may potentially promote transfer effects: (1) the trained tasks and the transfer tasks share some commonalities (e.g., underlying brain regions); (2) a focus on exercising fundamental functioning or improving basic cognitive efficiency (e.g., executive control, speed and accuracy); (3) engagement of cognitively stimulating activities involving executive control and promoting self-regulation (e.g., spontaneous problem solving); (4) an extensive practice schedule lasting at least for weeks.

Taken together, like most classical cognitive training studies (e.g., [13]), the retest learning in the domain of

fluid ability is relatively ability specific and only benefits similar tasks that involve the same processes as those being practiced. Nevertheless, the magnitude of transfer effects is comparable between self-guided retest practice and tutor-guided strategy-based training in the reasoning domain. The limited transfer effects tend to be narrower in scope and more restricted in older than in young adults. Promisingly, retest practice on executive functioning, working memory, and cognitively engaged life style produced encouraging far transfer benefits than retest practice on fluid intelligence tasks.

6. Limitations, Implications, and Future Directions

The inspiring findings reviewed above should be evaluated in light of limitations commonly observed in retest learning studies. First, most of the studies involve a highly selected sample that features high-functioning and well-educated older adults, thus we need to be careful to avoid overgeneralization of the results to low-functioning older population. Second, the exact mechanisms underlying the retest improvement are still poorly understood, although previous studies shed a light on potential contributions of item-specific memorization, self-discovered strategies with practice, familiarity with testing situation, and implicit procedural learning [31, 41]. It is also possible that the mechanisms of retest learning are different across tasks or ability domains. For example, Yang et al. [41] reported that retest learning in the reasoning domain, but not in the speed and attention domains, was partially driven by item-specific memorization. More studies are in need to pinpoint the underlying factors driving retest learning in different ability domains. Third, transfer tasks were individually selected in different studies, and they may not capture the optimal transfer effects. In any event, the generally limited and task-specific transfer effects revealed in most cognitive training studies (including the retest learning ones), especially in the domain of fluid abilities, greatly constrain the application of these interventions to benefit everyday life of older adults. Finally, retest learning does not directly help with learning new strategies or skills. In the situations where learning new strategies are required (e.g., learning how to play piano), trainer-guided strategy-based training would be most effective.

Despite the limitations, retest learning literature revealed significant and robust retest learning improvement in the performance of practiced tasks in older adults, even in oldest-old age. A growing body of studies suggests that interventions with practicing on activities engaging complex executive functioning have promising broad transfer effects to benefit older adults' general cognitive function. These findings provide empirical foundation for practical application of the development of self-guided cognitive retest programs that could be integrated into older adults' everyday life. In comparison with most trainer-guided and strategy-based training programs, retest learning program is most economic and convenient for older adults because it does not require a trainer and it does not necessarily follow a strict schedule. Older adults could do it at their convenient time and location. All they need is a well-designed program (either a computerized program or a series of booklets) that targets the complex executive functioning and cognitive efficiency (e.g., processing speed and accuracy) of central neural system. This approach has been implemented in some recent work, although their benefits on older adults' everyday functions are still unclear. For example, Schmiedek and colleagues [55] developed a web-based training program involving 100 daily practice sessions on tasks of perceptual speed, episodic memory, and working memory. The program was evidenced to be acceptable and feasible for older adults. Some community-based programs focusing on cognitively stimulating activities and engaged life style and thus have shown promising effect on enhancing older adults' general cognitive ability [49–51]. Despite retest learning does not directly help with learning of new strategies, it should serve as the comparison baseline to correctly evaluate the strategy-based cognitive training effects [9]. Finally, retest practice of complex executive functioning may promote general cognitive efficiency and thus facilitate learning of new strategies or skills.

In light of promising broad transfer effects of retest practice on tasks heavily loaded with requirement in frontal-lobe-dependent executive control functioning, future studies should expand and explore the optimal conditions, features, and scopes of training to be most beneficial to the daily functions of older adults. This is a challenging direction given the overall limited and task-specific transfer effect revealed in most cognitive training studies, including both strategy-based or retest learning paradigm. But it is extremely meaningful because the ultimate goal of training/practice is to improve older adults' daily functioning and reduce their independence. Future studies should also be directed to the beneficial effects of life-long practice of an engaged life-style or mental exercise materials/programs in improving older adults' cognitive functioning.

7. Summary and Conclusions

The existing retest learning and aging literature suggests that older adults, even those in advanced ages, reserve substantial basic form of cognitive plasticity. They demonstrate sizable retest learning benefits from practicing on tasks measuring fluid ability and executive functioning. The retest learning effects are substantial, robust, and endurable and could occur at a non-item-specific conceptual level. In the domain of fluid intelligence, older adults are also capable of transferring retest learning to other ability-specific tasks, with equivalent transfer effects from self-guided retest practice and tutor-guided training. Some recent studies show promisingly broad transfer effects from retest practice of tasks engaging executive functioning. Practice of executive functioning provides a valuable and promising self-guided intervention approach for enhancing older adults' brain functions in prefrontal cortex, general cognitive functioning, and hopefully daily activities and functions.

Acknowledgment

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (CCI-102930).

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 481745, 9 pages
doi:10.4061/2011/481745

Research Article

Diffusion Tensor Imaging Evaluation of Corticospinal Tract Hyperintensity in Upper Motor Neuron-Predominant ALS Patients

Venkateswaran Rajagopalan,[1] Didier Allexandre,[1] Guang H. Yue,[1,2] and Erik P. Pioro[3,4]

[1] *Department of Biomedical Engineering, ND2, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA*
[2] *Department of Physical Medicine and Rehabilitation S30, Neurological Institute S90, Cleveland Clinic, Cleveland, OH 44195, USA*
[3] *Department of Neurology, S90, Neurological Institute, Cleveland Clinic, Cleveland, OH 44195, USA*
[4] *Department of Neurosciences, NB2, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA*

Correspondence should be addressed to Erik P. Pioro, pioroe@ccf.org

Received 15 April 2011; Accepted 9 August 2011

Academic Editor: Ana Verdelho

Amyotrophic lateral sclerosis (ALS) patients with predominant upper motor neuron (UMN) signs occasionally have hyperintensity of corticospinal tract (CST) on T2- and proton-density-(PD-) weighted brain images. Diffusion tensor imaging (DTI) was used to assess whether diffusion parameters along intracranial CST differ in presence or absence of hyperintensity and correspond to UMN dysfunction. DTI brain scans were acquired in 47 UMN-predominant ALS patients with ($n = 21$) or without ($n = 26$) CST hyperintensity and in 10 control subjects. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were measured in four regions of interests (ROIs) along CST. Abnormalities ($P < 0.05$) were observed in FA, AD, or RD in CST primarily at internal capsule (IC) level in ALS patients, especially those with CST hyperintensity. Clinical measures corresponded well with DTI changes at IC level. The IC abnormalities suggest a prominent axonopathy in UMN-predominant ALS and that tissue changes underlying CST hyperintensity have specific DTI changes, suggestive of unique axonal pathology.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive degeneration of motor neurons in the brain and spinal cord whose cause is unknown [1]. It is also unclear whether the motor neuron degeneration begins in the perikaryon (cell body) as a neuronopathy and proceeds anterogradely, or along the axon as an axonopathy and proceeds retrogradely. Brain motor neurons, mostly in the primary motor cortex (upper motor neurons), project their axons caudally along the corticospinal tract (CST) through the brainstem to the spinal cord where they synapse onto anterior horn cells (lower motor neurons). Progressive degeneration of these two motor neuron pools results in ALS with upper motor neuron (UMN) signs (hypertonicity, hyperreflexia, and pathologic reflexes) and lower motor neuron (LMN) signs (muscle fasciculations, atrophy, and weakness) both being required for diagnosis [1]. A certain percentage of ALS patients present with UMN-predominant disease, and few or no clinically detectable LMN signs. By El Escorial criteria, they are usually initially categorized as possible or, at most, probable with laboratory support ALS because of the limited extent of LMN findings [2]. As disease progresses, their LMN dysfunction may remain either limited in degree or become more extensive.

Independent of this observation, a relatively small percentage of ALS patients display bilateral hyperintensity of the corticospinal tract (CST) on T2- and proton-density-(PD-) weighted MRI sequences [1]. Such hyperintensity can be seen anywhere along the rostrocaudal extent of the intracranial CST extent from just beneath the primary motor cortex, corona radiata, and centrum semiovale, but it tends to be most prominent in the posterior limb of the internal capsule and cerebral peduncles. A single study in 1994 of three ALS patients with CST hyperintensity who at postmortem had demyelination and Wallerian degeneration in the posterior limb of the internal capsule (IC) [3].

Unexpectedly, we have identified other ALS patients with similar UMN-predominant clinical presentations but without CST hyperintensity. The reason for this variability between presence or absence of CST hyperintensity and the essentially identical UMN-predominant clinical features is unclear but may reflect a different location of maximal pathology in the nonhyperintense group CST (e.g., in spinal cord), or intracranial pathologies with distinct axonal/periaxonal characteristics.

Previous brain MRI reports of hyperintensity of CST in ALS have been qualitative using conventional T2- and PD-weighted [1] as well as FLAIR-weighted [1] sequences. Because such qualitative approaches depend on several factors, including quality of MR image, limits of visual detectability, and the interpreter's experience, more quantitative MR methods would validate these findings and potentially provide insights into pathogenesis of the CST hyperintensity. Diffusion tensor imaging (DTI) is a relatively recently described modality based on the freedom with which water molecules (protons) move randomly within tissue, with intact myelinated axons providing the greatest restriction to movement between them (high anisotropy) [4]. Because DTI signal is dependent on microscopic-level events, it has the potential of detecting tissue pathology at/near submacroscopic levels, even before the changes are visible by conventional MRI. Identifying pathologic changes noninvasively at early stages would shorten time to diagnosis and allow timely therapeutic intervention. Because the CST and other subcortical white matter in ALS brain are usually normal in appearance by conventional MRI, DTI should still be able to detect abnormalities. Furthermore, the organization of subcortical myelinated motor axons as compact parallel bundles (e.g., CST) can be ideally interrogated by DTI because its metrics will reflect whether they are intact (healthy) or disrupted (degenerating).

Therefore, the main goal of this study was to use DTI obtained at 1.5T as part of routine clinical neuroimaging of ALS patients with predominant UMN signs for quantitative evaluation of the intracranial CST, which in one group was hyperintense on T2- and PD-weighted sequences. This would allow us to determine where abnormalities in DTI metrics occur along the CST in our UMN-predominant ALS patients, and whether quantitative differences are detectable corresponding to the qualitative presence or absence of CST hyperintensity. We hypothesized that motor neuron degeneration in UMN-predominant ALS is anterograde, arising primarily in the perikaryon as a neuronopathy. If this is the case, DTI metrics would be expected to be more abnormal at more rostral levels of the CST intracranially. Second, we hypothesized that CST hyperintensity in ALS brain reflects a more extreme case of such anterograde degeneration that has unique tissue characteristics detectable by DTI.

By testing these hypotheses, we hope to have a better understanding of CST degeneration in UMN-predominant ALS patients and to identify quantitative DTI measures which may be useful in objectively differentiating disease subtypes.

2. Methods

2.1. Demographics. 1.5T MRI data obtained as part of clinical neuroimaging evaluation was approved by the Institutional Review Board at the Cleveland Clinic to be stored and analyzed as deidentified images after patients had provided verbal consent. DTI data were analyzed in the following patient groups: (1) 10 neurological controls (7 men, 3 women) aged 51.1 ± 7.3 years (mean ± SD, range 28–80 years), (2) 21 UMN-predominant ALS patients (14 men, 7 women) with CST hyperintensity on T2/PD-weighted images (CST+) aged 52.3 ± 11.02 years (range 32–75 years), and (3) 26 UMN-predominant ALS patients (14 men, 12 women) without CST hyperintensity identified on T2/PD-weighted images (CST−) aged 59.5 ± 12.1 years (range 32–76 years). UMN-predominant ALS patients were defined as those with either no lower motor neuron signs or, if present, then restricted to only one neuraxial level (bulbar, cervical, or lumbosacral) at the time of MRI. Duration of symptoms prior to MRI in the CST+ group was 9.6 ± 5.5 months (mean ± SD) and in the CST− group was 36.4 ± 44.2 months; the large standard deviation is due to two outlier values in the latter group of 148 and 180 months. El Escorial diagnostic criteria [2] assigned to each patient after their clinical evaluation were converted to a numeric form as follows: possible = 1, probable with lab support = 2, probable = 3, and definite = 4. This El Escorial criteria score in the CST+ group was 1.81 ± 0.98 (mean ± SD) and in the CST− group was 1.37 ± 0.82.

2.2. DTI Data Acquisition. DTI data were obtained on a 1.5T system (Siemens Symphony, Erlangen, Germany) using echo planar imaging (EPI) sequence along 12 diffusion-weighted ($b = 1000\,s/mm^2$) directions and one $b_0 = 0\,s/mm^2$. Imaging parameters were 30 slices, 4 mm thick, with 1.9 × 1.9 mm in-plane resolution; pulse sequence parameters were TR = 6000 ms, TE = 121 ms, EPI factor = 128, number of averages = 6, and scan time = 7.54 minutes. Gradient-echo field map images were acquired to correct for geometrical distortion caused by susceptibility artifacts. Field map imaging parameters were 30 slices, 4 mm thick, 4 mm slice gap, TR = 500 msec, TEs = 6.11, and 10.87 msec. T2-and PD-weighted images were obtained using dual-echo FSE sequence whose imaging parameters were number of slices = 40, contiguous, slice thickness = 4 mm, and in-plane resolution = 0.9 × 0.9 mm; pulse sequence parameters were repetition time (TR) = 3900 ms, TE = 26 ms and 104 ms, echo train length or turbo factor = 7, and number of averages = 1; total scan time = 3.5 minutes.

2.3. Data Processing. DTI images were first corrected for susceptibility artifacts and eddy current distortions using FSL FUGUE and eddy current distortion correction algorithm in FSL (http://www.fmrib.ox.ac.uk/fsl/) [5–7]. The b-matrix was rotated in order to preserve the correct orientation information after eddy current and oblique angle corrections [8, 9]. The above preprocessed DTI

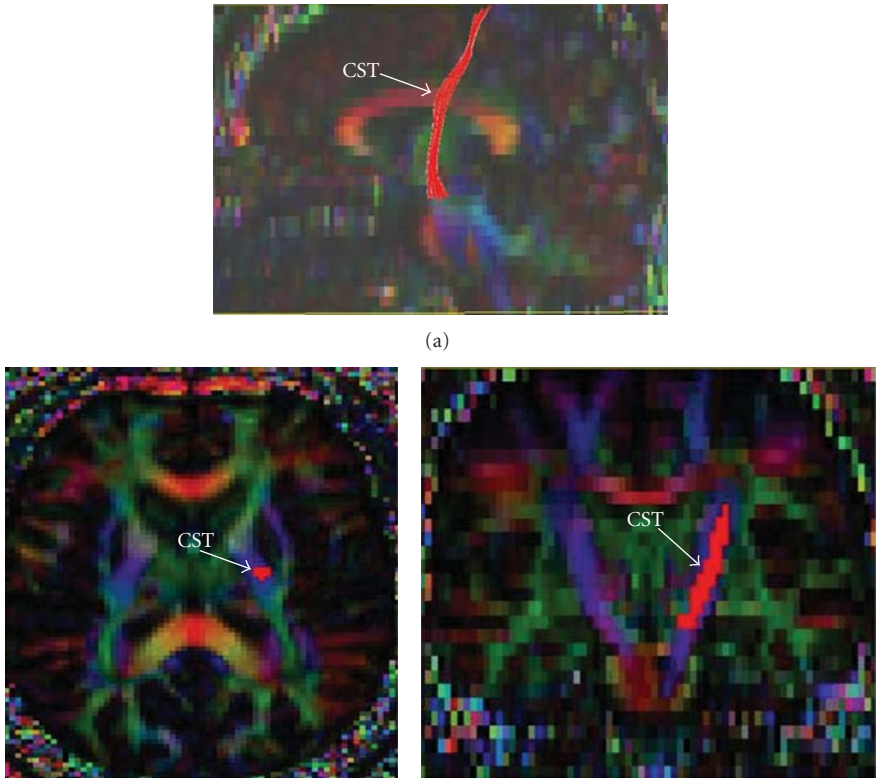

Figure 1: Corticospinal tract (CST) reconstructed using tractography superimposed on a subject's own FA color map in (a) sagittal, (b) axial, and (c) coronal planes.

images were then processed using DTI Studio open software (https://www.mristudio.org/) [10]. DTI matrix for each voxel element was calculated based on multivariate linear least square fit. The tensor matrix was then diagonalized to derive principal eigenvalues and eigen vectors. Maps of diffusion metrics, namely, fractional anisotropy (FA), mean diffusivity (MD mm^2s^{-1}), axial diffusivity (AD, $\lambda_{||}$ mm^2s^{-1}), and radial diffusivity (RD, $\lambda_{\perp}$ mm^2s^{-1}), were obtained. Virtual nerve fibers were reconstructed using the fiber assignment by continuous tracking (FACT) algorithm [10], described in detail elsewhere [11]. Fiber tracking parameters were initiated from every voxel with FA = 0 [12], threshold for termination 0.2 [12], and a bending angle of 41°.

After above steps, both control and ALS patients' CST fiber tracts on both left and right sides were reconstructed ("tractography") following Wakana et al.'s method [12] by placing first ROI caudally in cerebral peduncle and the second rostrally just below the primary motor cortex. Four regions of interest (ROIs) were identified *a priori* at specific levels along the CST (using $b_0 = 0$ and FA images) for DTI measures, including cerebral peduncle (CP), posterior limb of the internal capsule (IC), and centrum semiovale at top of lateral ventricle (LV) and subjacent to primary motor cortex (MC). DTI metrics of FA, MD, AD, and RD were measured in each ROI after superimposing each subject's own CST tractography mask on their DTI maps. Values were then compared between ALS patients and controls and also between the patient groups. Figure 1 shows the left CST mask superimposed on a subject's FA color map.

Statistical comparisons for each of the DTI metrics across control and patient groups were carried out using SPSS 16.0 (SPSS Inc., Chicago, Ill, USA). Based on data meeting the assumptions of ANOVA, one of the following statistical methods was used with a significance level of $P < 0.05$. One-way ANOVA followed by Tukey's post hoc test was carried out when the assumptions of both normality and equal variance were met. If the equal variance assumption was violated, the Welch ANOVA followed by the Dunnett T3 post hoc test was employed. When both normality and equal variance assumptions were violated, the Kruskal-Wallis nonparametric approach followed by the Bonferroni correction of the Mann-Whitney test was used. Clinical parameter of symptom duration prior to MRI was correlated with DTI metrics in all the 4 ROIs along the CST using Spearman's correlation method after correcting for multiple comparisons using false discovery rate (FDR).

Because abnormalities of DTI values along the CST may represent degeneration or related pathology, we assessed whether such metrics correlated with pathologic UMN signs (spasticity, hyperreflexia, and pathologic reflexes), as identified by neurologic examination performed at clinical evaluation near/at time of MRI. Because CST fibers decussate below the lowest ROI analyzed for DTI metrics (at the cervicomedullary junction), the intracranial CST corresponds to the contralateral side of body reflecting UMN signs. In order to detect a side-to-side asymmetry in CST DTI values which may correspond to asymmetry of UMN signs, ratios of side-to-side DTI values (right to left) for FA, MD, AD, and RD

Table 1: Percent correspondence of abnormal CST DTI metrics with appropriate side of body showing more UMN signs.

	CP		IC		LV		PMC	
Body predominance of UMN signs	Left (%)	Right (%)	Left (%)	Right (%)	Left (%)	Right (%)	Left (%)	Right (%)
FA	50	33.00	**85**	28	68.40	66.60	16.60	45.50
AD (mm^2s^{-1})	50	61.1	30	33	73.6	55.5	41.6	36.3
RD (mm^2s^{-1})	50	55.5	30	**94.4**	52.6	55.5	58.3	45.4
MD (mm^2s^{-1})	50	55.5	15	**94.4**	52.6	61.1	58.3	36.3

Key: AD: axial diffusivity, CP: cerebral peduncle, CST: corticospinal tract, FA: fractional anisotropy, IC: posterior limb of internal capsule, LV: centrum semiovale at top of lateral ventricle, MD: mean diffusivity, PMC: subjacent to primary motor cortex, RD: radial diffusivity, UMN: upper motor neuron.

in all the 4 ROIs along CST were calculated in control and in both the ALS patient groups. For this analysis, all ALS patients (both CST+ and CST− groups) were categorized by their UMN-predominant body signs being primarily right sided or left sided, based on their clinical evaluation at time of MRI. Patients with predominantly right body UMN signs should have more abnormal left CST DTI metrics, and vice versa. For example, a patient with mostly right body UMN signs would have lower FA and AD values in the left CST, so right to left ratio would be greater than or equal to 1, whereas a patient with mostly left body UMN signs would have lower FA and AD values in the right CST, so right to left ratio would be less than or equal 1. Because an inherent asymmetry was observed between right and left CST DTI values even in controls, the mean right to left ratio from control values was used as a threshold to estimate the number of ALS patients above (with more right body UMN signs) or below (with more left body UMN signs) it. A percentage value was then calculated of patients whose ratios were abnormal DTI values in CST relative to control, which represents the degree of correspondence between abnormal DTI values in CST and the appropriate side of body with pathologic UMN signs, as shown in Table 1.

3. Results

Mean FA, MD, AD, and RD values for each ROI along the CST and their significant differences (based on parametric and nonparametric tests depending on data meeting the assumptions of the test as described in Methods) between the 3 groups are given in Figures 2–5. FA showed significant difference between control and the ALS groups at right IC and left MC. However, no significant differences in FA values were observed between ALS subgroups in any of the 4 ROIs (Figure 2). MD values showed no significant differences in any of the 4 ROIs among the 3 groups (Figure 3). Axial and radial diffusivities showed significant differences only between control and ALS CST hyperintense groups and only at the level of IC (Figures 4 and 5).

The CST was found to be truncated above the LV level, most prominently on the right compared to left in 12 subjects (of 21, 57%) in the hyperintense CST group; of these, CST was truncated on the right in 8 (of 12) and bilaterally in 4. Similarly, CST truncation was observed in 6 subjects in the nonhyperintense CST group (of 26, 23%); of these, truncation was on the right in all. Truncation of CST was not observed in any of the 10 control subjects. Figures 6(a) and 6(b) show CST tractography extending to the cortex in a typical control subject and a truncated CST in an ALS subject. Because the same DTI processing methodology was employed across all subject groups, this could not have accounted for the observed differences in CST truncation. We also investigated whether the absence of CST above the LV level in some patients may have lead to not detecting significantly different DTI metrics in the MC. Since tractography was used to identify the fibers to be measured, missing values at the MC level in patients with truncated CST could underestimate differences in DTI metrics between groups. To correct for this possibility, Mori's CST atlas was superimposed on the FA map, and values were measured in all 4 ROIs along the CST. This did not change the results, and still no significant differences were observed in FA values at the MC level of control and ALS groups even when identifying the CST using Mori's atlas. We further investigated whether the quality of DTI scans obtained at 1.5T, that is, resolution, anisotropic voxel dimension, and signal/noise ratio, contributed to the CST truncation problem. 3T DTI data were collected on another set of ALS patients with (in 1 patient) and without (5 patients) CST hyperintensity and in control subjects (5 patients). Imaging parameters of 3T data were almost identical to those of our 1.5T data except that at 3T voxels they were isotropic ($2 \times 2 \times 2.5$ mm) resulting in improved resolution, and signal to noise ratio was higher. CST tractography and analysis on 3T data were performed in an identical manner to the 1.5T data. Although the number of studies at 3T was limited, we found similar CST truncation in 2 of 5 ALS CST hyperintense patients and none in controls.

Symptom duration prior to MRI was significantly shorter ($P < 0.0003$) in ALS patients with CST hyperintensity (median = 13 months) than in those without CST hyperintensity (median = 31 months). No significant difference was found in El Escorial criteria scores between either of these two groups. No significant correlation was obtained between symptom duration prior to MRI and DTI metrics on any of the 4 ROIs along CST.

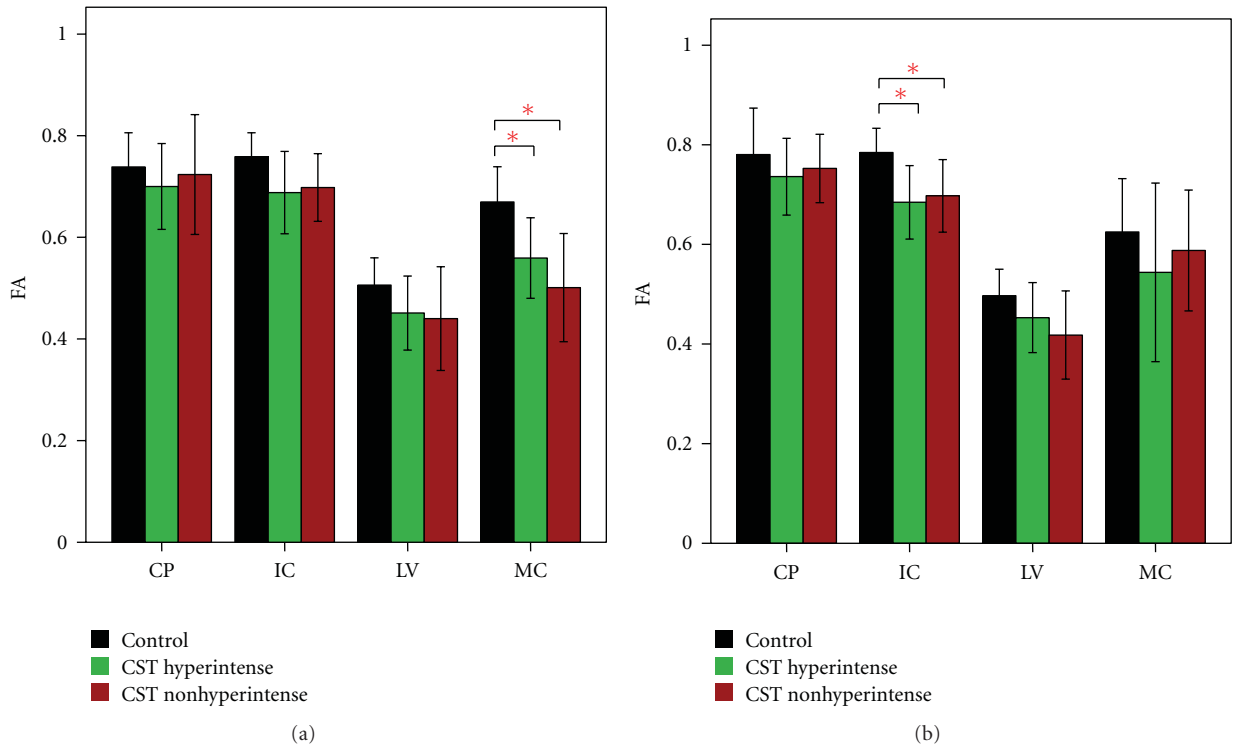

Figure 2: Fractional anisotropy (FA) at four CST levels on left (a) and right (b), revealing significantly lower values in right MC and left IC of CST hyperintense and CST nonhyperintense patients compared to controls. $^{*}P < 0.05$. Key: CP, cerebral peduncle; IC, posterior limb of internal capsule, LV, centrum semiovale at top of lateral ventricle, MC, subcortical to motor cortex.

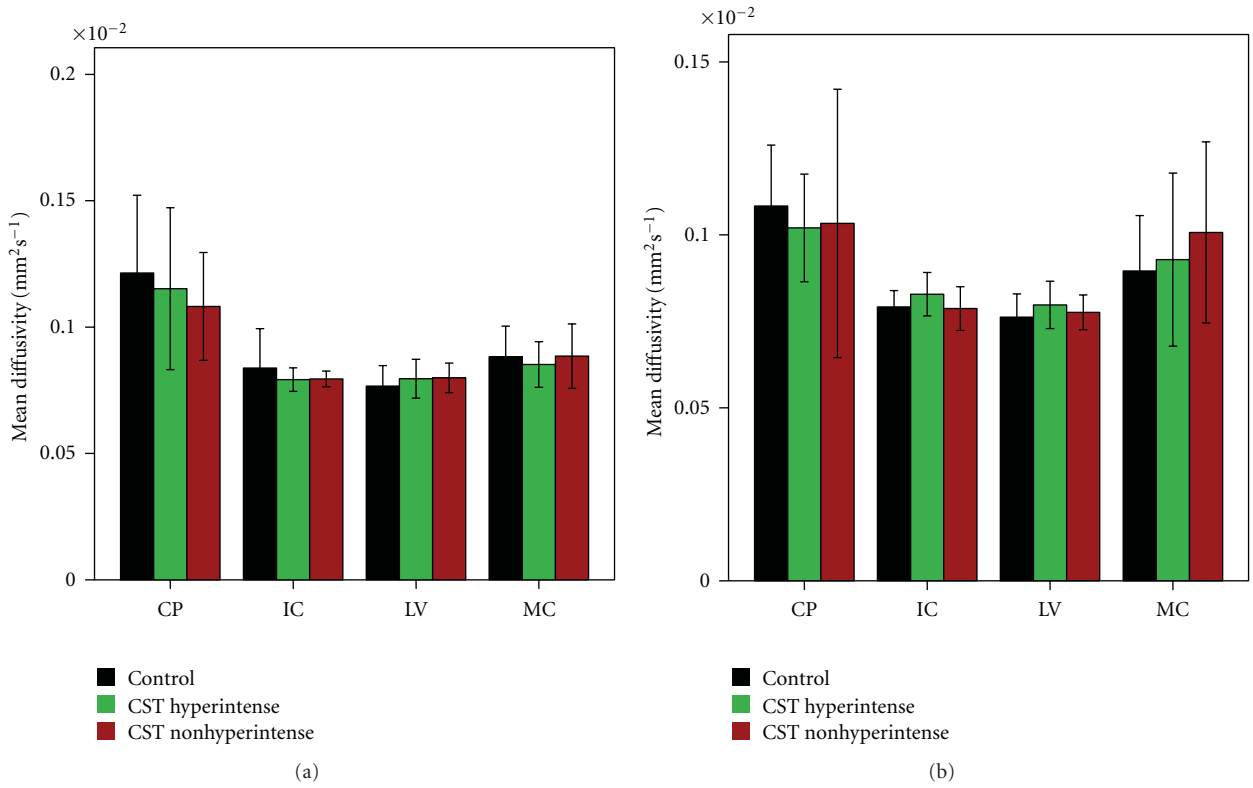

Figure 3: Mean diffusivity (MA) at four CST levels on left (a) and right (b), revealing no significant differences between groups. Key: CP, cerebral peduncle; IC, posterior limb of internal capsule, LV, centrum semiovale at top of lateral ventricle, MC, subcortical to motor cortex.

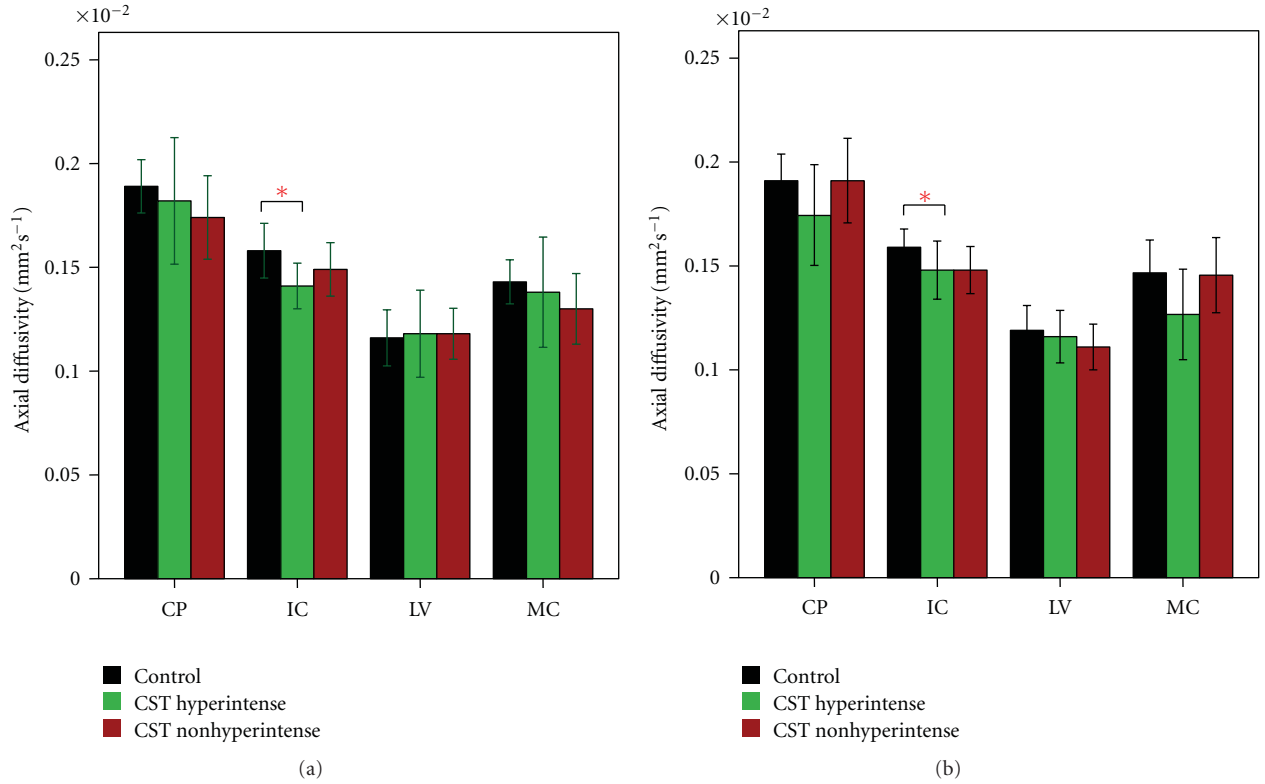

Figure 4: Axial diffusivity (AD) at four CST levels on left (a) and right (b), revealing significantly lower values at the IC level bilaterally in only CST hyperintense patients compared to controls. $^*P < 0.05$. Key: CP, cerebral peduncle; IC, posterior limb of internal capsule, LV, centrum semiovale at top of lateral ventricle, MC, subcortical to motor cortex.

4. Discussion

Fractional anisotropy (FA) abnormalities seen in this study of CST at the level of internal capsule agree well with previous ALS studies [8, 13–17], although reaching significance only on the right. We also found significantly lower FA values in ALS patients at the subcortical motor cortex level on the left. The reason for this side-to-side variability is unclear. In general, FA values were reduced in both CST hyperintense (CST+) and CST nonhyperintense (CST−) ALS groups when compared to controls, but they were not significantly different between the ALS groups. The CST+ group did, however, have lower FA values than the CST− group. Of note, axial diffusivity (AD) and radial diffusivity (RD) values were significantly different at the IC level only between controls and the CST+ group and not the CST− group. Such differences in AD and RD abnormalities may reflect microanatomical pathologic differences in the CST in these two groups of ALS patients. It is known that FA may not be as reliable a measure as individual AD and RD, from which FA is calculated. Beaulieu [18] showed that FA value changes result from either decreased AD, increased RD, or changes in both. He also demonstrated in animal and human studies that AD is reflective of axonal integrity and RD is reflective of myelin integrity, with abnormalities in these DTI metrics representing their degeneration [18]. Such interpretations should, however, be interpreted with caution in the present study, as they are likely oversimplifications, and further studies would be required for confirmation. Nonetheless, the aforementioned suggests that AD and RD are more representative of microanatomical integrity than is FA. In general, FA, AD, and RD values were different between CST+ and CST− ALS groups but failed to reach statistical significance.

Lack of significant differences in MD values in all ROIs along the CST in this study generally agrees with other ALS studies [19, 20], although some DTI studies in ALS have found MD abnormalities at the IC level [21–23]. Reasons for this discrepancy may include pooling ALS patients of multiple clinical phenotypes into a single group in other studies, variable DTI data acquisition and processing parameters (our processing included oblique angle and susceptibility artifact corrections, not used in previous DTI studies in ALS), and use of ROI-based approach (our study used tractography of patient's own CST which is more accurate and reproducible). Furthermore, since ALS results in not only axonal/myelin degeneration (which would result in decreased FA and increased MD) but also gliosis [14, 24], the net diffusion revealed by MD may not be higher than control values. If this is the case, MD values would be expected to be higher in patients with shorter disease duration (and presumably less time for gliosis) than longer disease duration.

The fact that we found FA, AD, and RD abnormalities in the CST primarily at the IC level and not caudally at

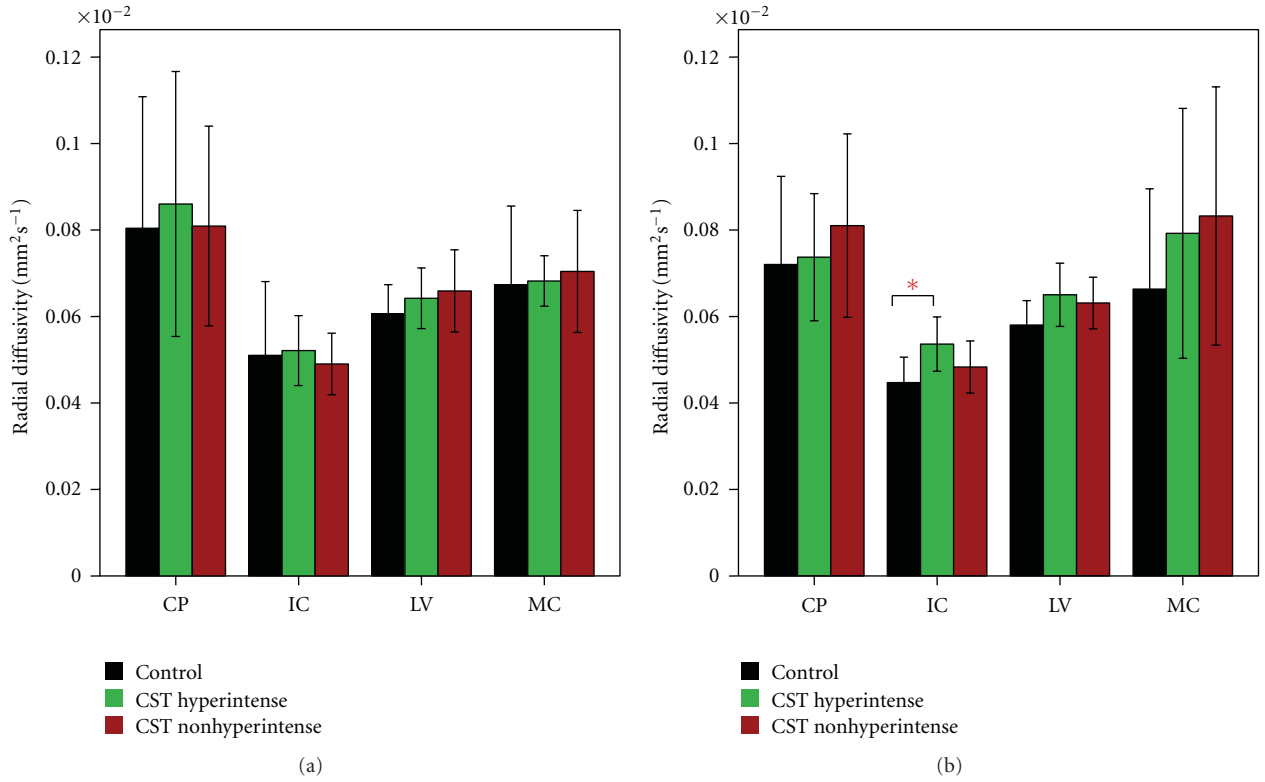

Figure 5: Radial diffusivity (RD) at four CST levels on left (a) and right (b), revealing significantly higher values at the IC level on the right in only CST hyperintense patients compared to controls. $^{*}P < 0.05$. Key: CP, cerebral peduncle; IC, posterior limb of internal capsule, LV, centrum semiovale at top of lateral ventricle, MC, subcortical to motor cortex.

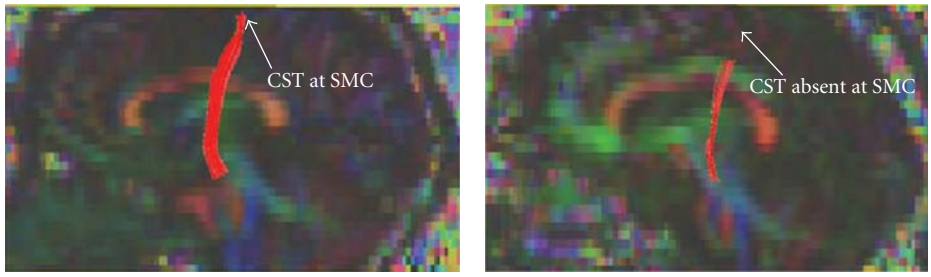

Figure 6: Sagittal views of FA color maps showing tractography-derived CST extending up to the submotor cortex (SMC) level in a control subject (a) but truncated in an UMN-predominant ALS patient with CST hyperintensity (b).

the CP level, and some abnormalities rostrally, suggests a degenerative process that may begin at midpoint along the subcortical myelinated motor axon. Although interpreting such DTI metrics as proof of axon/myelin loss is inaccurate, it indicates that CST microanatomy is sufficiently abnormal to result in perturbed water (proton) diffusion. Postmortem histopathologic studies of this region may elucidate the significance of these DTI findings. Supportive evidence that these DTI abnormalities at the IC level are related to the CST neurodegenerative process includes FA values showing 85% correspondence with left body UMN-predominant clinical signs and radial diffusivity as well as MD showing 94.4% correspondence with right body UMN-predominant clinical signs. Only correspondence with a 75% or greater threshold was chosen, to insure only a 25% correspondence occurred by chance.

Furthermore, the preponderance of CST virtual fibers truncated at the subcortical motor cortex (MC) level in the CST+ over the CST− ALS patients (and never in control subjects) also suggests disease-specific changes rather than artifact in data acquisition or processing. How this CST virtual fiber truncation corresponds to axonal pathology is unclear and invites further study. Failure to detect significant changes in DTI metrics of the CST at the LV level could be due to effects of the superior longitudinal fasciculus whose fibers cross perpendicularly at that level. This region in

control subjects also has decreased uniformity of fiber tracts (unpublished observations), consistent with crossing fibers (as measured by Westin's linear and planar indices [25]).

Duration of ALS symptoms prior to MRI was shorter in CST+ than in CST− group, which may reflect faster disease progression, although this is uncertain. In an early neuroimaging-pathologic study, Yagishtia et al. [3] found CST hyperintensity on MRI corresponds to demyelination and axon degeneration suggesting a different pathology from patients without CST hyperintensity. Other studies reported that ALS patients with CST hyperintensity had rapid clinical decline initially [1], shorter disease duration, and faster disease progression [26]. In a separate study evaluating 112 ALS patients over a 10-year period who had undergone at least one brain MRI, we found those with CST hyperintensity ($n = 35$) had significantly more rapid disease progression and shorter survival than those without CST hyperintensity ($n = 77$) [27]. It is therefore possible that ALS patients with CST hyperintensity have different underling pathology from those without CST hyperintensity, which results in faster disease progression. In the context of similar or slightly worse FA and AD/RD abnormalities at the IC level of the CST, this suggests a more rapidly evolving disease process in the CST+ group. Although correlations between duration of symptoms and individual DTI metrics at each of the ROIs along the CST did not reach statistical significance after correction for multiple comparisons (false discovery rate correction), there was a trend for correlation of FA, AD, and RD abnormality at IC and LV levels with disease duration when $P < 0.05$ is uncorrected for multiple comparisons.

Although the DTI changes we observe between these ALS patient groups are small, they support differences visualized by qualitative T2/PD images and suggest they result from real microanatomic pathologic changes, such as inflammation, demyelination, axon loss, or gliosis [28]. However, detailed studies correlating CST changes detected by DTI and T2/PD with postmortem histopathology will be required to determine what causes the imaging abnormalities we have found.

To our knowledge this is the first study to use DTI to quantitatively evaluate the CST after its virtual reconstruction by tractography and classify UMN-predominant ALS patients into groups based on the presence or absence of CST hyperintensity.

5. Conclusion

DTI performed at 1.5T as part of routine clinical brain MR imaging demonstrates abnormalities in FA and related parameters predominantly at the IC level of the CST in UMN-predominant ALS patients compared to control subjects. This correlates with the appropriate body side showing more prominent UMN signs clinically. In addition, patients with CST hyperintensity have abnormalities of the AD and RD components of FA, while those without CST hyperintensity do not, suggesting differences in tract microanatomy. Furthermore, subcortical truncation of virtual CST fibers generated by tractography occurs more frequently in ALS patients who have CST hyperintensity than those who do not, again suggesting divergent pathology. Predominance of DTI abnormalities at the IC level and rostrally suggests an anterograde process arising from a neuronopathy of motor neurons forming the CST. Identification of these DTI abnormalities in UMN-predominant ALS patients from routine clinical scans demonstrates feasibility of acquiring useful quantitative information with a 1.5T magnet system. Future DTI studies at 3T with larger control subject group will confirm these findings.

Conflict of Interests

None of the authors of this paper has any conflict of interests that may arise from being named as an author on the manuscript.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 362189, 11 pages
doi:10.4061/2011/362189

Research Article

Genetic Risk Factors for Longitudinal Changes in Structural MRI in Former Organolead Workers

Bryan D. James,[1,2] Brian Caffo,[3] Walter F. Stewart,[4,5] David Yousem,[6] Christos Davatzikos,[7] and Brian S. Schwartz[4,8,9]

[1] *Rush University Alzheimer's Disease Center, Rush University Medical Center, Room 1038, Chicago, IL 60612, USA*
[2] *Department of Internal Medicine, Rush University Medical Center, Chicago, IL 60612, USA*
[3] *Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA*
[4] *Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA*
[5] *Center for Health Research and Rural Advocacy, Geisinger Clinic, Danville, PA 17822, USA*
[6] *The Russell H. Morgan Department of Radiology and Radiological Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21278, USA*
[7] *Department of Radiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA*
[8] *Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA*
[9] *Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD 21278, USA*

Correspondence should be addressed to Bryan D. James, bryan_james@rush.edu

Received 21 February 2011; Accepted 31 July 2011

Academic Editor: Sofia Madureira

This study examined associations between polymorphisms in three genes, apolipoprotein E (*APOE*), angiotensin converting enzyme (*ACE*), and vitamin D receptor (*VDR*), and longitudinal change in brain volumes and white matter lesions (WML) as well as effect modification by cardiovascular factors and tibia lead concentrations. Two MRIs, an average of 5 years apart, were obtained for 317 former organolead workers and 45 population-based controls. Both regions-of-interest and voxel-wise analyses were conducted. *APOE* $\varepsilon 3/\varepsilon 4$ and $\varepsilon 4/\varepsilon 4$ genotypes were associated with less decline in white matter volumes. There was some evidence of interaction between genetic polymorphisms and cardiovascular risk factors (*ACE* and high-density lipoprotein; *VDR* and diabetes) on brain volume decline. The *VDR FokI* ff genotype was associated with an increase in WML (no association for *APOE* or *ACE*). This study expands our understanding of how genetic precursors of dementia and cardiovascular diseases are related to changes in brain structure.

1. Introduction

Brain volume loss [1] and increase in white matter lesions (WML) [2] are common consequences of aging, and both are related to worse cognitive function and risk of dementia [3–6]. Little is currently known about the genetic determinants of age-related brain volume loss or increase in WML or how genes may modify the effect of other environmental risk factors for these outcomes. Certain genetic polymorphisms associated with a greater risk for neurodegenerative diseases such as Alzheimer's disease (AD) in later life [7] are potential candidates. The apolipoprotein E (*APOE*) gene is the best-documented genetic risk factor for AD, with the $\varepsilon 4$ allele significantly increasing the risk for AD [8–10] and cognitive decline [11]. The angiotensin converting enzyme (*ACE*) gene is associated with AD [12, 13] and WML [14]. Certain vitamin D receptor (*VDR*) gene polymorphisms have also been associated with increased risk of AD [15], cardiovascular disease, and diabetes [16, 17], and these, in turn, have been linked to WMLs [18, 19]. These genes are, therefore, strong candidates for evaluation of genetic determinants of brain volume loss and increased WML in living persons through the use of neuroimaging technology. Furthermore, because it is hypothesized that exposure to certain external agents may induce upregulation of neurodegenerative disease-associated genes [20], it is appropriate to also examine the effect of these

genes in the light of gene by environment interaction. Specifically, well-known risk factors for neurodegenerative disease, including cardiovascular risk factors and occupational lead exposure, may modify the effect of certain genotypes on brain volume loss and WML.

Research has begun to evaluate relationships between genetic risk factors and structural differences in the human brain, the vast majority investigating *APOE* [21–31]. However, there are a number of limitations to this body of work, including a reliance on cross-sectional data and extrapolation of differences in structure across persons in different age ranges to within-person change. Many of these studies also involve small sample sizes, limiting power to detect differences, and some focus specifically on certain brain structures such as the hippocampus rather than structures across the whole brain. Furthermore, there has been little examination of the effect of gene by environment interaction on structural brain changes. In this paper, we report on the associations between *APOE*, *ACE*, and *VDR FokI* genetic polymorphisms and longitudinal change in brain volumes and WML from a cohort of over 350 older men who participated in two structural MRIs an average of 5 years apart. Additionally, because these genetic polymorphisms may not directly contribute to changes in brain structure, but rather may modify the effect of other risk factors (i.e., gene by environment interaction), we tested for interactions between these genes and cardiovascular risk factors as well as occupational lead exposure in determining changes in brain volumes and WML.

2. Methods

2.1. Study Design and Overview. As previously described [32–34], subjects were initially recruited during two study phases between 1994 and 2003. In phase I (1994–7), former employees of a chemical manufacturing plant in the eastern United States were identified and recruited. In phase II (2001–3), additional study participants were enrolled and the first MRI data was acquired. In phase III (2005–8), subjects who completed the first MRI were invited for a second MRI. All phases of the study were reviewed and approved by the Johns Hopkins Bloomberg School of Public Health Committee on Human Research and written informed consent was obtained from all participants.

2.2. Selection and Recruitment of Study Subjects. The selection, recruitment, and enrollment of former lead workers and controls (community-dwelling persons without occupational lead exposure) have been previously reported [3, 32–35]. During phase II, all participants were eligible for MRI measurement, and first MRIs were completed on 589 of 979 (60%) former lead workers and 67 of 131 (51%) controls. During phase III, a second MRI was obtained from a total of 377 persons: 317 of 589 (54%) former lead workers and 45 of 67 (67%) controls. Reasons for not obtaining a second MRI are reported elsewhere [36]. The analytic cohort herein includes the 309 former lead workers and 44 controls with two adequate MRIs ($n = 353$; 8 former lead workers and 1 control had inadequate first MRIs).

2.3. Data Collection. Detailed data collection methods for the first two phases of the study have been previously described [34]. The remaining description is confined to measures specifically used for the analysis presented herein.

2.3.1. Subject Interview. In phase III, the subject interview was expanded to include a number of additional study variables [37, 38]. Health outcomes (e.g., diabetes and heart disease) were ascertained by interview response to the question, "Has a doctor ever told you that you had (each condition)?" Only "yes" responses were counted; participants who answered "possible" were classified as negative for all outcomes in order to increase specificity of outcome classification. For educational attainment, information was obtained by interview on years of education, trade school, general educational development (GED) credential, and other educational certificates using previously published methods [38].

2.3.2. Tibia Lead. Tibia lead, an estimate of lifetime cumulative lead dose, was available from earlier phases of the study on all former lead workers and all but one control with two MRIs. For former lead workers, current tibia lead was back-extrapolated to peak tibia lead, the estimated level at the end of employment in the factory. The measurement of tibia lead and this extrapolation to peak tibia lead are described elsewhere [33].

2.3.3. Serum Tests. All serum assays were performed in the Core Laboratory of the General Clinical Research Center (Johns Hopkins Bayview Medical Center). C-reactive protein was measured by enzyme-linked immunosorbent assay (ELISA) using the American Laboratory Products Company (Salem, NH) kit, with a sample sensitivity of 0.5 ng/mL, an intrasample coefficient of variation (CV) of 6.33% and an intersample CV of 2.20%. The lipid profile was performed on a Medical Computer Systems analyzer with a sample sensitivity of 0.80%, an intrasample CV of 3.08% and an intersample CV of 3.72%.

2.3.4. Genotyping

APOE. Genotyping was completed using different methods in the different phases of the study as technology progressed. The method of Hixson [39] was used during phase I. In phase II, DNA was isolated using the Flexigene DNA Kit (Qiagen, Valencia, Calif, USA), and genotyping was performed using published PCR conditions [40, 41]. In phase III, DNA was isolated as for phase II. For genotyping, for determination of the C to T substitution causing the Arg112Cys and Arg158Cys polymorphisms, we performed allelic discrimination using TaqMan Probes as previously described [41] with the following modifications: (1) instead of using the nested PCR approach for the Arg112Cys polymorphism, 1X Genotyping Master Mix (Applied Biosystems, Foster City, Calif, USA) was used with 20 ng of genomic DNA and processed according to the manufacturer's recommended protocol, (2) the 1X Genotyping Master Mix was also used

for the *APOE* Arg158Cys polymorphism, and (3) plate reads were performed in the 7500 Real Time PCR system to capture fluorescence, and genotypes were determined by manual clustering (Applied Biosystems 7500 software v1.2.3). Of the subjects with two MRIs, *APOE* genotyping was performed with the phase II method in 39.9% of subjects and with the phase III method in 58.5% of subjects (the rest genotyped in phase I).

ACE. We used a published PCR method to determine the insertion/deletion polymorphism of the *ACE* gene [42] with the following modifications: annealing time of 30 seconds and the final concentrations: 0.4 μmol/L primers, 1.5 mmol/L MgCl, 200 μM/L dNTPs, and 0.5 U Taq. Fragments were resolved on 2.5% agarose/TBE gels stained with EtBr. Gels were imaged and photographed with a Fuji LAS 1000 system and analyzed with Fuji Multigauge version 3.0 software.

VDR. Genomic DNA was isolated as for a previous study [41] from stored blood using the Flexigene DNA Kit from Qiagen (Valencia, CA). For determination of the T to C substitution causing the *VDR* 12022 polymorphism (allowing identification of FF, Ff, and ff genotypes), we performed allelic discrimination using TaqMan Probes (Applied Biosystems, Foster City, Calif, USA) using previously published methods for single nucleotide polymorphisms [41]. Allelic discrimination assays, consisting of primers and allele-specific TaqMan MGB probes labeled with 6FAM and Vic, were designed with Primer Express 2.0 and custom-ordered from Applied Biosystems (sequences of primers and probes available upon request). All reactions contained 1X assay mix, 1X TaqMan Genotyping MasterMix, and 20 ng DNA in 25 microliters. Cycling was performed in the Applied Biosystems 7500 Real Time PCR system with the following conditions: 95°C for 10 minutes and 50 cycles of amplification at 95°C for 15 seconds and 60°C for 1 minute. Following amplification, plate reads were performed as described above.

MRI Acquisition. For the first MRI, all subjects were imaged at the same location on the same General Electric 1.5 T Signa model as previously described [34]. For the second MRI, a 3 T General Electric scanner was utilized. T1-weighted images were acquired using an SPGR sequence (TE = 8 ms, TR = 21 ms, flip angle = 30°, FOV = 24 cm). Axial PD/T2 (TR/TE/TE2 = 2,200/27/120) and FLAIR (TR/TE/T1 = 8,000/100/2000) images were also acquired for WML grading.

Clinical MRI Review and Assignment of WML Grade Scores. MRIs were reviewed to exclude urgent or emergent brain disease and subjects and their physicians were notified if present [43]. MRIs were assigned a WML grade score by a trained neuroradiologist using the Cardiovascular Health Study (CHS) ten-point (0 to 9) scale [44, 45], as previously reported [34], which allowed for analysis of change in ratings.

2.4. Image Analysis. The methods to obtain regional and voxel-wise volumes, including skull stripping, segmentation, registration, and transformation to regional analysis of volumes examined in normalized space (RAVENS), were completed using published methods [34, 36, 46–50]. Due to changes in scanner technology and pulse sequences, we employed specialized image analysis methods that minimized the discontinuity between the two scans. We used the CLASSIC algorithm [51], which employs a 4-dimensional segmentation framework in which the baseline and follow-up scans are considered jointly to minimize discrepancies between the two segmentations and better estimate longitudinal change. This algorithm has been previously validated [51].

2.5. Statistical Analysis. The purpose of the present analysis was to first determine if genotypes for three different candidate genes were associated with changes in brain volumes and WML and then evaluate whether the genes modified relations of cardiovascular factors and tibia lead with changes in brain volumes and WML. Multiple linear regression was used to evaluate associations of the polymorphisms with change in brain volumes using both ROI-based and voxel-wise approaches as well as change in CHS scores (WML). All regression models were adjusted for baseline age, duration of time between MRIs, control status (i.e., former lead worker versus control), height (cm), and education [38] and baseline ROI volume for ROI analysis or baseline CHS score for the WML analysis. Results were similar for models that did not include terms for baseline ROI volume or CHS score (not presented). Cross-product terms were used to evaluate effect modification.

Because lead is associated with smaller brain volumes [34], we first evaluated whether the association between genotypes and change in brain volumes or WML differed between former lead workers and population-based controls or, within former lead workers, the associations of genotypes with MRI outcomes differed by peak tibia lead (PTL) level. There was no evidence that associations of interest differed by control status, so we proceeded with our main analyses using data from both lead workers and controls. Furthermore, a separate analysis found no association between PTL and change in brain volumes [36]. We incorporated the results of PTL by gene interactions in former lead workers into our analyses as described below.

2.5.1. ROI-Based Approach. We modeled change in 20 previously selected ROI volumes consistent with our prior published reports (as listed in Table 2) [34]. For bilateral structures, the volume represented the sum of right and left structures to minimize multiplicity concerns, but analyses were also performed separately for change in left- and right-sided ROI volumes (data not reported). We did not formally adjust for multiple comparisons in the analysis, choosing instead to report unadjusted P values and the number of regressions.

We first examined the relationship between each genotype and change in ROI volumes (core models). We then separately examined the relationships between cardiovascular

risk factors (hypertension (HTN; yes versus no), cardiovascular disease (CVD; yes versus no), diabetes mellitus (DM; yes versus no), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and C-reactive protein (CRP)) and change in brain volumes. To evaluate effect modification by genotypes on relations of cardiovascular risk factors with change in volumes or WMLs, we added a term for cardiovascular risk factors (separately) and a cross-product term for genotype $*$ risk factor to the core models. Effect modification by genotypes on age relations were also examined in separate models with a cross-product term for genotype $*$ age at baseline. We then examined effect modification by genotype on the relationship of CHS score at baseline, as well as change in CHS score across visits, with change in ROI volumes. Finally, in former lead workers, we examined interactions of PTL and genotypes on change in ROI volumes. Model diagnostics were used to evaluate influence and normality.

2.5.2. Voxel-Wise Approach. The relationship between genotypes and change in voxel volumes was modeled controlling for the aforementioned covariates using multivariate permutation testing in the R statistical programming language (http://www.cran.r-project.org/). The SPM5 package (Statistical Parametric Software, Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, University College London, 2003) was used to perform smoothing using a 3D isotropic Gaussian filter and MRIcro [52] to display results. Statistical significance was evaluated using a permutation approach that controlled for confounding variables. The maximum cluster size and cluster peak above the threshold was used to define a conservative permutation distribution on cluster sizes and peaks that, when compared to the observed cluster sizes and peaks, controls for multiple comparisons.

2.5.3. White Matter Lesions. Linear regression was used to model change in WML grade scores controlling for covariates and evaluating the same effect modification variables. As in the ROI-based analysis, we examined the relationships between cardiovascular risk factors and change in WML, and then effect modification by genotype on relationships of cardiovascular risk factors with change in WML. Effect modification by genotype on the relationship of age and change in WML was also examined. In former lead workers, we examined interactions of PTL and genotypes on change in WML.

3. Results

3.1. Descriptive Summary of Study Subjects. Basic descriptive characteristics of the 353 subjects with two valid MRIs are presented elsewhere [36]. In short, the mean (SD) age was 65.1 (7.9) years (range: 48–82), 93% had a high school education, and 90% were white. Seven subjects were missing *ACE* genotyping, 6 subjects were missing *ACE* and *VDR* genotyping, and 1 subject was missing data for *ACE*, *VDR*, and *APOE*. With the exception of CRP by *APOE* genotype and diabetes by *VDR* genotype, there were no differences in distributions of cardiovascular risk factors by genotype (Table 1). There were no differences in *APOE*, *ACE*, or *VDR* genotypes by control status (data not shown). There were no differences in *APOE* or *VDR* genotypes by MRI status (i.e., zero versus one versus two MRIs; data not shown); we did not perform *ACE* genotyping on persons without two MRIs. Controls had significantly lower mean (SD) levels of total cholesterol (182.0 (31.5) versus 200.9 (40.8), $P = 0.004$) and LDL (97.5 (29.4) versus 114.5 (34.8), $P = 0.003$), and higher levels of CRP (3.3 (3.8) versus 2.4 (2.5), $P = 0.04$) than former lead workers.

3.2. Change in ROI Volumes. As presented elsewhere in more detail [36], the volumes of all ROIs except for occipital WM declined from the first to the second MRI over an mean (SD) time of 5.0 (0.4) years, with a more substantial decline in gray ($-24.4\,\text{cm}^3$) versus white ($-5.4\,\text{cm}^3$) matter. On average, total brain volume declined an average of $30\,\text{cm}^3$.

Cardiovascular Risk Factors and WML. There was little consistent evidence of a main effect of cardiovascular risk factors on change in ROI volumes. However, higher HDL was associated with more decline in 5 ROI volumes: total brain volume (β(SE) $= -0.183(0.078)$, $P = 0.02$), total WM (β (SE) $= -0.101$ (0.047), $P = 0.03$), parietal WM (β (SE) $= -0.038$ (0.011), $P < 0.001$), cingulate gyrus (β (SE) $= -0.009$ (0.004), $P = 0.01$), and hippocampus (β (SE) $= -0.004$ (0.001), $P = 0.006$). More WML at baseline and change in WML were not associated with change in ROI volume.

APOE. There were consistent associations of *APOE* genotype with change in ROI volumes (Table 2). Results are only presented for the $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$ genotypes combined, from a model that also included terms for $\varepsilon2/\varepsilon2$ plus $\varepsilon2/\varepsilon3$ and $\varepsilon2/\varepsilon4$ (with $\varepsilon3/\varepsilon3$ as the reference group). The positive beta coefficients indicate less decline for those with the $\varepsilon3/\varepsilon4$ or $\varepsilon4/\varepsilon4$ genotypes (e.g., the TBV for persons with the $\varepsilon3/\varepsilon4$ or $\varepsilon4/\varepsilon4$ genotypes declined an average of $23\,\text{cm}^3$ versus $31\,\text{cm}^3$ for persons with the $\varepsilon3/\varepsilon3$ genotype (Figure 1)). The differences in ROI volume declines by *APOE* genotype were largest and most consistent for changes in white matter volumes.

There was evidence that *APOE* genotype modified relations of age with change in ROI volumes. Persons who were older at baseline and had the $\varepsilon3/\varepsilon4$ or $\varepsilon4/\varepsilon4$ genotype experienced more decline in the following ROI volumes: frontal WM (β (SE) $= -0.207$ (0.086), $P = 0.02$), parietal WM (β (SE) $= -0.115$ (0.048), $P = 0.02$), corpus callosum (β (SE) $= -0.023$ (0.007), $P = 0.001$), hippocampus (β (SE) $= -0.013$ (0.006), $P = 0.02$), and amygdala (β (SE) $= -0.008$ (0.003), $P = 0.02$) (frontal WM displayed in Figure 2. There were no consistent interactions between cardiovascular risk factors and genotype for change in any ROI volume.

We next evaluated whether relations among change in WML and change in ROI volumes were modified by *APOE* genotype. In models that included terms for change in WML and a cross-product for *APOE* genotype $*$ change in WML,

Table 1: Distribution of cardiovascular risk factors by genotype.

Genotype	N (%)	HTN N (%)	CVD N (%)	Diabetes N (%)	Total cholesterol mean (SD)	HDL mean (SD)	LDL mean (SD)	CRP mean (SD)
APOE ($n = 352$)[1]								
ε2/ε3 + ε2/ε2	46 (14)	20 (43)	11 (24)	10 (22)	196.7 (49.4)	52.7 (15.6)	107.0 (41.9)	2.7 (2.6)
ε3/ε3	214 (61)	116 (54)	23 (11)	39 (18)	198.8 (39.7)	50.2 (13.9)	111.8 (34.4)	2.7 (2.9)
ε2/ε4	11 (3)	6 (55)	2 (18)	0 (0)	196.9 (39.8)	44.3 (14.8)	121.3 (27.6)	2.5 (2.6)
ε3/ε4 + ε4/ε4	81 (23)	42 (52)	9 (11)	15 (19)	199.2 (36.6)	48.3 (12.9)	116.2 (31.2)	1.7 (1.8)
		$P = 0.62$	$P = 0.094$	$P = 0.42$	$P = 0.99$	$P = 0.19$	$P = 0.43$	$P = 0.02$
ACE ($n = 339$)[2]								
I/I	64 (19)	38 (59)	8 (13)	10 (16)	200.2 (38.9)	52.5 (14.9)	111.9 (34.7)	2.1 (1.8)
I/D	137 (40)	67 (49)	12 (9)	26 (19)	196.7 (39.0)	49.8 (13.3)	112.0 (31.8)	2.4 (2.6)
D/D	138 (41)	74 (54)	22 (16)	25 (18)	198.9 (42.6)	48.7 (14.4)	112.5 (37.7)	2.7 (2.9)
		$P = 0.37$	$P = 0.20$	$P = 0.85$	$P = 0.82$	$P = 0.20$	$P = 0.99$	$P = 0.32$
VDR ($n = 346$)								
FF	125 (36)	60 (48)	17 (14)	15 (12)	198.6 (39.1)	49.4 (14.8)	111.9 (33.3)	2.4 (2.7)
Ff	168 (49)	90 (54)	19 (11)	34 (20)	199.1 (40.9)	49.5 (13.7)	114.0 (36.0)	2.6 (2.7)
ff	53 (15)	31 (58)	8 (15)	14 (26)	197.3 (42.6)	52.2 (13.1)	109.3 (34.4)	1.9 (2.2)
		$P = 0.40$	$P = 0.72$	$P = 0.047$	$P = 0.96$	$P = 0.43$	$P = 0.68$	$P = 0.21$

[1] Certain genotypes were combined for analysis, resulting in following analytic groups: (1) ε3/ε4 and ε4/ε4, (2) ε2/ε2 and ε2/ε3, (3) ε2/ε4, (4) ε3/ε3 (reference group). [2] I: insertion, D: deletion.

there was evidence of such effect modification. Persons who had *APOE* ε3/ε4 or ε4/ε4 genotype and an increase in WML experienced less decline in the following ROI volumes; total brain volume (β (SE) = −6.658 (2.402), $P = 0.006$), total WM (β (SE) = −3.727 (1.419), $P = 0.009$), frontal WM (β (SE) = −1.717 (0.611), $P = 0.005$), temporal WM (β (SE) = −0.806 (0.358), $P = 0.03$), parietal WM (β (SE) = −1.183 (0.341), $P = 0.001$), medial structures (β (SE) = −0.802 (0.304), $P = 0.009$), cingulate gyrus (β (SE) = −0.251 (0.116), $P = 0.03$), insula (β (SE) = −0.242 (0.081), $P = 0.003$), corpus callosum (β (SE) = −0.117 (0.052), $P = 0.03$), internal capsulate (β (SE) = −0.125 (0.048), $P = 0.01$), and hippocampus (β (SE) = −0.139 (0.040), $P = 0.001$) (see, e.g., Figure 3).

ACE. There were no associations between *ACE* genotype and change in ROI volumes. There was no evidence that *ACE* genotype modified relations of age with change in ROI volumes. There was evidence that *ACE* genotype modified relations of HDL with change in ROI volumes. Persons with greater HDL who had the I/I genotype experienced less decline in the following ROIs: total brain volume (β (SE) = 0.533 (0.211), $P = 0.01$), total WM (β (SE) = 0.342 (0.127), $P = 0.007$), frontal WM (β (SE) = 0.131 (0.055), $P = 0.02$), parietal WM (β (SE) = 0.080 (0.030), $P = 0.009$), occipital WM (β (SE) = 0.034 (0.017), $P = 0.04$), and hippocampus (β (SE) = 0.007 (0.004), $P = 0.04$). There was no evidence that *ACE* genotype modified relations of any other cardiovascular risk factors or WML scores with change in ROI volumes.

VDR. There were no associations between *VDR* genotype and change in ROI volumes. There was no evidence that *VDR* genotype modified relations of age with change in ROI volumes. There was evidence that *VDR* genotype modified relations of diabetes with change in ROI volumes. Persons who had diabetes and were heterozygous for the *VDR FokI* Ff genotype experienced less decline in the following ROIs: total WM (β (SE) = 9.873 (4.102), $P = 0.02$), frontal WM (β (SE) = 3.557 (1.755), $P = 0.04$), temporal WM (β (SE) = 2.412 (1.013), $P = 0.02$), occipital WM (β (SE) = 1.281 (0.537), $P = 0.02$), internal capsule (β (SE) = 0.336 (0.137), $P = 0.02$), and entorhinal cortex (β (SE) = 0.153 (0.062), $P = 0.01$). There was no consistent evidence that *VDR* genotype modified relations of any other cardiovascular risk factors with change in ROI volumes. There was evidence that *VDR* modified relations of change in WML scores and change in ROI volumes. Persons who were homozygous for the *VDR FokI* ff genotype and had an increase in WML experienced more decline in the following ROI volumes: total GM (β (SE) = −4,570 (2.089), $P = 0.03$), frontal GM (β (SE) = −1.402 (0.604), $P = 0.02$), and parietal GM (β (SE) = −0.696 (0.319), $P = 0.03$), but *less* decline in two ROIs: frontal WM (β (SE) = 1.513 (0.723), $P = 0.04$), and parietal WM (β (SE) = 0.895 (0.401), $P = 0.03$).

Lead By Gene Interaction. In former lead workers, there was little evidence that PTL modified the relationship of candidate genes with change in ROI volumes. A significant interaction of PTL with *APOE* ε3/ε4 or ε4/ε4 genotype was found in only one ROI, occipital GM (β (SE) = 0.031 (0.016), $P = 0.045$).

Change in Voxel Volumes. In a parallel analysis, results were substantively similar using a voxel-wise approach. The supra-threshold clusters for the association of *ACE* genotype and *VDR* genotype with change in GM and WM volume were

Table 2: Regression[a] results for delta ROI models for former lead workers and controls ($N = 352$), adjusting for confounding variables.

	APOE	*ACE*		*VDR*	
	$\varepsilon3/\varepsilon4 + \varepsilon4/\varepsilon4$[c]	I/D, I/I[d]		Ff, ff[e]	
ROI[b]	Beta (SE)	Beta (SE)		Beta (SE)	
TBV	8.045 (2.621)***	4.498 (2.477)	−2.709 (3.134)	2.188 (2.429)	0.537 (3.373)
TOTAL GM	3.320 (1.975)*	2.135 (1.839)	−1.236 (2.328)	1.733 (1.806)	−0.038 (2.508)
FRONT GM	1.076 (0.575)*	0.645 (0.534)	−0.591 (0.677)	0.585 (0.524)	0.288 (0.729)
OCCIP GM	0.241 (0.213)	0.187 (0.198)	−0.083 (0.251)	0.254 (0.194)	0.106 (0.271)
PARI GM	0.612 (0.301)**	0.636 (0.282)**	−0.041 (0.357)	0.104 (0.276)	−0.011 (0.383)
TEMP GM	0.619 (0.485)	0.677 (0.457)	0.046 (0.578)	0.709 (0.443)	−0.063 (0.616)
TOTAL WM	5.059 (1.554)***	2.402 (1.491)	−1.398 (1.887)	0.548 (1.451)	0.309 (2.012)
FRONT WM	2.331 (0.666)***	0.839 (0.639)	−0.825 (0.309)	−0.080 (0.623)	−0.248 (0.864)
OCCIP WM	0.818 (0.203)***	0.176 (0.195)	−0.051 (0.246)	0.140 (0.190)	0.052 (0.263)
PARI WM	0.921 (0.374)**	0.378 (0.358)	−0.291 (0.452)	0.071 (0.347)	0.111 (0.481)
TEMP WM	0.557 (0.389)	0.242 (0.367)	−0.477 (0.464)	−0.148 (0.358)	0.130 (0.495)
ERC	0.032 (0.024)	0.008 (0.023)	0.016 (0.029)	−0.001 (0.022)	−0.013 (0.031)
AMYG	0.035 (0.024)	0.033 (0.023)	−0.043 (0.029)	0.013 (0.022)	−0.039 (0.031)
HIPPO	0.070 (0.044)	0.044 (0.042)	−0.020 (0.053)	0.050 (0.040)	0.057 (0.056)
CEREB	0.102 (0.473)	0.158 (0.447)	−0.043 (0.568)	0.650 (0.432)	0.447 (0.598)
MEDIAL	0.898 (0.332)***	0.231 (0.315)	−0.361 (0.396)	0.207 (0.306)	0.044 (0.424)
INSULA	0.128 (0.088)	0.092 (0.083)	−0.146 (0.104)	0.010 (0.081)	−0.051 (0.113)
CINGULATE	0.236 (0.124)*	0.149 (0.116)	−0.047 (0.147)	0.050 (0.114)	−0.107 (0.159)
CORP CALL	0.055 (0.057)	0.005 (0.054)	0.039 (0.068)	−0.029 (0.052)	−0.033 (0.072)
INT CAPS	0.151 (0.052)***	−0.002 (0.050)	−0.059 (0.064)	−0.028 (0.049)	−0.058 (0.068)

* $0.05 < P < 0.10$; ** $0.01 < P < 0.05$; *** $P < 0.01$.

[a]Models adjusted for height, baseline ROI, control status, duration between MRIs, and education.

[b]ROI: region of interest; TBV: total brain volume (TBV1 = TBV at first MRI); GM: gray matter; FRONT: frontal; OCCIP: occipital; PARI: parietal; TEMP: temporal; WM: white matter; ERC: entorhinal cortex; AMYG: amygdala; HIPPO: hippocampus; CEREB: cerebellum; MEDIAL: medial structures (bilateral amygdala, cuneus, entorhinal cortex, hippocampal formation, lingual gyrus, medial front-orbital gyrus, medial frontal gyrus, medial occipitotemporal gyrus, parahippocampal gyrus, perirhinal cortex, precuneus, and uncus); CORP CALL: corpus callosum; INT CAPS: internal capsule.

[c]Compared to *APOE*3-3 as reference group; model also included terms for 22 + 23 and 24.

[d]Compared to D/D (homozygous for deletion) as reference group.

[e]Compared to FF as reference group.

within the range expected by chance (not shown). The adjusted association between *APOE* $\varepsilon3/\varepsilon4$ or $\varepsilon4/\varepsilon4$ genotype and change in voxel volumes identified large supra-threshold clusters in WM, whose sizes were above the distribution of the maximum cluster size under the null hypothesis (largest association cluster depicted in Figure 4).

3.3. Change in WML Grade Score. Summary statistics for change in CHS WML scores between the first and second MRI have been previously reported [36]. In brief, 74% of the sample showed increased WML over followup. The *APOE* and *ACE* genotypes were not associated with changes in WML scores. In adjusted analysis, controlling for age, duration between MRIs, control status, height, education, and baseline CHS score, the *VDR FokI* polymorphism was associated with increases in WML in a gene-dose-dependent fashion, with beta coefficients (SE, *P*-value) of 0.18 (0.12, $P = 0.13$) and 0.45 (0.16, $P = 0.006$) for Ff and ff genotypes, respectively. This indicates, for example, that, on average, subjects with the ff genotype had CHS scores that increased 0.45 categories higher than did those with the FF genotype (Figure 5). These associations did not change when baseline CHS score was removed from the model. In former lead workers, there was no evidence of interactions of genes with PTL.

4. Discussion

In this cohort of nondemented older men with two MRI scans an average of five years apart, we examined relations of three genetic polymorphisms with longitudinal change in brain volumes and WMLs, Our main findings were that the *APOE* $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$ genotypes were associated with less decline in brain volumes over time, especially in WM, and that these genotypes modified the relationship of age as well as change in WML with change in brain volumes. We also found that the *VDR FokI* ff genotype was associated with an increase in WMLs. There was some evidence that the genotypes modified relations of cardiovascular risk factors with change in both ROI volumes and WMLs, but these findings were not consistent across brain regions or consistent across risk factors. There was no evidence that genotypes modified relations of lead levels with change in ROI volumes and WMLs in former lead workers. These

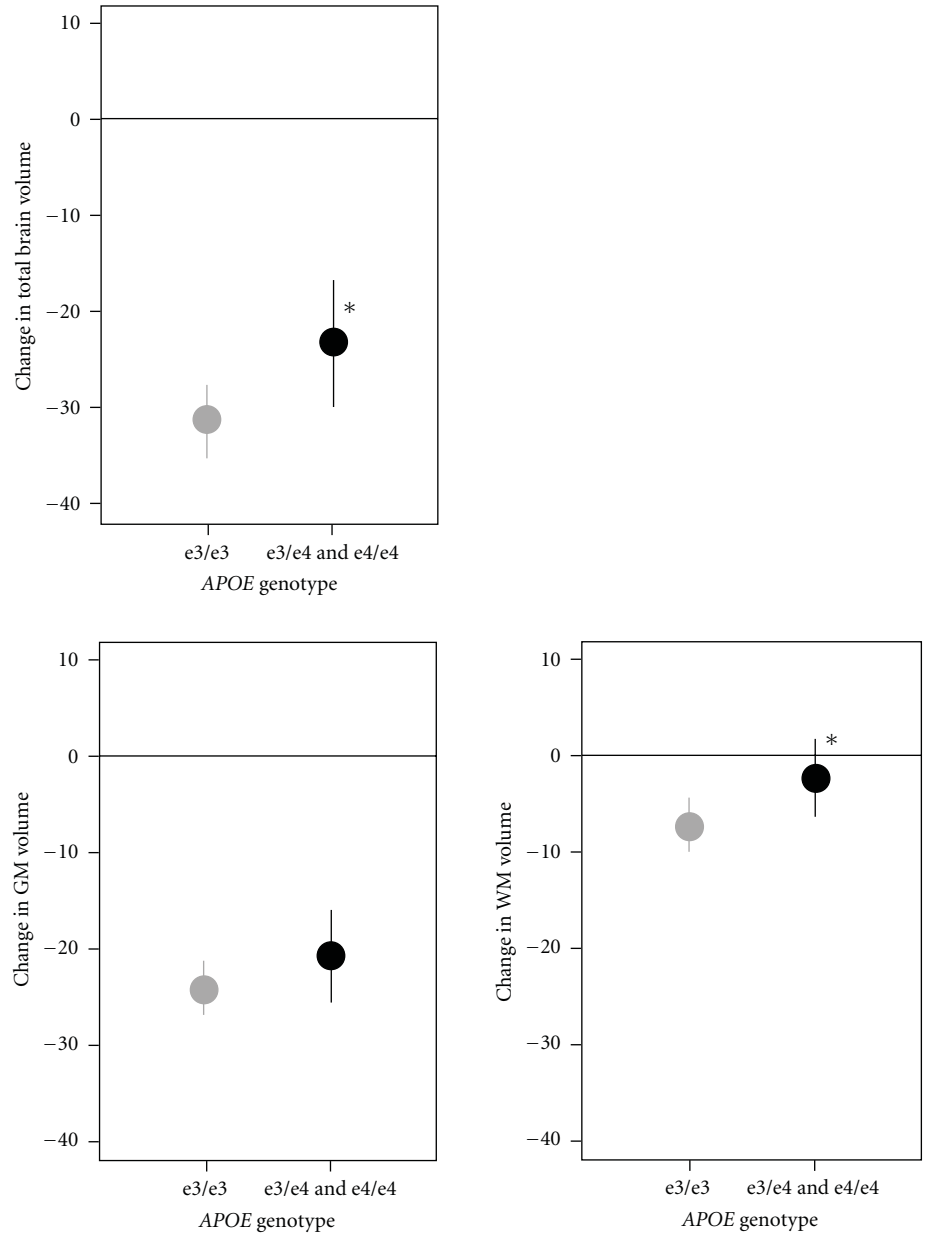

Figure 1: Change in total brain, GM, and WM volumes by *APOE* genotype. The grey and black lines are estimated change in volumes (mean ± 95% confidence interval) for the *APOE* ε3/ε3 and *APOE* ε3/ε4 + ε4/ε4 groups, respectively. The asterisk indicates that the estimated change for the ε3/ε4 + ε4/ε4 group is significantly different than for the ε3/ε3 group ($P < 0.05$).

findings give us some insight into the genetic determinants of structural changes in the brain that may contribute to cognitive impairments in later life.

A number of studies have examined the relation between *APOE* genotype and brain structure. The ε4 allele has been associated with smaller total brain, gray matter, hippocampus, amygdala, and corpus callosum volumes, and more WMLs [21–24]. However, there are a number of studies that have found no association between *APOE* and brain volumes [25, 26] or WML [26] in healthy samples, and at least three studies in AD patients have found an association between ε4 and *larger* volumes [27–29]. The majority of these studies used cross-sectional study designs in which change in structure volumes across age ranges is extrapolated from inter-individual differences in age. Very little research has focused on how these genetic risk factors relate to longitudinal intraindividual changes in brain structure. In nondemented cohorts, one longitudinal study found an association of the ε4 allele with greater hippocampus volume loss [30], while another found a nonsignificant trend for a relation between the ε4 allele and greater brain atrophy [31]. One longitudinal study on APOE genotype and WMLs has been conducted, which found an increase in WMLs in ε4/ε4 individuals only [53]. There has been little published

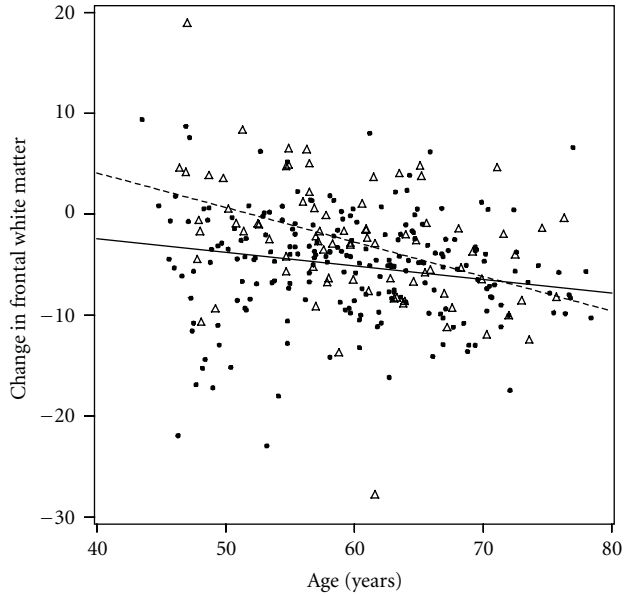

Figure 2: Effect modification by *APOE* genotype on relation of age with change in frontal WM volume for *APOE* ε3/ε3 (black dots, solid regression line) and ε3/ε4 plus ε4/ε4 groups (triangles, dashed regression line). The slopes of the two lines were different ($P < 0.05$).

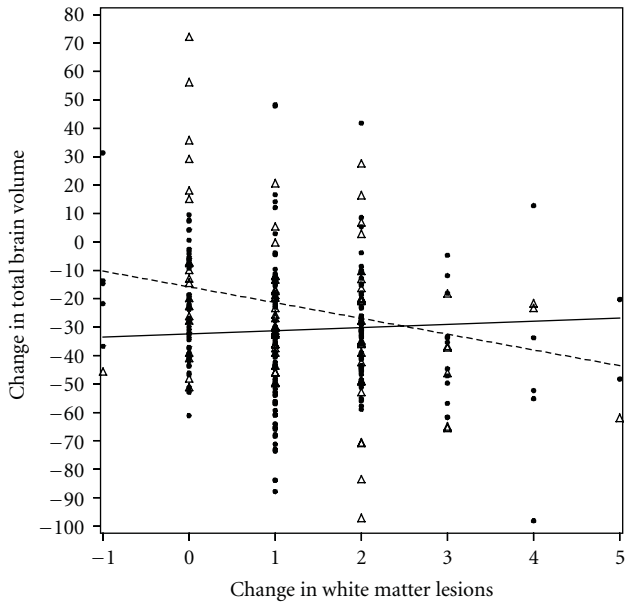

Figure 3: Effect modification by *APOE* genotype on relation of change in WML and change in total brain volume for *APOE* ε3/ε3 (black dots, solid regression line) and ε3/ε4 plus ε4/ε4 groups (triangles, dashed regression line). The slopes of the two lines were different ($P < 0.05$).

research on *ACE* polymorphisms and differences in brain structure. One study found an association between the I/I genotype and smaller hippocampus and amygdala volumes in women only, but no association with WML [54] and another found no relation to volume or WML [55]. A recent review found evidence of association between *ACE* I/D

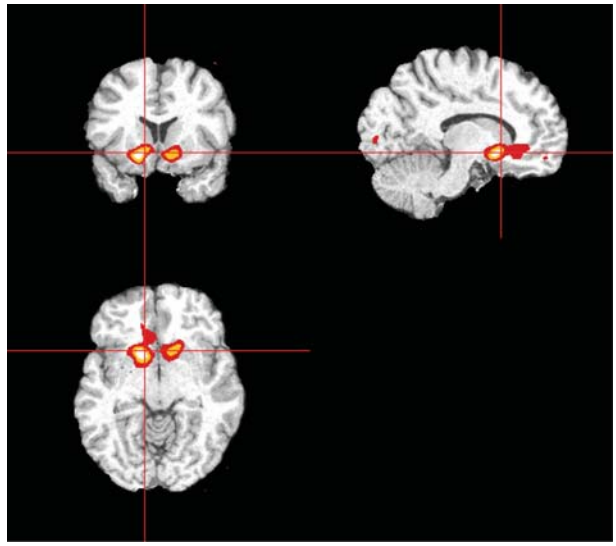

Figure 4: Largest significant clusters where *APOE* ε4/ε4 or ε3/ε4 genotypes were associated with less decline in WM volume for statistical maps based on family-wise error rate corrected *P* value thresholding from permutation testing. Results are shown for relevant coronal (upper left), sagital (upper right) and axial (lower) slices. Statistical significance was based on suprathreshold cluster-level permutation testing. Colors represent voxels satisfying *P* value thresholds of 0.001, 0.0001, and 0.00001, respectively.

polymorphism and WML from 9 cross-sectional studies, but cautioned against publication bias [56]. We were unable to identify any prior studies of *VDR* genotype and brain structure.

Our results are not consistent with the small number of studies that have previously examined the relations between the *APOE* ε4 allele and change in brain volumes in nondemented cohorts. We found that subjects with the ε4 allele had less rather than more decline in volumes of brain structures compared to those without the allele. This finding is unexpected in light of the established relation between the ε4 allele and neurodegenerative diseases such as AD and the concomitant brain atrophy experienced by individuals who have these diseases. However, given the paucity of evidence regarding this relationship, these findings should be treated as preliminary and may suggest a different pathway from gene to AD expression as mediated through structural changes in the brain than have been previously recognized. For example, the association between ε4 and less decline in volume was strongest in WM; this could be consistent with an adverse effect in persons with the ε4 allele if the slower rate of WM volume loss is due to inflammation, edema, swelling of cells, or other changes in WM that are present in early lesions in these relatively young study subjects [57]. This hypothesis may be supported by the accompanying finding that higher levels HDL, usually considered protective against vascular events, was associated with more WM decline as well. Alternatively, these findings may align with the emerging theory of *APOE* antagonistic pleiotropy in which the ε4 allele confers an advantage at younger ages while producing detrimental neurocognitive consequences in later

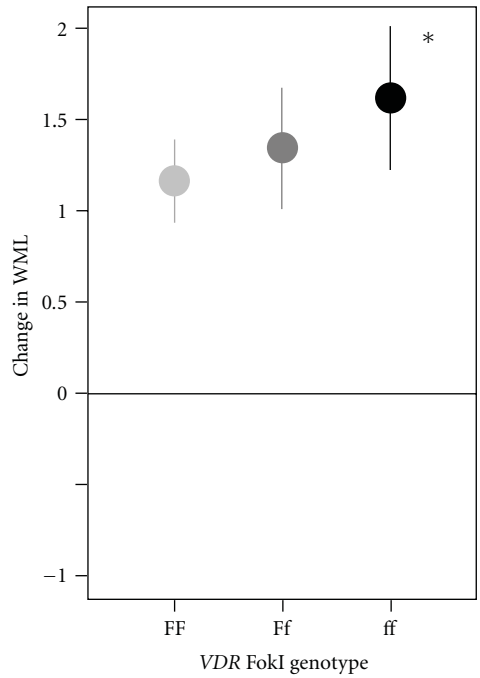

Figure 5: Change in WML grade score by *VDR FokI* genotype. The light gray, dark gray, and black lines are for groups with the FF, Ff, and ff genotypes, respectively. The asterisk indicates that the estimated change in WML scores for genotype was significantly different from the FF genotype.

life [58]. This is supported by our finding of an interaction between age and ε4 status on WM decline in which persons with ε3/ε4 or ε4/ε4 genotype experience less decline than ε3/ε3 carriers at earlier ages, but this difference attenuates and actually reverses after the age of 70, after which ε3/ε4 or ε4/ε4 experience more decline. Further, there is evidence that *APOE* antagonistic pleiotropy is related to integrity of the cholinergic system [59]; the most robust associations between ε3/ε4 or ε4/ε4 genotype and less decline was found in the basal forebrain (Figure 4), a region considered to be the major cholinergic output of the brain.

We also observed effect modification by *APOE* genotype on the relation of change in WML with change in brain volumes. Persons with ε3/ε4 or ε4/ε4 genotypes experience *more* decline in brain volumes with increases in WML. Notably, cardiovascular risk factors were not associated with increased WML, perhaps indicating that both decline in WM and progression of WML may not have linear relationships with traditional risk factors for cognitive impairment over the life course. Finally, our study is the first to report on an association between *VDR FokI* genotype and change in WML. This finding needs to be replicated before any conclusions can be drawn, but connections between *VDR*, cardiovascular disease, and WMLs gives this finding biological plausibility [16, 19].

The study had several strengths, including larger sample size than most prior studies, longitudinal design, use of ROI-based and voxel-wise analyses, relatively long duration between scans, and analysis of WMLs as determined by application of the CHS WML grading method. The main strength of this study was the ability to examine intraindividual change in brain structures over a 5-year period using longitudinal data. This provides a more valid measure of change and predictors of change than extrapolating an estimate of change from separate individuals across a range of ages using cross-sectional data.

A limitation of this study is the selected nature of the cohort, which was made up entirely of men, most of whom had histories of occupational lead exposure. However, general population samples have shown tibia lead levels similar to this cohort [60, 61], consistent with documentation that all Americans over the age of 50 years had significant environmental lead exposure [62]. Thus, our ability to adjust for and examine interactions with lead is also a strength of this study. The fact that prior studies of older Americans have not considered this ubiquitous neurotoxicant that influences brain volumes [34] could be an important source of confounding. The ubiquity of lead exposure could also mask a potential gene by lead interaction, resulting in a gene appearing to exert a main effect [63]. However, a gene by lead interaction was not observed for change in brain volumes or increase in WML.

An important consideration that could affect the internal validity of these results is selection bias, as persons who had an MRI scan may not be representative of the total cohort. In previous papers, we reported that there was unlikely to be meaningful selection bias, and if present, would likely mask rather than spuriously create associations [34, 36]. A methodological challenge was changes in the scanner technology between the two MRI scans. We attempted to minimize the problems introduced by these changes by using an image analysis technique that was specifically developed and validated for longitudinal studies that is more likely to underestimate rather than overestimate longitudinal brain changes [51].

In conclusion, this analysis adds to the emerging body of literature on genetic contributions to brain changes in later life. The findings suggest that early WM lesions in middle-aged persons with the *APOE* ε4 allele may initially be space-occupying, due to inflammation, edema, or swelling of cells, but that with advancing age and increases in WM lesions, persons with the ε4 allele experience more volume loss. This analysis is also one of the first to show an association between *VDR* genotype and changes in WM lesions.

Disclosure

None of the authors has any actual or potential conflicts of interest including any financial, personal, or other relationships with other people or organizations within 3 years of beginning the work submitted that could inappropriately bias their work.

Acknowledgment

This research was supported by R01 AG10785 (National Institute on Aging). Its content is solely the responsibility

of the authors. The authors thank Amanda Dziedzic and Anne Jedlicka for assay design and genotyping; facilities and instrumentation were supported by the Johns Hopkins Malaria Research Institute.

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Journal of Aging Research
Volume 2011, Article ID 280630, 8 pages
doi:10.4061/2011/280630

Research Article

Progression of White Matter Lesion Volume and Health-Related Quality of Life in Patients with Symptomatic Atherosclerotic Disease: The SMART-MR Study

Anne M. Grool,[1] Yolanda van der Graaf,[1] Theo D. Witkamp,[2] Koen L. Vincken,[3] Willem P. T. M. Mali,[2] and Mirjam I. Geerlings[1]

[1] *Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Stratenum 6.131, P.O. Box 85500, 3508 GA Utrecht, The Netherlands*
[2] *Department of Radiology, University Medical Center Utrecht, 3508 GA Utrecht, The Netherlands*
[3] *Image Sciences Institute, University Medical Center Utrecht, 3508 GA Utrecht, The Netherlands*

Correspondence should be addressed to Mirjam I. Geerlings, m.geerlings@umcutrecht.nl

Received 24 March 2011; Accepted 12 August 2011

Academic Editor: Leonardo Pantoni

Objectives. Mechanisms influencing the course of physical and mental functioning after an atherosclerotic event are unclear. We examined effects of white matter lesion (WML) activity on changes in functioning in patients with symptomatic atherosclerotic disease. *Methods*. In 486 patients (58 ± 9 years) of the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, volumetric WML measurements on 1.5T MRI were performed at baseline and 3.9±0.4 years followup. Functioning was assessed with the modified Short-Form 12 (SF-12) questionnaire. Associations of WML progression with changes in functioning were adjusted for age, sex, and vascular risk factors. *Results*. Physical functioning (baseline: 44, 10th–90th percentile 29–55) improved, whereas mental functioning (baseline: 51, 10th–90th percentile 32–60) declined during followup. WML progression (highest quartile versus rest) contributed to a stronger decline in mental functioning ($B = -1.76$, 95% CI -3.11 to -0.42), but did not influence changes in physical functioning. *Conclusions*. Progression of WML volume contributes to a decline in mental functioning in patients with symptomatic atherosclerotic disease.

1. Introduction

Ischemic heart disease and stroke are leading causes of disability and mortality worldwide [1]. As a result of improved survival and the lifelong aspect of these diseases, health-related quality of life (HRQoL), including physical and mental functioning, has become an increasingly important clinical and research outcome when evaluating burden of disease and treatment benefits. In addition, reduced physical and mental functioning not only interferes with daily living, but also increases the risk of incident ischemic vascular events and mortality [2–4]. Compared to the general population, HRQoL is substantially lower in patients with ischemic heart disease and stroke, especially in the domain of physical functioning [5–7]. A recent study indicated that HRQoL not only is lower in the acute phase of recovery from stroke, but also can decline up to five years after stroke in survivors free of recurrence or myocardial infarction [8]. Also, marked impairments in HRQoL have been observed in patients with other manifestations of atherosclerotic disease, including peripheral arterial disease [9, 10] and abdominal aortic aneurysm [11, 12].

Patients with symptomatic vascular disease frequently have atherosclerotic changes in the small vasculature in the brain, which are characterized by white matter lesions (WMLs) on magnetic resonance imaging (MRI) [13]. Although WMLs are often asymptomatic, they have been identified as a risk factor for functional decline [14], late-life depression [15, 16], and cognitive impairment [17–19]. It has been suggested that greater disease activity, characterized by an accelerated progression of WML volume, is an important

underlying mechanism contributing to this elevated risk [20, 21], but longitudinal studies are still relatively scarce. Whether greater progression of WML volume is also associated with a poorer HRQoL has not been studied yet, although it could be expected that more subtle impairments in physical and mental functioning could already be present in patients with greater WML disease activity, before the development of depression or functional decline. In addition, it is unknown whether the influence of WML progression on physical and mental functioning is comparable between patients with different locations of symptomatic atherosclerotic disease.

Our first aim was to investigate the course of physical and mental functioning in patients with different manifestations of atherosclerotic disease over four years of follow-up. Second, we examined whether greater progression of WML volume contributed to poorer physical and mental functioning in these patients and whether these associations depended on the location of symptomatic atherosclerotic disease.

2. Materials and Methods

2.1. Participants. Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed to investigate brain changes on MRI in 1309 independently living patients with symptomatic atherosclerotic disease. Details of the design and participants have been described elsewhere [13]. For the current study, data were used from 989 patients newly referred to the University Medical Center Utrecht between January 2002 and December 2005 with manifest peripheral arterial disease, coronary artery disease, cerebrovascular disease, or abdominal aortic aneurysm without MR contraindications and available data on the HRQoL questionnaire. During a 1-day visit to our medical center, an MRI of the brain, physical examination, blood, and urine sampling were performed. Risk factors, medical history, and functioning were assessed with questionnaires.

Between January 2006 and May 2009, all participants still alive were invited for follow-up measurements, including MRI of the brain, neuropsychological testing, a physical examination, blood and urine sampling, risk factors, medical history, and functioning. The SMART-MR study was approved by the ethics committee of our institution, and written informed consent was obtained from all participants.

In total, 585 of the surviving cohort (62% of $n = 943$) gave written informed consent; 346 (37%) persons refused, and 12 (1%) were lost to followup.

2.2. Magnetic Resonance Imaging Protocol. MR investigations were performed on a 1.5-tesla whole-body system (Gyroscan ACS-NT, Philips Medical Systems, Best, The Netherlands). The protocol consisted of a transversal T1-weighted gradient-echo sequence (repetition time (TR)/echo time (TE): 235/2 ms; flip angle, 80°), a transversal T2-weighted turbo spin-echo sequence (TR/TE: 2200/11 ms and 2200/100 ms; turbo factor 12), a transversal T2-weighted fluid attenuating inverse recovery (FLAIR) sequence (TR/TE/inversion time (TI): 6000/100/2000 ms), and a transversal inversion recovery (IR) sequence (TR/TE/TI: 2900/22/410 ms) (field of view 230×230 mm; matrix size, 180×256; slice thickness, 4.0 mm; no gap; 38 slices).

2.3. Brain Segmentation. We used the T1-weighted gradient echo, IR sequence, and FLAIR sequence for brain segmentation. The probabilistic segmentation technique has been described elsewhere [22, 23]. The segmentation program distinguishes cortical gray matter, white matter, sulcal and ventricular cerebrospinal fluid (CSF), and lesions. The results of the segmentation analysis were visually checked for the presence of infarcts and adapted if necessary to make a distinction between WML and infarct volumes. Total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WML and infarcts. All volumes cranial to the foramen magnum were included. As a result, the total brain volume includes the cerebrum, brainstem, and cerebellum. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the sulcal and ventricular CSF.

2.4. Infarcts and White Matter Lesions. The whole brain was visually searched for infarcts by a trained investigator and a neuroradiologist. Raters were blinded to history and diagnosis of the patient. Discrepancies in rating were reevaluated in a consensus meeting. Infarcts were defined as focal hyperintensities on T2-weighted images of at least 3 mm in diameter. Hyperintensities located in the white matter also had to be hypointense on T1-weighted and FLAIR images in order to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location, form, and the absence of gliosis. The location, affected flow territory, and type were scored for every infarct.

WML volumes obtained with the segmentation program were summed to obtain total WML volume. Volumes of WML were normalized for ICV and expressed as percentage of ICV.

2.5. Physical and Mental Functioning. At baseline and followup, patients completed the Short Form-12 (SF-12) [24], a shortened version of the Short Form-36 (SF-36) Medical Outcomes Study Health Survey [25], to measure HRQoL at baseline and followup. The SF-12 questionnaire includes 1 or 2 items from each of the 8 health summary scales of the SF-36 [26] and enables calculation of the Physical (PCS) and Mental Component Summary scales (MCS). The SF-12 summary scales are positively scored and normalized to a general population mean of 50 with standard deviation of 10. Higher SF-12 scores indicate better HRQoL; a positive change in SF-12 scores indicates an improvement, and a negative change a deterioration in HRQoL. Because of its brevity, the SF-12 is considered advantageous over the SF-36 for large studies focusing on overall physical and mental functioning [26].

2.6. Severity of Atherosclerotic Disease at Baseline. In patients with peripheral arterial disease, severity of vascular disease at baseline was assessed using the Fontaine scale [27]. Stage 1 (pain-free walking distance >200 m) and stage 2 (pain-free walking distance <200 m) were defined as mild or moderate ischaemia, whereas stage 3 (rest pain) and stage 4 (ulceration or gangrene) were defined as severe ischaemia. In patients with coronary artery disease, disease severity was rated according to the number of coronary arteries with marked atherosclerosis (>70% stenosis or fractional flow reserve <0.80 or treatment of the vessel). One-vessel, two-vessel, three-vessel, left main disease with or without right coronary artery involvement was rated in all coronary artery disease patients on the basis of coronary angiography reports. Information was incomplete in some patients, and additional information was obtained from percutaneous coronary intervention or coronary artery bypass grafting reports. For patients with cerebrovascular disease, disease severity was classified with a handicap scale, the modified Rankin Scale (mRS) [28].

2.7. Other Variables. During the visit to the medical center, an overnight fasting venous blood sample was taken to determine glucose levels. Systolic and diastolic blood pressures (mmHg) were measured twice with a sphygmomanometer and averaged. Hypertension was defined as mean systolic blood pressure ≥160 mmHg, mean diastolic blood pressure ≥95 mmHg, or self-reported antihypertensive drug use. Diabetes mellitus was defined as fasting glucose ≥7.0 mmol/L or self-reported use of oral antidiabetic drugs or insulin. Smoking habits and alcohol intake were assessed with questionnaires. Packyears of smoking was calculated, and alcohol use was categorized into never, past, and current.

2.8. Study Sample. Of the 585 patients participating at followup, data on baseline or follow-up MRI variables were missing in 74 patients (no MR ($n = 41$), irretrievable MR data ($n = 5$), missing FLAIR images ($n = 7$), or artefacts ($n = 21$)). Of these, HRQoL data at followup were missing in 17 patients. Of these 494 patients, data on vascular risk factors were missing in 8 patients. This resulted in a total study sample of 486 patients.

Compared to patients who were lost to followup ($n = 503$), patients who participated at followup ($n = 486$) were significantly younger (mean 57.5 versus 59.6 years) at baseline, had less often hypertension (50% versus 57%) and diabetes mellitus (16% versus 25%), more often reported current alcohol intake (79% versus 72%), had lower WML volume (median 1.3 versus 1.7 mL), had better mental functioning (median 51.0 versus 48.3), and were less often included with peripheral arterial disease (19% versus 26%) or abdominal aortic aneurysm (5% versus 11%) (Table 1).

Table 1: Baseline characteristics of patients with complete data at followup and of those lost to followup.

	Complete data at followup ($n = 486$)	Lost to followup ($n = 503$)
Age¥ (years)	58 ± 9.3	60 ± 10.2
Male gender (%)	80	79
Diagnosis of symptomatic atherosclerotic disease‡		
(i) Peripheral arterial disease	19	26
(ii) Coronary artery disease	65	61
(iii) Cerebrovascular disease	24	23
(iv) Abdominal aortic aneurysm	5	11
Severe atherosclerotic disease£	11	9
Smoking† (pack/years)	21 (0–53)	18 (0–50)
Alcohol use		
(i) Never	13	18
(ii) Former	7	11
(iii) Current	79	72
Hypertension (%)	50	57
Diabetes mellitus (%)	16	25
Total intracranial volume¥ (mL)	1467 ± 127	1457 ± 132
Absolute total WML volume† (mL)	1.3 (0.4–5.8)	1.7 (0.6–8.3)
Physical functioning†	44 (29–55)	43 (26–54)
Mental functioning†	51 (32–60)	48 (29–60)

WML: white matter lesions; mRS: modified Rankin Scale.
‡ The different groups of symptomatic atherosclerotic disease do not add up to the total study sample of 486, because various locations of symptomatic atherosclerotic disease can occur within one patient.
£Defined as patients with coronary artery disease and three-vessel or left main disease at inclusion, patients with cerebrovascular disease and a mRS grade ≥2 at inclusion, or patients with peripheral arterial disease with Fontaine grade ≥3 at inclusion.
¥ Mean ± SD
† Median, (10th–90th percentile).

2.9. Data Analysis. First, we calculated changes in physical and mental functioning after on average 4 years of followup in the total sample and then compared changes in physical and mental functioning between different locations of symptomatic atherosclerotic disease using generalized linear models with physical and mental functioning scores at followup as the dependent variables and location of symptomatic atherosclerotic disease, age, sex, baseline physical or mental functioning, and follow-up time as independent variables.

Second, linear regression analysis was used to investigate whether greater progression of WML volume was associated with changes in physical and mental functioning. Progression of WML volume was defined as the difference in WML volume (% of ICV) between baseline and followup. We divided WML progression into quartiles, and dichotomized WML progression (highest quartile ($n = 126$) versus lower quartiles ($n = 360$) to investigate whether patients with greatest progression showed a different course of physical

Table 2: Baseline characteristics.

	Total sample ($n = 486$)	Peripheral arterial disease ($n = 90$)‡	Coronary artery disease ($n = 318$)‡	Cerebrovascular disease ($n = 115$)‡	Abdominal aortic aneurysm ($n = 26$)‡
Age¥ (years)	58 ± 9.3	56 ± 10.2	58 ± 9.0	59 ± 9.9	62 ± 7.9
Male gender (%)	80	66	86	76	96
Smoking† (pack/years)	21 (0–53)	26 (1–56)	18 (0–51)	22 (0–53)	32 (7–76)
Alcohol use					
(i) Never	13	16	12	15	8
(ii) Former	7	10	8	4	4
(iii) Current	79	74	80	82	89
Hypertension (%)	50	58	47	61	54
Diabetes mellitus (%)	16	17	16	18	27
Total intracranial volume¥ (mL)	1467 ± 127	1437 ± 132	1474 ± 123	1467 ± 128	1507 ± 125
Absolute total WML volume† (mL)	1.3 (0.4–5.8)	1.4 (0.5–4.6)	1.3 (0.3–4.7)	2.2 (0.4–11.2)	1.8 (0.5–10.3)
Physical functioning†	44 (29–55)	40 (20–53)	44 (31–55)	46 (31–56)	43 (32–55)
Mental functioning†	51 (32–60)	50 (33–60)	51 (31–60)	51 (34–59)	52 (34–58)

WML: white matter lesions.
‡The different groups of symptomatic atherosclerotic disease do not add up to the total study sample of 486, because various locations of symptomatic atherosclerotic disease can occur within one patient.
¥Mean ± SD
† Median, (10th–90th percentile).

and mental functioning than patients with no or minimal WML progression. Analyses were first performed in the total sample, and because we expected that associations could be influenced by the type of underlying atherosclerotic disease, we repeated the analyses within strata of locations of atherosclerotic disease. In model I, associations were adjusted for age, sex, baseline physical or mental functioning and follow-up time. We additionally adjusted for smoking, alcohol use, hypertension, and diabetes mellitus in model II, because it is not clear to what extent these vascular risk factors are confounders or preceding factors in the pathway between WML volume and functioning, or both.

We repeated the analyses after excluding patients with severe atherosclerotic disease at baseline, defined as patients with coronary artery disease and three-vessel or left main disease at inclusion, patients with cerebrovascular disease and a mRS grade ≥2 at inclusion, or patients with peripheral arterial disease with Fontaine grade ≥3 at inclusion. This was done to assess to what extent the observed associations between small-vessel disease and functioning were influenced by the severity of macrovascular disease.

Further, to examine whether associations were independent of incident vascular events during followup, analyses were repeated after excluding patients who experienced a new vascular complication (nonfatal ischemic stroke or myocardial infarction) between baseline and followup. In all analyses, 95% confidence intervals are given. SPSS version 15.0 (Chicago, Ill, USA) was used to analyze our data.

3. Results

Baseline characteristics are summarized in Table 2. Mean age of the study population was 58 ± 9 years, and 80% was male. At baseline, median physical functioning was 44 (10–90th percentile 29–55) and mental functioning was 51 (10–90th percentile 32–60).

Mean elapsed time between the vascular event and screening date was 2.1 ± 1.4 months. In the total sample, physical functioning improved (median 3.8, 10th–90th percentile −6.5 to 18.3) and mental functioning deteriorated (median −4.0, 10th–90th percentile −14.0 to 13.0) after a mean followup of 3.9 ± 0.4 years. When different locations of atherosclerotic disease were identified, physical functioning improved in all groups (Figure 1). This improvement was significantly lower in patients with cerebrovascular disease compared to patients with other locations of symptomatic atherosclerotic disease ($B = -2.58$, 95% CI −4.29 to −0.87). Mental functioning deteriorated in all groups, without any significant differences between different locations of symptomatic atherosclerotic disease (Figure 1).

3.1. Progression of WML Volume. Patients with greatest progression of WML volume (highest quartile, >0.07% increase in WML volume as % of ICV) showed a significantly stronger deterioration in mental functioning than patients with lower WML progression ($B = -1.76$, 95% CI −3.11 to −0.42, Figure 2) in model I. Additional adjustment

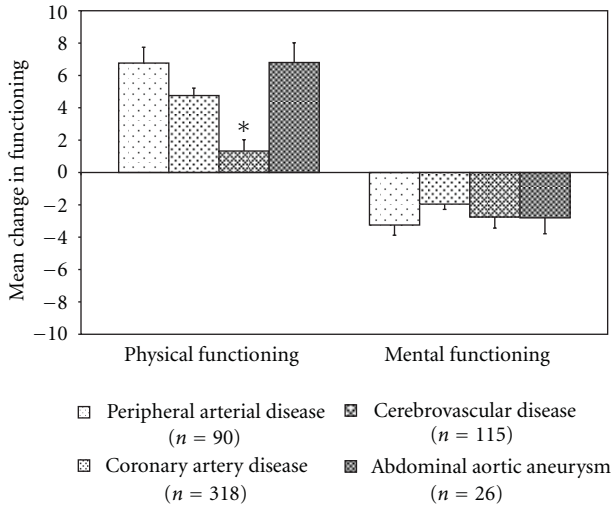

Figure 1: Mean changes in physical and mental functioning for different locations of symptomatic atherosclerotic disease, adjusted for age, sex, baseline functioning, and follow-up time. Significant differences, compared to other locations of symptomatic atherosclerotic disease, are indicated with an asterix.

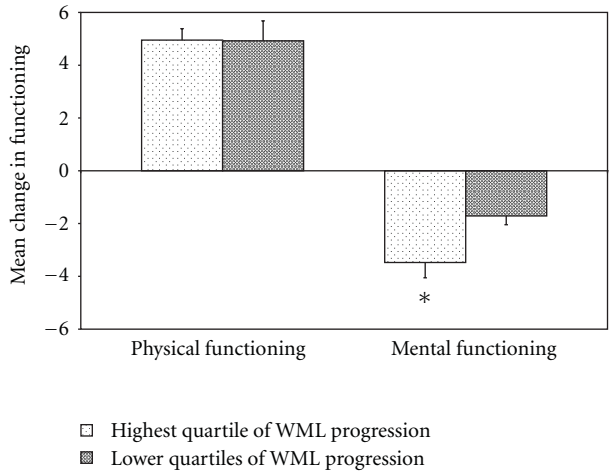

Figure 2: Mean changes in physical and mental functioning for patients with greatest progression of white matter lesion (WML) volume (highest quartile, >0.07% increase in WML volume as % of ICV) versus patients in the lower three quartiles of progression, adjusted for age, sex, baseline functioning, and follow-up time. Significant differences are indicated with an asterix.

for vascular risk factors did not change the results (data not shown). When analyses were repeated within different strata of locations of symptomatic atherosclerotic disease, greater WML progression was associated with a stronger deterioration in mental functioning in all patients except for patients with cerebrovascular disease (Figure 3), although the deterioration was statistically significant only in patients with coronary artery disease (model I, $B = -2.03$, 95% CI -3.61 to -0.45). Additional adjustment for vascular risk factors did not change these associations.

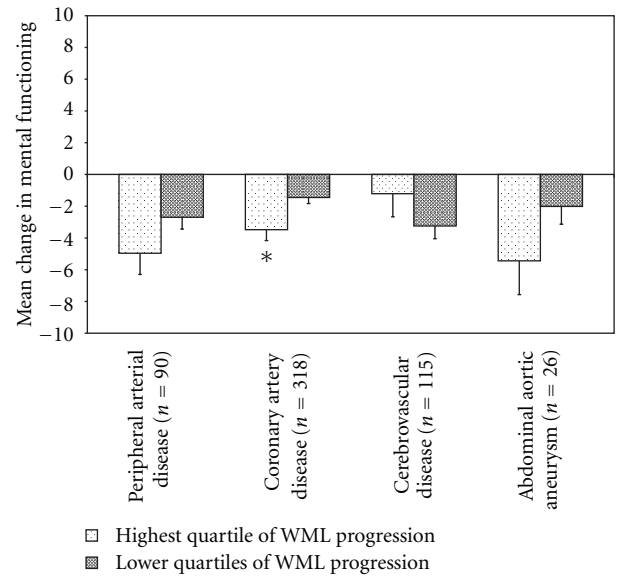

Figure 3: Mean changes in mental functioning for patients with greatest progression of white matter lesion (WML) volume versus patients in the lower three quartiles of progression, for different locations of symptomatic atherosclerotic disease, adjusted for age, sex, baseline functioning, and follow-up time. Significant differences are indicated with an asterix.

Greater progression of WML volume was not significantly associated with changes in physical functioning at followup in model I in the total sample ($B = -0.04$, 95% CI -1.79 to 1.72, Figure 2) or within strata of patients with coronary artery disease ($B = 0.02$, 95% CI -2.26 to 2.30), peripheral arterial disease ($B = 1.26$, 95% CI -3.65 to 6.16), cerebrovascular disease ($B = 0.00$, 95% CI -3.46 to 3.46), or abdominal aortic aneurysm ($B = -0.38$, 95% CI -7.07 to 6.32).

Excluding patients with most severe symptomatic atherosclerotic disease ($n = 46$) did not materially change the results. Greater WML progression was still significantly associated with a stronger deterioration in mental functioning (model I, $B = -1.82$, 95%-3.25 to -0.38).

Between baseline and followup, 17 patients experienced a nonfatal vascular event. Excluding these patients did not change the observed associations of greater WML progression with a stronger deterioration in mental functioning (model I, $B = -1.83$, 95% CI -3.20 to -0.46).

4. Discussion

In a cohort of patients with different manifestations of symptomatic atherosclerotic disease, physical functioning substantially improved in all patients after four years of followup, although the improvement was less in patients with cerebrovascular disease. Mental functioning declined in all types of symptomatic atherosclerotic disease. Greater progression of WML volume over four years of followup was associated with a stronger decline in mental functioning in all patients except for those with cerebrovascular disease.

To our knowledge, this is the first study directly investigating the influence of WML progression on the course of physical and mental functioning in patients with different manifestations of symptomatic atherosclerotic disease. A strength of this study is that by including patients with different locations of symptomatic atherosclerotic disease we could investigate whether the effect of WML progression on physical and mental functioning depended on the type of underlying vascular disease. Furthermore, volumetric WML assessment provided estimates that are more precise and less influenced by observer bias than visual rating scales [29–31] and enabled the measurement of relatively small volume changes over time. In addition, we included a large number of patients, and the extensive information available on cardiovascular risk factors and the extent of clinical and subclinical atherosclerosis made it possible to adjust for potential confounders.

A limitation of this study is that, despite the large sample size, relatively few patients had peripheral arterial disease, cerebrovascular disease, or abdominal aortic aneurysm. Although similar associations were found in patients with coronary artery disease, peripheral arterial disease and abdominal aortic aneurysm, the relatively low number of patients with locations of symptomatic atherosclerotic disease other than coronary artery disease contributed to large confidence intervals and possibly nonsignificant relations in these patients. Further, the largest impact on physical and mental functioning would be expected in patients suffering most severe atherosclerotic events. Because these patients are less likely to participate in our study, this could have contributed to a relative underestimation of the effect. Moreover, patients who participated at followup were healthier at baseline, with fewer vascular risk factors, lower WML volume, and higher mental functioning than patients lost to followup. Therefore, the changes in physical and mental functioning might have been less prominent in the total cohort. Also, because baseline mental functioning was higher in patients with complete data at followup, regression to the mean could have contributed to the observed decline in mental functioning after four years. On the other hand, the selection of relatively healthy patients could have resulted in a decreased contrast between those with greatest WML progression and those without, which could have led to an underestimation of the effect of WML progression on changes in mental functioning.

In recent years, HRQoL has become an increasingly important clinical and research outcome measure when evaluating burden of disease and treatment benefits in patients with atherosclerotic disease. Population-based studies have shown that patients with various manifestations of symptomatic atherosclerotic disease have a poorer HRQoL compared to the general population, with most pronounced effects on physical functioning [5–7, 9, 10]. It is unclear whether physical and mental functioning returns to population levels after the acute phase of recovery or whether functioning remains lower, or perhaps even further declines after the initial event.

Our data showed that physical functioning was substantially lower in patients with symptomatic atherosclerotic disease in the acute phase of recovery from an atherosclerotic event compared to previously published age-adjusted population norms [32]. In a previous population-based study, a prolonged decline in HRQoL was observed in stroke survivors free from recurrent stroke or myocardial infarction [8]. Although we found an improvement in physical functioning in our sample of patients with cerebrovascular disease after four years followup, this improvement was substantially lower compared to patients with other locations of symptomatic atherosclerotic disease. In line with our findings, another study also reported significant improvements in functioning in postoperative abdominal aortic aneurysm patients, which returned to population norms in long-term survivors [11].

In our study, mental functioning was similar to population norms in the acute phase of recovery from a vascular event, but declined during a four year follow-up period. Other studies reported an increased prevalence of mood disturbances already in the acute phase in patients hospitalized for ischemic cardiac or cerebrovascular events [33, 34]. One explanation for our findings could be that in the acute phase of an atherosclerotic event, subjective well-being is dominated by the substantial impairments in physical functioning, whereas awareness of the emotional consequences arises after recovery of physical functioning. An alternative explanation could be that the course of mental functioning in patients with symptomatic atherosclerotic disease depends on the severity of the atherosclerotic event. Relatively few patients were included with severe atherosclerotic disease in our study, which could contribute to the different findings in the course of mental functioning between our study and others.

The underlying mechanisms contributing to a lower HRQoL in patients with symptomatic atherosclerotic disease are unclear. It has been suggested that a lower HRQoL could result from direct complications of the disease or treatment of underlying vascular risk factors, or from raised awareness of the disease [35]. An alternative mechanism contributing to a lower perceived HRQoL could be the presence of co-occurring intracerebral atherosclerotic changes, characterized by WMLs on MRI. WMLs are strongly associated with the presence of common vascular risk factors, including increased age, hypertension, and diabetes mellitus [36–38]. Although the exact underlying pathophysiological mechanisms remain unclear, arteriosclerotic changes to the cerebral small vasculature, with consequent ischemia, apoptosis, and blood-brain barrier alterations, are thought to be involved in the formation and progression of WMLs [39]. Although WMLs are often asymptomatic MRI findings, increased volume and progression of WML have been previously associated with an increased risk of functional decline [14], depression [16], and cognitive impairment [18, 19]. WMLs are thought to account for the increased risks of functional decline and mood disorders by disrupting brain pathways that are involved in the regulation of physical and emotional responses [40]. Although we did not formally measure depression, our finding that increased WML activity was associated with a greater decline in mental functioning may be interpreted as being supportive of this "vascular

depression" hypothesis [40]. Greater progression of WML volume contributed to a stronger decline in mental functioning in all patients except for patients with cerebrovascular disease. This finding is somewhat counterintuitive but may be explained by our finding of little improvement in physical functioning in patients with cerebrovascular disease. It could be that as a result of the substantial impairments and disability already associated with stroke lesions, increased progression of WML volume does not substantially contribute to the decline in mental functioning in these patients.

5. Conclusion

In summary, in patients with different manifestations of atherosclerotic disease, we found that physical functioning was mainly impaired in the acute phase after a symptomatic atherosclerotic event and improved during four years of followup, although improvement in physical functioning remained substantially lower in patients with cerebrovascular disease. Mental functioning was relatively unimpaired in the early phase, but declined in the four years thereafter. Greater progression of WML volume contributed to an even stronger decline in mental functioning in patients with symptomatic atherosclerotic disease. Considering the substantial impact on well-being and previously reported increased risk of adverse events associated with lower mental and physical functioning, further research should investigate whether modification of WML through better control of vascular risk factors could influence the course of HRQoL in patients with symptomatic atherosclerotic disease.

Conflict of Interests

The authors report no conflict of interests.

Acknowledgments

The authors gratefully acknowledge the members of the SMART Study Group of UMC Utrecht: A. Algra, M.D., Ph.D., Julius Center for Health Sciences and Primary Care and Rudolf Magnus Institute for Neurosciences, Department of Neurology; P. A. Doevendans, M.D., Ph.D., Department of Cardiology; D. E. Grobbee, M.D., Ph.D. and G. E. H. M. Rutten, M.D., Ph.D., Julius Center for Health Sciences and Primary Care; L. J. Kappelle, M.D., Ph.D., Department of Neurology; F. L. Moll, M.D., Ph.D., Department of Vascular Surgery; F. L. J. Visseren, M.D., Ph.D., Department of Vascular Medicine. This study was supported by a program grant from the Netherlands Heart Foundation (NHF: project no. 2007B027). The NHF had no involvement in study design; in the collection, analysis, and interpretation of data; in writing of the report; in the decision to submit paper for publication.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 187219, 11 pages
doi:10.4061/2011/187219

Research Article

Striatal Hypodensities, Not White Matter Hypodensities on CT, Are Associated with Late-Onset Depression in Alzheimer's Disease

Jessica A. Brommelhoff,[1] Bryan M. Spann,[2] John L. Go,[3] Wendy J. Mack,[4] and Margaret Gatz[1,5]

[1] *Department of Psychology, University of Southern California, Los Angeles, CA 90089, USA*
[2] *Department of Neurology, University of Southern California, Los Angeles, CA 90033, USA*
[3] *Department of Radiology, University of Southern California, Los Angeles, CA 90033, USA*
[4] *Department of Preventive Medicine, University of Southern California, Los Angeles, CA 90089, USA*
[5] *Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, 17177 Stockholm, Sweden*

Correspondence should be addressed to Jessica A. Brommelhoff, jbrommelhoff@mednet.ucla.edu

Received 16 April 2011; Accepted 22 July 2011

Academic Editor: Ana Verdelho

This study examined whether there were neuroanatomical differences evident on CT scans of individuals with dementia who differed on depression history. Neuroanatomical variables consisted of visual ratings of frontal lobe deep white matter, subcortical white matter, and subcortical gray matter hypodensities in the CT scans of 182 individuals from the Study of Dementia in Swedish Twins who were diagnosed with dementia and had information on depression history. Compared to individuals with Alzheimer's disease and no depression, individuals with Alzheimer's disease and late-onset depression (first depressive episode at age 60 or over) had a greater number of striatal hypodensities (gray matter hypodensities in the caudate nucleus and lentiform nucleus). There were no significant differences in frontal lobe deep white matter or subcortical white matter. These findings suggest that late-onset depression may be a process that is distinct from the neurodegenerative changes caused by Alzheimer's disease.

1. Introduction

While some studies have suggested that a history of depression is a risk factor for dementia later in life (e.g., [1, 2]), a number of authors have concluded that depression is an early symptom of dementia [3–6]. Boland [7] proposed that prodromal depression arises from dementia-related neuropathology. Several studies of nondemented older adults have suggested an association between white matter lesions and depression, specifically late-onset depression [8–14]. In a longitudinal study, white matter changes pre-dated and independently predicted the onset of depressive symptoms in older adult participants [15], providing some evidence that white matter changes are an antecedent to depression. Furthermore, in their review, Schweitzer et al. [16] concluded that the white matter changes that are common in individuals with late-onset depression were associated with cognitive impairment, and thus, were indicative of a prodrome to dementia.

A greater amount of total brain white matter lesions has been associated with more severe depression or a greater number of depressive symptoms [9, 17, 18]. Other studies have suggested that white matter lesions in the frontal lobe are specifically associated with a higher rate of depressive symptoms among persons without dementia [8, 19]. A review of white matter lesions and clinical manifestations concluded that while periventricular white matter lesions are often associated with Alzheimer's disease (AD), lesions in the subcortical white matter are more often associated with late-onset depression [16]. Among 39 hospital inpatients over age 60 with severe depression, later age of first depressive episode was associated with a greater severity of subcortical deep white matter hyperintensities [20]. After a mean followup of 14 months, 27% of the original 39 inpatients had developed a probable dementia syndrome, which was predicted by a later age of depression onset and subcortical white matter hypodensities (WMH) [21].

In their review of structural brain abnormalities in affective disorders, Taylor and Krishnan [22] posit that for late-onset depression "evidence is strongest for a contributory effect of [subcortical hyperintensities], particularly as to hyperintensities of the basal ganglia" (page 61). In fact some of the earliest studies in this area dating back nearly 20 years noted the greater prevalence and severity of subcortical hyperintensities among depressed older adults. For example, Coffey et al. [23] found lesions in the subcortical gray matter nuclei (basal ganglia and thalamus) to be significantly more common in depressed than in nondepressed older adults (60 years of age and older). Greenwald et al. [24] sought to further specify the location of the subcortical lesions associated with late-life depression, comparing the subcortical gray matter hyperintensities in a group of older adults receiving treatment for depression to a group of older adult community controls. In this sample, left-hemisphere hyperintensities in the putamen, a part of the lenticular nucleus, which is a component of the striatum, were significantly more common among depressed individuals.

These findings of an association between frontal lobe, subcortical deep WMH, and striatal hypodensities and depression suggest that damage to frontal-subcortical circuitry may be responsible for late-onset depression. Such damage would be consistent with the frontostriatal hypothesis of depression [25, 26], which suggests that damage to the frontal lobe and striatum (input nuclei for the basal ganglia comprised of the caudate nucleus and the putamen) are associated with depression, though the exact nature of the association remains unclear. In her review focusing on subcortical ischemic vascular dementia, Chui [27] posits that deep white matter lesions can disrupt frontal-subcortical loops and the white matter tracts therein, which are important for cognition and emotion.

Increasing evidence suggests that the pathenogenesis of white matter lesions may be largely due to cerebrovascular disease (CVD). One study [28] indicated that cardiac insufficiency, one of the sequelae of cardiovascular disease, results in hypoperfusion (decreased blood flow) to subcortical regions of the brain. Frontosubcortical loops and the associated white matter tracts seem to be especially vulnerable to hypoperfusion and ischemia [27]. Thus, even in the absence of a stroke, white matter changes could occur secondary to CVD-related systemic hypoperfusion and ischemia. In conjunction with the vascular depression hypothesis [29], CVD may be considered as a potentially important antecedent in the relationship between late-life depression and white matter pathology.

The aim of the present study was to evaluate neuroanatomical alterations on the CT scans of individuals with dementia based upon a history of late-onset depression (LOD, first episode of depression at age 60 or older) or late-life depression (LLD, any episode of depression at age 60 or older). We hypothesized that individuals with dementia and LOD and/or LLD would be more likely than nondepressed individuals with dementia to exhibit hypodensities in the frontal lobe deep white matter and subcortical white matter (subinsular region and internal capsule), as well as the subcortical striatal gray matter (caudate nucleus and lentiform nucleus, which includes the putamen and globus pallidus) on CT scans. We further hypothesized that these patterns would be evident among all demented individuals and in Alzheimer's disease alone.

2. Methods

2.1. Participants. Study participants were part of the Study of Dementia in Swedish Twins (HARMONY) [30]. The HARMONY study population included all twins in the Swedish Twin Registry [31] who were aged 65 and older and alive during the telephone-screening phase. Although this study uses data from Swedish twins, it is not a twin study per se.

Dementia was ascertained through a two-step procedure that entailed an initial cognitive screening with a subsequent diagnostic workup of each suspected case. In brief, the TELE [32, 33] and the Blessed Dementia Rating Scale (BDRS) [34] were used to screen for cognitive dysfunction. Twins who screened positive for suspicion of dementia (and their twin partners) were evaluated in person by a physician and nurse. Final diagnoses of dementia were determined by a multidisciplinary consensus board. Dementia was diagnosed according to the criteria in the Diagnostic and Statistical Manual of Mental Disorders IV [35] and differentially diagnosed for AD versus vascular dementia using NINCDS/ADRDA [36] and NINDS-AIREN criteria [37]. The HARMONY study used two sources of information to estimate the age of dementia onset: informant reporting during an in-depth semistructured interview and medical records [38].

The sample for the present study ($N = 238$) consisted of all HARMONY twins who (1) were diagnosed as having dementia, (2) had a hard copy of their CT scan (performed as part of the clinical phase of dementia assessment) that could be assessed by the CT raters, and (3) did not meet any of the exclusion criteria (see Figure 1). Exclusion criteria included missing information on timing of depressive episodes ($N = 4$) or first depression onset more than six months after the scan date ($N = 2$).

Of the 238 CT scans scored by the raters, 56 were excluded during the rating process. Four scans were excluded due to the poor quality of the scan, 45 because there was evidence of major stroke (i.e., middle cerebral artery stroke, posterior cerebral artery stroke), 2 because of hydrocephalus, and 5 because of other major brain problems (e.g., evidence of major brain surgery or traumatic brain injury). Those with evidence of a minor stroke (i.e., small lacunar infarct) were not excluded. The final sample included white matter hypodensity and striatal hypodensity ratings for 182 individuals.

2.2. Neuroimaging. Diagnostic neuroimaging utilized CT scans because at the time of the clinical diagnostic assessment insufficient numbers of participants lived close to MRI centers. Twins completed CT scans at their most convenient participating CT center. All CT technicians were given standardized instructions to perform a CT of the brain with

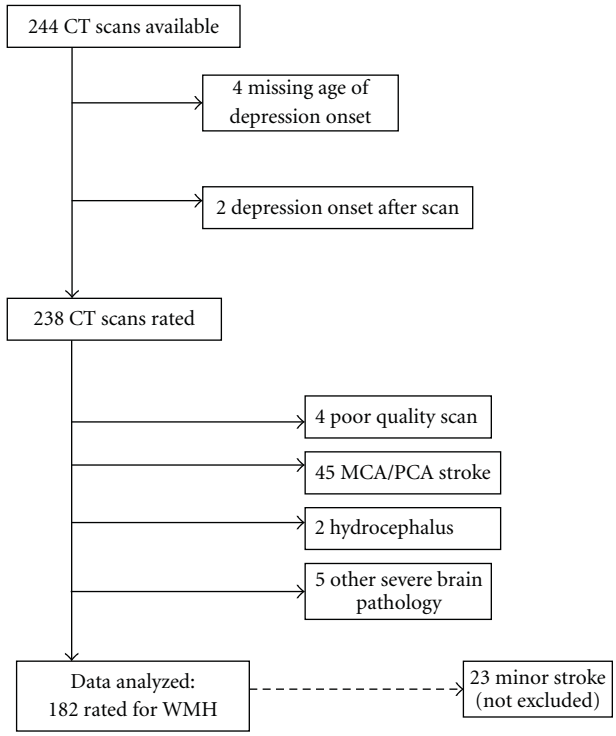

Figure 1: Study sample.

standard slices and noncontrast enhancement. The slices were to be four to five millimeters from the base of the skull and eight to ten millimeters from the pars petrosa ossis temporalis. Contrast enhancement was performed if needed for clinical reasons. The diagnostic protocol allowed individuals who had a CT within six months prior to the clinical workup to provide a copy of that scan instead. After obtaining a clinical read, CT scans were deidentified and made available for research purposes.

2.3. Visual Rating of CT Scans. B.M.S. and J.L.G. served as the CT raters. Raters were blind to clinical diagnosis and any demographic information, including which scans were from twin pairs, as well as the age, gender, and zygosity of the scanned individual. If either rater determined that an image quality was unacceptable, it was excluded from the analyses. If there was evidence of a major stroke, such as middle cerebral artery or posterior cerebral artery stroke, the CT was not included in the analyses. Individuals with small lacunar infarcts, however, were not excluded. CT scans that indicated hydrocephalus or other severe neuropathology, such as evidence of a brain tumor, major brain surgery, or traumatic brain injury, were also excluded from the analyses.

2.3.1. Frontal Deep White Matter Hypodensities. Deep white matter hypodensities in the frontal lobe were rated on a modified version of the Age-Related White Matter Changes (ARWMC) Scale [39], where 0 = an absence of hypodensities, 1 = one focal hypodensity (≥5 mm), 2 = more than one focal hypodensity, 3 = confluent hypodensities, and 4 = confluent hypodensities with additional discrete focal hypodensities. Focal hypodensities were defined as discrete hypodensities greater than five millimeters in size. Confluent hypodensities were present when discrete hypodensities could not be separately defined. Deep white matter hypodensities (DWMH) were defined as WMH that were located medially from the sulci. The ratings of frontal DWMH in the right and left hemisphere were highly correlated (Spearman's rho = 0.92, $P < 0.0001$). Therefore, summary scores for frontal DWMH used the maximum score for the right and left hemispheres.

2.3.2. Subcortical White Matter Hypodensities. The density of the white matter in the internal capsule and subinsular region (comprised of the external capsule, claustrum, and extreme capsule) was compared to the density of homogenous areas of white matter in the frontal lobe to determine if there were relative hypodensities evident in either of these components. The location, side (left versus right hemisphere), and rating of the hypodensities within these regions were indicated, with hypodensities rated on the modified version of the ARWMC Scale. The ratings of subcortical WMH in the right and left hemisphere were significantly correlated (Spearman's rho = 0.79, $P < 0.0001$); a summary subcortical WMH score was created using the maximum score for the right and left hemispheres.

2.3.3. Basal Ganglia-Thalamic Hypodensities. The raters also examined each scan for gray matter hypodensities in the basal ganglia and thalamic regions. The homogeny of the gray matter in the striatum (caudate nucleus and lentiform nucleus), substantia nigra, and thalamus were compared to one another to identify hypodensities. The total number of hypodensities in the striatum on both right and left hemispheres was summed.

2.3.4. Interrater Reliability. Each CT scan was scored separately by both raters. Interrater reliability for bilateral WMH was substantial (weighted kappa, right WMH = 0.92; weighted kappa, left WMH = 0.89) and slightly lower though adequate for bilateral basal ganglia-thalamic hypodensity ratings (weighted kappa, right = 0.73; weighted kappa, left = 0.62). In cases of disagreement, the scans were rerated conjointly, and the consensus rating was used for analysis.

2.4. Depression. History of depression, comorbid depression, and estimated age of depressive episodes were determined using information from four sources: (1) the national computerized Inpatient Discharge Registry (IDR), (2) the national registry of inpatient psychiatric hospital services, (3) medical history provided by an informant, and (4) medical records.

The Swedish Twin Registry is linked to the national computerized Inpatient Discharge Registry that records all inpatient hospital discharges in Sweden. Discharge diagnoses use International Classification of Disease (ICD) codes. If the discharge date was prior to 1969, the ICD-7 coded depression diagnoses as 302 (involutional melancholia), 314 (depressive neurosis), and 790.2 (other recurrent depressive disorder). If

the discharge date was 1969–1986, the ICD-8 coded depression diagnoses as 296.0 (involutional melancholia), 298 (reactive depressive psychosis), 300.4 (depressive neurosis), and 790.2 (other recurrent depressive disorder). If the date of discharge was 1987–1996, the ICD-9 coded depression diagnoses similar to the ICD-8, with 296.2 (depressive psychosis), 296.3 (recurrent depressive psychosis), 296.82 (atypical depression), 300.4 (dysthymia), and 311 (depression, NOS). From 1997, the ICD-10 depression diagnoses included F32 (depressive episode), F33 (recurrent depressive disorder), and F34.1 (dysthymia). There were 11 participants with discharge diagnoses of depression in the Inpatient Discharge Registry.

The Swedish Twin Registry has also been linked to a national registry of inpatient psychiatric hospital services that was maintained between 1967 and 1983. For each person entered in this registry, there is a record of the discharge diagnosis and the date of hospitalization. All diagnoses are given in terms of an ICD-8 diagnosis (see above). There were two participants with diagnoses of depression in the inpatient psychiatric hospital services registry, both of whom also had a diagnosis of depression in the Inpatient Discharge Registry.

Thus, a total of 11 participants in this study had at least one depression-related discharge diagnosis in the IDR between the years of 1964 and 2004, a span of 40 years, or between 1967 and 1984 in the inpatient psychiatric hospital services registry. The most common depression-related discharge diagnosis was depression not otherwise specified, followed by dysthymia, recurrent depressive disorders, and depressive episodes of mild, severe, or other characteristics. Discharge diagnoses in both the IDR and the psychiatric hospital discharge registry that were regarded as not depression-related included bipolar affective disorder and manic-depressive reaction, manic or unspecified type, schizoaffective disorder, and unspecified mood disorders.

Medical history reported by an informant was collected during the clinical evaluation. The history included whether the individual had any history of "major depressive disorder" or "reactive depression," and if so, the date or dates of onset. The first depressive episode according to the medical history used the earliest date recorded for the onset of a depressive disorder and the most recent episode of depression used the latest date recorded for a depressive episode.

Medical records, ordered during the clinical evaluation phase, were coded by the assessment team to reflect whether the twin had been diagnosed with depression. Records typically go back approximately ten years before the clinical evaluation for dementia. Thus, these records were most helpful in determining whether late-life depression was present. Data included the onset and dates of depression.

Use of antidepressant medications was also coded from the medical records and medical history. A total of 31 individuals were prescribed antidepressant medication, but did not have any other information indicating that they had ever received a diagnosis of depression. These individuals were coded as not having a history of depression, as it is possible that these medications were prescribed for reasons other than depression.

The age of the first episode of depression was determined using the age at the earliest reported occurrence of depression across all sources. Individuals with a first episode of depression at age 60 or older were considered to have late-onset depression (LOD). Individuals who had prior episodes of depression including at least one episode of depression at age 60 or over were considered to have a history of late-life depression (LLD). In sum, a total of 132 (72.5%) individuals did not have a history of a depression diagnosis and 50 (28.5%) individuals had a history of a depression diagnosis. Of these individuals, 36 had LOD, having their first depressive episode after age 60, with a mean (SD) age of first depressive episode of 74.2 (7.0) years. Of the 14 individuals who had their first depressive episode before age 60, nine individuals had an episode of depression occurring after age 60. Thus, 45 of the 182 participants were considered to have LLD. Those with an early episode of depression that did not recur in later life ($N = 5$) were not included in further analyses.

2.5. Cerebrovascular Disease. Cerebrovascular disease risk factors, as indicators of CVD risk, were examined as potentially important covariate. Data on hypertension, diabetes, atrial fibrillation, peripheral artery disease, transient ischemic attack, and coronary artery disease (CAD) indicators were extracted from the participant's medical records and coded by the assessment team to reflect whether the individual had a history of any of these risk factors. Coronary artery disease was considered present if the individual had a history of myocardial infarction, angina, or heart failure.

2.6. Other Covariates. Additional covariates included age at the time of the CT scan, dementia duration (i.e., age at the time of the scan compared with age of dementia onset), gender, zygosity, and number of years of education completed. Because of the cross-sectional study design, particular attention was paid to the duration of dementia at the time of the CT scan (calculated by subtracting age of dementia onset from the age at the CT scan).

2.7. Analyses. Associations between history of LOD (no depression versus LOD) and all potential demographic and medical confounders were initially examined to determine factors to include as covariates in multivariate models. Chi-square tests were used to examine whether gender, zygosity, or risk factors for CVD (hypertension, diabetes, atrial fibrillation, peripheral artery disease, transient ischemic attack, and coronary artery disease indicators) differed by history of LOD. One-way ANOVA was used to determine whether age at CT scan, age of dementia onset, duration of dementia at the time of the CT scan, or level of education differed by presence versus absence of LOD.

Kruskal-Wallis chi-square was used to examine whether frontal deep white matter or subcortical white matter score varied by age at CT scan or dementia duration. Fisher's Exact Test was used to determine whether frontal deep white matter or subcortical white matter score differed by gender. Simple linear regression was used to test whether number

of striatal hypodensities varied by age, gender, or dementia duration. We took a conservative approach to determining which potential confounders should be included in the final model, including covariates with an association of $P < 0.15$.

Logistic regression for dichotomous outcomes controlling for gender and history of TIA was used to examine the association between LOD and the presence of any frontal lobe deep WMH (focal and confluent WMH versus no WMH), as well as between LOD and the presence of confluent frontal lobe deep WHM (confluent WMH versus no WMH and focal WMH only). To analyze the relationship between LOD and level of subcortical WMH (0 = no WMH, 1 = one WMH, 2 = more than one WMH, 3 = confluent WMH), ordinal logistic regression was used, controlling for age at CT scan and zygosity. The association between numbers of striatal hypodensities and LOD was examined using one-way ANOVA; there were no demographic or medical covariates associated with striatal hypodensities. All of the analyses testing white matter associations with LOD were reanalyzed using LLD. Finally two sets of analyses were performed, the first using all participants with dementia and the second using only individuals with Alzheimer's disease.

Seven complete twin pairs were included in these analyses. To account for the possibility that these seven pairs had correlated data, we also used generalized estimating equations (GEE) to examine the association between the presence of frontal deep WMH and LOD, the amount of subcortical WMH and LOD, and the number of striatal hypodensities and LOD for both the total sample and the Alzheimer's disease only sample. GEE accounts for the lack of independence between twin observations.

3. Results

The most frequent type of dementia among these 182 individuals was Alzheimer's disease ($N = 127$, 69.8%), followed by vascular dementia ($N = 29$, 15.9%) and dementia not otherwise specified ($N = 16$, 8.8%). An additional five participants (2.7%) were diagnosed with dementia of a mixed type (both Alzheimer's disease and vascular dementia pathology) and another five (2.7%) individuals had other forms of dementia (e.g., frontotemporal dementia). Average duration of dementia (from dementia onset to date of CT scan) was 5.2 years (SD = 4.25, range 0–23 years). The majority of participants were female ($n = 116$, 63.7%) and the average age at CT scan was 80.6 years (SD = 6.7 years, range 56–96 years). Participants had 7.4 years (SD = 2.4) of education on average, and 51 participants (28.0%) were from a monozygotic twin pair. Demographic characteristics are presented in Table 1 for all dementias and for Alzheimer's disease alone.

3.1. Associations between Imaging Data and Sample Demographic Characteristics. Across all study participants, 39.6% ($N = 72$) had at least one hypodensity in the frontal lobe deep white matter and 51.6% ($N = 94$) had at least one subcortical white matter hypodensity. Among the 94 participants with subcortical white matter hypodensities, the majority ($N = 88$, 93.6%) had at least one hypodensity in the subinsular region, only six individuals had hypodensities solely within the internal capsule, and 30.8% ($N = 29$) had hypodensities both in the subinsular region and the internal capsule.

Age at the time of the scan, duration of dementia, and education were not associated with frontal deep white matter hypodensities. Male gender was associated with the presence of frontal deep white matter hypodensities (53.0% of males versus 34.5% females; $P = 0.01$); gender was therefore included as a covariate in the multivariate logistic regression analysis. Zygosity was not associated with presence of frontal lobe deep WMH.

Age at the time of the CT scan was significantly associated with subcortical white matter hypodensities, such that older individuals had more subcortical WMH ($\chi^2 = 1.59, \text{df} = 3, P = 0.03$). Individuals who were monozygotic twins were less likely than nonmonozygotic twins to have subcortical WMH (37.2% of monozygotic twins; 57.2% of dizygotic twins; $\chi^2 = 10.43, \text{df} = 3, P = 0.02$). Gender, education, and duration of dementia were not associated with subcortical white matter hypodensities. Thus, age at the time of the CT scan and zygosity were included as covariates in the multivariate ordinal logistic regression models.

Across all study participants, 30.8% ($N = 56$) had at least one hypodensity in the striatum. A simple linear regression analysis indicated that age at the time of the scan, education, and the duration of dementia were not associated with the number of striatal hypodensities. Gender and zygosity were also not associated with striatial hypodensities.

3.2. Associations between Imaging Data and Vascular Risk Factors. Presence of frontal deep WMH, level of subcortical WMH severity, and total number of striatal hypodensities did not significantly vary by history of CAD. History of a TIA was present for 14 individuals in the nondepressed group and no individuals with a history of depression. Controlling for gender, individuals with a history of TIA were 3.92 (95% CI = 1.16, 13.24, $P = 0.03$) times more likely than individuals without a history of TIA to have hypodensities in the frontal lobe deep white matter. Amount of subcortical WMH and striatal hypodensities did not significantly vary by history of TIA.

3.3. Associations between Imaging Data and Dementia Type. A logistic regression model controlling for gender indicated that individuals with vascular dementia were 2.67 times more likely to have frontal lobe deep WMH than individuals with other types of dementia (95% CI = 1.24, 5.76, $P = 0.012$). Gender also remained significant, and men were 1.85 times more likely to have frontal lobe deep WMH than women (95% CI = 1.08, 3.61, $P = 0.025$). Although individuals with vascular dementia were more likely to have a higher ranked amount of subcortical WMH, this association was not statistically significant ($P = 0.14$). Presence of striatal hypodensities did not differ according to dementia type.

3.4. Depression Status. Individuals with no history of depression and individuals with LOD did not differ significantly

Table 1: Sample demographics and covariates for all dementia and alzheimer's disease only.

N	All dementia	Alzheimer's disease
	182	127
Age at CT scan (SD)	80.6 (6.7)	80.8 (6.9)
Duration of dementia (SD)	5.2 (4.2)	5.1 (4.2)
Female, *n* (%)	116 (63.7)	86 (67.7)
Education, years (SD)	7.4 (2.4)	7.5 (2.6)
Monozygotic, *n* (%)	51 (28.0)	40 (31.5)
Hypertension, *n* (%)	153 (84.1)	104 (81.9)
Diabetes, *n* (%)	43 (23.6)	24 (18.9)
Arrhythmia, *n* (%)	36 (19.8)	25 (19.7)
PAD, *n* (%)	4 (2.2)	2 (1.6)
CAD, *n* (%)	59 (32.4)	38 (30.0)
TIA, *n* (%)	14 (7.7)	6 (4.7)
Any CVD, *n* (%)	166 (91.2)	113 (89.0)

PAD: peripheral artery disease; CAD: coronary artery disease; TIA: transient ischemic attack; CVD: cerebrovascular disease.

on most demographic variables (Table 2). Similarly, no differences were observed between the non-depressed and the LLD groups. Participants in the LOD group were more likely to be a member of a monozygotic twin pair compared to non-depressed participants ($P = 0.05$). Gender and years of education did not significantly differ by either history of LOD or history of LLD.

Among all study participants, the mean (SD) duration of dementia at the time of the CT scan was 5.2 (4.2) years. There was no significant difference between the no depression and the LOD group with respect to duration of dementia at the time of the CT scan. At the time of the CT scan, those with LOD had an average dementia duration of 4.2 years (SD = 3.5) and individuals with no history of depression had an average dementia duration of 5.2 years (SD = 4.2).

The presence of CAD and TIA differed by depression group (Table 2). Individuals in the non-depressed group were more likely to have had a transient ischemic attack than individuals in the LOD group ($P = 0.04$). Individuals with LOD were more likely than non-depressed individuals (RR = 1.43, 95% CI = 0.92, 22.33, $P = 0.13$) to have a history of CAD. CAD was not significantly more prevalent, however, in the LLD group (40.0%) compared to the no depression group (31.1%; *P* value for difference = 0.27).

3.5. White Matter Hypodensities and LOD/LLD. Among individuals with LOD, if there were hypodensities in the frontal lobe deep white matter, they were generally rated as confluent (92.9% of deep WMH were rated as confluent). Therefore, we examined whether LOD was associated with confluent hypodensities, testing frontal deep WMH (DWMH) as a dichotomous variable—confluent frontal lobe DWMH versus nonconfluent (no or only focal hypodensities) frontal lobe DWMH. Controlling for gender, LOD was not significantly associated with a greater risk of confluent frontal lobe DWMH for both the total dementia and Alzheimer's disease only analyses, although the pattern of the results was in the hypothesized direction (Table 3). A similar pattern was observed for LLD, where, after controlling for gender, the association between LLD and risk of confluent frontal lobe DWMH was nonsignificant for both the total dementia (OR = 1.38, 95% CI = 0.66, 2.88) and Alzheimer's disease only (OR = 2.12, 95% CI = 0.86, 5.28). There was no association between frontal lobe DWMH dichotomized as presence versus absence of hypodensities and dementia or AD (Table 4).

After controlling for age at CT scan and zygosity, no association was observed between subcortical WMH and LOD. The distribution of subcortical WMH is shown in Table 5. Results were similar for LLD versus no depression comparison. A greater age at the time of the CT scan and a greater amount of subcortical WMH (OR = 1.08 per year of age, 95% CI = 1.01, 1.12, $P = 0.006$) were the only statistically significant association in the multivariate model.

3.6. Striatal Hypodensities and LOD/LLD. Number of striatial hypodensities is shown in Table 6. One-way ANOVA indicated significantly more striatal hypodensities for individuals with late-onset depression compared with non-depressed individuals when AD was considered alone. The patterns when comparing LOD to total dementia and when comparing LLD to non-depressed were similar ($0.05 < P < 0.10$). GEE was used to account for the possible correlation between twin pairs. The relationship between a greater number of striatal hypodensities and LOD remained significant (OR = 2.27, 95% CI = 1.02, 5.05).

3.7. Source of Depression Information. Despite the demonstrated concordance between medical records and informant-reported depression, we examined in a post hoc analysis whether the relationships between hypodensities and depression differed based upon the source of information about depression. There was no significant difference in the relationship between frontal DWMH, subcortical WMH, striatal hypodensities and depression when informant-reported medical history information was disregarded. There were also eight individuals missing either

Table 2: Sample demographics by depression group.

N	No Depression	LOD	*P* value*
	132	36	
Age at CT scan (SD)	81.1 (6.1)	81.0 (7.0)	n.s.
Age at dementia onset (SD)	75.9 (6.9)	76.9 (6.7)	n.s.
Dementia duration, years (SD)	5.2 (4.2)	4.2 (3.5)	n.s.
% Female	63.6	63.9	n.s.
Education, years (SD)	7.5 (2.4)	7.4 (2.8)	n.s.
Monozygotic, *n* (%)	33 (25.0)	15 (41.7)	**0.05**
Hypertension, *n* (%)	113 (85.6)	30 (83.3)	n.s.
Diabetes, *n* (%)	32 (24.2)	6 (16.7)	n.s.
Arrhythmia, *n* (%)	25 (18.9)	8 (22.2)	n.s.
PAD, *n* (%)	3 (2.3)	1 (2.8)	n.s.
CAD, *n* (%)	41 (31.1)	16 (44.4)	**0.13**
TIA, *n* (%)	14 (10.7)	0 (0.0)	**0.04**

Associations significant at $P < 0.15$ are bolded.
LOD: late-onset depression; PAD: peripheral artery disease; CAD: coronary artery disease; TIA: transient ischemic attack.
*Group comparisons by ANOVA for continuous variables and by chi-square for categorical variables.

Table 3: Risk of confluent frontal lobe deep white matter hypodensities in late-onset depression compared with No depression for all dementia and alzheimer's disease.

	No depression		Late-onset depression		
	−Con WMH	+Con WMH	−Con WMH	+Con WMH	OR*
	N (%)	*N* (%)	*N* (%)	*N* (%)	(95% CI)
All dementia	97 (73.5)	35 (26.5)	23 (63.9)	13 (36.1)	1.58 (0.72, 3.46)
AD only	73 (76.8)	22 (23.2)	16 (64.0)	9 (36.0)	1.83 (0.70, 4.79)

−ConWMH: Confluent frontal deep white matter hypodensities absent; +ConWMH: Confluent frontal deep white matter hypodensities present; OR: Binary logit odds ratio estimate, controlling for gender; CI: confidence interval; AD: Alzheimer's disease.
*Late-onset depression group compared to no depression group.

Table 4: risk of frontal lobe deep white matter hypodensities in late-onset depression compared with no depression for all dementia and Alzheimer's disease.

	No depression		Late-onset depression		
	−WMH	+WMH	−WMH	+WMH	OR
	N (%)	*N* (%)	*N* (%)	*N* (%)	(95% CI)*
All dementia	75 (56.8)	57 (43.2)	22 (61.1)	14 (38.9)	0.96 (0.44, 2.08)
AD only	59 (62.1)	36 (37.9)	16 (64.0)	9 (36.0)	0.88 (0.35, 2.25)

−WMH: frontal deep white matter hypodensities absent; +WMH: frontal deep white matter hypodensities present; OR: binary logit odds ratio estimate, controlling for gender; CI: confidence interval; AD: Alzheimer's disease.
*Late-onset depression group compared to no depression group.

Table 5: risk of subcortical white matter hypodensities in late-onset depression compared with no depression for all dementia and Alzheimer's disease only.

	No depression				Late-onset depression				
Level of WMH*	0	1	2	3	0	1	2	3	OR (95% CI)**
All dementia	62 (47.0)	16 (12.1)	25 (18.9)	29 (22.0)	17 (47.2)	3 (8.33)	9 (25.0)	7 (19.4)	0.97 (0.48, 1.92)
AD only	48 (50.5)	11 (11.6)	18 (18.9)	18 (18.9)	12 (48.0)	2 (8.0)	5 (20.0)	6 (24.0)	1.16 (0.48, 2.58)

OR: ordinal cumulative logit odds ratio estimate, controlling for age at time of CT scan; CI: confidence interval; AD: Alzheimer's disease.
Level of WMH: 0 = none, 1 = one discrete hypodensity, 2 = more than one discrete hypodensity, 3 = confluent hypodensities.
*All numbers for Level of WMH given in terms of *N* (%). **Late-onset depression group compared to no depression group.

Table 6: number of striatal hypodensities in late-onset depression compared with no depression for all dementia and Alzheimer's disease only.

	No depression	LOD	No depression versus LOD*
All dementia			
N	132	36	
Striatum, mean (SD)	0.48 (0.96)	0.86 (1.53)	0.07
Alzheimer's disease only			
N	95	25	
Striatum, mean (SD)	0.42 (0.92)	0.96 (1.72)	**0.03**

LOD: late-onset depression.
*P value.

an informant report ($N = 6$) or coded medical records ($N = 2$). Dropping these individuals from our analyses did not affect the outcomes of our analyses.

4. Discussion

The aim of the present study was to investigate the underlying neural mechanisms linking depression and dementia, stemming from the idea that there could be a pathophysiological basis in the brain for the comorbidity of depression and dementia [1]. According to the fronto-striatal hypothesis of vascular depression, white and gray matter lesions in the fronto-striatal pathway resulting from cerebrovascular disease (as well as normal aging) may cause late-onset depression [25]. In line with the fronto-striatal hypothesis, we predicted that demented individuals with a LOD or a history of LLD would have a higher prevalence of WMH in the frontal lobe deep white matter and subcortical white matter, as well as a greater amount of gray matter hypodensities in the striatum, compared to individuals with dementia and no depression. While similar hypotheses have been tested among individuals with late-onset and/or late-life depression, they have rarely been considered among individuals with both dementia and late-onset and/or late life depression.

In support of one of the main hypotheses of the study, individuals specifically with Alzheimer's disease and late-onset depression had a significantly greater total amount of striatal hypodensities compared to individuals with Alzheimer's disease and no history of depression. Further analysis revealed that this association was not explained by any other medical or demographic covariate and remained significant even after accounting for the possibility that the twin pairs included in the study sample were not truly independent observations. According to the bivariate level analyses, there were no associations between striatal hypodensities and any of the demographic or medical history confounders. Only CAD differed between the LOD and non-depressed group in the individuals with AD ($\chi^2 = 3.89, \mathrm{df} = 1, P = 0.048$), with the LOD group more likely to have CAD than the non-depressed group (48.0% versus 27.4%, resp.). However, CAD was not associated with number of total striatal hypodensities. Thus, there were no other tested covariates that could explain the association we found between the LOD group and striatal hypodensities among the AD group. Although there was a consistent pattern of findings for striatal hypodensities, the finding was statistically significant only among those with Alzheimer's disease in particular, when comparing those with late-onset depression to those with no earlier history of depression.

Although the direction of some of the associations was consistent with our hypotheses, the presence (versus absence) of frontal lobe deep WMH did not differ significantly between the non-depressed and LOD or LLD groups, nor did severity of subcortical white matter hypodensities differ between the nondepressed and LOD or LLD groups. Male gender and a history of TIA were the only two significant predictors of presence of frontal lobe deep WMH. While the prevalence of ischemic neuropathology on CT in cognitively intact patients with TIAs has been estimated at approximately 40%, most abnormalities are diffuse throughout the cerebral white matter or basal ganglia [40]. Therefore, our finding that hypodensities were specific to the frontal lobe deep white matter may be specific to individuals with dementia. Severity of subcortical WMH was only associated with age at CT scan.

4.1. White Matter Hypodensities and LOD/LLD. Our lack of statistically significant findings with respect to the white matter components of the fronto-striatal pathway was surprising in light of the solid base of literature supporting an association with late-onset and late-life depression. Several differences between the present study and past studies may explain this discrepancy. Most of the study populations that found the association between WMH and LOD and LLD were comprised of cognitively intact older adults or older adults with mild cognitive impairment. All of the individuals in the present study had dementia, with approximately 70% diagnosed with Alzheimer's disease. Therefore, this study at least partially "controls" for the neuropathology related to dementia. However, it could be that by the time cognitive and functional impairment has progressed to the point of dementia, the neurological damage caused by dementia is widespread enough to subsume the neuropathology of late-onset depression such that the two neuropathologies become indistinct. In fact, this may even happen in the early stages of dementia, as suggested by a study of mildly demented patients both with and without a subsyndromal level of depressive symptoms, which failed to find any significant relationship between white matter changes and depressive symptoms [41]. At variance with this, however, Lavretsky et al. [18] found that higher lacunar volume in the white matter was associated with neuropsychiatric symptoms (including depressed mood, anhedonia, anergia, and apathy) in a group of both cognitively intact and cognitively impaired individuals, even after controlling for cognitive status.

4.2. Subcortical Gray Matter Lesions and LOD in Alzheimer's Disease. The finding that individuals with LOD and Alzheimer's disease had a greater number of striatal hypodensities compared to individuals without depression and Alzheimer's disease is consistent with several prior imaging studies of late-life depression in nondemented individuals demonstrating a greater prevalence of lesions in the putamen among individuals with geriatric depression [24] and reduced caudate nucleus volume among adults with depression [42]. The striatum represents the subcortical gray matter components of the fronto-striatal pathway. Thus, the finding that individuals with AD and LOD have a greater amount of striatal hypodensities compared to individuals with AD and no depression provides some support for the fronto-striatal hypothesis of depression among individuals with dementia.

Although Alzheimer's disease is a "cortical" dementia, the individuals with LOD had a greater prevalence of hypodensities in the subcortical gray matter compared to the individuals with Alzheimer's disease and no depression. As subcortical changes are not typically associated with Alzheimer's disease, the greater amount of striatal hypodensities evident in the individuals with Alzheimer's disease and LOD may represent a neuropathological process associated with late-onset depression that is distinct from the neurodegenerative changes caused by Alzheimer's disease. In fact, dementia characterized by the neurodegeneration of subcortical structures, or subcortical dementia, is often associated with depression-like symptoms, which may provide further support to the idea that the subcortical gray matter lesions may represent a process specific to LOD. Therefore, the individuals in the present study with Alzheimer's disease and LOD may have a "double dose" of neuropathology—one that is primarily cortical (Alzheimer's disease) and the other subcortical (LOD).

4.3. Limitations

4.3.1. Technological. This study has several limitations. First is the use of CT instead of MRI. CT has better specificity than MRI for white matter changes of clinical significance—95.5% versus 68.2%, respectively, for frontal white matter changes, and 95.5% versus 63.3% for whole brain white matter changes [43]. This was supported by the high interrater reliability for bilateral WMH ratings; whereas, the basal ganglia-thalamic hypodensity ratings were relatively less reliable. However, CT is known for being less sensitive in detecting subtle microvascular ischemic changes, especially in individuals with Alzheimer's disease, the diagnosis assigned to the majority of this study's participants. In one study comparing MRI to CT neuroimaging of individuals with Alzheimer's disease, white matter changes in the frontal lobe were evident in seven out of the 22 participants using MRI, but only one out of the same 22 participants using CT. Additionally, sensitivity to mild frontal lobe WMH was only 31.0% for CT imaging versus 75.9% for MRI [43]. Thus, it is possible that the white matter changes found by other studies are too subtle to be detected by CT. Nonetheless, white matter changes in the present study were found to increase with age, as would be expected, and to be more common in vascular dementia than in other dementias.

4.3.2. Cross-Sectional Study Design. A major limitation inherent in the study design is that CT data were available for only one point in time and on average five years after the age of dementia onset. Due to our lack of premorbid CT data, we can make no causal statements regarding depression and the presence of white or gray matter hypodensities, nor can we make any statements about change over time relative to progression of dementia. At the same time, somewhat surprisingly, there was not a significant relationship between duration of dementia and amount of white matter hypodensities in either the frontal lobe or subcortical white matter. For striatal hypodensities, there was no relationship when the entire sample was examined as a whole. Taken together with the greater amount of striatal hypodensities in the LOD/Alzheimer's disease group compared to nondepressed Alzheimer's disease group, this association, although cross-sectional, lends further support to the idea that there may be two separate neurodegenerative processes occurring among the individuals with comorbid Alzheimer's disease and LOD.

4.3.3. Depression Assessment. Another limitation was that the methods for ascertaining a history of depression were more sensitive to detecting recent episodes. Indeed, in our sample only 8.2% had a depressive episode prior to age 60, which is well below the estimated lifetime prevalence rate of 19.5% for major depression in Swedish twins [44]. Thus individuals in the late-onset group may have been misclassified due to unreported early episodes of depression, although they would have been correctly classified in analyses of late-life depression. In addition, findings from a recent study of the population from which the present study sample was drawn indicated that depression closer in time to dementia onset, and not earlier life depression, was associated with an increased risk of dementia [3]. Therefore, it is also possible that there was differential survival in individuals with early-onset depression, such that they were not as likely as individuals with LOD to develop dementia, and thus were underrepresented as a whole in this sample.

Another limitation stems from the fact that there is no consensus as to what age constitutes early-onset versus late-onset and late-life depression. A recent meta-analysis [45] of WMH in late-life depression noted that there was substantial variability across studies in the age cutoffs used to define late-onset depression, with ages ranging from 45 to 65 years of age. Whereas Alexopoulos et al. [29] proposed that vascular depression be defined by an age of onset after 65 years, Krishnan et al. [46] suggested a definition using an age cutoff of 50 years. Devanand et al. [47] recommended an age cutoff of 60 years because differences in cardiovascular risk factors between an earlier versus a later age of depression onset are greatest at this point.

Another reason for the differences in findings between our study and the findings of some previous studies of depression in those without dementia could be the nature

of our depression measures. Our depression measures were dichotomous, and there was no way to distinguish between levels of depression severity. As suggested by previous studies of late-life depression (e.g., [21, 23]) white matter changes are related to the severity of late-onset depression, with differences between non-depressed and late-onset depression groups being evident only when the level of depression is relatively severe. Therefore, as the late-onset depression group in our study likely represented a wide range of depression severity (and because there were not enough individuals in our sample for a separate analysis with depression severe enough to require hospitalization), differences between the non-depressed and LOD groups could have been obscured due to the number of individuals with milder levels of depression.

5. Conclusions

In support of the fronto-striatal hypothesis of depression, the results of the present study indicated that among individuals with Alzheimer's disease, there was a significant relationship between late-onset depression and a greater amount of striatal hypodensities (gray matter hypodensities in the caudate nucleus and lentiform nucleus) compared to people with no depression. As subcortical changes are not typically associated with Alzheimer's disease, the greater amount of striatal hypodensities evident in the individuals with Alzheimer's disease and LOD may represent a neuropathological process that is distinct from the neurodegenerative changes caused by Alzheimer's disease. We did not find an association between LOD/LLD and white matter hypodensities in the frontal deep white matter or subcortical white matter. Other studies in individuals without dementia finding such an association have typically used MRI. Thus, associated white matter changes may have been too subtle to be detected by CT imaging. However, as prior studies were among individuals without dementia, it is equally likely that the neurological damage in the white matter caused by dementia subsumes the neuropathology of late-onset depression, such that the two neuropathologies become indistinct with respect to white matter. Thus, for older adults with dementia and late-life depression, there are important public health implications for identifying the pathophysiologic mechanisms linking these two conditions. Given that many treatments for depression are mediated by neurobiological factors, the efficacy of these treatments may be compromised in individuals with neuroanatomical abnormalities. An understanding of the unique brain changes associated with dementia versus those associated with LOD may elucidate avenues for treatments with better efficacy in older adults.

Acknowledgment

Research and preparation of this paper was funded by the National Institutes of Health Grants R01 AG08724 and F31 MH078331.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 281274, 12 pages
doi:10.4061/2011/281274

Research Article

Fiber Tracts Anomalies in APPxPS1 Transgenic Mice Modeling Alzheimer's Disease

H. Chen,[1,2] S. Epelbaum,[3] and B. Delatour[1,3]

[1] *CNRS, Laboratoire NAMC, UMR 8620, Université Paris-Sud 11, 91405 Orsay, France*
[2] *Department of Neurology, Third Xiangya Hospital, Central South University, Changsha 410013, China*
[3] *"Alzheimer's and Prion Diseases" Team, ICM Brain & Spine Institute, UPMC/Inserm UMR-S 975, CNRS UMR 7225, Pitié-Salpêtrière Hospital, 47 Boulevard de l'Hôpital, 75013 Paris, France*

Correspondence should be addressed to B. Delatour, benoit.delatour@upmc.fr

Received 13 March 2011; Revised 30 May 2011; Accepted 28 June 2011

Academic Editor: Sofia Madureira

Amyloid beta (Aβ) peptides are known to accumulate in the brain of patients with Alzheimer's disease (AD). However, the link between brain amyloidosis and clinical symptoms has not been elucidated and could be mediated by secondary neuropathological alterations such as fiber tracts anomalies. In the present study, we have investigated the impact of Aβ overproduction in APPxPS1 transgenic mice on the integrity of forebrain axonal bundles (corpus callosum and anterior commissure). We found evidence of fiber tract volume reductions in APPxPS1 mice that were associated with an accelerated age-related loss of axonal neurofilaments and a myelin breakdown. The severity of these defects was neither correlated with the density of amyloid plaques nor associated with cell neurodegeneration. Our data suggest that commissural fiber tract alterations are present in Aβ-overproducing transgenic mice and that intracellular Aβ accumulation preceding extracellular deposits may act as a trigger of such morphological anomalies.

1. Introduction

Alzheimer's disease (AD) is a highly prevalent neurodegenerative disease accompanied by gradual and irreversible behavioral and cognitive impairments. Brain lesions observed during the course of AD involve two main aspects: extracellular amyloid-beta (Aβ) deposition in the senile plaques and intracellular tau accumulation forming neurofibrillary tangles and promoting cytoskeletal disorganization. Current research on AD is largely guided by a dominant pathogenic theory, the so-called amyloid cascade hypothesis [1]. Regularly commented on and amended [2], this model posits accumulation of Aβ in the brain as a key primary event that determines the onset of other brain alterations (e.g., synaptic and neuronal death), finally leading to the dementia. Early onset familial forms of AD are indeed associated with mutations in different genes (amyloid precursor protein (APP) and presenilins 1&2, (PS1&2)) involved in the biosynthesis of Aβ. Dysfunction of these genes is logically thought to alter the rate of APP cleavage, resulting in exaggerated Aβ production. High levels of brain Aβ and associated parenchymal amyloid plaques are key phenotypes described in transgenic mice overexpressing one or more of these mutated genes (see [3] for review). These mice subsequently develop neuropathological alterations and behavioral impairments mimicking AD phenotype [4, 5].

The exact impact of brain Aβ accumulation on clinical symptoms remains to date difficult to decipher, both in AD patients and in animal models of the disease. Clinicopathological correlative analyses have led to mitigated conclusions [6–10], and it is now considered that preplaques Aβ assemblies are the most deleterious species [11] while aggregated insoluble deposits have a reduced pathogenicity [12].

In addition white matter anomalies are described in AD patients, presumably in association with cerebrovascular impairments [13], and in transgenic mice modeling brain amyloidosis [14]. These lesions can be detected through conventional postmortem neuropathological examination, but also in vivo by means of dedicated techniques such as diffusion tensor imaging [15, 16], and it has even been proposed that white matter defects are potent diagnostic

biomarkers for AD [17, 18]. The integrity of axonal bundles constituting white matter fiber tracts is particularly compromised during the course of AD, even at the very early stages of the disease [19, 20]. Alterations of fiber tracts have obvious functional consequences: disconnection of neural networks occurs following fibers loss, leading to diaschisis and cortical disorganized activity [21, 22]. Also, disruption of myelin in axonal bundles might have deleterious effects on neuronal communication by altering propagation of action potentials and increasing brain energy expenditure (see [23, 24] for reviews).

White matter alterations in fiber tracts are related to demyelination and also to other axonal morphological defects that can ultimately lead to degeneration and loss of fibers. It has been demonstrated that AD patients show concurrent decreased axonal densities [25] and myelin breakdown [23]. Interestingly, experimental studies in AD animal models have shown that axonal pathology can be dri-ven by Aβ deposition in the brain [26–28].

The aim of the present study was to further evaluate fiber tracts changes in a double APPxPS1 mouse transgenic model with aggressive Aβ-related pathology [29–31]. Two commissural fibers tracts (corpus callosum and anterior commissure) that show significant alterations in AD patients [32, 33] were selected and analyzed: axonal integrity was quantified by assessing antineurofilament immunostainings. Myelin density was evaluated by histochemistry using a gold chloride staining that provides high contrast and spatial resolution [34, 35] and that has previously been used to underline myelin alterations in AD transgenics [36]. Age-dependent changes were detected in both fiber tracts of APPxPS1 mice. We tentatively correlated the relationship between these morphological anomalies and Aβ deposition.

2. Materials and Methods

2.1. Transgenic Mice. Female transgenic APP/PS1 mice (Thy1 APP751 SL (Swedish mutation KM670/671NL, London mutation V717I introduced in human sequence APP751) $\times$ HMG PS1 M146L) modeling early onset and progressive cerebral amyloid deposition were used [29, 30, 37–39]. Heterozygous APP/PS1 mice were obtained by crossing APP(+/wt)/PS1(wt/wt) with APP(wt/wt)/PS1(+/+) mice established on a C57bl6/CBA background. Note that, due to the use of homozygous PS1(+/+) mice, no wild-type mice could be generated from the breeding scheme. PS1 littermates were therefore used as control animals. PS1 mice have been described in previous studies to be devoid of neuropathological alterations [30, 37] and hence constitute good controls (age-matched littermates with no overt phenotypes) for analyzing APPxPS1 transgenics. A total of 28 mice were evaluated. Two age groups were analysed: "young" (2 months: 8 APP/PS1 and 7 PS1 mice) and "old" animals (24 months: 6 APP/PS1 and 7 PS1 mice).

2.2. Histology

2.2.1. Brain Processing. Following decapitation, the brains were extracted, fixed in 10% buffered formalin, and stored overnight in a solution of 20% glycerin and 2% dimethylsulfoxide in 0.1 M phosphate buffer for cryoprotection. Brains were subsequently sectioned into 40 μm thick coronal sections on a freezing microtome. Twelve series of sections ranging from the frontal to the occipital poles were collected and stored at -20°C in cryoprotectant before use.

2.2.2. Myelin Staining. Myelin staining was performed on a batch of serial sections using a protocol derived from [34] with some modifications. Brain sections were rinsed with 0.1 M phosphate buffer (PB) mounted on Superfrost+ slides and dried overnight at 50°C. The slides were then rinsed in 0.02 M phosphate saline buffer (PBS). Two milliliters of freshly prepared 0.2% gold chloride (Carlroth, Karlsruhe, Germany) dissolved in 0.02 M PBS was added on each glass slides to completely cover the section. Incubation was carried out at room temperature (RT) for 2 hours in obscure humid chambers. The tissue was briefly rinsed in water, fixed in 2.5% sodium thiosulfate for 5 minutes, and finally rinsed in running tap water for 30 minutes. Sections were coverslipped with Eukitt after dehydration in graded alcohols and clearing in xylene.

2.2.3. Axonal Immunostaining. Free-floating sections were rinsed in PB and pretreated with 0.3% H_2O_2 in methanol for 10 minutes to block endogenous peroxidase activity. Nonspecific antigenic sites were blocked by 5% goat serum in PBS-Triton X-100 (1 hour at RT). Sections were then incubated overnight at RT with a C-terminal antineurofilament M (145KD) primary antibody (AB1987; Chemicon, Temecula, Calif) diluted 1 : 1000 in serum. The following day sections were incubated in a secondary biotinylated antirabbit antibody solution (Sigma, St. Louis, Mo; 1 : 200, 1.5 hours at RT) and then in an avidin-biotin peroxidase complex (ABC) reagent (Vectastain Elite ABC-Kit, Vector Laboratories, USA, 1 : 800, 1.5 hours at RT). The sections were rinsed 3 $\times$ 10 min between all incubation steps. The immunoreaction was visualized with 3,3′-diaminobenzidine (DAB) under microscopical control. The reaction was stopped when the signal-to-noise ratio was considered optimal. The sections were finally mounted on superfrost slides, dried, and processed in alcohols/xylene before being coverslipped.

2.2.4. Amyloid Plaques and Intracellular Aβ. One series of section was stained by Congo red according to the standard technique (30 minutes in a 80% ethanol solution saturated with Congo red and sodium chloride) [30, 40]. Another series of sections was immunostained with the primary 4G8 monoclonal anti-Aβ antibody (Signet Laboratories, Dedham, Mass; 1 : 10000, incubation overnight at RT). A high dilution of the biotinylated 4G8 antibody was used to avoid cross-reaction with APP and to solely immunodetect Aβ [41]. Thanks to biotin complexion, no secondary antibodies were used and tissue was directly incubated in the ABC Kit solution before being developed in DAB.

2.2.5. Evaluation of Neurodegeneration. Staining of degenerating neurons was performed using the Fluoro-Jade B dye

[42] with a slightly modified protocol [43]. Glass-mounted sections were passed through absolute ethanol and 75% ethanol followed by a 1-minute rinse in distilled water. Tissue was then incubated in 0.06% potassium permanganate solution for 15 minutes with slight agitation and rinsed before staining in Fluoro-Jade B (Histo-Chem., Jefferson, Ark; 0.001% solution prepared in 0.1% acetic acid; 30 minutes at RT). After extensive rinsing in distilled water, sections were dehydrated, cleared in xylene, and coverslipped.

2.3. Quantitative Image Analysis and Statistics

2.3.1. Myelin Densities. All slides were digitized using a Super CoolScan 8000 ED scanner (Nikon, Champigny sur Marne, France) with a 4000 dpi in-plane digitization resolution (pixel size 6.35 μm^2). Quantification of gold chloride staining was performed, in two regions of interest (corpus callosum and anterior commissure), by relative optical density (ROD) analysis, a method that has already been used to evaluate myelin stainings [44]. In the rostral brain, 3 sections were selected to analyze myelin at the level of the anterior commissure (bilateral countings, i.e., $n = 6$ samples / mouse). For the analysis of myelination of the corpus callosum, 7 sections per animal were selected, spanning the rostrocaudal extent of the region; the ROD analysis was performed on the total corpus callosum volume and also separately in its anterior and posterior parts (see below).

Measure of mean grey value was semiautomatically performed in the two regions of interest using Photoshop CS2 software (Adobe, Paris, France). ROD was obtained after transformation of mean grey values using the formula ROD = $\log_{10}$ (256/(mean grey value)). Values of background staining were taken in adjacent tissues with no myelin staining (upper cortical layers or striatum) and subtracted to get a final densitometric evaluation of myelin stain.

While 2-month-old APPxPS1 mice were devoid of amyloid plaques, Aβ deposition was very extensive in aged APP/PS1 transgenics. Notably many plaques were observed in the corpus callosum and in the anterior commissure. Obviously the gold chloride negatively stained areas corresponding to plaque occupancy might artificially decrease the local mean grey values. To correct for this bias in old mice, plaques surfaces were manually removed from analyzed regions of interest before processing ROD calculations.

2.3.2. Fiber Tracts Atrophies. The sizes (surface area from coronal sections) of both the corpus callosum and the anterior commissure were evaluated by manually outlining the structures on digitized scans of myelin-stained brain sections (see Figure 1). Three measures of fibers tracts size were taken: (1) in the anterior corpus callosum (sampling between 4.9 and 3.22 mm/interaural reference in the Paxinos and Franklin atlas [45], $n = 4$ sections/mice), (2) in the posterior corpus callosum (3 sections taken between 1.5 and 2.58 mm/interaural reference), and (3) in the anterior commissure (sampling between 4.18 mm and 4.9 mm/interaural reference, $n = 3$ sections analyzed bilaterally/mice). For each region of interest, area size measurement was calculated by summing outlined areas across serial sections.

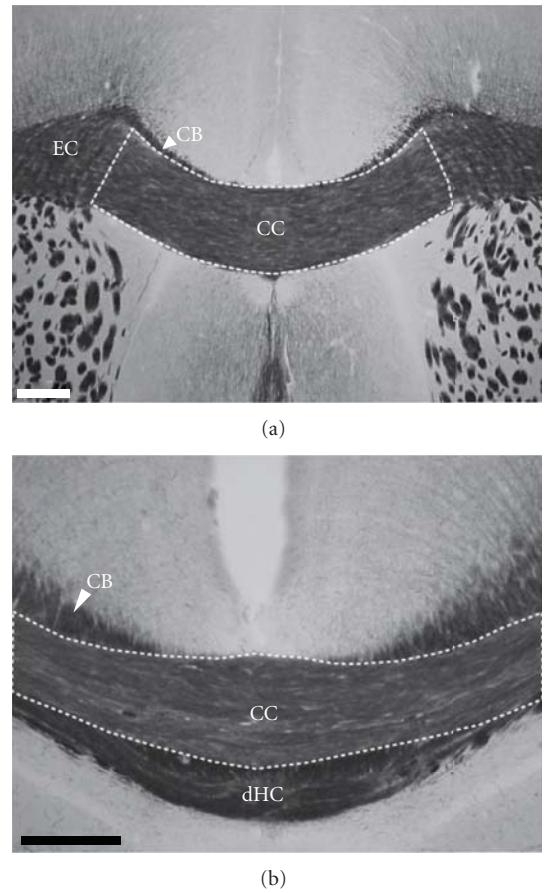

Figure 1: Myelin stain of the corpus callosum. On frontal sections, staining of the myelin using the gold chloride method allows to delineate the perimeter of the corpus callosum (CC) at the rostral (a) and caudal (b) levels. On the basis of axon orientation and staining intensities, adjacent fiber tracts (CB: cingulate bundle; dHC: dorsal hippocampal commissure; EC: external capsule) are clearly outlined allowing accurate visualization of the corpus callosum borders. Scale bars: 200 μm.

2.3.3. Axonal Neurofilament Densities. Neurofilament immunostained sections were digitized as described above for myelin-stained sections. Assessment of neurofilament immunoreactivity was performed in the same two regions of interest (corpus callosum, $n = 7$ sections/mouse; anterior commissure, $n = 3$ sections/mouse) by means of ROD analysis.

2.3.4. Extracellular Amyloid Loads in Old Mice. Evaluation of the extracellular amyloid load was only performed in the 24-month-old APP/PS1 mice as almost no Congo red positive deposits were observed in young double transgenic mice.

Plaques loads were quantified using computer-based thresholding methods [46]. Scans were prepared using Photoshop CS2 to outline selected regions of interest. Images were then processed with ImageJ freeware (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, Md, USA, http://rsb.info.nih.gov/ij/, 1997–2006) using a dedicated macrocommand that extracts amyloid deposits from

background tissue with no user inputs. Regional amyloid loads were expressed as percent of tissue surface stained by the Congo red dye that corresponds to the proportion of plaques volume. Amyloid loads were evaluated (1) in the whole brain [30], (2) in the rostral isocortex that is richly innervated by axons passing through the anterior corpus callosum, and (3) directly in the two fiber tracts that were investigated in the present study (anterior commissure, corpus callosum).

2.3.5. Intracellular Amyloid Loads in Young Mice. Intraneuronal Aβ immunoreactivity was not found in aged mice (as already reported for this mouse line [38]) and was quantified only in the young APPxPS1 mice. Two sections/animals were selected at the level of the frontal cortex, and a semiquantitative analysis, based on a four-point scale, was performed on both hemispheres to evaluate levels of neuronal immunostainings in the cortex: 0: no obvious positive staining; (1): weak intracellular staining; (2): moderate staining; (3): strong staining. For each animal semi-quantitative analyses were hence performed in four fields (2 slices; bilateral countings).

To evaluate the connection properties of Aβ-containing neurons and their possible participation to commissural tracts we performed an axonal tracing study in two wild-type mice (3 month-old): biotinylated dextran amine (BDA), an anterograde tracer, was stereotactically injected in the cortical regions where Aβ-positive neurons were concurrently observed in APPxPS1 transgenics, allowing fine visualization of cell bodies and of neurites. Surgery and immunolabeling of BDA stained axons were performed using standard protocols as previously described [47, 48].

2.3.6. Statistics. Student's t-tests were performed using Statistica 7 (StatSoft, Inc., Tulsa, USA). Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Altered Volumes of Fiber Tracts in APPxPS1 Mice. Gold chloride myelin staining, as compared to standard stains (e.g., HE or Nissl stains), allowed to precisely outline the area of the corpus callosum. In particular, delineating the corpus callosum from adjacent white matter tracts (e.g., cingulate bundle, dorsal fornix, and dorsal hippocampal commissure) was greatly facilitated by the myelin staining (Figure 1). Lateral limits of the corpus callosum and borders of the external capsule were identified by a horizontal-to-vertical shift in fiber orientation. Also, the anterior commissure was easily identified and outlined in the gold chloride-stained sections (see Figure 5(b)).

3.1.1. Young Mice. The size of the anterior commissure was similar in 2-month-old PS1 mice and in age-matched APPxPS1 transgenics ($t(13) = -0.58$; Figures 2(b) and 5(b)). On the contrary, the corpus callosum size was significantly decreased in young APPxPS1 as compared to controls ($t(13) = 3.501$, $P < .005$; Figures 2(a) and 5(a)). Subregional analysis indicated a significant reduction in the size of the rostral corpus callosum of young APPxPS1 mice ($t(13) = 3.743$, $P < .005$) while there was no difference between genotypes in the size of the posterior corpus callosum ($t(13) = 0.136$, ns).

3.1.2. Old Mice. With aging, a significant increase in the size of axonal bundles was observed in PS1 control mice (corpus callosum: $t(12) = 3.858$, $P < .005$; anterior commissure: $t(12) = 4.275$, $P < .005$). From pilot studies, we have observed the same developmental traits in wild-type C57bl6 mice (increased volumes of the corpus callosum and of the anterior commissure with aging; data not shown) precluding the possibility that the PS1 transgene by itself triggered abnormal (increased) fiber tract growth in PS1 transgenic mice.

On the other side, age-dependent enlargement of commissural bundles was clearly not evidenced in the double APPxPS1 transgenics: in this genotype, the size of the corpus callosum remained constant between 2 and 24 months ($t(12) = 1.850$, ns) and the surface area of the anterior commissure even undergoes slight atrophy with aging ($t(12) = 2.284$, $P < .05$). Hence, strong differences between genotypes were observed in 24-month-old mice with APPxPS1 transgenics showing, in comparison to PS1 controls, decreased white matter surface areas. This was observed at the level of the anterior commissure ($t(11) = 6.388$, $P < .0001$) and of the corpus callosum (total corpus callosum: $t(11) = 4.653$, $P < .001$; anterior part: $t(11) = 5.404$, $P < .0005$). The only posterior region of the corpus callosum did not show significant atrophy in old APPxPS1 mice ($t(11) = 1.492$, ns).

3.2. Potentiation of Axonal Neurofilament Loss in Old APPxPS1 Mice. Densities of axonal neurofilaments in the corpus callosum and in the anterior commissure were quantified using ROD analysis of immunostainings. In 2-month-old mice axonal densities were similar in both genotypes whatever the fiber tract considered (all P's $> .35$). With aging, a severe decrease in neurofilament density was observed, both in APPxPS1 and PS1 mice, in the corpus callosum (Figures 2(c) and 3(a)) and in the anterior com-missure (Figures 2(d) and 3(b)) (all P's $< .0001$). Age-related reduction of neurofilament density was, however, largely more prominent in old APPxPS1 mice than in age-matched PS1 controls. This accelerated loss of axonal neurofilaments in old APPxPS1 mice was further confirmed by statistical analysis in the different subregions of the corpus callosum (APPxPS1 < PS1: all P's $< .0001$) and 2) and also at the level of the anterior commissure ($t(10) = 4.16$; $P < .005$).

3.3. Abnormal Myelination in Old APPxPS1 Mice. ROD analysis of gold chloride stainings in 2-month-old mice indicated comparable myelin densities in PS1 and APPxPS1 transgenics (corpus callosum: $t(13) = 0.318$, ns, Figures 2(e) and 5(a); anterior commissure: $t(13) = 1.277$, ns, Figures 2(f) and 5(b)).

The myelin density of the anterior commissure was not affected by aging (PS1: $t(12) = 1.292$, ns; APPxPS1: $t(12) = 0.556$, ns), and myelin densities in this fiber tract were similar

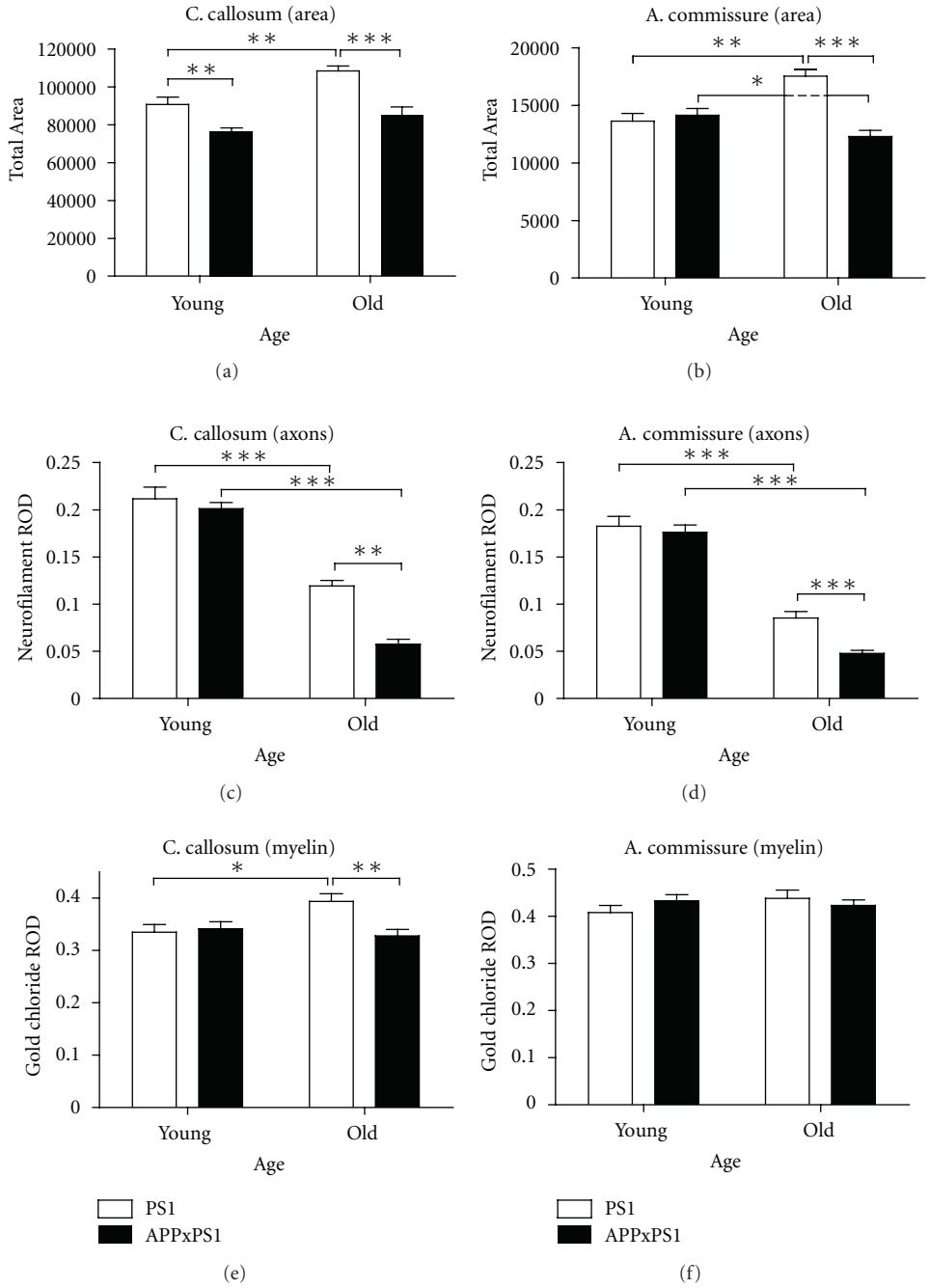

Figure 2: Summary of quantitative morphological analysis. The analysis was focused on the corpus callosum (left column) and on the anterior commissure (right column). Morphological data were collected in old and in young mice from PS1 and APPxPS1 genotypes. (a)-(b). Size of fiber tracts (pixels). (c)-(d). Relative optical densities (ROD) of neurofilament M145Kd (axons) immunostainings. (e)-(f). Relative optical densities of gold chloride (myelin) stainings. See text for details. All measures are illustrated by means + SEM in each experimental groups. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

in 24-month-old PS1 and in age-matched APPxPS1 mice ($t(11) = 0.679$, ns).

On the other hand, an increase of the myelination of the corpus callosum was observed when comparing 2-month- and 24-month-old PS1 mice ($t(12) = 2.823$, $P < .05$). Noticeably, this increase in myelin density was significant in the rostral corpus callosum ($t(12) = 4.171$, $P < .005$) but not in its posterior part ($t(12) = 0.461$, ns). Contrarily to PS1 mice, such an age-related increase of callosal myelination was not observed in APPxPS1 mice ($t(12) = 0.7$, ns). Consequently decreased myelin staining was evidenced in 24-month-old APPxPS1 mice when compared to old PS1 controls (total corpus callosum: $t(11) = 3.332$, $P < .01$). Differences between genotypes were further confirmed at the level of the anterior

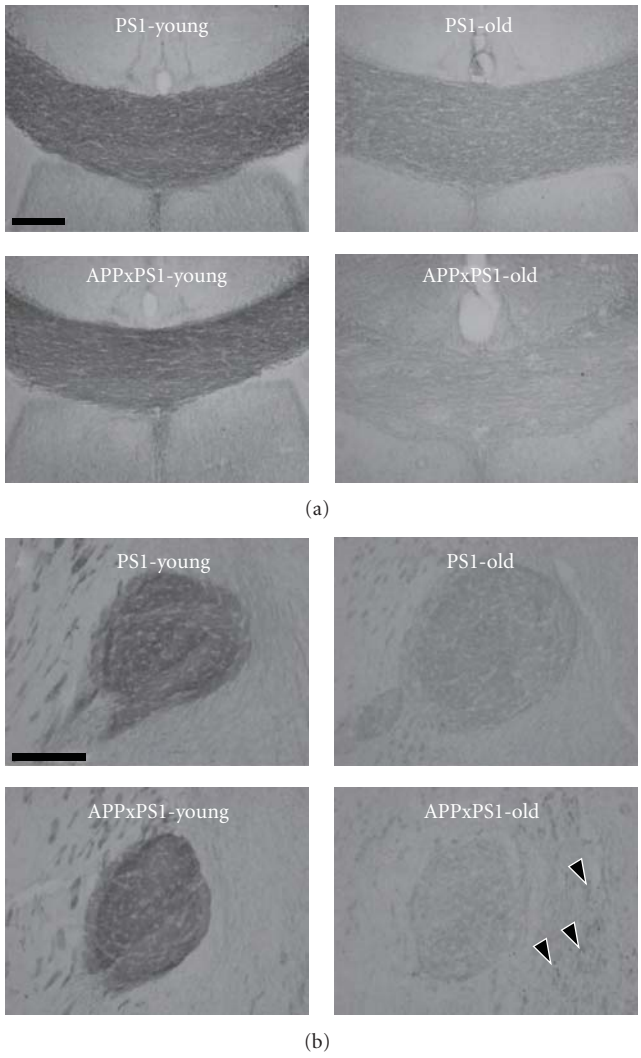

Figure 3: Representative illustration of neurofilament immunostainings. Immunodetection of axonal neurofilaments is illustrated at the level of the corpus callosum (a) and anterior commissure (b) in both young and old PS1 and APPxPS1 transgenics. See text for details concerning age and genotype effects. Black arrow heads point to positive axonal enlarged varicosities in old APPxPS1 mice. Scale bars: 100 μm.

corpus callosum ($t(11) = 3.512$, $P < .005$), while no difference between PS1 and APPxPS1 mice were evidenced in more caudal regions ($t(11) = 1.9$; ns).

Qualitative examination of myelin-stained sections was then performed in old APPxPS1 mice. No evidence of myelin breakdown (debris) was found in the large myelinated bundles of the corpus callosum. However, in comparison to control animals, myelin appeared to be fragmented in the isocortex and hippocampus of APPxPS1 mice (Figure 4). Myelin material was often detected under the form of small tortuous segments with bead-like varicosities. These morphological anomalies, absent in young APPxPS1 mice, were found at the vicinity of Aβ deposits but also in the parenchyma in areas distant from plaques.

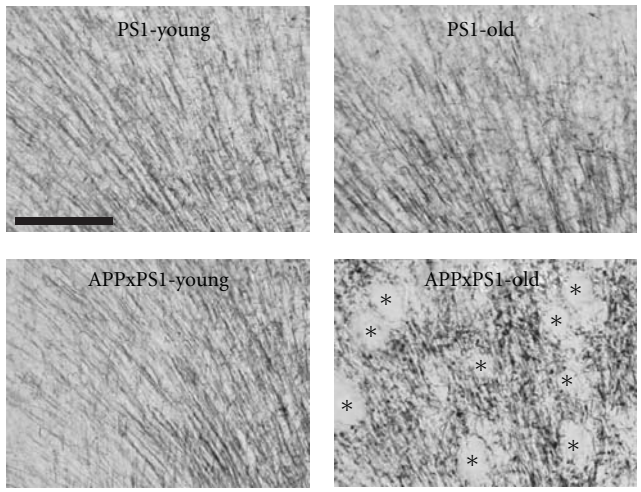

Figure 4: Myelin stains in the cingulate cortex. In young PS1 and APPxPS1 mice myelinated axons in the cingulate cortex have a radial organization that is preserved in old PS1 mice but is severely disorganized in old APPxPS1 transgenics (lower right photo: optically empty areas correspond to amyloid plaques and are identified by black asterisks). Scale bar: 100 μm.

3.4. Relationship with Aβ Pathology and with Neurodegeneration. Congo red positive aggregates were detected and quantified in the anterior commissure (mean load= 2.8%; min = 1.6%; max = 6%) and in the corpus callosum (mean load = 2.2%; min = 1.6%; max = 2.7%) of old APPxPS1 mice (Figures 6(a) and 6(b)). Correlative analysis did not reveal significant associations between local amyloid loads in fiber tracts and decreased neurofilament immunoreactivity/myelin densities (all P's $> .111$). Also there were no correlations between the sizes of the corpus callosum/anterior commissure and total or cortical amyloid loads (all P's $> .196$).

In addition to amyloid plaques loads, intraneuronal Aβ was semiquantitatively assessed in the isocortex of young APPxPS1 mice. As expected from previous observations, positive labeling was detected using the 4G8 antibody in a subset of cells. Staining was mainly observed in deep cortical layers (V) involving a distinctive band of large pyramidal cells (Figures 6(c) and 6(d)). There were no direct correlations between levels of intracellular Aβ that may significantly vary from one animal to the other (mean = 7.8; min = 4.5; max = 11.5) and neurofilament and myelin densities (all P's $> .119$). Interestingly, tracing experiments underlined that the deep layers of the cortex, where specifically Aβ-positive neurons were observed in the double transgenics, are the source of dense callosal projections (Figure 6(e)). Therefore, although no tight correlations were stressed between the density of intracellular Aβ and the magnitude of axonal anomalies, we evidenced that callosal fibers still originate from a subpopulation of neurons overproducing toxic Aβ species.

The Fluoro-Jade B dye was used to assess neurodegeneration in APPxPS1 mice. No positive neurons were detected in the studied animals (data not showed). In particular no degenerating neurons were observed in the cortical layers

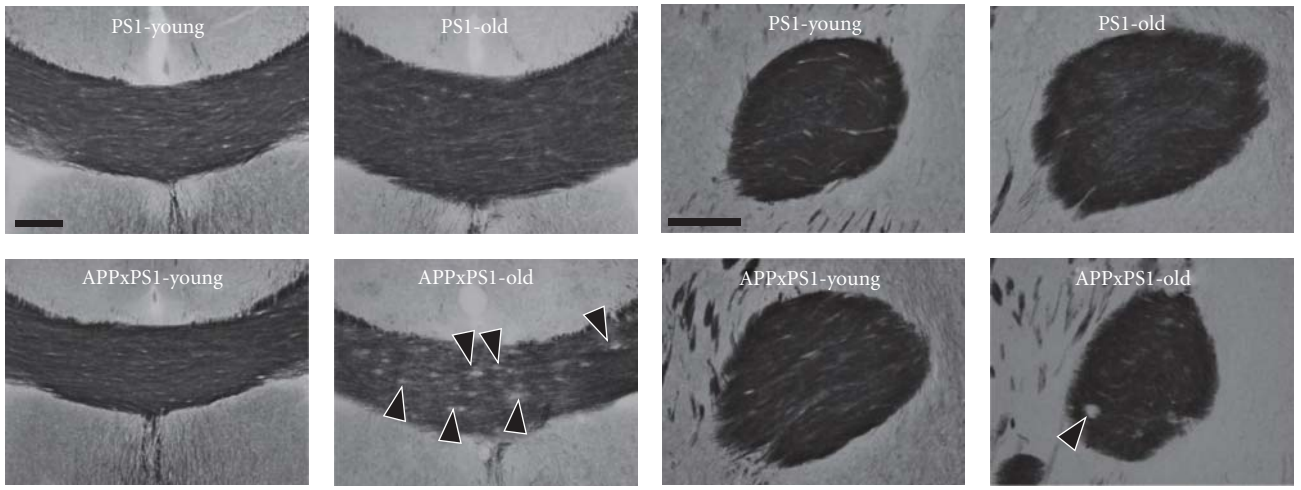

Figure 5: Representative illustration of myelin stains of fiber tracts. Myelin (gold chloride) stains are illustrated at the level of the corpus callosum (a) and anterior commissure (b) in both young and old PS1 and APPxPS1 transgenics. See text for details concerning age and genotype effects. Black arrow heads ((a) lower right photo) point to circular unstained areas corresponding to amyloid plaques in the corpus callosum of old APPxPS1 mice. Scale bars: 100 μm.

with high densities of Aβ-positive neurons (see above). Only the core of amyloid deposits and the surrounding degenerating dystrophic neurites as well as reactive astrocytes were detected with Fluoro-Jade B in old APPxPS1 mice (see [49, 50] for similar observations).

4. Discussion

Neuroimaging assessment repeatedly underlined white matter changes in AD patients. Evidence from diffusion tensor imaging of fiber tracts suggests for instance that during the course of the disease there is a loss of barriers that restrict water motion and tissue anisotropy of white matter. These observations have obviously a neuropathological counterpart: anomalies of diffusion could reflect either loss of axons, demyelination, and/or oligodendroglial pathology. To date only a few studies have investigated the source of white matter/fiber tracts impairments in AD patients and in aged nondemented subjects [51, 52]. Recent attempts to decipher the origin and mechanisms of such morphological alterations have been performed in animal models of the disease [36, 53, 54] but a systematic screening of fiber tracts anomalies related to AD pathology is still lacking in these models.

The goal of our work was therefore twofold: (1) to assess in an AD mouse model the severity of fiber tracts alteration across aging and (2) to refine the understanding of their histological substratum and in particular to evaluate the relation between Aβ peptide deposition and the occurrence of the white matter anomalies.

4.1. Fiber Tract Atrophies in APPxPs1 Mice. Our quantitative analysis underlines the decreased size of forebrain fiber bundles in old APPxPS1 mice displaying concurrent severe brain amyloidosis. Volume reductions were observed in the corpus callosum, as already reported in AD transgenics [14, 30, 55] but also, as a novel finding, at the level of the anterior commissure. These anomalies do not purely mimic an age-related atrophy and might also reflect a lack of normal development of fiber tracts in APPxPS1 mice (see also [30] for similar conclusions).

As mentioned above, atrophy of the corpus callosum has previously been reported in AD transgenic mouse models but more interestingly is also classically depicted in AD patients (e.g., [33]). We found that atrophy of the corpus callosum, in our APPxPS1 transgenics, largely predominates in its anterior part. Although not constantly reported, similar findings concerning regional atrophies of the corpus callosum have been described in human patients [21, 56]. In addition to callosal atrophy we also evidenced, in old APPxPS1 mice, a reduction in size of the anterior commissure that might find a parallel with recent observations in AD patients [32].

Supporting size reduction of fiber tracts we evidenced a very strong decrease of axonal neurofilament densities in the double transgenics, outclassing the "normal" loss of neurofilament we observed in aged control PS1 mice (and in wild-type mice; data not shown). We also substantiated a significant myelin breakdown in APPxPS1 mice. The loss of myelin in aged APPxPS1 mice appears however to be limited in comparison to the high and accelerated decrease of neurofilament densities these mice undergo. As evidenced by correlative analysis, there were no strict linear relationships between fiber tracts atrophies and neurofilament or myelin markers. For instance, we found a reduced size of the corpus callosum in young APPxPS1 mice while these animals showed the same neurofilament and myelin densities as control PS1 mice—such observations emphasizing similar densities of axonal markers in an overall reduced volume of

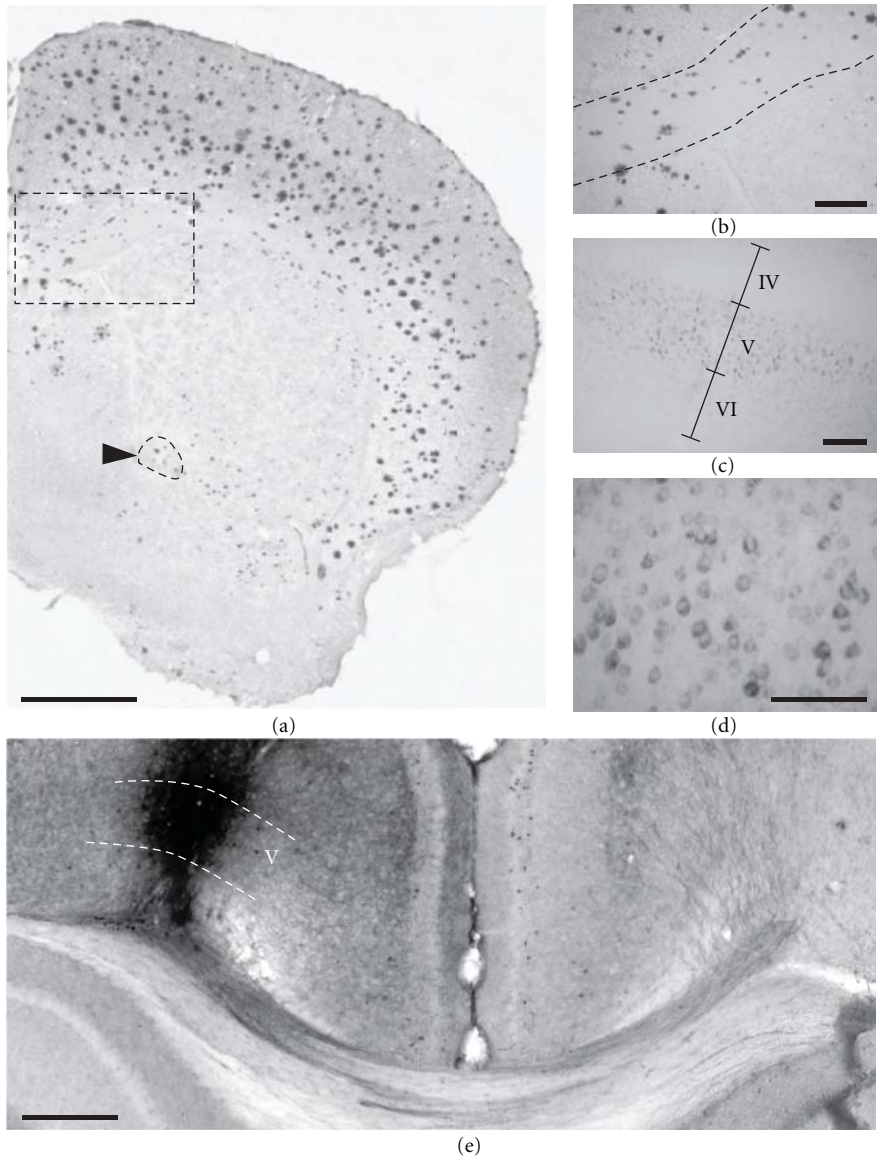

Figure 6: Aβ pathology in APPxPS1 mice. 24-month-old APPxPS1 mice display heavy cortical amyloid burden as evidenced by Congo red staining (a). Congophilic plaques were also observed in white matter tracts such as the corpus callosum (outlined rectangular area in magnification in (b)) and the anterior commissure (outlined in a and pointed by a black arrow head). In young APPxPS1 mice dense core amyloid plaques were virtually absent but strong Aβ intraneuronal accumulation, as revealed by 4G8 immunostainings, was detected in infragranular cell layers ((c), magnified view in (d)). (e) Biotinylated dextran amine, an anterograde tracer, was injected in the deep layers of the isocortex (left hemisphere) at the locus where Aβ-containing neurons were found in APPxPS1 transgenics. These neurons send their axons through the corpus callosum and cingulum bundle, cross the midline, and innervate the opposite (right) hemisphere. Scale bars: 1000 μm (a), 100 μm ((b), (c), and (d)), and 500 μm (e).

tissue might indeed testify for an early net loss of callosal fibers in young APPxPS1 mice. Also we found that fiber tracts continue to grow and pursue their myelinisation in normal mice between 2 and 24 months of age while neurofilament densities in the same mice concurrently display a 50% drop—this observation can be explained (1) by a progressive enlargement of myelin sheets with aging that will maximize space around individual axons and consequently decrease their density and (2) by a concurrent diminution of the expression of axonal neurofilament proteins that has previously been described in aged animals [57].

4.2. Substratum of Fiber Tract Anomalies: Altered Myelinisation. We have investigated various sources of neuropathological alterations that could explain fiber tracts atrophies in our APPxPS1 model. It has been claimed that a demyelination process is observed during the course of AD and is a trigger in the physiopathogeny of this disease [23, 24]. It has even been proposed that myelin breakdown in AD initiates a vicious circle as release of iron after demyelination might promote Aβ production and toxicity which in turn affects myelin integrity [58]. In the present study we indeed observed qualitative anomalies of axonal myelination in old

APPxPS1 mice accumulating Aβ. Similar observations, that might deserve confirmation and further characterization at the ultrastructural level using electron microscopy, have been recently reported in AD Tg2576 and APPxPS1 mice but only in the neuropil in close association with amyloid plaques [54]. In our study myelin alterations, including fragmentation and spheroid-like pathological enlargements, were observed in axons passing through the gray matter, even at distance of amyloid deposits, but not easily in dense fiber tracts under light microscopy. At the quantitative level, the overall myelination of the corpus callosum was found to be significantly decreased in old APPxPS1 mice. However no differences were observed between genotypes at the level of the anterior commissure, suggesting that loss of myelin is heterogeneous and does not affect similarly all axonal bundles. Actually, myelination kinetics during ontogenesis might considerably vary from one brain region to the other [44, 59]. It is hence possible that brain areas and fiber tracts with late or early myelination are differentially impeded during (pathological) aging [60, 61]. In addition, it is known that the corpus callosum, in terms of phylogeny, is a neural pathway that is only found in placental mammals while the anterior commissure is a more ancestral axonal tract [62], adding supplementary differences between the two fiber bundles that could explain regional discrepancies in age-dependent (de)myelination processes. Interestingly, it has been shown that oligodendrocytes have a reduced myelin turnover in regions lately myelinated during ontogenesis [63]. This would imply a diminished capacity for myelin repair in these regions, especially under challenging conditions (oxidative stress, excitotoxicity, inflammation, etc.) because oligodendrocytes are a strikingly vulnerable population of cells [23]. Toxicity of Aβ on oligodendrocytes has been evidenced in several reports [36, 64] with effects ranging from mitochondrial dysfunction to apoptosis and cell death. The involvement of oligodendrocytes in the myelin anomalies depicted in our APPxPS1 transgenics would deserve further analysis. In particular, it would be interesting to document possible anomalies at the level of myelin basic proteins.

4.3. Substratum of Fiber Tract Anomalies: Axonal Pathology. Decreased sizes of the corpus callosum and of the anterior commissure in APPxPS1 mice might be related to defects in fiber myelination but, according to our observations, can also be linked to an accelerated loss of axonal neurofilaments, possibly reflecting loss of fibers.

We have previously reported vascular alterations (vessel voids and reduction of vessel lengths) by means of in vivo magnetic resonance angiography in the APPxPS1 transgenic mouse model used in the present study [65]. These lesions, closely associated with amyloid angiopathy, could contribute to the described axonopathies [66].

Anomalies in regulatory proteins involved in axonal structural plasticity during AD could also be causative [67] but remain to be firmly established in our transgenic mouse model.

Axonopathy has previously been reported in the APPxPS1 mouse line but was originally evidenced at the spinal level [31]. Our results highlight that axonal pathology in APPxPS1 mice also concerns forebrain fiber tracts. Interestingly, it has been demonstrated that AD patients sustain axon loss [25, 68] but the magnitude of the effects we reported (large drop in neurofilament densities, first in aged PS1 control mice and second, greatly accentuated, in aged double APPxPS1 transgenics) has not been reported up to now to the best of our knowledge. Bussières and collaborators [69] have demonstrated that neurons from AD brains expressing neurofilament proteins with medium- and heavy- molecular-weight subunits (SMI-32 immunoreactive neurons) constitute an exquisite vulnerable subpopulation of cells during disease process. These neurons have a specific laminar distribution in the cortex compatible with their participation to corticocortical connections relying on fiber tracts and axonal fasciculi. Back to our experimental data, it would be appealing to speculate that loss of axonal neurofilaments in the anterior commissure and corpus callosum indeed implies pathological events at the level of efferent neurons in specific brain regions (e.g., isocortex area for callosal neurons). We did not find however any evidence of cell degeneration in old APPxPS1 mice (see also [50] for similar report). On the other side we cannot totally preclude that APPxPS1 mice have decreased neuronal densities in projections regions resulting from earlier insults, with no overt signs of active degeneration in the oldest animals. A substantial (30%) loss of neurons has been described in the hippocampus of old APPxPS1 mice [37, 70]. It is not yet known if a similar loss occurs in isocortical areas and further analysis will be required to ascertain this point.

4.4. Substratum of Fiber Tract Anomalies: Role of Aβ-Positive Lesions. In accordance with previous studies (e.g., [37, 71]), we did not find evidence of a clear pathogenicity of aggregated (Congo red positive) insoluble Aβ deposits. The density of amyloid plaques did not predict, in old APPxPS1 mice, the magnitude of fiber tracts anomalies these mice sustained. In a previous study we evidenced that axon terminals of cortico-cortical fibers endorse degeneration when contacting amyloid plaques [26] but this local synaptotoxicity was not associated with clear retrograde degeneration. In another study of Jantaratnotai [27] axonal damage and demyelination were reported following in vivo Aβ injections in the corpus callosum. However these effects, obtained in a context of sudden and potentiated toxicity, cannot be considered as conclusive. Furthermore, Stokin and collaborators [72] in a recent study have confirmed that axonal defects induced by APP/PS1 mutations are not directly caused by Aβ overproduction and amyloid accumulation. These late results strengthen our observations.

While amorphous insoluble amyloid deposits do not appear to be deleterious per se, our results support the hypothesis of a pathogenicity of Aβ when it accumulates intraneuronally [73, 74]. As described by others [75], young APPxPS1 mice display intracellular Aβ accumulation, several weeks before the occurrence of amyloid plaques. In the present study, Aβ-positive neurons were observed, in 2-month-old APPxPS1 mice, in specific layers of the cortex (mostly

layer V). In primates, including humans, it is known that pyramidal neurons projecting through the corpus callosum belong to lamina III and V of the association cortex [76–78]. In mice also the origins of callosal afferents have been traced back to cell bodies in layer V [79]. By means of anterograde tracings we confirmed that the Aβ-containing neurons in the deep layers of the cortex participate to commissural fibers passing the midline through the corpus callosum. One may infer from these data that, early during aging, APPxPS1 mice accumulate Aβ in a subset of cortical neurons that later become dysfunctional, develop axonal pathology (loss of neurofilament immunoreactivity and of myelin), possibly through a dying-back mechanism [80], and eventually die [41], leading to a definite loss of axons in brain commissures.

To summarize, we found, in APPxPS1 mutant mice modeling AD brain amyloidosis, evidence of fiber tracts atrophies. These anomalies are potentiated in aged mice but might partly be explained by some neurodevelopmental defect. Axonal pathology developed by APPxPS1 mice does not appear to be caused by amorphous insoluble amyloid deposits but intracellular Aβ that accumulates in projecting neurons could be a causative factor. The atrophies in fiber bundles we depicted in APPxPS1 mice resemble those described in AD patients and are concomitant with a loss of axonal neurofilaments and a myelin breakdown. These results renew the importance of white matter impairments in Alzheimer's disease as these anomalies, with clear functional impact, can be reproduced in mice overexpressing Aβ, a molecular actor that is the principal target for current therapeutical assays in this devastating disease.

Acknowledgments

The authors thank the Sanofi-Aventis Neurodegenerative Disease Group for the generous gift of the animals that were involved in this study. They also want to acknowledge Pr. Duyckaerts for stimulating discussions of the results as well as for critical reading of the paper.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 391783, 5 pages
doi:10.4061/2011/391783

Research Article

Validation of Automated White Matter Hyperintensity Segmentation

Sean D. Smart, Michael J. Firbank, and John T. O'Brien

Institute for Ageing and Health, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne NE4 5PL, UK

Correspondence should be addressed to Michael J. Firbank, m.j.firbank@ncl.ac.uk

Received 4 May 2011; Revised 1 July 2011; Accepted 3 July 2011

Academic Editor: Ph Scheltens

Introduction. White matter hyperintensities (WMHs) are a common finding on MRI scans of older people and are associated with vascular disease. We compared 3 methods for automatically segmenting WMHs from MRI scans. *Method*. An operator manually segmented WMHs on MRI images from a 3T scanner. The scans were also segmented in a fully automated fashion by three different programmes. The voxel overlap between manual and automated segmentation was compared. *Results*. Between observer overlap ratio was 63%. Using our previously described in-house software, we had overlap of 62.2%. We investigated the use of a modified version of SPM segmentation; however, this was not successful, with only 14% overlap. *Discussion*. Using our previously reported software, we demonstrated good segmentation of WMHs in a fully automated fashion.

1. Introduction

Magnetic resonance imaging (MRI) is now widely used in the diagnosis of diseases by doctors and is particularly useful for scanning images of the brain and detecting cerebrovascular disorders. White matter hyperintensities (WMHs) are a common finding in elderly people which are associated with vascular risk factors and an increased risk of decline in cognitive and motor function [1]. A number of methods have been used to quantify the hyperintensities to correlate to clinical data such as visual ratings, volumetric measuring, and WMHs pattern [2–6].

An investigation with the LADIS study cohort found that volumetric measurement was more sensitive than visual rating to detect differences in WMHs between groups with versus without memory symptoms although both volumetric measurement and visual rating detected differences in WMHs relating to age and gait disturbance [7].

Currently, there is no accepted gold standard for a fully automated WMHs segmentation program. The SPM package (http://www.fil.ion.ucl.ac.uk/spm/software/) has a widely used segmentation tool which classes brain tissue into grey, white matter, and CSF, using a combination of image intensity and a priori knowledge regarding distribution of tissue types. The default does not include information about WMHs, and these can be misclassified as grey matter [8]. Adding information regarding the a priori distribution of WMHs may help to improve the segmentation of WMHs in SPM.

The study aims to investigate the ability of SPM to segment WMHs from (a) T1 weighted and (b) T1 + FLAIR images using a priori information about WMHs distribution. Results will be compared to manual segmentation of WMHs from FLAIR images, an in-house WMHs segmentation program [9], and a different previously reported program [6].

2. Materials and Method

2.1. Subjects. We used 30 MRI scans of subjects randomly selected from a previously published study [10]. We included 10 older subjects with no evidence of dementia as well as 20 subjects with mild-to-moderate severity dementia. Of these, 16 fulfilled criteria for probable Alzheimer's disease according to NINCDS/ADRDA [11], and 4 cases met criteria for probable dementia with Lewy body according to the consensus criteria [12]. The scans were acquired from a Phillips 3T MRI system (Intera Achieva scanner), using the integrated RF body coil for transmission and signal detection

through an 8 channel SENSE head coil. All the participants were aged 60+, and basic demographic information was collected, along with a minimental state examination (MMSE) which is used to screen cognitive function and can indicate if a person shows signs of cognitive impairment (Table 1). The study was approved by the local ethics committee. Images acquired included a T1 weighted volumetric sequence covering the whole brain (MPRAGE, sagittal acquisition, slice thickness 1.2 mm, pixel size 1.15×1.15 mm; TR = 9.6 ms; TE 4.6 ms; flip angle = 8°) and FLAIR images to demonstrate white matter hyperintensities (TR 11000; TE 125; TI 2800 ms, data out as 1.016×1.016; 60 slices 2.5 mm). The FLAIR and T1 weighted images were spatially registered together using SPM's "coregister" tool.

2.2. White Matter Hyperintensity Segmentation. Figure 1 gives an overview of the steps for each of the automated segmentation processes.

2.3. In-House Programme. We have previously described the in-house segmentation routine [9]. Briefly, SPM5 (http://www.fil.ion.ucl.ac.uk/spm/) was used to segment grey matter (GM), white matter (WM), and cerebral spinal fluid (CSF) of the T1-weighted images. A brain mask was then created from GM + WM. The mask was used to remove nonbrain regions from the fluid attenuated inversion recovery (FLAIR) image. The images were then segmented. To perform the segmentation, (a) on the skull stripped FLAIR image, the modal pixel intensity was determined. (b) A threshold-based segmentation was then performed, using a threshold of 1.45 times the modal pixel intensity. (c) Isolated pixels were then removed from the segmentation.

2.4. Wu Programme. The programme code was obtained from the author Minjie Wu. This is a brief summary of the process; for more details, see Wu et al. [6]. The programme's automated WMHs segmentation used three main steps; image preprocessing which included (a) coregistering the FLAIR with the T1 image, (b) using the Brain Extraction Tool (part of FSL (http://www.fmrib.ox.ac.uk/fsl/)) on the T1-images to create a brain mask, (c) the brain mask was then applied to the FLAIR images to remove nonbrain tissue. Next, an automated procedure identifies lesion seeds by using an intensity histogram for the image, using the mean plus 3 SD for the minimum threshold and labels these seeds. Afterwards, it uses a fuzzy connected algorithm to segment lesions while iteratively updating the seeds. When the process can no longer detect any seeds, it combines the clusters and is able to produce a mask of WMHs. The code was changed to mean +2.5 standard deviations to see if this would improve the accuracy of the program due to a low overlap with the manual selections with the original code.

2.5. Statistical Parametric Mapping (SPM). Severe WMHs appear as hypointense areas on T1-weighted images, having a similar intensity to grey matter. For this reason, the standard SPM segmentation sometimes misclassifies WMHs as grey matter [8]. The SPM segmentation segments images into a number of separate channels, utilising information regarding the a priori probability distribution of those channels. We investigated the efficacy of including an extra channel for WMHs into the SPM segmentation using an a priori probability distribution of WMHs to improve the accuracy of the segmentation.

Table 1: Subject demographic details. WMHs volume is from manual segmentation.

	Control	AD	DLB
Age: mean (SD)	77.2 (9.0)	77.3 (8.9)	75.5 (5)
Sex F : M	2 : 8	8 : 8	2 : 2
MMSE mean (range)	28.9 (27–30)	21.1 (16–27)	17.8 (15–22)
Hypertension *N* (%)	4 (40%)	6 (33%)	1 (25%)
WMHs volume median mL (range)	4.0 (0.8–46)	4.2 (1.0–34)	5.4 (1.9–28)

2.6. Creation of WMHs a Priori Map. We used a different, previously published group of 60 subjects aged over 65 [9] to create an a priori probability distribution of WMHs. FLAIR images from these subjects were segmented using the in-house WMHs segmentation program. The FLAIR images were then affine-transformed into MNI space, using the registration tools in SPM, and the transformation applied to the segmented WMHs. An average of the transformed segmented WMHs images was calculated as the a priori probability distribution of WMHs.

2.7. SPM Segmentation. We performed segmentation using both the SPM5 unified segmentation [13] and the multimodal segmentation "new segment" in SPM8. The SPM5 segmentation was performed on the T1 weighted images, whilst the SPM8 was done as a multimodal segmentation using both the T1 and FLAIR images. In both cases, we added an extra segmentation channel, using the a priori probability distribution of WMHs just described.

2.8. Manual Segmentation. Manual selection was done using in-house software written in java language by a trained biomedical sciences undergraduate student (SS). Using a mouse, the operator selected WMHs individually and then altered the intensity threshold to best outline the area of the WMHs. The program labelled these as regions of interest (ROI). Accuracy and validation of manual selection was done by self-comparison, two weeks after the original selection, and comparison to another operator with extensive experience of WMHs segmentation (MJF) over a random selection of scans from the cohort. These segmentations were compared to the original masks to find the overlap between the accuracy checks and the original selections.

2.9. ROI Overlaps and Quantitative Results. To compare segmentation methods, we calculated the overlap using

$$\text{Overlap} = 100 \times \frac{\left(\text{ROI}^1 \cap \text{ROI}^2\right)}{\left(\text{ROI}^1 \cup \text{ROI}^2\right)}, \tag{1}$$

utilising the *fslmaths* and *fslstats* tools in FSL.

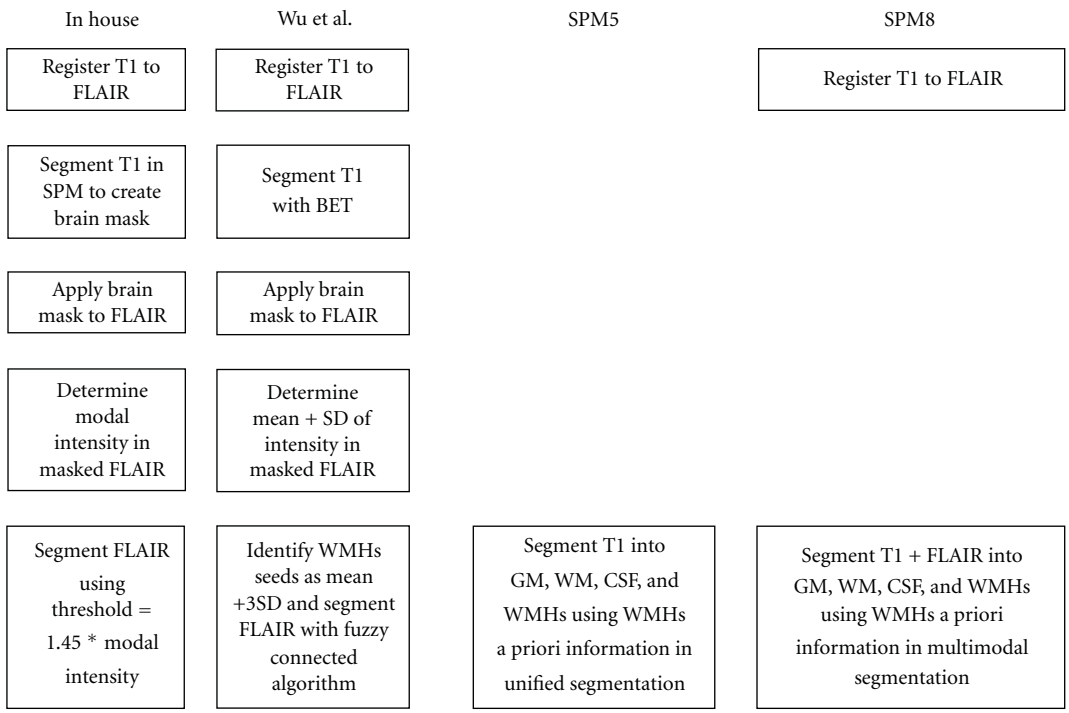

Figure 1: Overview of segmentation procedures.

We calculated the overlap of the manual segmentation versus all the automated methods.

3. Results

The results are summarised in Table 2. Figure 2 shows examples of typical segmentation.

3.1. In-House Program. The in-house program produced an average overlap of 62.24% (S.D. ± 11.45%) with the manually selected regions chosen by the operator similar to the disagreement between the two operators. On visual inspection, causes of disagreement were that the in-house program does tend to misclassify where blood flow can be seen on an image or on brain slices that display bright grey matter. This occurs on mostly on scans that do not have a large volumetric mass of WMHs to begin with and possibly caused by the modal intensity of the whole scan being low. In scans that appear to show large volume of WMHs (over 10,000 mm^3) the in-house program was very accurate over 70% overlap with the manual selection. These scans had easily definable WMHs to be marked, and the large masses of the lesions usually covered any errors misclassifying tissue.

3.2. Wu Program. The Wu program produced a mean overlap of 36.81% (S.D. ± 18%) which is markedly lower than the in-house program and also differs from the original work which appears to show good ability to identify WMHs accurately from other matter. The highest was 83.07%, while the lowest was 11.11%; the actual lowest was 2.19% but this was caused due to the preprocessing stage not properly stripping the skull from the image rather than an error in how the images were segmented. On one scan, there was an abnormally low segmentation of WMHs, on visual inspection the program appeared to be outlining small regions within the WMHs and not the whole WMHs itself. Altering the number of standard deviations to change the minimum threshold did not improve accuracy; instead, the program became more inaccurate as more non-WMHs were included in the segmentation. There was a tendency for deep WMHs and smaller clusters of WMHs to be not included in the segmentation (see Figure 2).

Table 2: Percent overlap of segmentation methods against manual segmentation.

	Mean (SD)	Range
Within rater repeat ($N = 5$)	71.4 (12)	
Between rater repeat ($N = 5$)	63.3 (18)	
In-house segmentation	62.2 (12)	38–85
Wu segmentation	36.8 (18)	11–83
SPM5 T1 segmentation	14.4 (21)	1–69
SPM8	11.9 (19)	1–65

3.3. Statistical Parametric Mapping. The SPM5 segmentation using just the T1 weighted images produced a mean overlap of 14.4%. The SPM8 segmentation using both T1 and FLAIR produced an average overlap of 11.9% (S.D. ± 18.5%).

Visual inspection showed the errors on the segmentation with just the T1 images to be from a variety of causes, both misclassification of non-WMHs regions and underestimation of WMHs. The segmentation worked best when

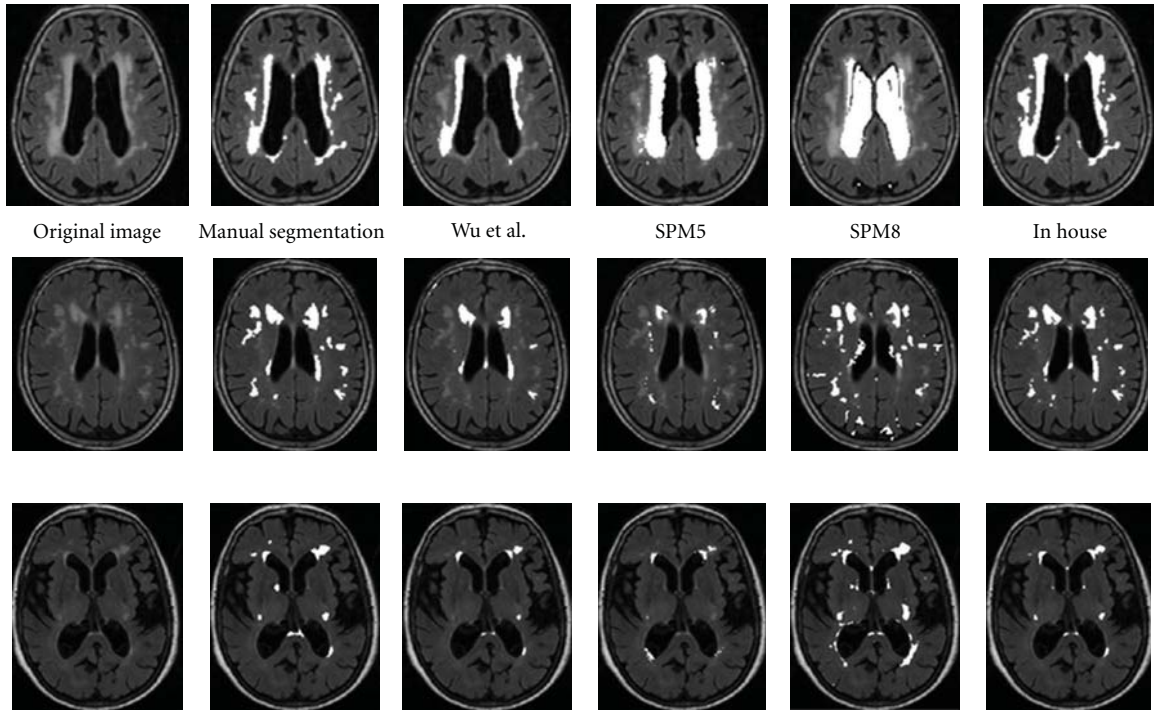

Figure 2: Segmentation results. From left: raw image, manual segmentation, segmentation with Wu et al. software, SPM5 segmentation with WMHs a priori probability included, SPM8 multimodal segmentation with WMHs a priori probability included, and segmentation with in-house software.

WMHs were large in extent. The presence of large ventricles (which overlapped with the a priori WMHs distribution) or of subtle WMHs reduced the accuracy of the segmentation. There was a tendency for the WMHs segmentation to include regions of CSF (see Figure 2) with the SPM5 segmentation including typically the ventricles, whilst the multimodal SPM8 often included sulcal CSF. The SPM5 software also often missed deep WMHs, which were located in regions of low expectation of WMHs in the a priori map.

We had expected adding the FLAIR to the segmentation to improve the results, however, this was not the case. Visual inspection showed that the SPM8 segmentations using T1 and FLAIR was very nonspecific—while regions of WMH were identified correctly, a large amount of non-WMHs were also misclassified, leading to an overall poor performance. For example, in the middle row of Figure 2, whilst the majority of the WMH have been segmented correctly, there are regions in the ventricles and around the sulci which have been incorrectly segmented as WMHs. In the lower row, the SPM8 segmentation has included a much larger region of white matter than the manual segmentation.

4. Discussion

Out of all the programs, the in-house program produced the most favourable results as a fully automated method to identify WMHs. Although only producing a reasonable accuracy for the majority of the scans, it is currently only reliable to give sufficiently accurate measurements on scans with large masses of WMHs visible. A possible method to improve accuracy would be to provide a template that can mask arteries within the brain to prevent segmentation in the area because blood flow appears as bright pixels, brighter than the WMHs, and is often selected in the segmentation process. Wu et al. reported a high success in accurately identifying WMHs in the original work [6]; however, we were unable to replicate this. Their segmentation used the SD of histogram to determine the threshold for segmenting WMHs and possibly differences in SNR between scanners affected results. Our in-house segmentation seems relatively robust to scanner differences, as the original development and testing was done on a 1.5T scanner, and it still produced a reasonable accuracy on the images from the Phillips 3T scanner. The SPM programs could segment large easily defined WMHs masses in images with reasonable accuracy (approx 67%); however, the segmentations were poor in other cases. For SPM5, the segmentation uses T1-weighted images for segmentation, and WMHs may not be as clear on these images, as they are on FLAIR images although SPM8 segmented using a dual channel of T1-weighted and FLAIR images and was on average less accurate. The dual channel approach was nonspecific, and although regions of WMHs were correctly identified, large regions of other tissue were misclassified as WMHs. One difficulty with segmenting the WMHs with the SPM approach is that WMHs are not always present, unlike GM and WM which are always present in a brain, albeit in subject dependent morphology. Hence an FLAIR image of a subject with no WMHs has a very different intensity distribution to that of a subject with a large volume of WMHs. Including information regarding the

likely local spatial and intensity characteristics of WMHs may help improve the segmentation.

We confirmed the accuracy of our in-house segmentation code on 3T images, with 70% overlap against manual segmentation. The code is suitable for large-cohort investigations of WMHs in aging.

Acknowledgments

The authors thank Dr. Wu for providing the segmentation code. They are grateful for funding from the Alzheimer's Research Trust. This work was supported by the UK NIHR Biomedical Research Centre for Ageing and Age-related Disease Award to the Newcastle upon Tyne Hospitals NHS Foundation Trust.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 617927, 13 pages
doi:10.4061/2011/617927

Review Article

Age-Related White Matter Changes

Yun Yun Xiong and Vincent Mok

Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, Shatin 999077, Hong Kong

Correspondence should be addressed to Vincent Mok, vctmok@cuhk.edu.hk

Received 5 May 2011; Revised 28 June 2011; Accepted 28 June 2011

Academic Editor: Leonardo Pantoni

Age-related white matter changes (WMC) are considered manifestation of arteriolosclerotic small vessel disease and are related to age and vascular risk factors. Most recent studies have shown that WMC are associated with a host of poor outcomes, including cognitive impairment, dementia, urinary incontinence, gait disturbances, depression, and increased risk of stroke and death. Although the clinical relevance of WMC has been extensively studied, to date, only very few clinical trials have evaluated potential symptomatic or preventive treatments for WMC. In this paper, we reviewed the current understanding in the pathophysiology, epidemiology, clinical importance, chemical biomarkers, and treatments of age-related WMC.

1. Introduction

Age-related white matter changes (WMC) are prevalent findings among the elderly. WMC are considered to be etiologically related to cerebral small vessel disease and are important substrates for cognitive impairment and functional loss in the elderly [1]. Although extensive studies have investigated various aspects on WMC, controversies still exist in the pathophysiology and clinical phenotypes, and consensus regarding to treatments for WMC has not been reached. In this paper, we aimed to provide an update review on the epidemiology, pathophysiology, neuroimaging, clinical importance, chemical biomarkers, and treatments of age-related WMC.

The literature search was conducted using the National Center for Biotechnology Information (NCBI) PubMed/Medline to identify relevant articles related to WMC that were published until June 2011. We used the following keywords for the search: white matter, white matter changes, white matter lesions, leukoaraiosis, white matter hyperintensities, and small vessel disease.

The articles were included in this paper if (1) the journal article was published in English and (2) they were related to epidemiology, pathophysiology, neuroimaging, genetics, clinical phenotypes, biomarkers, and treatment of WMC. Further searches on bibliographies in the main articles and relevant papers were performed.

2. Prevalence and Risk Factors

WMC are almost endemic in community elderly with prevalence ranging from 50% to 98% [2–6]. In stroke patients, prevalence of WMC varies from 67% to 98% [7–10]. In Alzheimer's disease, WMC are also common with prevalence ranges from 28.9% to 100% [11–13]. About 30–55% of patients with Parkinson's disease (PD) also harbor WMC [14–16]. Age [2, 4, 6, 17–20] and hypertension [3, 18, 20–30] are established risk factors for WMC. A recent Manhattan study in community elderly found that compared with individuals with low blood pressure (BP) and low fluctuations in BP, the risk of WMC increased with higher BP and BP fluctuations [31]. Associations of diabetes mellitus (DM), cholesterol, smoking, and homocysteine are less consistent between studies. Although past studies had suggested that WMC are highly heritable [32] and that several polymorphisms in various candidate genes, such as apolipoprotein E (epsilon 4±), methylenetetrahydrofolate reductase (677 cytosine/thymine polymorphism (C/T)), and angiotensinogen (Met235Thr), were found to be associated with WMC, [33–35] a recent meta-analyses failed to show

convincing evidence for an association between WMC and the candidate genetic polymorphisms [36].

3. Progression of WMC

Age-related WMC are not static lesions. The lesions may progress, or even regress, over time. Several longitudinal studies have investigated the rate and predictors for progression of WMC [37–47]. Perhaps the most consistent predictor for progression of WMC is the baseline severity of WMC [44, 47, 48]. Patients with punctate WMC usually have minimal progression of WMC, whereas those with early confluent and confluent WMC at baseline have rapid progression of WMC [44, 49]. In the Austrian Stroke Prevention Study, the median (interquartile range) volume increase over the 6-year period was 0 cm^3 in subjects with no lesions, 0.2 (0.0–1.1) cm^3 in subjects with punctuate lesions, 2.7 (0.5–5.9) cm^3 in subjects with early confluent lesions, and 9.3 (7.1–21.0) cm^3 for individuals with confluent WMC at baseline [44]. In AD and PD patients, the baseline severity of WMC also predicted lesion progression, AD median WMC progression was 0.08%, while PD dementia was 0.07% [50]. Sachdev et al. study in 51 healthy subjects with follow-up duration of 6 years found that increase in DWMC volume (43.8%) was greater than that of PVWMC (29.7%) [47]. Furthermore, female may have more lesion progression than male. A longitudinal study in 554 elders (313 men, 241 women) aged 70 to 82 years indicated that women had significantly higher DWMC volume than men at baseline; after 3 years followup, they had accumulated approximately twice as much DWMC as men, whereas their progression of PVWMC was similar to men [51]. Other factors associated with faster decline in WMC are higher age, cigarette smoking, and elevated BP [48].

4. Pathology and Physiology

Pathologically, WMC are characterized by partial loss of myelin, axons, and oligodendroglial cells; mild reactive astrocytic gliosis; sparsely distributed macrophages as well as stenosis resulting from hyaline fibrosis of arterioles and smaller vessels [52]. Nowadays, the most accepted opinion is that WMC represents incomplete ischemia mainly related to cerebral small vessel arteriolosclerosis [53].

Another mechanism is blood-brain barrier dysfunction. Small vessel alterations could lead to damage of the blood-brain barrier and chronic leakage of fluid and macromolecules in the white matter [53]. Increased concentration of cerebrospinal fluid albumin and IgG values were found in patients with CT-detected WMC [54, 55]. A recent MRI study even found that blood-brain barrier permeability increased in normal-appearing white matter in patients with WMC and its presence in normal-appearing white matter would be consistent with it playing a causal role in disease pathophysiology [56]. Moreover, a pathological study showed that albumin extravasation was widespread in the ageing brain and enhanced in WMC [57]. According to the location of the lesions, WMC can be divided into periventricular WMC (PVWMC) and deep WMC (DWMC). Pathological studies have shown that PVWMC were related to disruption of the ependymal lining with subependymal widening of the extracellular space resulting from disruption of the blood brain barrier, whereas the DWMC were mainly related to incomplete ischemic arteriolosclerosis [58, 59].

Vascular risk factors, especially hypertension, cause lipohyalinosis of the media and thickening of the vessel walls, which attributes to narrowing of the lumen of the small perforating arteries and arterioles nourishing the deep white matter [60]. The perforating vessels, which originate from cortical and leptomeningeal arteries, have a relatively poor anastomotic system, which makes the white matter vulnerable to cerebral ischemia. Hypertension can also cause disturbances in the blood-brain barrier and lead to WMC by cerebral edema, activation of astrocytes, or destructive enzymes or other poisons which pass through the damaged vessel walls [60]. DM alters the glucose and insulin transfer across the blood-brain barrier, thus affects regional metabolism and microcirculation. Chronic hyperglycemia, which further alters membrane permeability and decreases regional blood flow, might lead to permanent cell damage. Therefore, DM seems to be associated with progressive metabolic disturbance in the cerebrovascular bed that may affect blood flow and accelerate the white matter ischemia [61, 62].

Recently, postmortem Medical Research Council Cognitive Function and Ageing Study using RNA microarray and pathway analysis found that 8 major pathways in which multiple genes showed altered RNA transcription (immune regulation, cell cycle, apoptosis, proteolysis, ion transport, cell structure, electron transport, metabolism) and WMC represented areas with a complex molecular phenotype [63]. Xu et al. study revealed that 241 genes specific for WMC expression were associated with inflammation, oxidative stress, detoxification, and hormonal responses, included genes associated with brain repair, long-term potentiation, and axon guidance, and included genes associated with oligodendrocyte proliferation, axon repair, long-term potentiation, and neurotransmission [64]. These neurogenetic findings support the ischemia, blood-brain barrier dysfunction, systemic oxidative stress, and inflammation in the pathogenesis of WMC, as well as other potential processes in the pathogenesis which warrant future research.

Other mechanisms hypothesized to be involved in the pathophysiology of WMC encompassing dysfunction of vasomotor reactivity and autoregulation [65–69], chronic edema [70, 71], apoptosis [72], and endothelial dysfunction [73, 74].

Therefore, the pathophysiology of WMC is complex and may be multifactorial. Further studies should address more on how these different pathways interact with each other.

5. Neuroimaging Assessment

WMC are ill defined hypodensities on CT. On MRI, which is more sensitive than CT on delineating the lesions, they appear as hypointensities on T1-weighted imaging and hyperintensities on T2-weighted imaging, proton density and fluid-attenuated inversion recovery sequences (FLAIR) (Figure 1). The FLAIR sequence is probably the best to assess the severity of WMC because of clear distinction

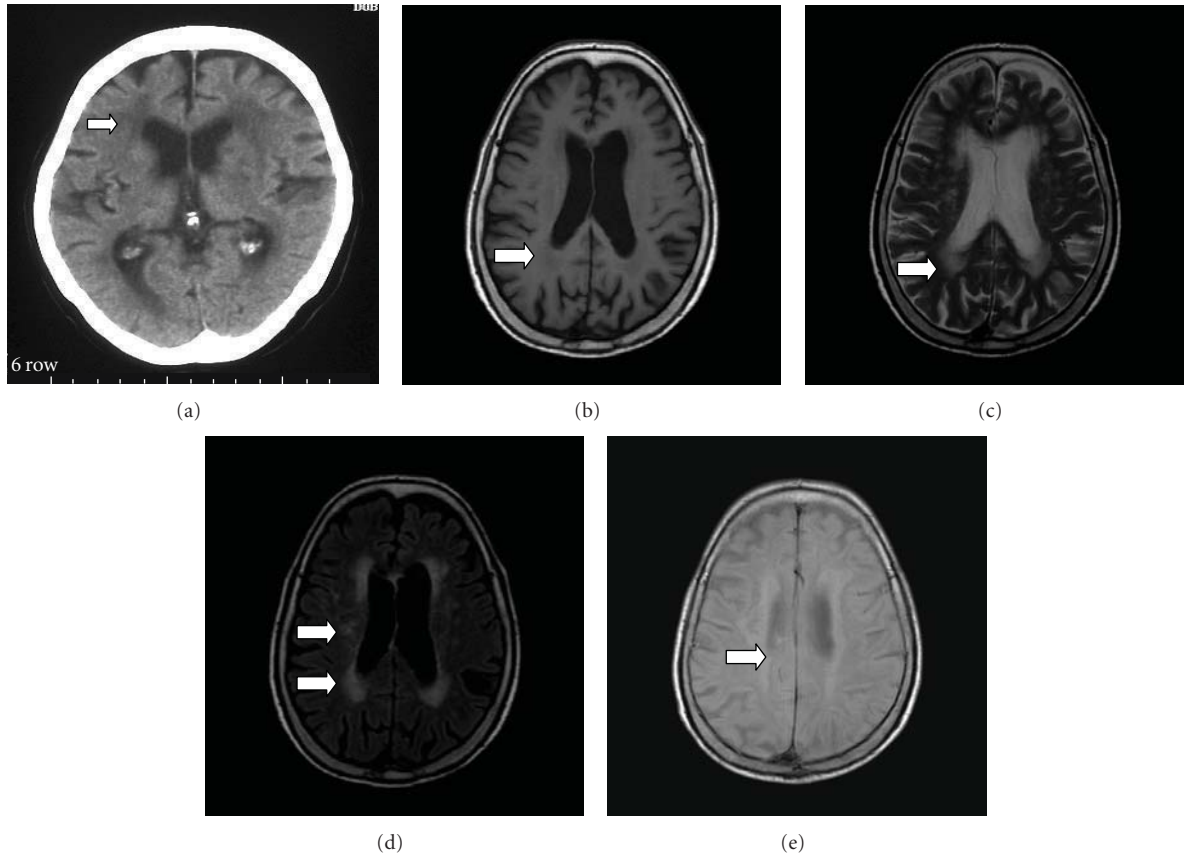

Figure 1: WMC on CT and MRI. (a) Hypodensities on CT; (b) hypointensities on T1-weighted MRI; (c, d, e) hyperintensities on T2-weighted MRI, proton-density, and FLAIR sequences, respectively. (d) The lower arrow shows the PVWMC, and the upper one shows the DWMC.

between ventricles and the PVWMC. Lesions are defined as PVWMC when their largest diameters are adjacent to the ventricles, otherwise they are considered as DWMC [75]. The more recent diffusion tensor imaging (DTI) technique provides information on the integrity of white matter tracts by estimation of the diffusion trace (mean diffusivity) and the directionality-fractional anisotropy (FA). In WMC, mean diffusivity is elevated and FA is reduced [76], which suggests impaired white matter integrity. Measures of DTI are probably more sensitive than WMC volume in detecting cognitive changes over time [77].

As to the assessment of WMC severity, various visual rating scales have been proposed. The visual rating scales are quick and easy to perform on different quality of scans [78]. However, they varied from each other, and data is not quantitative and has ceiling effect. In addition, discrepancies between the various scales may lead to inconsistent findings. Two most popular visual rating scales are Fazekas scale [79] which has been validated histopathologically and Scheltens scale [80] which is detail with good reliability but relatively time-consuming. To unite the visual rating for WMC, the vascular cognitive impairment harmonization standard recommends the age-related WMC (ARWMC) scale [81] as the preferred visual rating scale [82]. This scale can be applied to both MRI and CT with moderate to excellent reliability, and it has been validated against volumetric measurement and cognitive impairment [83]. The operationalized ARWMC scale further gave operational definitions on ARWMC scale and improved interrater reliability on CT [84]. Noteworthy is that visual rating scale for assessing the progression of WMC is lacking. Both the Rotterdam progression scale [85] and Schmidt progression scale [42] were correlated with volumetric measurements. The Rotterdam progression scale had moderate to good reliability (weighted Cohen's = 0.63 (intraobserver), 0.59 (interobserver)), whereas the Schmidt progression scale was less reliable [85, 86].

Nowadays, fully automated techniques and semiautomated segmentation methods become increasingly available. Different from the visual rating scales, volumetric measurement is more accurate and provides continuous data without ceiling effect. However, it is more time-consuming with higher requirement of expertise and excellent quality of MRI, which limits its use for research purpose. Overall, volumetric method is preferred over visual rating scales for longitudinal studies.

6. Clinical Importance

6.1. Cognitive Impairment and Decline. Large amounts of evidence shows that WMC are associated with cognitive

impairment (executive function [2, 87, 88], mental processing speed [89, 90], and global cognition [2, 91]) and long-term cognitive decline in both community and stroke patients [92]. Although some inconsistent results exist, part of the discrepancies stem from different sensitivities of rating scales for WMC, small sample size, and use of different neuropsychological tests [60]. Longitudinal studies have demonstrated that WMC progression parallels cognitive decline [92]. Baseline PVWMC volume was longitudinally associated with reduced mental processing speed [93] and increased the risk of dementia [94, 95]. In some studies, when brain atrophy was added into the predicting models, influence of WMC became insignificant whereas global and/or regional atrophy (i.e., medial temporal lobe atrophy, cortical gray matter (cGM) and hippocampus atrophy) exerted greater influence upon cognitive decline [96–98]. These studies also showed that whole brain or cGM atrophy was related to severity of WMC. It was thus proposed that cognitive decline in patients with WMC was mediated by brain atrophy [96]. The hypothesized mechanisms of how WMC induce cortical atrophy include demyelination of axons leading to cortical-subcortical deafferentation and subsequent secondary cortical neuronal loss, [99, 100] hypoperfusion, and hypometabolism [101, 102], as well as concomitant cortical microinfarct, which its detection is beyond the ability of current neuroimaging techniques [102]. A recent study also showed that severe WMC were associated with hippocampus atrophy [103]. Moreover, Smith et al. study in community residents revealed that WMC progression might predict normal to mild cognitive impairment, whereas global atrophy predicted mild cognitive impairment to dementia [104]. With the development of DTI technique, studies indicated that microstructural integrity of both WMC and normal-appearing white matter was associated with cognitive function, regardless of white matter atrophy, WMC volume, and lacunar infarcts [105, 106].

Certain factors need to be considered in the evaluation of the relationship between WMC and cognitive impairment. First, the relationship between WMC and cognitive impairment may not be linear, and a threshold effect was proposed [107]. Second, cognitive impact of WMC may vary with its location. PVWMC may affect cognition more than DWMC. Thus, studies evaluating total WMC may dilute the cognitive influence of region-specific WMC [108]. Third, psychometric tests in studies may not be comprehensive for the assessment of executive function; hence, the impact of WMC may be underestimated [108]. Forth, silent brain infarcts and microbleeds were reported to be associated with cognitive impairment as well [109–113], and brain atrophy (cGM, hippocampus and medial temporal lobe atrophy) may be a confounder between WMC and cognitive impairment, these neuroimaging measures were not assessed in some studies. Last, future studies should utilize DTI in exploring the mechanisms of cognitive decline and as a surrogate marker for disease progression in therapeutic trials.

6.2. Gait Disturbance and PD. Both cross-sectional and longitudinal studies have found that WMC were associated with gait disturbance and falls [114–121]. Tasmanian Study of Cognition and Gait study showed that the risk of incident falls was doubled in people with WMC volumes in the highest quintile of its distribution compared with the lowest (adjusted relative risk 2.32, 95% confidence interval: 1.28–4.14) [122]. Regardings the WMC location on gait, Tasmanian Study of Cognition and Gait study found that bilateral frontal and periventricular WMC-affected voxels corresponded to major anterior projection fibers (thalamic radiations, corticofugal motor tracts) and adjacent association fibers (corpus callosum, superior frontooccipital fasciculus, short association fibers) showed the greatest covariance with poorer gait [123]. A DTI study also found white matter integrity in the genu of corpus callosum was an important marker of gait in the elderly [124]. Another two recent DTI studies revealed that in elderly subjects with small vessel disease, widespread disruption of white matter integrity, predominantly in the normal-appearing white matter, was involved in gait disturbances [125, 126]. Iseki et al. study using single-photon emission-computed tomography suggested that abnormalities in the basal ganglia-thalamocortical loops partly explained gait disturbance in WMC [127]. Thus, accumulating evidence suggests that the disruptions in motor network may account for gait disturbance in WMC.

WMC correlate with gait disturbance in community residents, and some of these abnormalities overlap with features of PD. In postmortem study with 700 parkinsonism cases, 27 brains (3.9%) showed WMC and/or lacunes in the basal ganglia, white matter, or brainstem, without significant nigral lesions [128]. Studies also found that WMC contributed to dementia in PD patients [16, 129, 130]. Albeit inconclusive, evidences accumulating that cognitive effects of WMC tend to preferentially affect executive functions and may reflect frontal lobe WMC [129].

6.3. Urinary Incontinence. Studies had shown that WMC were associated with urgency urinary incontinence [131–135]. A community study found that among 100 residents, 64% of them had urinary incontinence. The presence of WMC in right inferior frontal regions and selected WM tracts predicted incontinence, incontinence severity, and degree of bother. The study confirmed a critical role for the cingulum in bladder control and suggested potential involvement of anterior corona radiata and superior frontooccipital fasciculus [132]. A study in old women indicated that the presence of WMC in specific pathways (anterior thalamic radiation and superior longitudinal fasciculus) might affect continence control [136].

6.4. Depression. Lines of evidence suggested that WMC are associated with late life depression [45, 137–144]. In poststroke patients, severe deep WMC predicted poststroke depression [145]. And concurrent atrophy of left inferior frontal gyrus was associated with depressive symptoms in poststroke patients with severe WMC [146]. Vascular depression hypothesis proposed that WMC causes depression by disrupting fiber tracts within frontostriatal circuits [142]. Because of their involvement in the regulation of

mood, disruption of frontostriatal circuits might lead to a disconnection syndrome that corresponded to the clinical and neuropsychological profile of depression [142]. A DTI study also found that frontolimbic neural pathways might contribute to the pathophysiology of depression [147].

6.5. Stroke and Death. The WMC increased the risk for stroke [8, 95, 148, 149] and death [149–151]. A recent meta-analysis revealed that stroke yielded a significant association of WMC with incident stroke (HR 3.5 (2.5–4.9), $P < 0.001$) and increased risk of death (HR 2.0 (1.6–2.7), $P < 0.001$) [92]. In patients treated with thrombolysis for acute stroke, the rate of symptomatic intracerebral hemorrhage increased by 10% in patients with severe WMC and multiple lacunes [152]. WMC were found to be independent determinants for intracerebral hemorrhage after controlling for age and other risk factors [152, 153].

WMC were not benign but predict poor clinical and functional outcomes. Some studies also indicated that they, especially DWMC, were associated with migraine [154, 155]. And brain stem WMC were associated with dizziness [156]. More studies are in need to explore the clinical significance of WMC.

7. Chemical Biomarkers

Homocysteine is a dietary sulphur-containing amino acid derived as an intermediate during the metabolism of methionine [157]. Nutritional deficiencies in the vitamin cofactors (folate, vitamin B_{12}, and vitamin B_6) required for homocysteine metabolism may promote hyperhomocyst(e)inemia [158]. Perini et al. study found that the hyperhomocysteinemia in acute stoke stage was associated with higher risk of small artery disease subtype of stroke [159]. Many studies had indicated that hyperhomocysteinemia was an independent predictor for WMC independent of smoking, hypertension, or age [160–172].

Inflammatory biomarkers as intercellular adhesion molecule-1 (ICAM-1) [173, 174], and high sensitive C-reactive protein (Hs CRP) [175–177] were also reported to be associated with WMC load. However, these studies were cross-sectional so that causal relationship cannot be determined. Longitudinal studies are needed to study the relationship between progression of inflammatory factors with progression of WMC.

8. Treatment of WMC

The WMC are predictors for poor clinical outcomes and important substrates for vascular dementia. The European Task Force on age-related WMC recommended that clinical trials on cerebral small vessel disease should target those with severe WMC and use its progression as surrogate marker in clinical trials [49]. Albeit WMC are clinically important, very few clinical studies had been conducted so far to evaluate treatments for WMC. In this part, studies on treatments for WMC- and WMC- related vascular dementia are reviewed.

8.1. BP Lowing Therapy. The Epidemiology of Vascular Ageing MRI study has shown a positive linear relationship between BP and severity of WMC [28]. Dufouil et al. study retrospectively found subjects receiving regular treatment of hypertension had less severe PVWMC than those receiving no or irregular treatment of hypertension. Due to its cross-sectional design, the treatment effect of hypertension on WMC progression cannot be examined in this study. The Perindopril Protection against Recurrent Stroke Study (PROGRESS) MRI substudy [178] was a longitudinal randomized placebo-controlled trail investigating the BP lowing therapy using perindopril or perindopril plus indapamide on WMC progression. 192 participants were followed up for 36 months, the mean total volume of new WMC was significantly lower in the active treatment group compared with the placebo group, and the difference was greatest for patients with severe WMC at entry [178].

An open-label study [179] and a post hoc analysis of a randomized trial [180] showed some beneficial effects of nimodipine in patients with subcortical vascular dementia. A recent randomized placebo-controlled trial also found that the calcium antagonist nimodipine could slow down global cognitive decline in patients with small vessel disease related vascular dementia [181]. A recent review in vascular cognitive impairment indicated that nicardipine has been investigated in more than 6000 patients, with improvement of cognitive deterioration in more than 60% of patients treated [182]. The antihypertensive activity of nicardipine and its safety and effectiveness in cognitive domain suggested reconsidering this drug in the treatment of cognitive impairment of vascular origin and for reducing the risk of recurrent stroke in patients at high risk of it [182]. Further randomized double-blind placebo-controlled trail is needed to explore the efficacy and safety of nimodipine and nicardipine upon WMC progression.

Although most studies paid attention to lower BP in hypertension, noted that low BP was a risk factor for WMC as well [60]. Another study found that nocturnal BP dipping was associated with WMC [183]. Thus, a randomized controlled study dedicated to examine the effects of BP lowering therapy upon progression of WMC is needed.

8.2. Statins. Statins have long been demonstrated to reduce cardiovascular events and ischemic stroke among patients with coronary heart disease [184]. Whether statins affect progression of WMC is still controversial. The PROSPER (Prospective Study of Pravastatin in Elderly at Risk) study examined the effect of pravastatin 40 mg daily on the progression of WMC in 270 placebo-treated subjects and 265 active subjects within a period of 33 months. The study failed to demonstrate an overall beneficial effect of statins upon WMC progression. However, data on proportions of subjects having different WMC severity are lacking, and stratified analysis based on WMC severity was not performed in the study. In the Cardiovascular Health Study, 3334 community participants were followedup over an average observational period of 7 years [185]. Patients treated with statins were observed to have slightly less cognitive decline than untreated subjects. This significant cognitive benefit was associated

with reduced progression in cerebral infarcts among the treated subjects, whereas progression of WMC was not statistically different between these two groups. Although the findings may suggest that statins exert cognitive benefits independent of WMC progression, the visual rating scale used in that study was unlikely to be sensitive in detecting WMC progression [38]. The ROCAS (Regression of Cerebral Artery Stenosis) study evaluated simvastatin on WMC progression in patients with asymptomatic middle cerebral artery stenosis [38]. Two hundred and eight randomized subjects were assigned to either placebo ($n = 102$) or simvastatin 20 mg daily ($n = 106$) for 2 years. Simvastatin group did not slow the progression of WMC volume compared with the placebo group, but in those with severe WMC at baseline, the median volume increase in the simvastatin group ($1.9\,cm^3$) was less compared with that in the placebo group ($3.0\,cm^3$; $P = 0.047$). However, in this study, treatment probably prevented WMC progression among those with severe WMC at baseline was based only on subgroup analysis upon a small subset of subjects. Furthermore, the subjects of this study belonged to a high-risk group in that all our subjects had concurrent MCA stenosis. Hence, the findings may not be applicable to patients with less vascular burden or to those with WMC but without concurrent MCA stenosis.

Furthermore, a recent cross-sectional study showed that low cholesterol had more severe WMC in acute stroke patients [10]. Other studies found that low cholesterol was associated with intracerebral hemorrhage and high mortality in these patients [186, 187]. Hence, effects of stains and cholesterol control upon WMC progression are still uncertain, and randomized clinical trials are needed to address this issue.

8.3. Acetylcholinesterase Inhibitors and N-Methysl-D-Aspartate (NMDA) Receptor Antagonists. Acetylcholinesterase inhibitors (donepezil, galantamine, and rivastigmine) and *N*-methyl-D-aspartate (NMDA) receptor antagonists (memantine) have been approved for treatment of AD. Kavirajan and Schneider reviewed three donepezil, two galantamine, one rivastigmine, and two memantine placebo-controlled, randomized, double blinded trials, it showed that cognitive effects on the AD assessment scale-cognitive subscale (ADAS-cog) were significant for all drugs, and post hoc analyses of donepezil tails suggested greater improvement in patients with cortical and territorial lesions compared with those with predominantly subcortical small vessel disease related lesions [188]. By contrast, cognitive benefits in the memantine trials appeared to be more pronounced for patients with small vessel disease than for those with large vessel disease and such benefits derived largely from worsening in patients in the placebo-treated groups who predominantly had small vessel disease [188, 189]. With regards to safety profile, use of cholinesterase inhibitor significantly increased the odds of having adverse events (e.g., anorexia, nausea, vomiting, and diarrhea), while memantine was found to be well tolerable and safe. Overall, the data is insufficient to support the widespread use of acetylcholinesterase inhibitors (donepezil, galantamine, and rivastigmine) and memantine in patients with vascular dementia [188]. Yet, given the potential benefit of memantine upon subcortical vascular dementia based on post hoc analysis and its favorable safety profile, conducting a randomized study evaluating its efficacy in subcortical vascular dementia may be worthwhile.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is a genetic form of subcortical vascular dementia. A recent multicentre, 18-week, placebo-controlled, double-blind, randomized parallel-group trial using donepezil in 168 CADASIL patients revealed that donepezil had no effect on the vascular ADAS-cog score in CADASIL patients with cognitive impairment. Improvements were noted only on several measures of executive function, which might possibly suggest that cholinergic pathways were involved in the executive function [190]. Overall, findings of this study are similar to that of previous studies using acetylcholinesterase inhibitors in that acetylcholinesterase inhibitors might only induce subtle cognitive benefits among patients with vascular dementia.

Another more recent randomized, international, multicenter, 24-week trial in 974 probable or possible vascular dementia patients who received donepezil 5 mg/d or placebo found that donepezil improved the vascular ADAS-cog score but not global function [191]. However, subgroup analysis on the effects of donepezil upon subcortical type of vascular dementia was not performed in this study.

8.4. Homocysteine Lowering Therapy. Lines of evidence have shown that hyperhomocysteinemia was associated with WMC through endothelial dysfunction [160–172]. Whether homocysteine lowering therapy by means of multivitamins retards the progression of WMC or not is uncertain. A randomized double-blind, parallel, placebo-controlled trial on homocysteine lowering therapy is the VITAmins TO Prevent Stroke (VITATOPS) study. 8164 patients with recent stroke or transient ischaemic attack (within the past 7 months) received one tablet daily of placebo ($n = 4089$) or B vitamins (2 mg folic acid, 25 mg vitamin B_6, and 0.5 mg vitamin B_{12}, $n = 4075$) with a median followed-up duration of 3.4 years. Although vitamin treatment was not significantly more effective than placebo in reducing the incidence of the composite primary endpoint of stroke, myocardial infarction, or vascular death, in the subgroup analyses, homocysteine lowering might have preferential benefit in small vessel disease patients (risk ratio 0.80 (95% CI : 0.67–0.96)) [192]. The VITATOPS MRI substudy is currently underway to evaluate whether vitamins can slow WMC progression and/or cognitive decline.

9. Conclusion

WMC are common in elderly, and they are not benign. More extensive WMC are associated with a host of poor clinical outcomes. Although WMC have been shown to be associated with small vessel disease, age, and other vascular risk factors, the exact mechanisms explaining such association are still uncertain. To date, dataon the effectiveness of various

treatments (e.g., BP lowering, statins) in preventing WMC progression were derived mainly from subgroup analyses. Randomized studies dedicated in evaluating treatments for preventing WMC progression and its clinical correlates are thus urgently needed. Although some studies have suggested the efficacy of nimodipine, nicardipine, and memantine in subcortical vascular dementia, further randomized controlled studies are needed to clarify their effectiveness and safety.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 438978, 7 pages
doi:10.4061/2011/438978

Review Article

Connecting Cerebral White Matter Lesions and Hypertensive Target Organ Damage

Cristina Sierra,[1,2] Alfons López-Soto,[2] and Antonio Coca[1]

[1] *Hypertension Unit, Department of Internal Medicine, Institute of Medicine and Dermatology, Hospital Clinic of Barcelona, IDIBAPS, University of Barcelona, Villarroel 170, 08036 Barcelona, Spain*
[2] *Geriatric Unit, Department of Internal Medicine, Institute of Medicine and Dermatology, Hospital Clinic of Barcelona, IDIBAPS, University of Barcelona, Villarroel 170, 08036 Barcelona, Spain*

Correspondence should be addressed to Cristina Sierra, csierra@clinic.ub.es

Received 4 March 2011; Accepted 2 June 2011

Academic Editor: Ana Verdelho

Chronic hypertension leads to concomitant remodeling of the cardiac and vascular systems and various organs, especially the brain, kidney, and retina. The brain is an early target of organ damage due to high blood pressure, which is the major modifiable risk factor for stroke and small vessel disease. Stroke is the second leading cause of death and the number one cause of disability worldwide and over 80% of strokes occur in the elderly. Preclinical hypertensive lesions in most target organs are clearly identified: left ventricular hypertrophy for the heart, microalbuminuria for the kidney, fundus abnormalities for the eye, and intima-media thickness and pulse wave velocity for the vessels. However, early hypertensive brain damage is not fully studied due to difficulties in access and the expense of techniques. After age, hypertension is the most-important risk factor for cerebral white matter lesions, which are an important prognostic factor for stroke, cognitive impairment, dementia, and death. Studies have shown an association between white matter lesions and a number of extracranial systems affected by high BP and also suggest that correct antihypertensive treatment could slow white matter lesions progression. There is strong evidence that cerebral white matter lesions in hypertensive patients should be considered a silent early marker of brain damage.

1. Introduction

Chronic hypertension leads to concomitant remodeling of the cardiac and vascular systems, and various organs, especially the brain, kidney, and retina [1, 2]. Early detection of hypertensive target organ damage is important for more-successful prevention of cardiovascular diseases and to improve outcomes [1, 2]. The brain is an early target for organ damage due to high blood pressure (BP) [1, 2], which is the major modifiable risk factor in men and women for ischemic and hemorrhagic stroke [3], as well as small vessel disease [1, 2, 4] predisposing to lacunar infarction, white matter lesions (WML), and cerebral microbleeds, which are frequently silent [1, 2, 5].

Stroke is the second leading cause of death and the leading cause of disability worldwide [6]. For each decade of life after the age of 55 years, the stroke rate doubles in both men and women, and >80% of strokes occur in people aged ≥65 years [6]. Because of the aging population, the burden of stroke will increase greatly in forthcoming years. The increased vulnerability of elderly people to stroke is associated with changes in the aging brain and also with a higher prevalence of well-documented risk factors for stroke such as hypertension, atrial fibrillation, carotid stenosis, and cardiovascular disease [3].

The clinical significance and pathological substrate of WML are incompletely understood. It is known that WMLs are an important prognostic factor for stroke, cognitive impairment, dementia and death [7]. Cerebral WML are more common and extensive in patients with cardiovascular risk factors, such as hypertension and diabetes mellitus, heart disease, and symptomatic cerebrovascular disease [7–10]. However, it is controversial whether BP still is associated with WML in patients manifesting vascular disease. Vlek et al. [11] showed in 1030 patients manifesting vascular disease (including cerebrovascular disease, coronary

heart disease, peripheral arterial disease, abdominal aortic aneurysm), that BP was not associated with the presence of WML, irrespective of the presence of diabetes mellitus or the localisation of vascular disease.

Older age and hypertension are constantly reported to be the main risk factors for cerebral WML [4, 10]. Hypertensive patients have a higher rate and extension of cerebral WML compared with normotensives [10, 12], but treated, controlled hypertensive patients have a lower prevalence of WML than both untreated and treated but uncontrolled hypertensives [12]. A magnetic resonance imaging (MRI) substudy of the randomized PROGRESS trial of BP lowering with perindopril versus placebo in normotensive and hypertensive subjects with cerebrovascular disease found that the mean total volume of new WML was significantly reduced in the active treatment group compared with placebo [13]. A *post hoc* analysis found that greatest beneficial effect of antihypertensive therapy on WML progression was observed in patients with severe WML at study entry. Godin et al. [14] have recently shown that, in a prospective population-based cohort of 1319 subjects aged ≥65 years, BP at baseline and changes in BP over a 4-year follow-up are strong predictors of WML volume progression independently of potential confounders and suggest that correct antihypertensive treatment could slow WML progression. Interestingly, the Cardiovascular Health Study [15] has been recently shown that diastolic BP had no effect on ischemic stroke incidence in elderly patients with low WML levels but had a marginally significant J-curve relationship with ischemic stroke in elderly with high WML levels. Indeed, in elderly individuals with low-grade WML, low DBP may not pose a risk for ischemic stroke. However, in high-grade WML, ischemic stroke risk may increase in diastolic BP less than 69 mmHg but is highest more than 80 mmHg. The main current hypothesis concerning the association between high BP and WML is that long-standing hypertension causes lipohyalinosis of the media and thickening of the vessel walls, with narrowing of the lumen of the small perforating arteries and arterioles that nourish the deep white matter. The perforating vessels, which originate in the cortical and leptomeningeal arteries, have a relatively poor anastomotic system, which makes the white matter particularly vulnerable to cerebral ischemia. Low BP has also been reported to be a risk factor for WML [4]. Vuorinen et al. [16] showed in a 20-year follow-up study that risk of late-life WML was related to midlife hypertension, and hypertension from midlife to late life also increased the risk of WML. In addition, an association with WML was seen for decreasing BP (hypertension at midlife but not at late life) even after controlling for antihypertensive treatment. Authors speculate that the decline in BP could be secondary to dementia-related processes, when structures involved in BP regulation become affected. It has been shown in elderly people that severe WMLs frequently coexist with medial temporal lobe and global brain atrophy [17].

There is strong evidence that cerebral WML in hypertensive patients should be considered a silent early marker of brain damage [2]. Early atherosclerotic changes in the cerebrovascular system, ultimately leading to incident stroke and cognitive impairment or dementia [4, 7], could be mirrored by hypertensive target organ damage in the cardiac, renal, retinal, and vascular and other systems. Therefore, assessment of hypertensive target organ damage in these systems may be indicative of the extent of vascular disease in less easily accessible sites such as the brain.

2. Cerebral WML, Left Ventricular Hypertrophy, and Geometry

Several forms of heart damage, such as heart failure, coronary heart disease, and cardiac arrhythmias have been associated with WML [10] although the underlying pathogenetic mechanisms are not clear. Studies have reported that echocardiographically determined left ventricular hypertrophy (LVH) is an independent risk factor for cardiovascular morbidity and mortality in essential hypertensive patients [18, 19]. Bikkina et al. [20] demonstrated that left ventricular mass (LVM) was associated with an increased risk of cerebrovascular events such as stroke and transient ischemic attack in an elderly cohort from the Framingham Heart Study. It is suggested that left ventricular geometric patterns add prognostic information on the development of cardiovascular disease [21] and the presence of extracardiac target organ damage in essential hypertension [22, 23]. Hypertensive patients with concentric LVH have more-advanced target organ damage, including renal [22, 23] and retinal [23] involvement, than patients with other patterns of left ventricular geometry. Some studies have found an association between LVH and cerebral WML [23–28], but others have not [29]. We found a close relationship between silent WML and concentric LVH in middle-aged untreated essential hypertensive patients, with WML being more common among patients with concentric LVH [27]. This association was independent of the degree of BP elevation. The mechanisms connecting LVH and cerebrovascular damage are unclear and might reflect long-term exposure to genetic, hormonal, or metabolic factors in addition to BP [30]. It is difficult to differentiate the relative role of high BP from the direct contribution of LVH to the increased risk of cerebrovascular disorders, and longitudinal studies are necessary. However, detection of cardiac hypertrophy may help to identify patients at risk of cerebrovascular injury [2].

3. Cerebral WML and Early Renal Alteration

The brain and the kidney are highly vascular structures that respond to diseases such as hypertension and diabetes mellitus in similar ways at the microscopic level. In nephrosclerosis, gradual alterations in the renal endothelial cells, glomeruli, and interstitial spaces lead to glomerular leakage of serum proteins into the urine. If a similar process were occurring at the endothelial level in cerebral microvessels, serum proteins would pass into the cerebral extracellular space. Neuropathological studies show that white matter hyperintensities may represent enlarged perivascular spaces and perivascular demyelination, among other mechanisms [31]. These changes are what might be expected if the cerebral extracellular spaces were exposed to proinflammatory

proteins which, in healthy individuals, should remain within the vascular space. While there is no direct proof that this process occurs, some studies have shown that WMLs are associated with microalbuminuria [32–34].

It has been suggested that the brain and the kidney have a common, unique way of reacting to fluctuations in BP and flow due to similar low-resistance vascular beds. High-pressure fluctuations in the carotid, vertebral, and renal arteries, together with the turbulent flow, expose the small vessels of these two organs to pressure and flow fluctuations that may explain the microvascular damage and the resulting renal failure and neurological and cognitive alterations [35]. In addition, chronic renal disease (CRD) has been linked with proinflammatory and procoagulant states [36], which may contribute to WML [37, 38]. Therefore, information on renal microvascular damage may provide information on cerebral damage. Small vessel disease is a systemic condition which is induced by aging and exacerbated by vascular risk factors, especially hypertension, and affects the brain and other systems.

In the kidney, the damage markers are albuminuria/proteinuria and the estimated glomerular filtration rate (eGFR), which shows the functional reduction. Khatri et al. [39] studied 615 stroke-free subjects (mean age: 70 years) and found that the prevalence of silent WML was independently associated with CRD (people with eGFR between 15–60 mL/min per 1.73 m^2 when compared to people with 60–90 mL/min, and >90 mL/min) even after adjustment for age, sex, ethnicity, education, and vascular risk factors [24]. The Rotterdam study [40] of 484 subjects (mean age: 73.4 years; mean systolic BP: 145.7 mmHg; mean eGFR: 54.8 mL/min/1.73 m^2) found that individuals with a lower eGFR had more WML.

Takahashi et al. [41] recently studied 2,103 asymptomatic individuals with a younger mean age of 56 years and found that the prevalence of subcortical WML and periventricular WML correlated significantly with lower eGFR; in subgroups with eGFR ≥90, 60–89, and <60 mL/min/1.73 m^2, respectively, the prevalences were subcortical WML: 18%, 21%, and 37%, respectively; and periventricular WML: 7%, 10%, and 21%, respectively. Mean age differed significantly between groups (mean 51, 55, 63 years, resp.), and BP ≥ 130/85 mmHg was significantly more prevalent as eGFR rose.

Wada et al. [33], in a study of 625 Japanese individuals aged >61 years, found that subjects with lower eGFR had higher grades of silent WML, that mean grades of WML were greater in subjects with albuminuria than in those without, and that age and the prevalence of hypertension were significantly higher in individuals with higher grades of WML. Knopman et al. [32] also found an independent association between microalbuminuria and WML in 1251 asymptomatic individuals (mean age: 63.8 years, 78% with hypertension).

4. Cerebral WML and Retinal Microvascular Abnormalities

The retina offers a unique, noninvasive, and easily accessible window to study the microvascular etiology of cerebrovascular disease. Retinal and cerebral small vessels share similar embryological origins, anatomical features, and physiological properties [42].

Retinal microvascular abnormalities, such as microaneurysms, retinal hemorrhages, soft and hard exudates, arteriovenous nicking, and retinal focal arteriolar narrowing, usually result from small-vessel damage due to aging and high BP [43].

Arteriolar narrowing may be a sign of hypertension, a history of hypertension in the last three to six years, or a risk factor for the onset of hypertension in normotensive individuals [44]. Large prospective studies (the ARIC, Beaver Dam, Blue Mountains Eye, and Rotterdam studies) have found that reduced arteriolar diameter is an independent risk factor for developing hypertension within 3 to 10 years in normotensive individuals [43].

Studies have shown that retinal microvascular flow is reduced in persons with WML and lacunar infarction [45] and that retinal and cerebral arterioles share a similar histopathology in patients dying from stroke [46]. In the ARIC study, retinal abnormalities were associated with concurrent BP [47]. In a cohort of 1684 asymptomatic people aged 51–72 years from the ARIC study, individuals with WML were more likely to have retinal microvascular abnormalities [48]. In general, in this study, WMLs were significantly associated with increasing age, black ethnicity, and—after adjusting for age, sex, and ethnicity—with higher BP and increased carotid intima-media thickness (IMT). Retinopathy was significantly associated with black ethnicity and, after similar adjustment, with higher systolic BP, fasting glucose level, diabetes mellitus, and increased carotid IMT [48]. After a follow-up of 10 years, retinal microvascular abnormalities measured at baseline were prospectively associated with a long-term risk of subclinical cerebrovascular disease on MRI, independent of conventional risk factors in this population-based cohort of middle-aged persons without clinical stroke [49]. The authors suggested that retinal microvascular abnormalities are early and, possibly, more sensitive markers of subclinical cerebral small-vessel disease before radiological and clinical manifestations become apparent. In a cohort of 1717 people from the Cardiovascular Health Study [50] (mean age: 78.3 years; hypertension: 56.3%; diabetes mellitus: 13%; previous cardiovascular disease, including stroke: 23.8%) associations were found between WML grade, prevalent lacunar infarct, and a lower arteriovenous ratio. In 174 patients without a history of transient ischemic attack or stroke before follow-up, WMLs worsening on MRI 5 years later and incident lacunar infarct were associated with a lower arteriovenous ratio [50].

In light of data reporting that persons with a smaller arteriolar-to-venular ratio tended to have more WMLs on MRI, the Rotterdam study evaluated whether this could be due to arteriolar narrowing or venular dilatation [51]. In a population-based cohort study of 490 people without dementia (age 60–90 years; mean age: 68.4 years; mean systolic BP: 136.7 ± 19.9 mmHg; diabetic patients: 6.3%), cerebral small vessel disease was evaluated at baseline. A mean of 3.3 years later, 279 persons had a second MRI. Lacunar infarcts and WML at baseline, and incident infarct and changes in periventricular and subcortical WML and progression, were

evaluated in the follow-up. Neither venular nor arteriolar diameters were related to the severity of cerebral small vessel disease. Larger venular diameters were, however, associated with a marked progression of cerebral small vessel disease [51]. It has been hypothesized that retinal venular dilatation occurs in response to retinal hypoxia and venular dilatation has been also described as one of the earliest changes in diabetic retinopathy.

Retinal microcirculation abnormalities, including retinopathy, reduced arteriolar diameter and, increased venular caliber are widely observed in the general population [43]. The abnormalities observed in retinopathy may reflect disorders of the retinal vascular wall, endothelial dysfunction, and inflammation secondary especially to diabetes mellitus, age, hypertension, obesity, and metabolic disorders. The decreased arteriolar diameter signals the presence of hypertension (current or old) and the risk of hypertension onset. An increased venular diameter has been associated with diabetes mellitus, obesity, and metabolic disorders. All these abnormalities have been associated with WML.

5. Cerebral WML and Blood Vessels

Ultrasonographic findings of increased atherosclerotic plaques and carotid artery IMT are regarded as the subclinical markers of early atherosclerosis and are associated with nonmodifiable and modifiable risk factors and the subsequent risk of new or recurrent stroke [52]. Some studies have found that the severity of IMT and the presence of plaques in the carotid arteries are also predictive of WML [53–56], although another study in the elderly did not [57]. This association between large and small vessel disease may well be mediated via common intermediary risk factors such as hypertension.

It is known that the arterial system gradually stiffens due to the combined effects of aging, high BP, and other vascular risk factors. Increased arterial stiffness results in characteristic increased impedance and pulse wave velocity (PWV) in the aorta, which increases systolic and pulse pressure (PP) centrally. Stiff arteries cause high PP and pulsatile flow to be transmitted to distal organs during systole, damaging the cerebral microvasculature. It has been hypothesized that cerebral microvascular disease results from the damaging forces of abnormal flow pulsations extending into small cerebral arteries as a consequence of arterial stiffening.

In a homogeneous sample of never-treated hypertensive patients aged 50–60 years, after exclusion for known risk factors for cerebrovascular damage, such as diabetes mellitus or significant alcohol intake, Sierra et al. [58] found an association between higher PP (including office, ambulatory 24 hours, daytime and nighttime estimates) as a measure of arterial stiffness and WML. In the elderly, it has recently been shown that brachial PP is associated with WML [59].

Laurent et al. [60] found that aortic stiffness, assessed by carotid-femoral PWV using applanation tonometry, the gold standard for arterial stiffness measurement, was an independent predictor of fatal stroke in patients with essential hypertension. Whether the risk of stroke is mediated by large- and/or small-vessel disease is not clear, but the previously reported increased risk of stroke when preclinical cerebral microvascular disease (WML, silent lacunar infarcts, and/or cerebral microbleeds) is present suggests small-vessel disease involvement [4, 7]. Henskens et al. [61] studied 167 hypertensive subjects (mean age: 51.8 years) and found that higher PWV was significantly associated with a greater volume of WML and lacunar infarcts, but not with cerebral microbleeds after multivariate analyses adjusted for age, sex, brain volume, mean BP, and heart rate. This suggests that aortic stiffness is independently associated with the manifestations of cerebral small-vessel disease in hypertensive patients and links systemic large- to cerebral small-artery disease. With respect to radial arterial pulse wave analysis, Shrestha et al. [62] studied 179 unselected patients with a mean age of 66 years and found that central systolic BP values, measured by radial applanation tonometry, were more closely associated with WML than brachial systolic BP. In this study, the augmentation index did not correlate with the presence of WML.

6. Cerebral WML, Inflammation, and Endothelial Function

The role of inflammation in atherosclerosis and stroke has received increasing attention as basic and clinical research has provided evidence that inflammatory mechanisms play a central role in the pathogenesis and progression of atherosclerosis, plaque rupture, thrombosis, and stroke [38]. Endothelial dysfunction contributes to the initiation of atherosclerotic lesions. Whether inflammatory processes, apart from their involvement in large-vessel disease, are also involved in the development and consequences of cerebral small-vessel disease is still poorly understood.

Some studies have shown a relationship between WML and markers of inflammation, such as C-reactive protein [63], while others have not [64, 65]. A similar relationship has also been found with plasma homocysteine levels [66], lipoprotein-associated phospholipase A2 (Lp-PLA2), and myeloperoxidase (MPO) [67]. Plasma markers of endothelial dysfunction such as intercellular adhesion molecule-1 (ICAM-1) and P-selectin have also been associated with WML [68, 69]. It has recently been suggested that endothelial activation associated with small vessel disease is accompanied by enhanced levels of tissue plasminogen activator (tPA) and low levels of plasminogen activator inhibitor type 1 (PAI-1) when lacunar strokes are associated with WML, suggesting that differences in the activity of components of the fibrinolytic system might contribute to the development of WML [70].

Inspite of their small simple size one study has reported that altered vascular function and structure in subcutaneous small arteries of patients with late-life depression are related to cerebral small vessel disease, including WML, and basal ganglia and infratentorial changes [71]. The study was performed in 16 patients with late-life depression (68.8% hypertensives) who were compared with 15 controls (80% hypertensives). Small arteries were isolated and studied using pressure myography after subcutaneous gluteal fat biopsy.

In summary, there is some evidence to show that inflammation, impaired endothelial function, and abnormal wall growth are involved in the pathogenesis of WML.

Rizzoni et al. [72] found that the small cerebral arteries of patients with essential hypertension had an increased media/lumen ratio compared with normotensive individuals, similar finding to those previously observed in subcutaneous small arteries. Structural alterations of cerebral small vessels were assessed in 13 hypertensive patients and 15 normotensive individuals undergoing neurosurgery for benign or malign tumors. A small portion of morphologically normal cerebral tissue was excised from surgical samples and examined, and cerebral small resistance arteries were dissected and mounted on an isometric and isobaric myograph. An increased media-to-lumen ratio of subcutaneous small resistance arteries has also been shown to predict the development of cardio-cerebrovascular events in hypertensive patients [73].

7. Conclusion

Evidence on the prognostic role of subclinical organ damage continues to grow. Early detection of hypertensive target organ damage is important in order to prevent cardiovascular diseases more successfully and improve patients' outcomes [1, 2].

After age, hypertension is the most-important factor for the development of cerebral WML [4, 10], which are an important prognostic factor for stroke, cognitive impairment, dementia, and death [7]. Various studies have shown an association between WML and a number of extracranial systems affected by high BP. There are findings that suggest that correct antihypertensive treatment could efficiently slow WML progression [11–13]. Strong evidence suggests that cerebral WML in hypertensive patients should be considered a silent early marker of brain damage.

The latest Reappraisal of the European Guidelines on hypertension management [2] reports that, in a group of 192 untreated hypertensive patients (aged 18–90 years) without overt cardiovascular disease, silent cerebrovascular lesions (WML, lacunar infarcts, cerebral microbleeds) were even more prevalent (44%) than cardiac (21%) and renal (26%) subclinical damage and frequently occur in the absence of other signs of organ damage [5]. Similarly, 58% of patients with demonstrable cardiac or renal damage or both had silent cerebrovascular lesions.

With MRI being increasingly used for diagnostic procedures, investigation of silent cerebrovascular disease will become more frequent in prognostic and therapeutic studies in hypertension. In the meantime, target organ damage in other systems may aid the evaluation of early hypertensive brain damage.

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SAGE-Hindawi Access to Research
Journal of Aging Research
Volume 2011, Article ID 950341, 6 pages
doi:10.4061/2011/950341

Clinical Study

Motor Dysfunction Correlates with Frontal White Matter Ischemic Changes in Patients with Leukoaraiosis

Miguel Viana-Baptista,[1,2] Paulo Bugalho,[1] Constança Jordão,[3] Olga Ribeiro,[4] José António Esperança-Pina,[5] and José Ferro[6]

[1] *Neurology Department, Hospital de Egas Moniz (CHLO), 1349-019 Lisbon, Portugal*
[2] *CEDOC, Faculty of Medical Sciences, New University of Lisbon, 1169-056 Lisbon, Portugal*
[3] *Ressonância Magnética de Caselas, 1400-045 Lisbon, Portugal*
[4] *Neuropsychology Department, Hospital de Egas Moniz (CHLO), 1349-019 Lisbon, Portugal*
[5] *Department of Anatomy, Faculty of Medical Sciences, New University of Lisbon, 1200-249 Libson, Portugal*
[6] *Neurology Department, Faculty of Medicine, Hospital de Santa Maria, 1649-035 Lisbon, Portugal*

Correspondence should be addressed to Paulo Bugalho, paulobugalho@sapo.pt

Received 30 December 2010; Revised 3 March 2011; Accepted 11 March 2011

Academic Editor: Sofia Madureira

Objectives. To test the relation between white matter lesions (WML) location and physical performance, in aged patients. *Methods*. Subjects: 29 patients (17 males), aged >65 (mean age 72.6 ± 5.2), with leukoaraiosis. WML was quantified with a visual scale; Apparent Diffusion Coefficient (ADC) was measured bilaterally in frontal periventricular lesioned white matter and frontal and parieto-occipital normal appearing white matter (NAWM). Motor performance was studied using the Short Physical Performance Battery (SPPB), single leg stand time, finger tapping and grooved pegboard tests (GPT). *Results*. There were significant correlations between the frontal region visual scale scores and SPPB chair stands ($r = -0.379$; $P = .039$) and Grooved Pegboard ($r = 0.393$; $P = .032$); frontal NAWM ADC values and SPPB standing balance ($r = -0.450$; $P = .014$) and SPPB 4 meter walk ($r = -0.379$; $P = .043$). *Conclusion*. Frontal WML are negatively related to motor performance in patients with leukoaraiosis. DWI results suggest that this may be true even for NAWM.

1. Introduction

Leukoaraiosis was the first term introduced to characterize areas of loss of density of white matter observed on computerized tomography of the brain [1]. Later recognized as hyperintense areas on T2-weighted MR images, these findings, although probably related to vascular risk factors, are common in advanced age and thus usually designated age-related white matter changes (ARWMC). Several clinical features have been related to ARWMC, including cognitive impairment, depressive symptoms, mood disturbance, urinary dysfunction, and motor deficits. Among these, gait disturbance probably deserves further attention on account of related functional impairment [2, 3].

The pathophysiology of motor impairment in old age patients with leukoaraiosis is not fully understood. Although frequently attributed to frontal lobe dysfunction, related to disconnection effects of the lesions on white matter tracts [4], few studies have devoted their attention to the relation between ARWMC localization and motor deficits. Recently, conventional MRI studies have suggested that gait disturbance [5] and postural control [6] could be related not only to frontal but also to parieto-occipital (PO) lesions. Others have found association mainly with frontal lesions, when compared with basal ganglia and infratentorial lesions [7].

Difficulty in locating the cause of motor disturbance in patients with leukoaraiosis could be due to the low sensitivity of conventional imaging techniques to more subtle ischemic changes, occurring even in normal appearing white matter (NAWM). This could explain some mismatch between clinical and imaging data. Average apparent diffusion coefficient

(ADC), a measure of water diffusion in tissues, presents higher sensitivity to white matter structural changes. It is increased in chronic ischemic WML, probably due to breaking of the anatomic barrier formed by the myelinated axons and also in surrounding NAWM [8]. It could thus be a useful technique to study vascular damage in leukoaraiosis and also in tissue not visibly damaged in conventional MRI, but potentially altered at a microstructural level, permitting to analyze more accurately the relation between white matter vascular alterations and motor function in patients with leukoaraiosis.

On a previous study, we presented data regarding the association between age-related white matter changes and cognitive function, on a sample of old age, community-dwelling subjects with leukoaraiosis [9]. In the present analysis, we present data concerning the relation between motor function and location of ischemic white matter changes.

2. Materials and Methods

2.1. Subjects. We included 30 subjects, according to the following criteria: age above 65 years; no (or mild) disability assessed by Instrumental Activities of Daily Living scale (IADL); presence of ARWMC on CT-Scan, defined as periventricular or subcortical areas of hypodensity (all criteria needed). We exclude patients who presented with leukoencephalopathy from other identified cause, neurological or general incapacitating chronic disease, Modified Rankin Scale >2, dementia, aphasia, and general contraindications for undergoing MRI. Patients were referred to Egas Moniz Hospital Outpatient Neurology Clinic by general practitioners for reasons not directly related to the presence of ARWMC: cognitive complaints (6), stroke (12), gait disturbance (1), depression (2), incidental CT/MRI findings (4), vertigo (2), and other neurological symptoms (3). Stroke was confirmed, by history taking and revision of imaging findings, in 9 patients. Ictus occurred at least three months before study inclusion, leaving no neurological signs. They were all minor strokes, with the following classification: lacunar (5), cardioembolic (2), large artery disease (1), and other cause (1) strokes, occurring. All patients underwent MRI scanning, according to a standardized protocol. All patients provided informed consent, and the local ethic committee approved the protocol. The same sample was also investigated for cognitive impairment. Data related to neuropsychological variables is discussed elsewhere [9].

2.2. MRI Protocol

2.2.1. Imaging Acquisition Protocol. the head was imaged from foramen magnum to superior convexity with General Electric Sigma CV/i 1.5T equipment. Structural acquisition consisted in localizing sequence Sagital T1(FOV 24 cm, thickness 5 mm, Gap 1.5 mm, Matrix 256 × 224, Nex 2); Proton Density and T2 FSE (ET = 4, TR 4000, TE 102, FOV 22 cm, thickness 4 mm, Gap 0, Matrix 320 × 256, Nex 2 and ET = 16, TR 4000, TE 102, FOV 22 cm, thickness 4 mm, Gap 0, Matrix 320 × 256, Nex 2); T2 Fast FLAIR (TR 10000, TE 130, FOV 24 cm, thickness 4 mm, Gap 0-Double acquisition, Matrix 256 × 192, Nex 2); DWI (TR 10000, TE min, FOV 32 cm, thickness 5 mm, Gap 0, Matrix 128 × 128, Nex 1, $B = 0$ and $B = 1000\,\text{s/mm}^2$). Diffusion gradients were applied sequentially along six noncollinear directions.

2.3. Image Processing. Two consecutive slices were selected to study average ADC, one for LWM and parieto-occipital NAWM and another for frontal NAWM, respectively. The size of the ROI and the selected planes were previously decided in order to obtain consistent measures and avoid regions with both LWM and NAWM. The observer was unaware of motor test results and ARWMC rating results.

Average ADC was measured on circular, 58 voxel (standard deviation ≤10%) regions of interest (ROI), directly on ADC maps. They were placed bilaterally in parieto-occipital and frontal NAWM, avoiding areas with white matter lesions. We also measured ADC on lesioned frontal periventricular white matter (LWM), on 30 voxel oval ROI, placed near but at least 4 voxels apart from the tip of the anterior horns. (Figure 1).

2.4. White Matter Lesion Load. White matter lesions were scored on FLAIR sequences, bilaterally in frontal, parieto-occipital, temporal, basal ganglia, and infratentorial regions. They were rated retrospectively, by an experienced neuroradiologist (C. J.), blinded to clinical evaluation and DWI results, using a validated visual rating scale [10]. Mean regional scores were used, derived from the average of left and right values.

2.5. Motor Function Assessment

2.5.1. Subjects Underwent the Following Motor Function Tests

Short Physical Performance Battery [11]. This widely used scale tests lower extremity function, by measures of standing balance (including tandem, semitandem, and side-by-side stands), walking speed (fastest of two times to walk 4 meters), and ability to rise from a chair (time to rise to stand and sit five times). There are five performance scores, ranging from 0 (inability to complete the test) to 4 (highest level of performance). A total score is derived from adding the three partial scores. Based on this score, patients can be divided in three categories: higher mobility (SPPB = 11 or 12), intermediate mobility (SPPB = 9 or 10), or lower mobility (SPPB < 9).

Single Leg Stand Time Best Trial in Seconds. the subjects were instructed to balance as long as possible on one leg; two trials for each leg were performed, the best time of the four trials being used for analysis.

Finger Tapping from the Unified Parkinson's Disease Rating Scale [12]. In this section of the Unified Parkinson's Disease Rating Scale the patient is required to tap his/her thumb with index finger in rapid succession. The test is rated as follows: 0 = normal; 1 = mild slowing and/or reduction in amplitude;

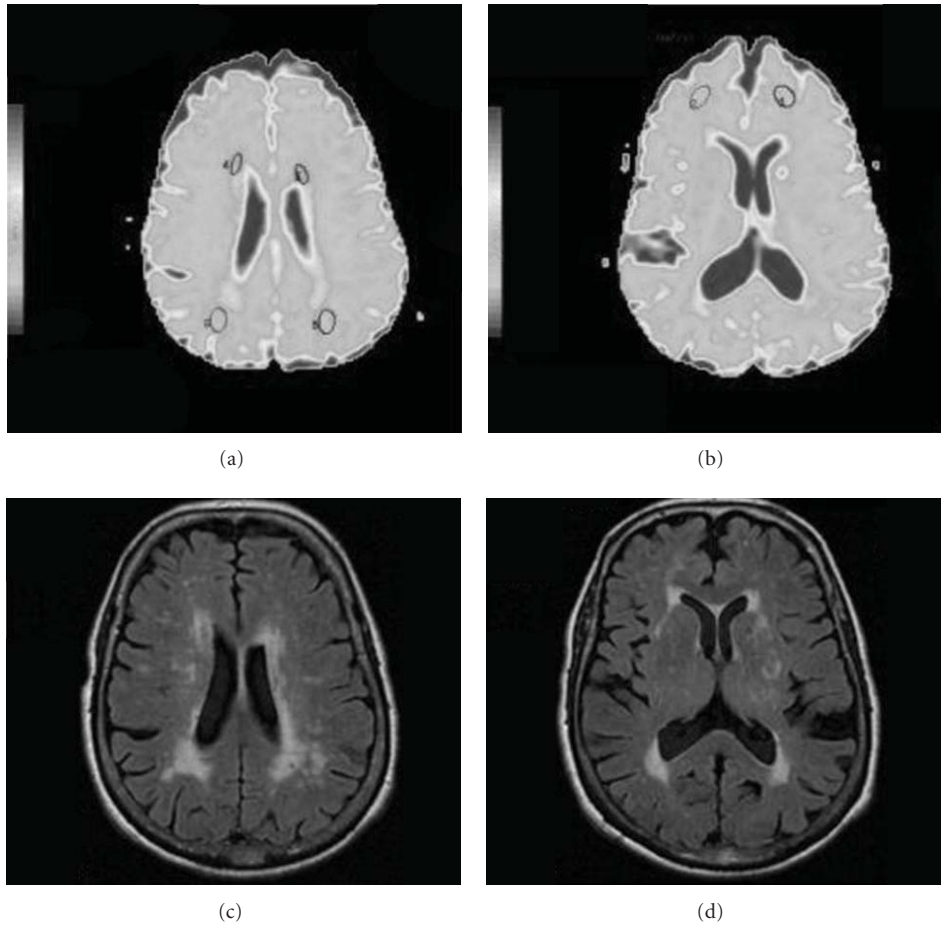

Figure 1: (a) and (b) ADC Map with representation of Regions of interest in Parieto-Occipital Normal Appearing White Matter (NAWM) and lesioned white matter (LWM) (a) and Frontal Normal Appearing White Matter (NAWM) (b). (c) and (d) Corresponding planes in FLAIR sequences.

2 = moderately impaired; definite and early fatiguing; may have occasional arrests in movement; 3 = severely impaired; frequent hesitation in initiating movements or arrests in ongoing movement; 4 = can barely perform the task. For correlation analysis, right and left scores were averaged.

Grooved Pegboard [13]. The Grooved Pegboard is a manipulative dexterity test, involving eye-hand coordination capacities. It consists of 25 holes with randomly positioned slots. Pegs with a key along one side must be rotated to match the hole before they can be inserted. The subjects are timed to determine how quickly they can insert grooved pegs into 25 holes in the pegboard, with both hands. For correlation analysis, left and right scores were averaged.

2.6. Statistical Analyses. Differences between ARWMC scores in the various regions and between mean ADC values in different ROI were tested using ANOVA followed by Bonferroni post hoc comparisons analysis.

We performed a data reduction study, using principal component analysis, in account of the high number of motor variables. Eigen values greater than 1 were considered as loading on a factor. Two rotated principal components accounted for 60.07 % of the total variance: Factor 1 (F1) was related mainly to Grooved Pegboard time ($r = -0.835$), SPPB standing balance ($r = 0.764$), and SPPB 4 meter walk ($r = 0.753$); Factor 2 (F2) was related mainly to SPPB chair stands ($r = 0.764$). Each patient was given a score on these factors. The relation between ADC and Visual Scale scores and motor tests, including F1 and F2, was tested with bivariate correlation analysis (Spearman or Pearson correlation coefficient, as appropriated). $P < .05$ was considered significant.

3. Results

Of the 30 subjects selected, 1 was excluded from analysis, because technical problems precluded utilization of MRI data. 17 subjects were male. Age ranged from 66 to 85 (mean 72.6 ± standard deviation 5.2).

Motor tests results are shown in Table 1. Regarding SPPB total score, most patients were on the higher (10) and intermediate (17) mobility group, with just two patients in the lower mobility group.

Table 1: Motor performance results.

	Minimum	Maximum	Mean	Standard deviation
SPPB standing balance	2	4	3.87	0.43
SPPB 4 meter walk	3	4	3.90	0.30
SPPB chair stands	1	4	2.33	0.99
SPPB total score	7	12	10.10	1.21
Single leg stand time best trial (seconds)	5	60	34.87	19.83
Finger tap	0	1.50	0.75	0.54
Grooved pegboard	80.50	417.00	126.98	60,50

SPPB: short physical performance battery.

Visual scale regional scores were significantly different ($F = 39.54$, $P < .0001$), with Bonferroni post hoc comparison analysis showing higher scores for frontal and PO regions ($P < .0001$), with no statistical difference between them. Basal ganglia, temporal, and subtentorial regions were excluded from correlation analysis because there were too many null values. ADC means were significantly higher in LWM ($P < .0001$). There was no significant difference between PO and frontal means (imaging data comparisons are discussed elsewhere [9]).

We found a global trend to negative correlations between the frontal lobe visual scale scores and ADC and motor performance. Correlations were significant between frontal visual scale scores and Factor 1 ($r = -0.468$; $P = .012$). There were significant correlations between the frontal region and SPPB chair stands ($r = -0.379$; $P = .039$) and Grooved Pegboard ($r = 0.393$; $P = .032$) (Table 2). Frontal NAWM ADC values were significantly correlated to Factor 1 ($r = -0.379$; $P = .043$). Significant correlations were found between frontal NAWM and SPPB standing balance ($r = -0.450$; $P = .014$) and SPPB 4 meter walk ($r = -0.379$; $P = .043$) (Table 2).

To account for the potential influence of age, correlations between imaging and motor variables were repeated, using partial correlation analysis, controlling for age. Correlations between SPPB and frontal ADC remained significant for NAWM and became significant for LWM. Correlation between frontal visual scale scores and SPPB became less significant, but still showed a trend to significance ($P < .1$) (Table 3).

To avoid the potential bias produced by the inclusion of stroke patients in our subject group, we compared imaging and physical performance results between patients with and without a history of stroke (independent samples Student's t-tests). We found no significant differences.

4. Discussion

We have investigated the relation between ischemic changes and motor function in a sample of 29 community-dwelling old age patients, with relatively good motor performance, as shown by high scores on the SPPB (most patients were in the higher/intermediate mobility categories). Significant correlations were found between ischemic white matter changes identified by conventional and DWI imaging techniques and several motor performance variables, including a rotated principal component (F1), related both to lower and upper limb function. These results are in accordance with several studies [14–26] and confirm the influence of white matter changes on old age subjects' motor function. Single variable analysis was in accordance with principal factor analysis, showing significant correlations between imaging data and SPPB items (standing balance, chair stands, walking speed) and Grooved Pegboard, suggesting a relation between white matter damage and gait velocity, balance and upper limb dexterity.

Some findings may additionally contribute to a better understanding of the pathophysiology of motor disturbance caused by leukoaraiosis. Patients with leukoaraiosis and those with frontal lobe lesions (or, in which, basal-ganglia/frontal lobe connections are deranged) show some similarities in gait disturbance. This leads to the concept that gait disturbance in leukoaraiosis could be due to disconnection between the motor and premotor frontal regions and the basal ganglia, related to frontal ARWMC [4]. In the present study we found significant correlation between gait dysfunction and frontal but not PO white matter lesions, although both regions showed similar degrees of white matter damage, thus corroborating the frontal lobe hypothesis.

As far as we are aware, few studies have investigated the relation between white matter lesions in particular regions and motor function, none of them using DWI techniques. Using the same gait measures (SPPB), despite different imaging methods, Benson and coworkers [5] found frontal and parieto-occipital lesions to be related to lower mobility in patients with leukoaraiosis. While the first location showed high sensitivity, despite low specificity, to predict gait impairment, the latter showed high specificity but low sensitivity. Contrary to Benson et al., we did not find any significant correlation between PO ischemic changes and motor function. This is true also for PO NAWM, as shown by the DWI study, which has higher sensitivity for subtle white matter changes. Thus, these results do not support the hypothesis put forward by the authors that PO WML low sensitivity for gait impairment could be due to underestimation of tissue damage caused by microscopically, nonvisible alterations. We should say, however, that our patients generally presented with higher mobility than those reported in that study, and, therefore, they could be in a less advanced stage of the disease. This would suggest that frontal lesions could have a more important role in gait

Table 2: Correlations between imaging data and motor performance tests.

	VS: frontal		VS: PO		DWI: LWM		DWI: frontal NAWM		DWI: PO NAWM	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Factor 1	−0.468	.012*	−0.364	.052	−0.364	.052	−0.379	.043*	−0.048	.804
Factor 2	−0.176	.353	−0.017	.931	−0.017	.931	0.212	.270	−0.007	.972
SPPB standing balance	−0.251	.180	−0.333	.078	−0.333	.078	−0.450	.014*	−0.335	.076
SPPB 4 meter walk	−0.189	.316	−0.108	.576	−0.108	.576	−0.379	.043*	−0.230	.230
SPPB chair stands	−0.379	.039*	−0.156	.420	−0.156	.420	0.269	.158	−0.116	.548
Single leg stand time best trial (seconds)	−0.057	.767	−0.152	.430	−0.152	.430	−0.234	.221	−0.072	.711
Finger Tap	0.358	.052	0.258	.176	0.258	.176	0.279	.142	0.092	.636
Grooved Pegboard	0.393	.032*	0.291	.126	0.291	.126	0.274	.151	0.131	.500

Values are as follows: *r*: spearman coefficient; *P*: level of significance; $*P < .05$; SPPB: short physical performance battery; VS: visual scale score; DWI: diffusion-weighted imaging; LWM: lesioned white matter; NAWM: normal appearing white matter; PO: parieto-occipital.

Table 3: Correlations between imaging data and motor performance tests, controlling for age.

	Frontal		PO		LWM		Frontal NAWM		PO NAWM	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Factor 1	−0.107	.582	0.259	.174	−0.433	.021*	−0.382	.045*	−0.155	.430
Factor 2	−0.107	.582	0.259	.174	−0.011	.957	0.197	.316	−0.027	.893
SPPB standing balance	−0.083	.669	−0.006	.975	−0.458	.014*	−0.465	.013*	−0.217	.267
SPPB 4 meter walk	0.062	.749	0.406	.029*	−0.113	.566	−0.199	.310	−0.136	.491
SPPB chair stands	−0.341	.070	−0.199	.300	−0.099	.616	0.400	.035*	−0.114	.564
Single leg stand time best trial (seconds)	0.036	.853	0.327	.083	−0.148	.453	−0.107	.588	−0.066	.740
Finger Tap	0.271	.155	0.034	.861	0.245	.209	0.129	.513	−0.012	.952
Grooved Pegboard	0.086	.656	0.255	.181	−0.325	.092	−0.283	.144	0.001	.997

Values are as follows: *r*: partial correlation coefficient, controlling for age; *P*: level o0f significance, $*P < .05$; SPPB: short physical performance battery; DWI: diffusion-weighted imaging; LWM: lesioned white matter; NAWM: normal appearing white matter; PO: parieto-occipital.

impairment on the initial stages of the disease. Novak and collaborators' study [6] found that PO lesions also correlated significantly with postural control measures. This study used automated measures of gait and posture and assessed a great number of patients, which may have permitted to detect more subtle relations between motor variables and WML.

Results from visual scale scoring and DWI point roughly to the same conclusions, that is, a relation between motor function and frontal lobe ischemic changes. However, the significant correlations found between frontal NAWM and several motor variables suggest that nonvisible white matter damage can have influence on patients' motor ability, as has been already shown for cognitive functions [9, 27]. More recently, a study using magnetic transfer imaging also showed relation between motor dysfunction in old age subjects and microstructural tissue damage, not seen in conventional MRI [28].

Our study has some limitations, one being not using automated methods to quantify WML. We should say, however, that in a recent analysis both automated methods and the visual scale used in this study had comparable power in discriminating between leukoaraiosis patients with and without gait complaints [29]. The large number of motor performance variables and the relatively low level of significance of the correlations found ($P < .05$) could have made separate variables correlation analysis less reliable. However, we should point that these results were mostly concordant with the results from data reduction analysis. Besides, they were similar in both imaging techniques. We also did not use diffusion tensor imaging (DTI) techniques, which are considered to be more specific than ADC in detecting white matter structural changes, and we did not perform inter- or intraobserver reproducibility tests for image analysis. As main advantages of the present study, we underline the small number of diffusion studies addressing motor disturbances in patients with white matter lesions, ADC measurement in both LWM and NAWM, and use of regional scores to quantify lesion load. In conclusion, our findings suggest that white matter changes are negatively related to balance, gait velocity, and hand dexterity in old age patients with leukoaraiosis and that this could be due mainly to frontal lobe derangement. DWI results suggest that this may be true even for NAWM.

Acknowledgment

This investigation was partially supported by a grant from Bristol-Myers Squibb, Portugal.

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