Dissertation

Magnetization Transfer Imaging for Detection of Microstructural Brain Tissue Changes in Aging: Associations with Cognition and Gait Disturbances.

Submitted by

Dr. med. univ. Stephan Seiler

For the academic degree of

Doctor of Philosophy (PhD)

At the **Medical University Graz**

Department of Neurology

Under the supervision of

Prof. Dr. Reinhold Schmidt

Prof. Dr. Stefan Ropele

Dr. Manfred Windisch

2015

Declaration

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of „Good Scientific Practice“.

Parts of this dissertation have already been published (own work) and may therefore resemble in content and syntax.

Graz, DEC 2nd, 2015 Stephan Seiler

Acknowledgement

I thank my supervisor Prof. Dr. Reinhold Schmidt for sharing his knowledge with me and for his patience.
I thank Dr. Manfred Windisch for his efforts as member of the thesis committee.
My special thanks go to my colleagues and fellow researchers in- and outside the department, especially Prof. Dr. Petra Schwingenschuh and Prof. Dr. Stefan Ropele.

I thank my family, friends and all other supporters.

Je me souviens.

Index

Abbreviations

AD	Alzheimer's disease
ANCOVA	Analysis of Covariance
ASPS	Austrian Stroke Prevention Study
CSF	Cerebrospinal Fluid
cSVD	cerebral Small Vessel Disease
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Imaging
Eq.	Equation
FA	Fractional Anisotropy
FLAIR	Fluid Attenuated Inversion Recovery
FSL	Functional Magnetic Resonance Imaging of the Brain Software Library
IDL	Interactive Data Language
LADIS	Leukoaraiosis and Disability in the Elderly Study
MB	Microbleed
MCI	Mild Cognitive Impairment
MD	Mean Diffusivity
MMSE	Mini Mental State Examination
MPRAGE	Magnetization Prepared Rapid Gradient Echo
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MTI	Magnetization Transfer Imaging
MTR	Magnetization Transfer Ratio
NAGM	Normal Appearing Gray Matter
NAWM	Normal Appearing White Matter
RF	Radio Frequency
SIDAM	structured interview for the diagnosis of dementia of the Alzheimer type, multi-infarct dementia and dementias of other etiology according to ICD-10 and DSM-III-R
SPSS	Statistical Package for the Social Sciences

VaD	Vascular Dementia
VCI	Vascular Cognitive Impairment
VIF	Variance Inflation Factor
VLSM	Voxel-Based Lesion Symptom Mapping
VMTRSM	Voxel-Based MTR Symptom Mapping
VRF	Vascular Risk Factor
WMH	White Matter Hyperintensities

List of figures

List of tables

List of equations

This page intentionally left blank

Abstract in German

Abstract auf Deutsch

Die vorliegende Dissertation besteht aus 2 nacheinander durchgeführten Projekten mit dem Schwerpunkt Magnet Resonanz Tomografie (MRT). Es war unser Ziel, die Rolle von mikrostrukturellen Hirnveränderungen und deren Assoziation mit kognitiver Leistungsfähigkeit (Studie #1) und Ganggeschwindigkeit (Studie #2) zu untersuchen. Dafür bedienten wir uns des Magnetisierungs Transfer Imagings (MTI), einer MR-Modalität zur Visualisierung von mikrostrukturellen Hirnveränderungen.

In Studie #1 untersuchten wir die Assoziation zwischen der Magnetisierungs Transfer Ratio (MTR) in der grauen und weißen Hirnsubstanz und kognitiver Leistungsfähigkeit an 355 Teilnehmern der Österreichischen Schlaganfall Vorsorge-Familienstudie (ÖSVS-Fam). Die Teilnehmer waren zwischen 38 und 86 Jahre alt. Jeder Teilnehmer wurde mittels 3 Tesla MR inklusive MTI durchuntersucht. MTR wurde im Cortex, der tiefen grauen Substanz, Hyperintensitäten der Weißen Substanz (WMH) und normal aussehendem Hirngewebe berechnet. Als signifikant erachteten wir einen p-wert <0.05. Adjustierte gemischte Modelle ergaben einen direkten und signifikanten Zusammenhang zwischen totaler und lobärer kortikaler MTR und Gedächtnisfunktion, exekutiven Funktionen und motorischer Geschicklichkeit. Die Dosis-Effekt Beziehung folgte einem linearen Trend. Die MTRs der tiefen grauen Substanz, sowie jene der normal aussehenden weißen Substanz, korrelierten mit exekutiver Dysfunktion. Alle Zusammenhänge waren unabhängig von demographischen Daten, vaskulären Risikofaktoren, fokalen Hirnläsionen und Cortexvolumen.

In Studie #2 benützten wir voxel-basierendes Läsions-Symptom Mapping (VLSM) und voxel-basierendes MTR-Symptom Mapping (VMTRSM) um die Zusammenhänge zwischen makro- und mikrostrukturellen Hirnveränderungen im Alter und Gangstörungen zu untersuchen. Wir wählten jene 230 Teilnehmer der ÖSVS-Fam aus, die 60 Jahre alt oder älter waren, da wir uns auf alters-assoziierte Veränderungen fokussierten. Die Ganggeschwindigkeit wurde mittels einer Stoppuhr gemessen. Jeder Teilnehmer wurde mittels 3 Tesla MR, inklusive MTI, durchuntersucht. VLSM und VMTRSM wurden verwendet um voxel- weise die Lokalisation von White Matter Hyperintensitäten und MTR Veränderungen des Gehirns, welche mit Ganggeschwindigkeit korrelieren, zu identifizieren.

Alle Analysen wurden für Störvariablen und multiple Vergleiche kontrolliert. Als signifikant erachteten wir einen p-wert <0.05.
In der VLSM Analyse konnten wir keine WMH Cluster identifizieren, welche mit Ganggeschwindigkeit korrelierten. In der VMTRSM Analyse waren mehrere MTR Cluster mit Ganggeschwindigkeit korreliert. Die signifikanteste Assoziation fand sich im Bereich des Forceps Minor. Eine regionale Analyse (ROI) zeigte einen signifikanten Zusammenhang zwischen der MTR des Forceps Minor und Ganggeschwindigkeit (Beta=0.160; 95%CI 0.025-0.295; p=0.02). Dieser Zusammenhang war unabhängig von demografischen Daten, Hirnvolumen und vaskulären Risikofaktoren. In der Kovarianz Analyse fanden wir, dass die Assoziation zwischen MTR in Quartilen und Ganggeschwindigkeit einem dosisabhängigen, linearen Trend folgte (p =0.016).

Zusammenfassend liefert diese Dissertation neue Evidenz für die Bedeutsamkeit von mikrostrukturellen Hirnveränderungen im Kontext von Kognition und Gangstörungen im Alter. Weiterführende Forschung ist nötig um die Basis dieser Veränderungen auf Gewebsniveau zu verstehen und um die Bedeutung von MTR zur Vorhersage des Krankheitsverlaufes bei kognitivem Abbau, Demenz und Gangverschlechterung zu definieren. Dies ist insbesondere wichtig, da sich die MTR als Biomarker eignen und somit in Interventionsstudien Verwendung finden könnte.

Abstract in English

The present PhD thesis consists of 2 separate magnetic resonance imaging (MRI) studies that are based on each other. Our aims were to elaborate the role of microstructural brain tissue damage associated with cognitive performance (study #1) and gait disturbances (study #2). To accomplish this, we used Magnetization Transfer Imaging (MTI). MTI can detect microstructural brain tissue changes beyond what is visible on standard MRI and may be helpful in determining age-related cerebral damage.

In study#1, we investigated the association between the magnetization transfer ratio (MTR) in gray and white matter and cognitive functioning in 355 participants of the Austrian Stroke Prevention Family Study (ASPS-Fam) aged 38 to 86 years. Every participant underwent 3T MRI including MTI. MTR maps were generated for the neocortex, deep gray matter structures, white matter hyperintensities, and normal appearing white matter. Adjusted mixed models determined whole brain and lobar cortical MTR to be directly and significantly related to performance on tests of memory, executive function and motor skills. There existed an almost linear dose-effect relationship. MTR of deep gray matter structures and normal appearing white matter correlated to executive functioning. All associations were independent of demographics, vascular risk factors, focal brain lesions and cortex volume.
In study #2, we used voxel-based lesion symptom mapping (VLSM) and voxel-based MTR-symptom mapping (VMTRSM) to assess the macrostructural and microstructural determinants of gait velocity in aging. We chose those 230 non-disabled participants of the ASPS-Fam who were over or equal to 60 years old. Gait velocity was calculated at normal pace. Every participant underwent 3T MRI including Magnetization Transfer Imaging (MTI). VLSM and VMTRSM were carried out to correlate white matter hyperintensity (WMH) location and Magnetization Transfer Ratio (MTR) of each voxel with gait velocity. All analyses were adjusted for possible confounders. To account for multiple comparisons, a Family-Wise Error (FWE) corrected p-value <0.05 was considered statistically significant. VLSM did not find any significant clusters where WMH were associated with slower gait. In the VMTRSM analysis, MTR in several voxels was significantly associated with gait velocity. Most significant voxels were located in the forceps minor. Region of interest (ROI) analysis revealed a significant association between forceps minor MTR and gait velocity ($\beta=0.160$; 95%CI 0.025-0.295; $p=0.02$), independent of demographics, brain volume and vascular risk factors.

Analysis of covariance (ANCOVA) demonstrated that the association between quartiles of MTR within the forceps minor and gait velocity was dose-dependent (p for linear trend =0.016).

Our studies provide new evidence for the importance of microstructural brain tissue changes in cognition and gait disturbances at older age. Further research is needed to understand the basis of these associations at the tissue level, and to determine the role of MTR in predicting cognitive decline, dementia and gait impairment.

Publication Status

Study #1 has been published in *Frontiers in Aging Neuroscience* (1)

Study #2 has been submitted to *Neurobiology of Aging*

Introduction

General background

The benefits of modern medicine unceasingly increase life expectancy. As a consequence of a fall in mortality rates coupled with a fall in birth rates, the population of high-income countries gets constantly older (2). With higher age, dangerous neurologic conditions occur, including neurodegenerative- and cerebrovascular diseases, and impaired mobility. One weakness of health-care systems is early detection and prevention of such age-related illnesses. Early detection may help to considerably reduce costs and is highly warranted, but a major challenge for researchers (3).

Magnetic Resonance Imaging (MRI) is a noninvasive method suitable to study age-associated brain changes in a large number of subjects and relate them to clinical outcome. However, visible changes like atrophy or ischemic infarcts insufficiently explain the variety of trajectories in cognition and mobility in aging. There is evidence that microstructural brain tissue changes that elude standard MRI might precede atrophy, ischemic brain lesions and first clinical symptoms for years (1,4,5).

Newer imaging methods, developed to detect microstructural brain tissue changes are Diffusion Weighted Imaging (DWI) and Magnetization Transfer Imaging (MTI). These sequences make it possible to study the brain's microstructure beyond what can be expected from the routinely performed, standard MRI sequences (5-7). With help of these sequences, we may detect microstructural brain tissue alterations that are associated with subtle cognitive and functional changes in normal aging long before overt disease occurs. Early detection may lead to a better prevention of risk factors like hypertension and diabetes and might ease the development of treatment strategies.

In the present PhD thesis, MTI has been used to characterize brain tissue alterations on a microstructural level. We assessed their relationship to cognitive impairment and gait performance in a well-defined, large cohort of healthy community-dwelling adults.

The first section will give an overview of the project-relevant literature including standard MRI, DWI and MTI and their ability to detect age-associated brain changes. Associations of

macro- and microstructural brain changes with cognition and mobility will be elaborated. MTI will be discussed in more detail.

Standard MRI for the study of age-related brain tissue changes

Standard MRI is the state of the art method for the in-vivo study of brain changes during aging. The following subchapters will give a brief technical description of standard MRI and a short literature review of study findings in aging.

A brief technical introduction to standard MRI

MRI can detect signals from protons (1H) of water molecules (H2O), which is the most frequent molecule in the human body. The 1H nucleus rotates ("spins") and produces a magnetic field parallel to the axis of rotation. When this magnetic field is placed inside of an external static magnetic field ("B0") it changes the orientation of the rotational axis alongside B0 ("precession"). The frequency of this precession can be estimated with the Larmor equation.

$$f = \gamma B0 \qquad \text{(eq.1)}$$

As can be seen from the equation, the relationship between the frequency of precession (f) and B0 is linear. γ is the so-called gyromagnetic ratio (i.e. the ratio of its magnetic dipole moment to its angular momentum) and is (in the context of MRI) a specific property of the H1 nucleus.
Any signals received during an MRI acquisition are the result of the spins of up to 10^{26} nuclei. When an external, static magnetic field is applied, a small number of spins favorably align with it, instead of aligning with the opposite direction. The sum of spins aligned with the magnetic field, results in the net magnetization (M0). M0 actually produces the MR signal. A higher M0 increases the signal. By agreement, the external magnetic field runs parallel to the z-axis of a Cartesian coordinate system. In the next step, the magnetization is moved from the z-axis to the xy-plane (perpendicular to z) via a high-frequency radiofrequency (RF) pulse

("excitation"). The so-called "saturation" of spins occurs, as soon as their net magnetization has moved entirely to the xy plane. Immediately after excitation, the net magnetization vector dephases and returns to the z-axis. The dephasing is called transverse relaxation or T2. The consecutive increase of magnetization alongside the z-axis is longitudinal relaxation or T1. Via further processing including recording of these signals by a receive coil and feeding into a computer workstation, translation into the MR image can be achieved. Going into further detail would extent the scope of the dissertation, but a comprehensive review on MRI can be found in (8).

To understand basic MRI contrasts, T1 and T2 relaxation times are briefly described:

T1 relaxation time

The T1 relaxation time is a time constant that describes the rate at which the magnetization returns from the z-axis to M0 when the magnet is turned off. Terms commonly used are longitudinal relaxation time or spin-lattice relaxation time. In images with a T1 weighted contrast, tissue with short T1 appears bright and tissue with long T1 appears dark. T1 weighted images are part of every standard MRI examination and especially suited to depict anatomical structures (8).

T2 relaxation time

T2 is called transversal relaxation time or spin-spin relaxation time. It represents a loss of magnetization in terms of the loss of phase coherence in a plane perpendicular or transverse to M0. Commonly, tissue with a long T2 appears bright and tissue with a short T2 appears dark. Brain pathologies, like WMH, are usually hyperintense on T2-weighted images (8).

Standard MRI sequences used in aging research

High resolution T1 weighed scans with whole brain coverage provide excellent gray-white matter contrast and are thus best suited to study morphological brain changes like brain atrophy and changes in cortical thickness. These sequences are also the basis for automatic evaluation of structural MRI provided by FSL (9) and FREESURFER (10), as used in part within this dissertation.

White matter pathology including WMH and lacunes are best seen on FLAIR weighted images. FLAIR is a T2-weighted sequence with the signal of CSF suppressed.

Determinants of brain aging seen on standard MRI

Measurements of brain atrophy

High-resolution T1-weighted MRI sequences are best suited to study brain atrophy. The main feature of these sequences is very good contrast between gray matter, white matter and CSF. This allows delineation of the cortex, the deep gray matter structures and ventricles. Also cortical thickness and surface area can be calculated (8,11).

Several cross-sectional and longitudinal studies have reported global, but also regional brain volume loss during aging. In the Framingham Heart Study, 2200 individuals aged 30 to 90 were examined. Age explained 50% of total brain volume differences. The most prominent changes were found after age 50 (4). Numerous recent studies evidenced prominent age-related atrophy in a region-specific manner. Atrophy affects seemingly first and above other regions the gray- and white matter of frontal lobes, followed by the temporal lobes (12-14). Atrophy rates in the white- and gray matter are non-uniform. Aging is particularly associated with a loss of gray matter volume (4). In community-dwelling individuals, annual loss of gray matter volume across lifespan has shown to range from 0.45% to 0.83% (15,16). The loss of gray matter seems to be paralleled by a loss of white matter volume that is non-linear and again most pronounced in the frontal lobes (13). As a consequence, increasing ventricular volumes can be observed with advancing age (4,13). Another brain structure, the hippocampus, plays an important role for cognitive performance with aging. Loss of hippocampus volume has been described with advancing age in cross-sectional (17-19) and longitudinal (20,21) studies and seems to accelerate with aging (2.8% in a recent study) (19). Patients with Alzheimer's disease show significantly higher annual hippocampal atrophy rates (up to 5.8%) relative to their healthy counterparts (1.7%; $p < 0.001$) (22).

Cortical thickness and surface area are measures for gray matter loss with aging that can be evaluated automatically. Cortical thinning associated with aging was found in the frontal (23), prefrontal (24) and temporal lobes (25) with an annual decrease of about 0.35% (26).

Gray matter atrophy, atrophy of the hippocampus and cortical thinning are associated with cognitive performance in healthy adults (27-29) and dementia (27,30-32). Within the white matter, especially atrophy of the corpus callosum showed correlations with cognition (33). Associations between brain atrophy and gait performance have been reported. Volume loss of primary sensorimotor and medial temporal areas was associated with gait disturbances in community-dwelling older adults (34). Atrophy of the corpus callosum correlated with walking speed in a recent 3-year follow-up study (33).

Measurements of white matter damage

White matter hyperintensities (WMH)

WMH are areas of high signal intensity on T2- and FLAIR weighted images on standard MRI of older adults (5,35). Population-based studies report that 45 to 95% of participants exhibit some amount of WMH. 12–33% present with severe alterations (5,36). According to their distribution within the brain parenchyma, we can distinguish between periventricular and deep/subcortical WMH (37). Periventricular WMHs include caps around the frontal horns and a thin lining or so-called “halo” adjacent to the lateral ventricles. Deep changes are graded according to their severity into either punctate or beginning confluent or confluent (35). Microscopically, periventricular WMH are seemingly nonvascular in their origin, most probably resulting from transition of CSF from the ventricles through the ependyma. Punctate lesions were identified as widening of Virchow-Robin spaces, accompanied by reduced myelination around fibrohyalinotic arteries (5,38-41). Conversely, early confluent and confluent white matter hyperintensities are true ischemic lesions ranging from perivascular rarefaction of myelin and gliosis to complete infarcts. Punctate white matter lesions show low rates of progression, while their early confluent and confluent counterparts progress fast (42).

WMHs are associated with cognitive decline (43-48) dementia (49,50) and progression from mild cognitive impairment (MCI) to AD (51,52). Furthermore, higher volumes of WMH were seen in patients with poorer gait performance (53-56), but there have also been reports that did not corroborate these findings (57-59).

Lacunes

Lacunes are small cerebrospinal fluid (CSF) - filled cavities in the basal ganglia or white matter, best observed on FLAIR images in older adults (60). Lacunes are vascular in their origin, caused by ischemia in an area supplied by a small perforating artery (61). Prevalence of lacunes ranges from 7.8% (62) to 47% (63), depending on the examined study population. Incidence rates between 1.6% and 19% (48,62,64) have been reported. Lacunes correlate with cognitive performance (65,66). Results from the LADIS study showed that lacunes in the thalamus were associated with poorer MMSE, speed, motor control and executive function scores, independent of WMH burden (63). Incident lacunes seem to go along with a higher rate of executive dysfunction and lower psychomotor speed (67). In longitudinal studies, they related to decline in MMSE scores, general cognitive function, executive function and information processing speed (43,49,67). Most likely, the respective white matter tracts involved determine the pattern of cognitive dysfunction by disruption of the respective fiber bundles (66). Associations with gait disturbances have been reported (68,69).

Microbleeds (MB)

Cerebral microbleeds appear as hypointense susceptibility artifacts on T2* weighted MRI sequences. These artifacts are caused by the paramagnetic properties of hemosiderin at the location of the microbleed (70). Microscopically, they are of vascular origin as fibrohyalinosis and amyloid deposits have been found in the respective vessel walls (71). The prevalence of MBs ranges between 5% (72) and 24.4% (73), dependent on the examined cohort. Incidence of new MBs varies from 10 to 12% and appears to be age-related (73). In cross-sectional (74-

80) and longitudinal studies (81-83), microbleeds were related to cognitive performance. There is evidence that presence of microbleeds relates to gait instability (84).

Figure 1 shows representative examples of WMH, lacunes and microbleeds.

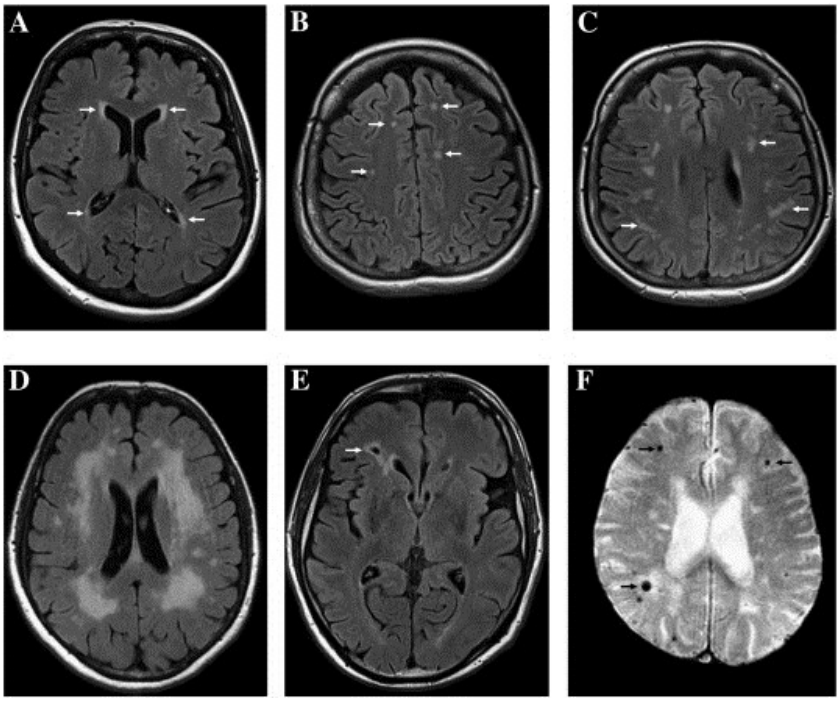

Figure 1. Representative examples of age-associated brain changes. A. Periventricular WMH; B–D. Deep/subcortical WMH. B. Punctate; C. Early confluent; D. Confluent; E. Lacune; F. Microbleeds. WMH=White Matter Hyperintensities. Image is own work, published in (52).

Microstructural brain changes associated with aging

Diffusion Weighted Imaging (DWI)

DWI measures the diffusion properties of water molecules and is able to assess the microstructure of brain tissue in vivo. In the brain, free water diffusion is constrained by the properties of its tissue compartments (e.g. lipid bilayers and axons). As a consequence, the diffusion of water molecules within brain white matter tissue is anisotropic (85). This anisotropy can be calculated within defined brain regions using modern post-processing methods (7). Measurements of diffusion are performed voxel-wise (a voxel is a 3-dimensional pixel) along (at least) 6 axes. By doing so, the so-called "diffusion tensor" can be determined. The diffusion tensor is an ellipsoid that is defined via the length of its longest, middle, and shortest axes (eigenvalues) and their orientations (eigenvectors). These six parameters can be obtained at each voxel and the degree of diffusion calculated. Diffusion values range across a continuum from isotropic to anisotropic. The most widely used metric of diffusion anisotropy is the ‘‘fractional anisotropy" (FA), scaled from 0 (isotropic diffusion) to 1 (anisotropic diffusion). A color-coded orientation map (7) can be used to visualize the main direction of the diffusion tensors within the image. Analysis is possible either voxel-wise or region of interest (ROI)- based. It is also possible to reconstruct streamlines corresponding to white matter fiber tracts ("tractography") (86-90). There are also limitations. The echo-planar sequence that DTI is based on is susceptible to artifacts. Especially subject movement can lead to a significant signal phase shift and signal loss (85). However, good correlations with cognitive performance have been described (see next section).

DTI findings in aging

Much about the tissue changes that underlie the DTI signal are unknown. However, post-mortem studies indicate that DTI changes correlate with axonal loss and demyelination (91). DTI measures get worse with aging and changes are non-uniformly distributed throughout the brain. One recent study in 203 adults between 20 and 84 years of age found that annual change of the usually used DWI markers fractional anisotropy (FA), axial diffusivity, radial

diffusivity, and mean diffusivity (MD) was greatest within the frontal and parietal lobes. Annual change increased with age, particularly within frontal regions, with age-related decline estimated to begin in the fifth decade. Longitudinal declines were mainly found in association and projection fibers (92). Vascular risk factors (VRF) seem to mediate the effects of age on diffusivity changes (93). Interesting data come from Maillard et al. Longitudinally, compared with individuals with no VRF, individuals with 1 VRF did not exhibit significantly different change in FA. However, those with ≥ 2 VRFs had greater decrease in FA within multiple white matter regions including the splenium of the corpus callosum (94).
Accelerated and earlier deterioration of frontal lobe diffusivity measures has been discussed (95,96), but results are controversial (97). Diffusion measures are more affected in age-related white matter lesions than in normal appearing brain tissue (98,99). Notably, DTI measures in the normal appearing brain tissue seem to be more closely related to the patients' clinical presentation than visible WMH load alone (5,99-102).
Correlations with cognitive test scores were described and include MMSE, executive function and IQ (99,101,103). DTI measures are also related to gait disturbances in older age. Two studies reported higher mean diffusivity (MD) and lower fractional anisotropy (FA) in the genu of the corpus callosum to correlate with poorer gait performance independently of visible WMH burden (59,104).

Magnetization Transfer Imaging (MTI) and Magnetization Transfer Ratio (MTR)

This section largely resembles the paper by Seiler S, Ropele S and Schmidt R "**Magnetization transfer imaging for in vivo detection of microstructural tissue changes in aging and dementia: a short literature review**", published in the *Journal of Alzheimer's disease* 2014;42 Suppl 3:S229-37. doi: 10.3233/JAD-132750 (105)

Basic principles of MTI

As mentioned above, standard MRI directly detects only signals from mobile water protons with adequately long T2 relaxation times. In human brain tissue, this signal comes from free intra- and extracellular tissue water ("free water pool"). Conversely, protons bound to macromolecules have too short T2 relaxation times (about 10 µs) to be detected directly. Macromolecules that bind protons in brain tissue are myelin proteins and lipids. This bound proton fraction ("bound water pool") can be imaged indirectly by exploiting the transfer of

magnetization between both proton pools, which is caused by dipolar coupling and chemical exchange mechanisms. In MTI, an off-resonance radio frequency (RF) pulse saturates the bound pool magnetization. Subsequent magnetization transfer shifts this magnetization to the magnetic resonance (MR) visible "free water pool". As a consequence, longitudinal magnetization decreases and causes a reduction of signal intensity. The degree of the magnetization transfer induced signal decrease is usually assessed by MTR (106,107). The MTR scales with the amount of magnetization exchange and with the extent of the bound pool and is expressed through the formula

$$\text{MTR}=\frac{M0-MSS}{M0} \qquad \text{(eq.2)}.$$

M0 characterizes the signal intensity of a voxel without RF saturation. MSS is the signal intensity of the identical voxel, acquired with the RF saturation pulse (6,108,109). A common approach to assess global MTR changes is histogram analysis. Regional MTR changes such as in white matter hyperintensities or (semi-) automatically segmented brain tissue compartments can be determined using a region of interest (ROI) analysis (110). Studies on post-mortem tissue of multiple sclerosis (MS) brains have evidenced that demyelination is most likely the correlate of MTR lowering within the white matter, but MTR reductions have also been related to axonal loss (111). There is evidence for MTR-lowering correlating with cortical demyelination in MS (112).

MTI findings in aging and dementia

Magnetization Transfer Imaging in Aging

MTR parameters changed with increasing age in several studies (113-115). Such associations have been described for normal appearing white matter (NAWM), i.e., all white matter outside T2-visible lesions (115), and the cortex in histogram- (110,116) and voxel-based analyses (106). According to study data, MTR lowering starts at the age of 40. MTR reductions seem to proceed more rapidly in men (113,117) and presence of vascular risk factors seemingly accelerates progression (113). Figure 2 shows a voxel-wise MTR map

where lower MTR and higher age correlate in community-dwelling older adults (Data from ASPS-Fam, preliminary results).

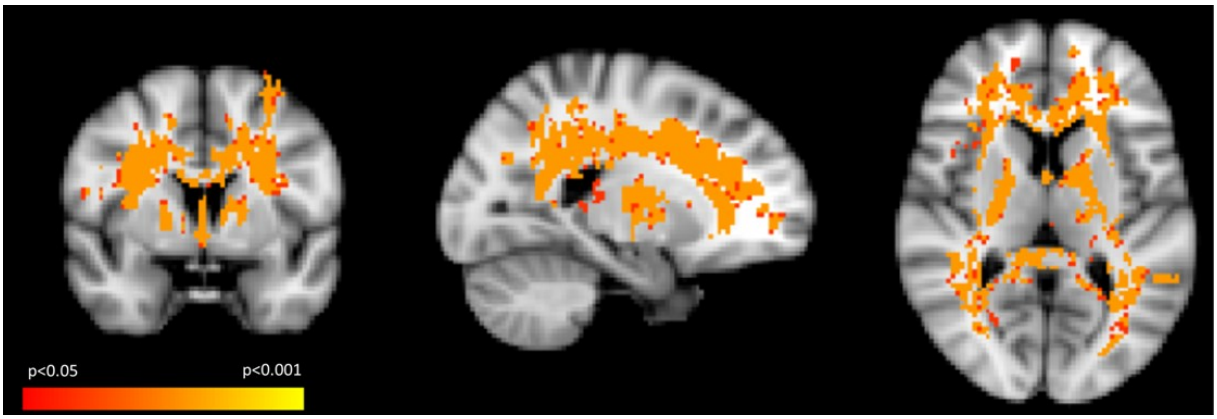

Figure 2 Associations between MTR and age. Yellow/red areas represent significantly lower MTR associated with higher age. Colour bar indicates statistical significance. Analyses were performed with FSL (9). MTR=Magnetization Transfer Ratio. Image is own work, published in (105)

MTI and Cognition

Studies on MTR metrics and their correlations with cognitive performance are summarized in Table 1.

In several investigations, lower MTR related to measures of executive dysfunction (114,118,119). One study on 272 older adults reported lower MTR in the NAWM but not the normal appearing gray matter, i.e., all gray matter outside T2-visible lesions, to be associated with worse information processing, independent of demographics, gray matter volume and WMH volume (118). Rather weak associations between lower MTR and impaired memory performance were reported. Whole brain MTR correlated with episodic memory (114) and frontal white matter MTR with verbal and working memory (120). In one study of our own group, we found a direct relationship between lower MTR in the frontal white matter and worse fine motor dexterity (110). Summing up, former studies on MTR and cognition were small and followed a cross-sectional design. Future studies will preferably assess these changes and their influence on cognition longitudinally, making causal inference possible.

Table 1. Studies on MTR metrics and correlations with cognitive performance in normal aging

Study	Cohort (N)	Brain region	Findings
Schiavone et al. 2009 (114)	64 healthy subjects	MTR whole brain histograms,	Correlations with information processing speed (r=0.486;p<0.0001), executive function (r=0.412;p<0.001) and episodic memory (r=0.376;p<0.003)
Fazekas et al. 2005 (110)	198 neurologically asymptomatic	MTR of the frontal NAWM	Correlation with fine motor dexterity (Purdue's Pegboard Test) only (p=0.04)
Venkatraman et al. 2011 (118)	272 elderly	Relative peak-height MTR of NAWM and NAGM	Correlation with DSST in NAWM (r=0.29;p<0.0001) and NAGM (r=0.18;p<0.004)
Lee et al. 2004 (119)	35 healthy community-dwelling elderly	MTR whole brain histograms	Correlation with MMSE (r=0.68,p<0.001), language (r=0.43;p<0.05), visuopspatial skills (r=0.66;p<0.001), verbal memory (r=0.66, p<0.001), visual memory (r=0.49,p<0.05), executive function r=0.68,p<0.001)
Düzel et al. 2010 (120)	86 healthy older and 24 young adults	MTR of substantia nigra, frontal white matter, and hippocampus	Correlation of basal forebrain and frontal white matter MTR with a factor combining verbal learning and memory and working memory
Seiler et al. 2011 (121)	16 healthy adults	MTR of cingulate, prefrontal region, thalami	Prediction of 55% of variance in accuracy in a test of visual selective attention
Seiler et al. 2014 (1)	355 healthy adults	MTR of the cortex, NAWM and deep gray matter	Whole brain cortical MTR significantly related to performance on tests of memory, executive function, and motor skills. MTR of deep gray matter structures and NAWM correlated with executive functioning

MTR=Magnetization Transfer Ratio; MMSE=Mini Mental State Examination;

NAWM=Normal Appearing White Matter; NAGM=Normal Appearing Gray Matter;

DSST=Digit Symbol Substitution Task; r=correlation coefficient

MTR in subjects with age-related vascular changes

MTR and white matter hyperintensities (WMH)

Across WMH types, there is evidence that MTR within T2-visible lesions is approximately 10% lower than that within NAWM (110,113). MTR of periventricular WMH is lower than that of deep lesions, suggesting more widespread microstructural tissue damage in periventricular than in deep abnormalities (115). This is in line with studies that showed more pronounced cognitive impairment in patients with periventricular WMH as compared to those who exhibited predominantly deep lesions (45,122-125). The finding that MTR in periventricular WMH of demented patients with Binswanger's disease (a form of subcortical arteriosclerotic encephalopathy including dementia, bilateral white matter abnormalities on MRI, and at least two of the following clinical findings: a vascular risk factor or evidence of systemic vascular disease, focal cerebrovascular disease, or "subcortical" cerebral dysfunction (126)) was significantly lower than that in periventricular WMH of similar severity of non-demented is in line with this observation (127). Tanabe et al. (128) presented comparable results and described reduced periventricular WMH MTR in demented subjects with small vessel disease as compared to control subjects.

MTR and normal-appearing brain tissue in patients with small vessel disease

As already stated, normal appearing brain tissue (including NAWM and NAGM) is defined as all brain tissue outside T2-visible lesions. In patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), the most common heritable origin of cerebral small vessel disease caused by mutations in the NOTCH3 gene, significantly lower MTRs in the NAWM of patients, relative to controls, were described (129). Higher WMH load correlated significantly with worse NAWM MTR (129). Lower NAWM MTR was significantly associated with poorer results on the SIDAM (a test to screen for dementia and for evaluation of dementia severity) and Rankin scale (assessment of the degree of disability and dependence in daily activities). This indicates widespread microstructural tissue damage outside visible WMH in patients with CADASIL. Maybe visible lesions are only the tip of the iceberg while more widespread tissue damage invisible to standard MRI is present. This study evidences the importance of these microstructural tissue abnormalities as diffuse tissue destruction of NAWM correlated better

with the clinical status of patients than the degree of visible abnormalities. Comparable relations between WMH severity and NAWM MTR of the frontal lobes were reported in clinically asymptomatic older adults (110).

MTI in the differential diagnosis of Alzheimer's disease

The diagnostic value of hippocampus MTR has been assessed by one small study. In this work, mean MTR of the hippocampus better discriminated (sensitivity 61%, specificity 90%) between AD and controls than total brain volume (sensitivity 50%, specificity 90%). However, hippocampal mean MTR was not superior to hippocampal volume (sensitivity 89%, specificity 61%) (130). Concerning the value of mean hippocampal MTR compared to visual atrophy rating, the discrimination rate reported by one recent study between AD and other dementias was 77% for mean MTR and 65% for the visual atrophy rating (131). Numbers for the discriminative power of DTI –based classifiers compared to MTI are not yet available. A voxel-based study by Giulietti et al. (107) measured MT differences Between AD patients and controls by using non-linear registration and inclusion of a volumetric map to minimize partial volume effects resulting from atrophy and subsequent CSF contamination. The authors identified reduced MTR values in AD patients mainly in the hippocampus, temporal lobe, posterior cingulate, and parietal cortex. They show that MTR abnormalities in AD occur in a disease-specific pattern, independent of cortical atrophy. Figure 3 shows exemplarily the results of a preliminary analysis where reduced MTR in AD patients were compared to controls (preliminary data from the prospective dementia registry in Austria – PRODEM).

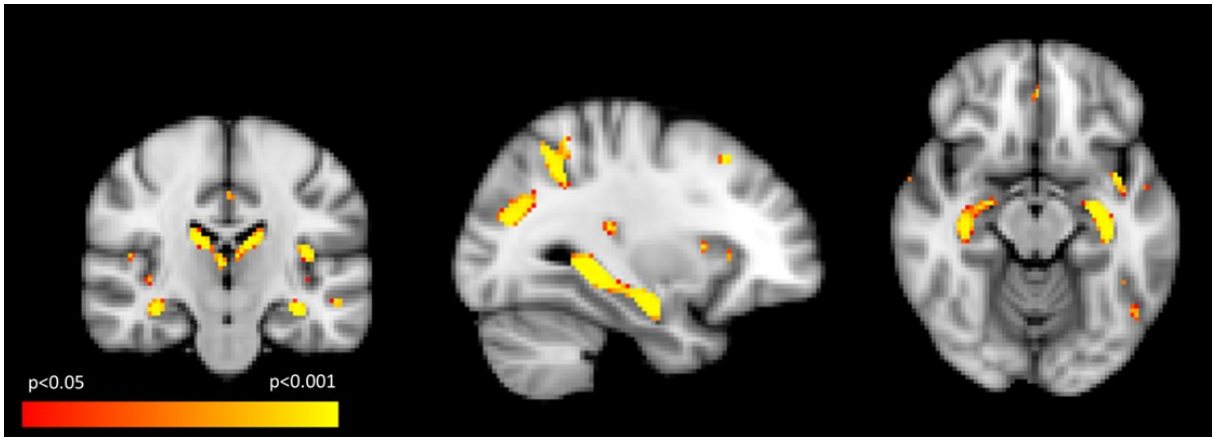

Figure 3. Voxel wise analysis. Red/yellow areas represent voxels with significantly lower MTR in AD patients, as compared to healthy controls. Colour bar indicates statistical significance. Analyses were performed with FSL (9). AD=Alzheimer's disease. Image is own work, published in (105)

MTI for prediction of MCI-AD conversion

Patients with mild cognitive impairment (MCI) show cognitive deficits in any domain, but do not yet fulfil dementia criteria (132). The individuals' activities of daily living are not troubled by these deficits. Annual conversion rates from MCI to AD are situated between 6.8 and 8.1% (133). Stable and even reversible courses have been reported. One study found MCI patients returning to normal cognition in 42% over a period of 5 years (134,135). Recent data suggest that both MCI and AD patients have more microstructural brain damage than healthy controls (136). Therefore, microstructural brain tissue changes may be present early during the disease process and most likely precede overt atrophy (137). Few cross-sectional studies have found MT classifiers to differentiate AD from MCI. Automatic model-based multiparameter MTI in the entorhinal cortex differentiated de novo AD from MCI with a sensitivity of 100% and a specificity of 94% (138). Similarly, quantitative MTI of the hippocampal head differentiated AD from MCI patients (139). One MTR study assessed whether MCI patients with a single domain deficit differ from patients with deficits in multiple domains (140). They found shared MTR reduction in the medial temporal lobe and posterior white matter between patients with exclusive memory impairment and additional executive dysfunction. Only those patients with additional executive dysfunction showed a spread of MTR reduction to prefrontal white matter and the insula.

Associations between MTR and cognition in AD

Previous studies on the association between MTR and cognitive functioning in AD were small. Results can be seen in table 2.

A number of studies reported associations with MMSE scores (130,141,142). Two studies evaluated whether regional MTR changes related to specific cognitive constellations in AD patients (142,143). The results of these studies are conflicting. While Fornari et al. (142) found lower MTR in the superficial white matter to be associated with worse performance on memory and naming tests, the study by Van der Flier et al. (143) failed to show any relationships between MTR and different cognitive domains. The authors reported non-specific associations between global and regional MTR values with all cognitive domains, indicating rather widespread microstructural brain damage in AD brain. One longitudinal analysis of MTR histograms of AD patients showed a constant and significant decline of global- and regional MTR over an observational period of 1 year (141). The authors found no correlation between the MTR histogram parameter changes over time with cognitive decline.

Table 2. Studies on MTR metrics and correlations with cognitive performance in Alzheimer's disease

Study	Cohort (N)	Brain regions	findings
Ropele et al. 2012 (141)	28 AD 19CO	Hippocampus Thalamus Putamen Caudate Nucleus	Left hippocampus ($r=0.57;p<0.01$), right hippocampus ($r=0.38;p=0.048$), left thalamus ($r=0.52;p<0.01$), right thalamus ($r=0.42;p=0.029$), left putamen ($r=0.7;p<0.001$), right putamen ($r=0.60;p<0.01$) MTR significantly correlated with MMSE; stronger associations in the left hemisphere
Van der Flier et al. 2002 (143)	22 AD 13 MCI 28 CO	Whole Brain Temporal lobe Frontal lobe	Strong association of all regions with MMSE and scores for memory, orientation, language, praxis, gnosis, executive functioning.
Fornari et al. 2012 (142)	15 AD 15 CO	WM (short association U-fibers)	correlation with MMSE, Lexis, and memory tests

MMSE=Mini Mental State Examination; MT=Magnetization Transfer; MTR=Magnetization Transfer Ratio; WM= White Matter; r=correlation coefficient; AD=Alzheimer's disease; MCI=Mild cognitive Impairment; CO=Controls

Cognitive domains and measures of mobility

Comprehensive neuropsychological testing procedures are available to assess patients' performance in different cognitive domains. Recent studies have mainly distinguished between memory, executive function and motor skills (1,144). As a measure of mobility, gait velocity has been found suitable (58,145). Test procedures used in this PhD thesis will be reviewed in the methods section.

Studies and hypotheses

Within this PhD project, 2 studies were performed. In study#1, we were interested whether microstructural tissue alterations detected by MTI were associated with cognition. In study#2, we tested for the association between MTR in the white matter of the brain and gait disturbances. For better readability, the two studies will be discussed separately.

1st study: MTR and cognition

This section resembles the "introduction" of the paper by Seiler S, Pirpamer L, Hofer E, Duering M, Jouvent E, Fazekas F, Mangin JF, Chabriat H, Dichgans M, Ropele S, Schmidt R **"Magnetization transfer ratio relates to cognitive impairment in normal elderly"** published in *Frontiers in Aging Neuroscience* 2014 Sep 25;6:263. doi: 10.3389/fnagi.2014.00263. eCollection 2014 (1)

Diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI) are MR based neuroimaging methods that allow the detection and quantification of microstructural tissue changes in white- but also gray matter of the brain. By these methods, we can quantify cerebral brain damage beyond what we can expect from standard MRI techniques like T1- and T2-weighted sequences (99,101,146,147). DTI measures the diffusion properties of tissue water (7). Conversely, MTI is based on the exchange of magnetization between tissue water and protons bound to macromolecules (6,108). This exchange is expressed by the magnetization transfer ratio (MTR). Histopathologically, lower MTR correlates with reduced myelin content and lower axonal density in both the white- and gray matter (111,112,148). Several studies showed MTR reductions in NAWM and the cortex with age (106,110). Fazekas et al. found a significant age dependency of MTR lowering in the frontal and parieto-

occipital gray matter. Draganski et al. found additional gray matter MTR changes in the basal ganglia and cerebellar cortex. In AD brains, decreased MTR has been described in the hippocampus (130,131), temporal lobe, posterior cingulate and parietal cortex. MTI changes were present in a disease-specific distribution, independent of gray matter atrophy (107). Concerning their correlations with cognitive decline, results of DTI and MTI studies are non-uniform. DTI measures in the NAWM strongly correlate with cognitive function in normal aging (97,146), and various diseases (147,149-151). However, little is known about the clinical significance of MTR measures. Previous studies reported conflicting results. One investigation in 64 healthy adults aged 50-90 years (114) reported associations of lower MTR in the NAWM with poorer scores in tests of processing speed, executive function and episodic memory. Another MTR study included 55 Alzheimer's disease (AD) patients, 19 patients with mild cognitive impairment (MCI) and 43 healthy controls. They found a significant and direct relationship between temporal gray matter MTR and MMSE across disease groups (136). Interesting longitudinal data come from Ropele et al. The authors describe an association between deep gray matter MTR of AD patients with MMSE scores, constantly over a one-year time period (141). However, studies also failed to evidence significant associations between both cortical- and white matter MTR and cognitive functions (110,152).

Hypotheses/Aims

The current investigation examined a large cohort of 355 community dwelling subjects over a wide age range. We hypothesized that MTR scales with the severity of tissue changes and relates to worse cognitive performance even when controlling for vascular risk factors and brain abnormalities on conventional MRI. We also hypothesized that the regional distribution of MTR changes determines the pattern of cognitive impairment.

2nd study: MTR and gait abnormalities

Gait abnormalities in older adults are a common clinical finding and incidence of abnormal gait increases with advancing age (153). A recent study on 488 older adults aged 70 to 99 years showed that 35% of study participants exhibited gait disturbances to some extent (154). Gait disturbances, including slower gait velocity, have been associated with falls in several studies (155-159). The hospitalizations and care-giving expenses associated with falls are serious public health issues (153,160).

A complex higher-level network including the primary and supplementary motor cortex, basal ganglia, cerebellum and brain stem manages the supraspinal control of motor performance (161). Damage of white matter tracts might lead to disconnection of this network resulting in disruption of function (57,162,163). In line with this assumption several studies found higher volumes of white matter lesions (WMH) to be associated with poorer gait performance (53-55), but there have also been reports that did not corroborate these findings (57-59). Factors that might be responsible for these conflicting results are different measures of WMH severity and gait performance between studies. Also sample sizes vary. Likewise, it is conceivable that similar as in cognitive dysfunction, WMH do not represent the full extent of tissue damage and age-or small vessel disease-related widespread microstructural abnormalities in normal appearing white matter are more important for gait abnormalities than WMH volume per se. Support for this hypothesis comes from two DTI studies that described higher mean diffusivity (MD) and lower fractional anisotropy (FA) in the genu of the corpus callosum to correlate with poorer gait performance independently of visible WMH (59,104).

Complementary information on microstructural brain tissue alterations may be obtained from magnetization transfer imaging (MTI). MTI probes the magnetization exchange between tissue water and protons bound to macromolecules. Other than DTI, which offers information on brain tissue organization by probing the mobility of water protons in axons (7), MTI offers information on tissue composition (6,108). Magnetization transfer ratio (MTR) is currently

the only MR measure that has been validated in postmortem comparisons to represent a direct marker of myelin content (111).
So far, only one study used MTI to determine the role of microstructural brain abnormalities for gait dysfunction in the elderly. The authors used a histogram-based MTR analysis across the whole brain parenchyma. They found lower whole-brain histogram peak-height MTR to be associated with poorer gait performance, independent of WMH load (164).

Hypotheses/Aims

The approach is hypothesis-free ("hypothesis generating") and relies on a minimum of prior assumptions. We used for the first time a combination of voxel-based lesion symptom mapping (VLSM) and voxel-based MTR symptom mapping (VMTRSM) to identify those brain areas where white matter lesions or MTR-determined microstructural tissue alterations relate to gait velocity.

Methods

There is some, but not substantial overlap between the methods sections of the 2 studies. For the most part, methods are presented for both studies separately to enhance readability and understandability.

1st study: MTR and cognition

This section resembles the "methods" of the paper by Seiler S, Pirpamer L, Hofer E, Duering M, Jouvent E, Fazekas F, Mangin JF, Chabriat H, Dichgans M, Ropele S, Schmidt R **"Magnetization transfer ratio relates to cognitive impairment in normal elderly"** published in *Frontiers in Aging Neuroscience* 2014 Sep 25;6:263. doi: 10.3389/fnagi.2014.00263. eCollection 2014 (1)

Subjects

The study cohort is from the Austrian Stroke Prevention Family Study (ASPS-Fam), a prospective single-center, community-based study on the cerebral effects of vascular risk factors in the normal elderly population of the city of Graz, Austria. The ASPS-Fam

represents an extension of the Austrian Stroke Prevention Study (ASPS), which was established in 1991(165,166). Between 2006 and 2013, study participants of the ASPS and their first grade relatives were invited to enter ASPS-Fam. Inclusion criteria were no history of previous stroke or dementia and a normal neurologic examination. A total of 381 individuals from 169 families were included into the study. The number of members per family ranged from 2 to 6.

The entire cohort underwent an extended diagnostic work-up including clinical history, blood tests, cognitive testing and a thorough vascular risk factor assessment. All individuals underwent MRI, except for 26 who had contraindications. Thus, magnetization transfer imaging (MTI) was available in a total of 355 subjects. The study protocol was approved by the ethics committee of the Medical University of Graz, Austria, and written informed consent was obtained from all subjects.

Vascular risk factors

Assessment of vascular risk factors included arterial hypertension, diabetes mellitus, cardiac disease, hypercholesterolemia, hypertriglyceridemia, hyperuricemia, peripheral vascular disease and venous thrombotic disease and was determined based on history and measurements at the examination as previously described (165).

Neuropsychological testing

We used a test battery assessing memory, executive function and motor skills as described previously (165). The tests are well established and were always applied in the same order and under identical laboratory conditions. The “Bäumler’s Lern- und Gedächtnistest” (LGT-3) (167), a highly demanding paper-pencil procedure consisting of six subtests, assessed intermediate memory recall and learning ability. Three subtests (word and digit association tasks and story recall) screen for verbal memory. Two subtests (trail and design recall) assess visuospatial memory. The total memory score used in this PhD thesis results from the sum of weighted scores from the aforementioned subtests and of an image recognition paradigm (165). Executive functions were tested by the Wisconsin Card Sorting Test (168), part B of the Trail Making Test (169), and Digit Span Backwards, which is part of the Wechsler Adult Intelligence Scale, revised (170). Adhering to Milner's criteria (171), the measures computed

for the Wisconsin Card Sorting test were categories completed, perseverative errors, and total errors (165).
Motor skills were evaluated by the Purdue Pegboard Test (172).
To reduce floor and ceiling artifacts and other sources of measurement error, we used summary measures of cognitive function in the analyses rather than the results of individual tests. We formed composite measures of the specific domains of cognitive function. Each summary measure was calculated by converting individual test scores to z-scores within the group and by computing the average of the scores in each cognitive domain (165).

Magnetic resonance imaging

MR imaging was performed on a 3T whole body scanner (TimTrio; Siemens Healthcare, Erlangen, Germany) and included conventional imaging and MTI. The MT sequence was based on a spoiled 3D gradient-echo sequence (TR=40ms, TE=7.38ms, flip angle=15°, number of slices=40, slice thickness=3mm, in-plane resolution=0.86x0.86mm) that was performed with and without a Gaussian shaped MT saturation pulse.
The conventional protocol included an axial FLAIR sequence (TR=10000ms, TE=69ms, inversion time=2500ms, number of slices=40, slice thickness=3mm, in-plane resolution=0.86x0.86 mm^2) and a high resolution T1 weighted 3D sequences with magnetization preparation (MPRAGE) and whole brain coverage (TR=1900ms, TE=2.19ms, inversion time=900ms, flip angle=9°, isotropic resolution of 1 mm).
White matter hyperintensities (WMH), silent non-lacunar and lacunar infarcts were recorded on FLAIR images as previously described (165) (Figure1). Non-lacunar infarcts were lesions with typical signal characteristics of infarcts following a typical vascular territory or being located in a border zone between two vascular territories. Lacunes were focal lesions involving the basal ganglia, the internal capsule, the thalamus, the brainstem or the white matter not exceeding a maximum diameter of 20 mm. All lesions were outlined using a custom written IDL program (Exelis Visual Information Solutions, USA) (Figure4). Lesion areas were segmented by combined region growing and local thresholding following manual selection (173). The total lesion volume (mm^3) was calculated using the program FSLMATHS (FSL, Oxford, www.fmrib.ox.ac.uk) by multiplying the lesion area with the slice thickness and normalized by head size. Cortex volume, normalized for the subject head

size, was calculated from the T1weighted MPRAGE images using the fully automated structural image evaluation of atrophy (FSL, Oxford, www.fmrib.ox.ac.uk) (9).

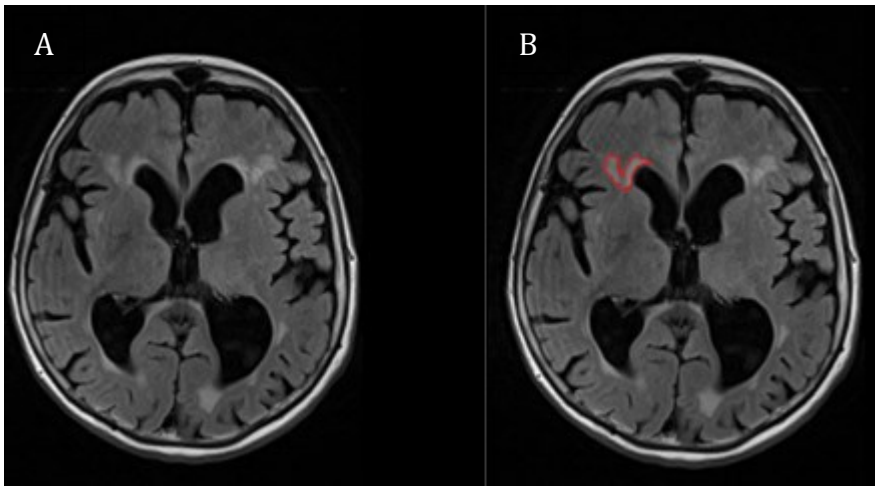

Figure 4. Example of lesion area segmentation. (A): Native FLAIR weighted scan with WMH. The red outline in (B) shows a segmented periventricular WMH. WMH=White Matter Hyperintensity; FLAIR=Fluid Attenuated Inversion Recovery

Image processing and MTR analysis

MTR metrics were assessed separately for the cortex, deep gray matter structures, white matter hyperintensities (WMH) and normal appearing white matter (NAWM). MTR maps were calculated according to (eq2). The MTR maps were registered to the corresponding FLAIR and T1 scans by using an automated affine registration tool (FLIRT as part of FSL, http://www.fmrib.ox.ac.uk/fsl/flirt/index.html) (174). WMH masks were generated from the outlined lesions using our custom written IDL tool (173). A mean MTR was calculated in MTR space for each WMH by masking the registered MTR maps with the WMH masks. The MTRs of all WMHs were averaged to obtain a mean lesion MTR for each subject's whole brain-, periventricular- and deep WMH.

White matter (WM) and cortex masks were generated using the program FAST (FSL, Oxford, www.fmrib.ox.ac.uk) (175). Both NAWM and cortex masks were generated separately and comprised all WM and cortical tissue outside WMHs, silent non-lacunar infarcts and lacunes, i.e. it comprised all neocortical gray matter and WM with normal signal intensity on FLAIR images. Corresponding MTR maps were produced by overlying both the cortex and NAWM masks separately on the MTR maps and a mean MTR for total NAWM and cortex was calculated for each subject. Calculation of lobar MTRs comprising frontal, parietal, occipital and temporal lobes was done by applying a home-written atlas tool. The atlas tool was generated in MNI 152 space by manually delineating neuroanatomical borders of the 4 lobes. The atlas was then registered to the respective patient's native space and overlaid on the total NAWM/cortex MTR maps to obtain lobar MTR values.

Deep gray matter structures including the thalamus, putamen, pallidum, hippocampus, caudate nucleus, amygdala and accumbens nucleus were generated using the program FIRST (FSL, Oxford, www.fmrib.ox.ac.uk) (9,176) . Corresponding MTR maps were produced by overlying the deep gray matter structure masks on the MTR maps and subsequently a mean MTR of each structure was calculated for each subject. To reduce partial volume effects, which might have occurred due to image registration and subsequent interpolation, we eroded all masks by 1 pixel before overlying on the MTR maps.

Statistical analysis

Assumptions of normal distribution were tested with the Kolmogorov-Smirnov test. Normally distributed variables are reported as mean +/- standard deviation and non-normally distributed variables as median and interquartile range. For bivariate correlations, Pearson's correlation

analysis has been performed. Due to the bimodal distribution of age (figure 5) we used age as an ordinal variable categorized into three categories (38 years to 60 years; 61 years to 70 years; 71 years to 86 years) in all statistical models.

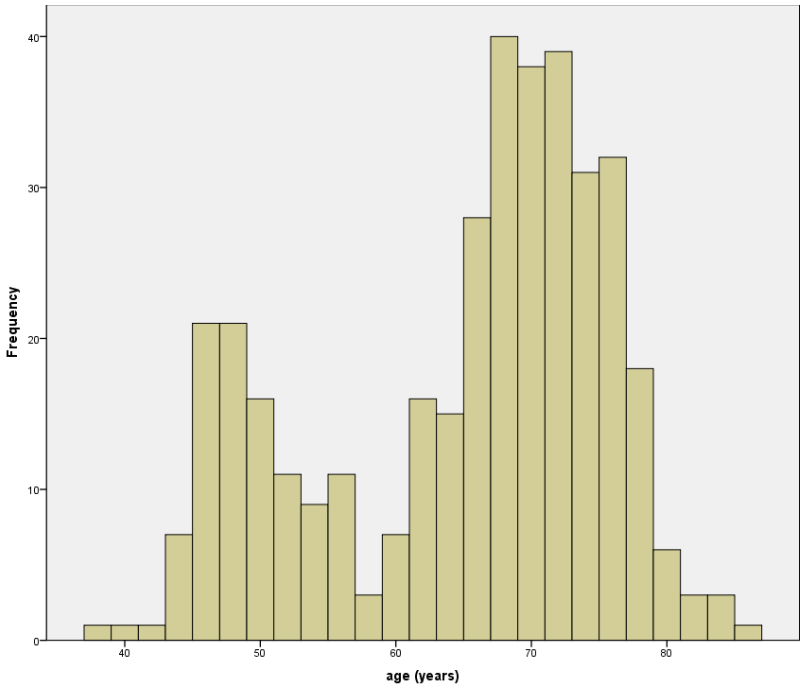

Figure 5 Bimodal age distribution of study participants.

WMH volume had a skewed distribution containing zero values and therefore the value 2 was added to the volumes before natural log transformation.

Multivariate linear and logistic regression analyses were used to correlate visible brain changes (WMH volume, presence of silent non-lacunar infarcts, presence of lacunes) with global and regional MTR metrics in the cortex and NAWM. These models were adjusted for age, sex, vascular risk factors and normalized cortex volume. We use cortex volume as a covariate because it is widely accepted that it strongly correlates with cognitive changes across the aging and dementia spectrum. To assess the associations between global and regional mean MTRs in the cortex, NAWM and WMH and domain-specific neuropsychological test performance, mixed models were calculated. The cognitive variable was the dependent and MTR was the predictor variable. In addition, we performed age- and sex stratified sub analyses. These models were adjusted for potential confounders to evaluate the independent effect of MTR on cognitive functions. Selection of confounders was a priori defined according to previous literature (177,178). We considered age, sex, education, vascular risk factors, silent non-lacunar infarcts, lacunes, WMH volume and cortex volume as possible confounders. Multicollinearity was assessed between the independent variables of the models using the variance inflation factor (VIF). A VIF value > 10 is an indicator of multicollinearity. There was no indication for multicollinearity in the models. The mean VIF for predictor variables in our study was 1.34 (range 1.03 – 3.48).

To account for the sample relationships in current family-based study, a random effect was added to each model using a kinship matrix describing the family structure (179,180).

To determine possible dose-effect relationships of significant associations between MTR and cognitive functioning, subjects were categorized into quartiles according to MTR value distribution. Linear regression analyses with MTR quartiles being the explanatory variables and cognitive test results being the dependent variable adjusted for age, sex, education, risk factors, lacunes, non-lacunar infarcts, WMH and normalized cortex volume were done. The highest quartile of MTR values served as the reference.

For each regression coefficient, the 95% confidence interval and the p-value were determined. A two-sided p-value <0.05 was considered to be statistically significant.

The Multicollinearity check and t-tests were performed with SPSS (IBM Statistics for Windows, Version 19, Armonk, New York). The mixed models and the kinship matrices were calculated using the coxme (Therneau, 2012) and kinship2 (Therneau, 2011) packages in R (R: A language and Environment for Statistical Computing, Vienna, Austria. www.R-

project.org). We assessed if age effects on cognitive function were mediated by MTR variables. Estimation of mediator effect sizes was calculated using bootstrapped models (181). According to Preacher and Hayes, "significant mediation can be determined via the 95% bootstrapped confidence interval. If "zero" lies within the interval range, it is possible with 95% confidence that the true indirect effect would be zero (no mediation). If "zero" does not occur between the interval boundaries then we can conclude that the indirect effect for this mediator is significant" (182). Mediation was assessed for cortical and NAWM MTR individually. We corrected the mixed model analyses and sub-analyses for multiple comparisons within each domain using the Benjamini–Hochberg false discovery rate (FDR, $p < 0.05$) (183).

2nd study: MTR and gait abnormalities

Subjects

The study population is basically the same as in the 1st study. However, because we focused on age-related decline in gait velocity, we included all 230 subjects aged 60 years and older in the current analysis. Table 1 provides subjects' characteristics.
The ethics committee of the Medical University of Graz, Austria, approved the study protocol and written informed consent was obtained from all subjects.

Measurement of gait velocity

Study participants were asked to walk a total distance of eight meters with three turns at their usual, self-selected pace on level ground. Time was measured with a stopwatch. The faster out of two trials was used for the subsequent analyses. We chose gait velocity, because it can be measured quickly and in a clinical setting without apparative efforts. It has been shown to be a good measure of mobility in elderly people (58).

Magnetic Resonance Imaging

MRI was performed on a 3T whole body scanner (Magnetom TimTrio; Siemens Healthcare, Erlangen, Germany) and included conventional imaging and MTI. The MT sequence was

based on a spoiled 3D gradient-echo sequence (TR=40ms, TE=7.38ms, flip angle=15°, number of slices=40, slice thickness=3mm, in-plane resolution=0.86x0.86mm) that was performed with and without a Gaussian shaped MT saturation pulse.
The conventional protocol included an axial FLAIR sequence (TR=10000ms, TE=69ms, inversion time=2500ms, number of slices=40, slice thickness=3mm, no interslice-gap, in-plane resolution=0.86x0.86 mm²) and a high-resolution T1-weighted 3D sequence with magnetization preparation (MPRAGE) and whole brain coverage (TR=1900ms, TE=2.19ms, inversion time=900ms, flip angle=9°, isotropic resolution of 1 mm).

Generation of WMH- and MTR maps

WMH maps were generated using a custom written IDL program (Exelis Visual Information Solutions, Boulder, CO) as described previously (173). RS and SS segmented WMHs on FLAIR images by combined region growing and local thresholding following manual selection (173). The total lesion volume in mm3 was calculated by multiplying lesion area with slice thickness. WMH volumes in white matter tracts were calculated by overlying the probabilistic white matter tract atlas (25% probability) provided within the FMRIB software library (184), on the normalized WMH maps.

MTR maps were calculated according to (eq2).

For the subsequent steps, tools from the Functional Magnetic Resonance Imaging of the Brain (FMRIB) software library (FSL) (9) were used.
Because we found that MTR provides good contrast to perform tissue segmentation, gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) partial volume maps were derived from the MTR weighted scans using FSL FAST (FMRIB's Automated Segmentation Tool; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FAST) (175).
The T1, FLAIR and MTR weighted scans were brain extracted using FSL BET (Brain Extraction Tool; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET) (185).
The resulting skull-stripped T1 weighted images were non-linearly registered to the Montreal Neurological Institute 152 standard space template (MNI 152) using FSL FNIRT (FMRIB's Non-Linear Image Registration Tool; (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT) (9).

Then, the brain extracted FLAIR and MTR scans were linearly registered to the corresponding brain extracted T1 weighted images using FSL's FLIRT (FMRIB's Linear Image Registration Tool; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT (174).
We used the transformation matrices from these steps to warp FLAIR and WM MTR maps to the MNI 152 standard space template. WMH maps were transformed to the MNI 152 standard space template in the same way.
The resulting WM MTR maps in standard space were eroded by 1 voxel to reduce partial volume effects resulting from "edge" voxels. To produce more normally distributed data, reduce noise and account for the inter-subject and registration variability (186), we smoothed the MTR maps with a 4mm Gaussian kernel.
A mean WM MTR mask was created and thresholded to exclude MTR values below 20% (105). We chose this threshold to be sure to exclude voxels from CSF and to further reduce the spurious effects of partial volume effects caused by the white matter-gray matter transition zone.
Anatomical structures containing clusters of voxels in which MTR related significantly to gait velocity were localized by overlying the probabilistic white matter tract atlas (25% probability) provided within the FMRIB software library (184), on the normalized WM MTR maps. As we did it in voxel-wise analysis, the segmentations of these tracts were eroded by 1 voxel to reduce CSF artifacts at "edge" zones. The resulting "core" white matter-mean MTR was used in subsequent analyses.

Statistical analysis

Basic statistical analysis

Assumptions of normal distribution were tested with the Kolmogorov-Smirnov test. Normally distributed variables are reported as mean +/- standard deviation and non-normally distributed variables as median and interquartile range. WMH volume had a skewed distribution containing zero values and therefore the value 2 was added to the volumes before natural log transformation. To relate demographic, clinical and imaging characteristics of the study participants to gait velocity, subjects were categorized into quartiles according to gait velocity distribution. One-way analysis of variance (ANOVA) and Chi2 tests were performed to test for significant associations with normally- and non-normally distributed variables, respectively. Variables significantly ($p<0.05$) associated with gait speed in these analyses

were entered as covariates in the VMTRSM and ROI analyses described in the subsequent paragraphs.

Voxel-based lesion symptom mapping (VLSM)

Non-parametric mapping (NPM) was used to relate WMH location to gait velocity (187). 1000 permutations were carried out to build the null distribution and the Brunner-Munzel test was applied to test for statistical significance (187,188). Briefly, permutation testing is a procedure that compares a test statistic to a null distribution derived from the dataset of interest itself. Permuting how the dependent and independent variables are paired typically derives the permutation null distribution. When the null hypothesis is true (no effect), the observed pairings should be no more likely to generate an extreme test statistic than any other (189). Voxels affected in less than seven subjects were not considered for analysis. Correction for multiple testing was achieved by permutation generated family-wise error (FWE) thresholds. Age, sex, height and brain volume were entered as covariates. To identify the localization of significant voxels within major white matter tracts, we used the probabilistic white matter tract atlas (184) provided within the FMRIB software library.

Voxel-based MTR symptom mapping (VMTRSM)

To find associations between MTR values within a voxel and gait velocity, we used the permutation-based statistical interference tool for non-parametric testing (RANDOMISE) as a part of FSL(9). 5000 permutations were carried out to build the null distribution and significant associations were determined selecting the threshold-free cluster- enhancement (TFCE) option. To correct for multiple comparisons, a family-wise error (FWE) adjusted p-value <0.05 was considered statistically significant. Age, sex, height and brain volume as well as variables that were univariately associated with walking speed were entered as covariates.

Region of interest (ROI) analysis

To identify white matter tracts in which mean MTR correlates with gait velocity, we overlaid the probabilistic white matter tract atlas (184) provided within the FMRIB software library on significant voxels from the VMTRSM analysis.

Mean MTR within identified tracts was calculated in standard space using FSLMATHS. To reduce partial volume effects from "edge" voxels, identified tracts were eroded by 1 voxel to exclude possible CSF contamination. The mean MTR in the resulting "skeletonized" tracts was used in the subsequent linear multiple regression analysis.
A possible dose-effect relation of MTR within identified tracts and gait velocity was investigated by means of analysis of covariance (ANCOVA) with MTR in quartiles.
Age, sex, height and brain volume as well as variables that were univariately associated with walking speed were entered as covariates in the linear multiple regression analysis and the ANCOVA. A p-value <0.05 was considered statistically significant.

Results

This chapter lists the results for each of the two studies separately.

1st study: MTR and cognition

This section resembles the "results" of the paper by Seiler S, Pirpamer L, Hofer E, Duering M, Jouvent E, Fazekas F, Mangin JF, Chabriat H, Dichgans M, Ropele S, Schmidt R **"Magnetization transfer ratio relates to cognitive impairment in normal elderly"** published in *Frontiers in Aging Neuroscience* 2014 Sep 25;6:263. doi: 10.3389/fnagi.2014.00263. eCollection 2014 (1)

Demographics, frequency of vascular risk factors, neuropsychological test results and MRI findings of the study participants are displayed in table 3.

Table 3. Demographics, risk factors, neuropsychological test performance and MRI findings of study participants.

A. Basic demographics	
Age, years (median, IQR)	68.00 (56.00-72.00)
Age category 1: 38-60 years, N(%)	100 (28.2)
Age category 2: 61-70 years, N(%)	131 (36.9)
Age category 3: 71-86 years, N(%)	124 (34.9)
Women N(%)	214 (60.3)
Education N(%)	
primary school N(%)	70 (19.7)
apprenticeship N(%)	157 (44.2)
high school diploma N(%)	72(20.3)
university degree N(%)	56 (15.8)
B. Risk factors	
Arterial hypertension N(%)	229 (64.5)
Diabetes N(%)	38 (10.7)
Heart disease N(%)	186 (52.4)
Hypercholesterolemia N(%)	272 (76.6)
Hypertriglyceridemia N(%)	60 (16.9)
Hyperuricemia N(%)	104 (29.3)
Peripheral vascular disease N(%)	5(1.4)
Venous embolic disease N(%)	38(10.7)
C. Neuropsychological testing (z-values)	
Memory, z-values (range)	-1.14-3.51
Executive function, z-values (range)	-4.15-1.35
Motor skills, z-values (range)	-2.49-3.12
D. MRI variables	
Lacunes N(%)	33(9.4)
Silent non-lacunar infarcts N(%)	19(5.5)
Cortex volume, cm3 (mean, +/-SD)	599.69 (40.11)
WMH volume, cm3 (median, IQR)	5.63 (2.97-10.76)

MRI: Magnetic Resonance Imaging, WMH: White Matter Hyperintensities, SD: Standard Deviation, IQR= interquartile range.

Lacunes and non-lacunar infarcts were inversely related to cortical MTR (ß = -0.55; 95%CI -1.003, -0.112; p= 0.014 and ß= -0.53; 95%CI -0.89, -0.17; p=0.003), and NAWM MTR (ß= -0.603; 95%CI -0.958, -0.248; p=0.0009 and ß= -0.44; 95%CI -0.90, 0.01; p=0.06) after adjustment for age, sex and vascular risk factors.

There existed no significant association between WMH volume and cortical or NAWM MTR (ß= 0.015; 95%CI -0.037, 0.067 p=0.570 and ß=-0.002, 95%CI 0.054-0.050, p=0.940, respectively).

MTR of cortex and NAWM correlated significantly and independently of subject characteristics and MRI findings with each other (ß = 0.68; 95%CI 0.594-0.757; p <0.001).

There existed a strong correlation between cortical MTR and cognitive functions (figure 6).

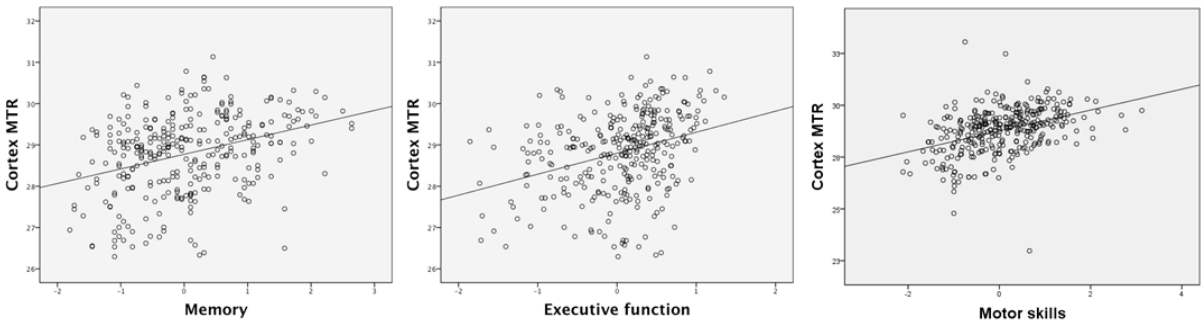

Figure 6. Scatterplots showing the correlation between cortex MTR and domain-specific neuropsychological test scores.

Abbreviations: MTR = Magnetization transfer ratio. x-axis displays z-values of neuropsychological test scores, y-axis gives MTR values of whole brain cortex.

Pearson's correlation analysis showed significant associations of cortex MTR with scores of memory ($r=0.338$, $p<0.01$), executive function ($r=0.326$, $p<0.01$) and motor skills ($r=0.472$, $p<0.01$

Table 4. Independent relationship between cortical MTR and cognitive functioning.

Mean MTR	Memory			Executive function			Motor skills		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
Cortex									
Whole brain	0.129	[0.038;0.220]	0.0366	0.118	[0.051;0.192]	0.0044	0.111	[0.033;0.189]	0.0418
Frontal lobe	0.119	[0.030;0.209]	0.0366	0.116	[0.049;0.188]	0.0044	0.097	[0.020;0.173]	0.0616
Parietal lobe	0.129	[0.042;0.217]	0.0366	0.123	[0.058;0.194]	0.0033	0.095	[0.020;0.171]	0.0616
Occipital lobe	0.121	[0.035;0.208]	0.0366	0.129	[0.066;0.199]	0.0022	0.104	[0.030;0.178]	0.0418
Temporal lobe	0.108	[0.022;0.194]	0.0409	0.086	[0.022;0.155]	0.0229	0.111	[0.038;0.184]	0.0418
Deep gray matter									
Thalamus	0.023	[-0.027;0.073]	0.4515	0.044	[0.006;0.082]	0.0421	0.038	[-0.008;0.078]	0.2514
Putamen	0.033	[-0.020;0.085]	0.3457	0.057	[0.017;0.097]	0.0183	0.036	[-0.013;0.077]	0.3025
Pallidum	0.023	[-0.022;0.068]	0.4262	0.034	[-0.001;0.068]	0.0864	0.045	[0.007;0.084]	0.0770
Caudate nucleus	0.024	[-0.025;0.074]	0.4400	0.062	[0.024;0.100]	0.0057	0.027	[-0.016;0.070]	0.4400
Amygdala	0.018	[-0.023;0.060]	0.4515	0.029	[-0.003;0.061]	0.1173	0.019	[-0.017;0.055]	0.5316
Accumbens nucleus	-0.011	[-0.046;0.024]	0.5830	0.020	[-0.007;0.047]	0.1941	0.011	[-0.019;0.041]	0.5988

Adjusted for age, sex, years of education, vascular risk factors, Cortex volume, thromboembolic infarcts, lacunes, and WMH volume. MTR=Magnetization Transfer Ratio. p-values adjusted for multiple testing.

As can be seen from table 4, whole brain, and all lobar cortical MTRs were significantly and directly associated with performance in memory and executive function. For motor skills only the temporo-occipital MTRs were significantly related and there was a non-significant trend for fronto-parietal lobe MTR. The MTR of deep gray matter structures correlated with executive function, but not with scores of memory and motor skills.

Table 5 displays the associations between MTR in NAWM and cognitive status. Significant direct relationships were found with memory and executive function. This was seen for NAWM MTR in the whole brain and for NAWM MTR in all lobes with the exception of the temporal lobe.

Table 5. Multivariate linear regression analysis: Normal Appearing White Matter (NAWM) and cognitive functioning

	Memory			Executive function			Motor skills		
Mean NAWM MTR	β	95% CI	p	β	95% CI	p	β	95% CI	p
Whole brain	0.112	[0.022;0.202]	0.0412	0.085	[0.015;0.155]	0.0340	0.030	[-0.048;0.108]	0.5988
Frontal lobe	0.100	[0.016;0.184]	0.0462	0.073	[0.007;0.139]	0.0524	0.023	[-0.050;0.096]	0.6252
Parietal lobe	0.121	[0.033;0.209]	0.0366	0.091	[0.022;0.160]	0.0229	0.032	[-0.044;0.108]	0.5988
Occipital lobe	0.114	[0.027;0.202]	0.0366	0.094	[0.026;0.161]	0.0201	0.047	[-0.028;0.122]	0.4400
Temporal lobe	0.062	[-0.015;0.139]	0.2200	0.036	[-0.024;0.096]	0.2778	0.047	[-0.019;0.113]	0.3911

Adjusted for age, sex, years of education, vascular risk factors, cortex volume, thromboembolic infarcts, lacunes and WMH volume.
NAWM=Normal Appearing White Matter, MTR=Magnetization Transfer Ratio. p-values adjusted for multiple testing.

Lower mean MTR in WMH related to poorer performance in executive function tests (ß=0.038; 95%CI 0.008-0.069; p=0.033). The associations with test results in the other cognitive domains were not significant.

Figure 7 demonstrates the associations between quartiles of mean MTR distribution in the cortex and in NAWM of the whole brain and domain-specific cognitive test results. There was a significant almost linear relationship between decreasing cortical MTR values and impairment in memory and executive function. The association with motor skills was of borderline significance and it was non-linear. For NAWM MTR a significant dose-effect relationship existed for memory performance and executive function.

Mixed model analysis stratified by sex demonstrated that the relationship between MTR in the cortex and memory as well as executive function scores was significant only in men but not in women (table 6)

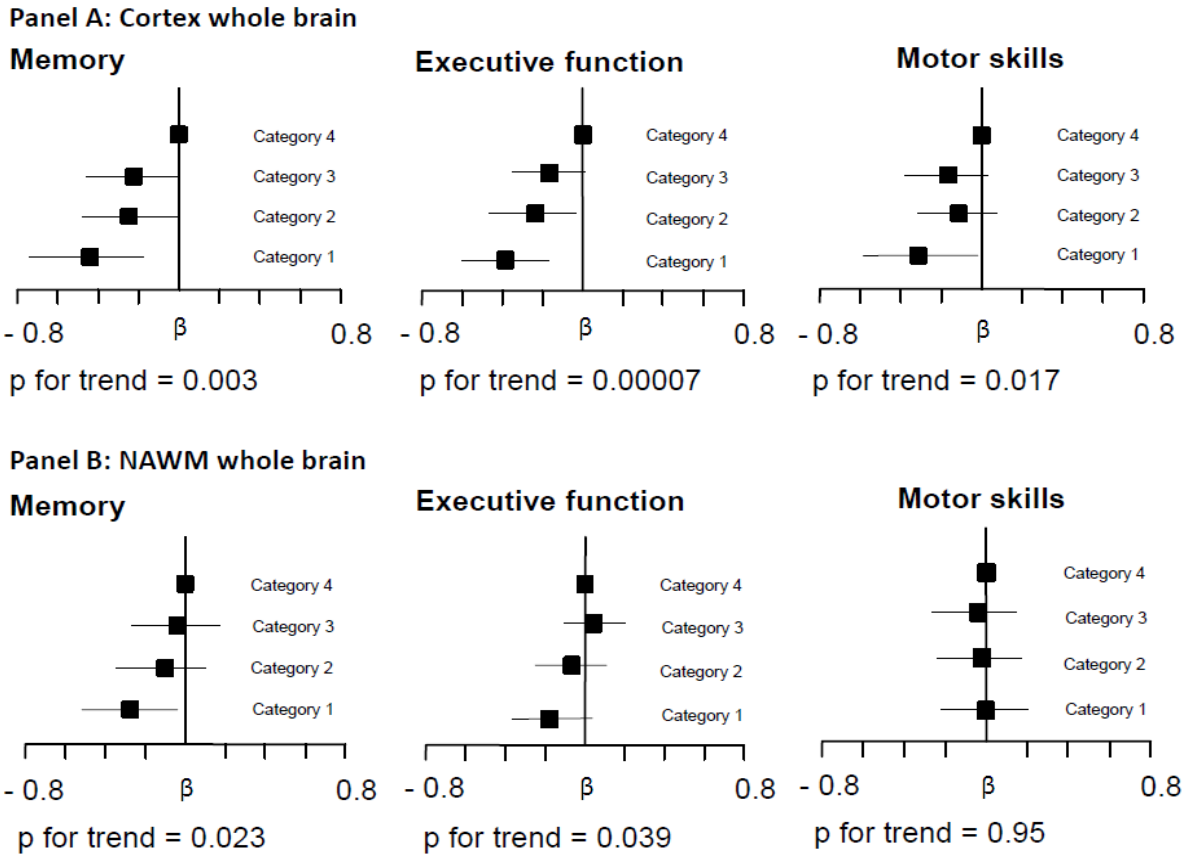

Figure 7. Relationship between whole brain cortical (A) and normal appearing white matter (NAWM) MTR (B) and domain-specific cognitive performance. Multiple regression analysis adjusted for age, sex, educational level, vascular risk factors, cortex volume, silent non-lacunar infarcts, lacunes and white matter hyperintensity volume compares the effect between MTR quartiles on performance on tests of memory, executive function and motor skills with the highest quartile of the MTR distribution serving as the reference. Squares on the x-axis indicate the β-coefficients and bars give the 95% confidence intervals. (A) demonstrates that decreasing MTR in whole brain cortical MTR related to poorer performance in memory, executive function and motor skills tests. With decreasing MTR quartile distribution there was an almost linear decline in memory and executive function performance. The association for motor skills was non-linear. (B) demonstrates that also decreasing whole brain NAWM MTR was significantly related to memory performance and executive function. The relative dose-dependent effect of MTR in NAWM was less pronounced than that seen for cortical MTR.

Table 6. Multivariate linear regression analysis stratified for sex:

	Males						Females					
MTR	Memory		Executive function		Motor skills		Memory		Executive function		Motor skills	
	β	p	β	p	β	p	β	p	β	p	β	p
Cortex												
Whole brain	0.265	0.007	0.193	0.002	0.133	0.154	0.048	0.660	0.077	0.203	0.092	0.314
Frontal lobe	0.253	0.007	0.180	0.003	0.107	0.254	0.028	0.809	0.077	0.203	0.090	0.314
Parietal lobe	0.257	0.007	0.209	0.000	0.133	0.154	0.051	0.623	0.072	0.212	0.066	0.462
Occipital lobe	0.250	0.007	0.216	0.000	0.133	0.154	0.063	0.480	0.075	0.203	0.084	0.314
Temporal lobe	0.225	0.013	0.145	0.024	0.131	0.154	0.040	0.707	0.060	0.284	0.092	0.314
Deep gray matter												
Thalamus	0.063	0.416	0.071	0.061	0.045	0.440	-0.013	0.809	0.031	0.316	0.028	0.566
Putamen	0.093	0.176	0.047	0.203	0.046	0.440	-0.001	0.980	0.060	0.097	0.027	0.566
Pallidum	0.089	0.176	0.037	0.279	0.033	0.566	-0.005	0.901	0.034	0.203	0.049	0.254
Caudate nucleus	0.044	0.623	0.048	0.203	0.010	0.880	0.024	0.707	0.076	0.024	0.039	0.440
Amygdala	0.072	0.228	0.050	0.173	0.038	0.440	-0.010	0.809	0.011	0.633	-0.002	0.960
Accumbens nucleus	-0.020	0.763	-0.006	0.760	0.010	0.854	0.005	0.901	0.043	0.097	0.016	0.645
NAWM												
Whole brain	0.263	0.007	0.089	0.203	0.067	0.503	0.005	0.952	0.071	0.220	-0.002	0.960
Frontal lobe	0.225	0.011	0.087	0.203	0.043	0.622	-0.011	0.901	0.051	0.360	0.002	0.960
Parietal lobe	0.248	0.008	0.112	0.108	0.081	0.440	0.025	0.809	0.067	0.243	-0.009	0.960
Occipital lobe	0.276	0.007	0.118	0.097	0.096	0.314	0.026	0.809	0.074	0.203	0.012	0.949
Temporal lobe	0.204	0.020	0.029	0.616	0.097	0.254	-0.020	0.809	0.040	0.414	0.005	0.960

Adjusted for age, years of education, vascular risk factors, cortex volume, thromboembolic infarcts, lacunes, and WMH volume.
MTR=Magnetization Transfer Ratio; NAWM=normal appearing white matter; WMH=white matter hyperintensities; p values adjusted for multiple testing

When assessing a possible mediating effect of cortex and NAWM MTR on the relationship between age and cognition, we found a significant mediation of cortical and NAWM MTR on the relationship between age and executive function, but not on memory or on motor skills (table 7).

Table 7. Analysis of mediating effects of MTR variables on the relationship between age and cognitive test results:

	MTR cortex whole brain			
	Total effect	Direct effect	Indirect effect	Bootstrapped CI
memory	-.0395	-.0344	-.0051	-.0114; .0006
executive function	-.0211	-.0157	-.0055*	-.0103; -.0016
motor skills	-.0408	-.0371	-.0036	-.0084; .0005
	MTR NAWM whole brain			
	Total effect	Direct effect	Indirect effect	Bootstrapped CI
memory	-.0397	-.0363	-.0034	-.0081; .0008
executive function	-.0211	-.0183	-.0027*	-.0059; -.0006
motor skills	-.0413	-.0414	.0001	-.0026; .0031

Predictor variable=age; outcome variable=memory, executive function, motor skills; mediator variable=MTR cortex whole brain, MTR NAWM whole brain. Abbreviations: MTR=magnetization transfer ratio; NAWM=normal appearing white matter. Adjusted for age, sex, years of education, vascular risk factors, cortex volume, thromboembolic infarcts, lacunes, and WMH volume.

Significant indirect effects are marked (*)

2nd study: MTR and gait disturbances

Demographic, clinical and imaging characteristics of the study participants are summarized in table 8. People who walked slower were significantly older and shorter as compared to their faster counterparts. They were more often diabetics and suffered more frequently from hyperuricemia.

Table 8. Characteristics of the study population by quartiles of walking speed.

Characteristics (N)	Entire group (230)	WSP Quartile 1 (slowest) (58)	WSP Quartile 2 (72)	WSP Quartile 3 (50)	WSP Quartile 4 (fastest) (50)	Sig.
Walking speed with 3 turns (m/s), mean (SD)	0.40 (0.10)	0.31 (0.33)	0.39 (0.19)	0.44 (0.17)	0.53 (0.51)	0.001* [a]
Age (years), mean (SD)	70.2 (4.9)	71.8 (5.0)	70.1(4.7)	68.8(4.7)	69.7(4.7)	0.013* [a]
Female, N(%)	153 (66.5)	44(75.9)	41(56.9)	34(68.0)	34(68.0)	0.149 [b]
Height (cm), mean (SD)	164.3 (8.3)	161.4(8.0)	166.0(8.7)	165.0(7.3)	164.6(8.5)	0.015* [a]
Subjects with hypertension, N(%)	165 (71.7)	47(81.0)	46(63.9)	37(74.0)	35(70.0)	0.182 [b]
Subjects with diabetes, N(%)	30 (13.0)	17(29.3)	8(11.1)	2(4.0)	3(6.0)	0.001* [b]
Subjects with hypercholesterolemia, N(%)	189 (82.2)	47(81.0)	58(80.6)	44(88.0)	40(80.0)	0.682 [b]
Subjects with hypertriglyceridemia, N(%)	40 (17.4)	14(24.1)	14(19.4)	7(14.0)	5(10.0)	0.226 [b]
Subjects with hyperuricemia, N(%)	69 (30)	15(25.9)	30(41.7)	15(30.0)	9(18.0)	0.036* [b]
Subjects with peripheral vascular disease, N(%)	1 (0.4)	0(0.0)	1(1.4)	0(0.0)	0(0.0)	0.531 [b]
Subjects with venous embolic disease, N(%)	33 (14.3)	10(17.2)	7(9.7)	12(24.0)	4(8.0)	0.069 [b]
Normalized brain volume (cm3), mean (SD)	990.3 (97.4)	974.0(95.8)	1000.0(100.4)	985.9(87.3)	999.7(104.2)	0.411 [a]
Normalized WMH volume (cm3), median (IQR)	13.3 (7.0;25.8)	11.7 (3.6;32.6)	12.7 (7.0;29.2)	14.7 (8.2;23.8)	14.4 (8.1;22.5)	0.663 [b]
Lacunes, N(%)	32 (13.9)	10(17.2)	11(15.3)	6(12.0)	5(10.0)	0.696 [b]
Silent, non-lacunar infarcts, N(%)	13 (5.7)	5(8.6)	4(5.6)	2(4.0)	2(4.0)	0.689 [b]

WSP=walking speed; WMH=White Matter Hyperintensities; SD=Standard Deviation; IQR=Interquartile Range.
[a] Analysis of Variance (ANOVA)
[b] Chi2 Test

There was no significant association between total WMH volume and gait velocity (ß = 0.118, 95%CI -0.011-0.248, p= 0.073). Voxel-based lesion-symptom mapping (VLSM) identified no voxel clusters in which WMH were significantly related to slower gait speed.

In the Voxel-based MTR symptom mapping (VMTRSM) analysis, we found significant clusters of MTR voxels that were positively correlated with gait velocity bilaterally within the frontal white matter (figure 8A). The association remained significant after correction for multiple comparisons and adjustment for age, sex, height, brain volume and presence of vascular risk factors that were significantly associated with walking speed (diabetes and hyperuricemia). To examine the spatial relationship between these clusters and major white matter tracts, we projected significant clusters from the VMTRSM analysis on the probabilistic white matter tract atlas in MNI space. As shown in figure 8B, there was substantial overlap with the forceps minor.

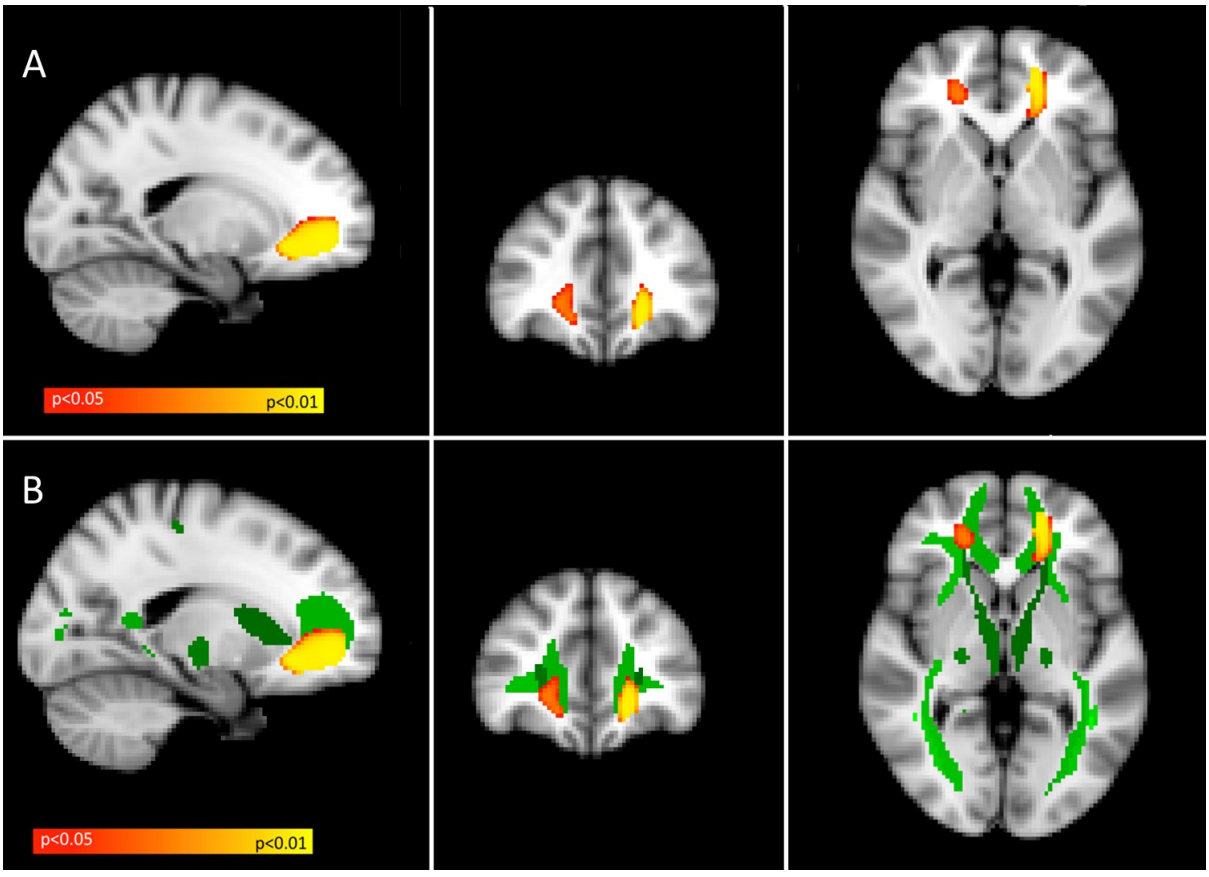

Figure 8. Voxel-wise analysis of the WM MTR positively associated with gait velocity. Adjusted for age, sex, height, diabetes, hyperuricemia and brain volume. Thresholded at p<0.05 and FWE-corrected for multiple comparisons. (A) The statistical map is superimposed on the MNI 152 standard space template, (B) superimposed on the JHU DTI-based white-matter tract atlas. WM=White Matter; MTR=Magnetization Transfer Ratio; FWE=Family-Wise Error; MNI=Montreal Neurological Institute; DTI=Diffusion Tensor Imaging; image orientation follows radiological convention.

Given the prominent association of MTR voxels and walking speed within the forceps minor, we assessed the mean MTR of the forceps minor in standard space and used a linear regression model, adjusted for age, sex, height, brain volume and vascular risk factors that were significantly associated with walking speed (diabetes and hyperuricemia), to determine the association between forceps minor MTR and gait velocity. Higher mean MTR within the forceps minor was positively related with gait velocity (β=0.160; 95%CI 0.025-0.295; p=0.02) (figure 9).

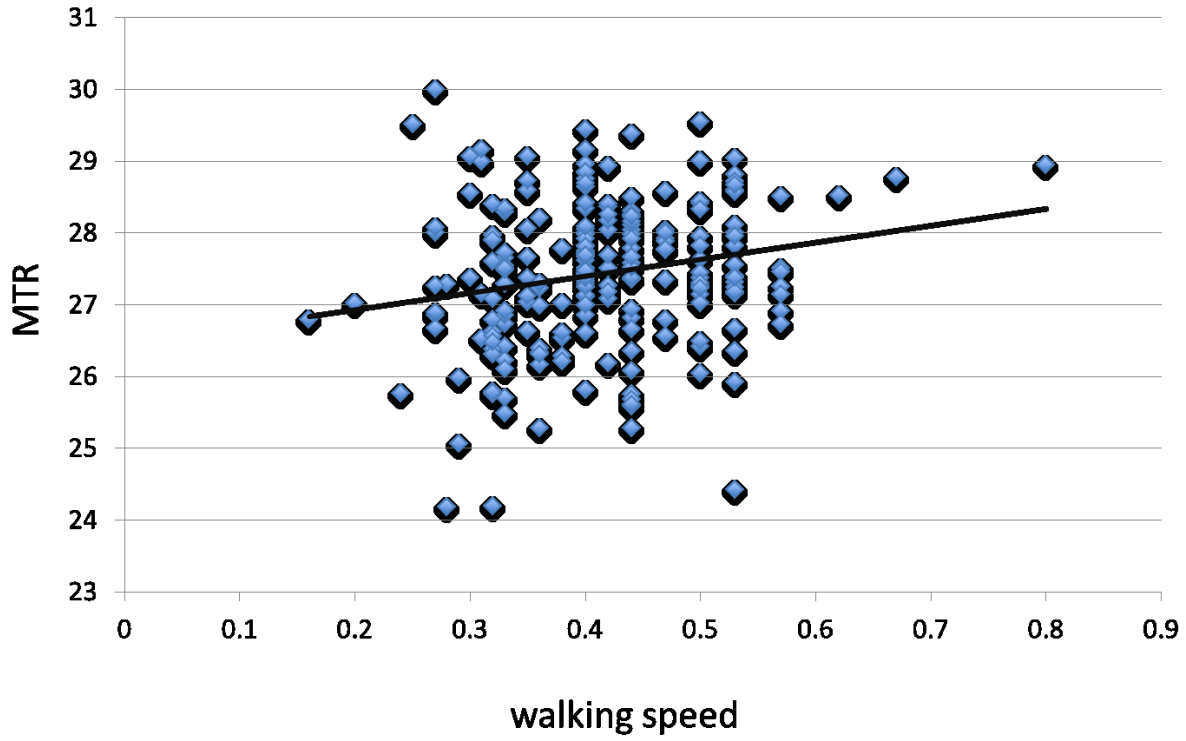

Figure 9. Correlation between walking speed (x-axis) and MTR of the forceps minor (y-axis). MTR=Magnetization Transfer Ratio

This association remained virtually unchanged when global WMH volume or WMH volume within the FM was added to the analysis (ß=0.151,95%CI 0.015-0.286; p=0.029) and (ß=0.161,95%CI 0.026-0.296; p=0.026), respectively.

Figure 10 illustrates the associations between quartiles of mean MTR distribution in the forceps minor and gait velocity. Analysis of covariance (ANCOVA), which we corrected for age, sex, height, brain volume and vascular risk factors that were significantly associated with walking speed (diabetes and hyperuricemia), showed an independent linear dose-effect relationship between forceps-minor-MTR quartiles and gait velocity (p for linear trend =0.016).

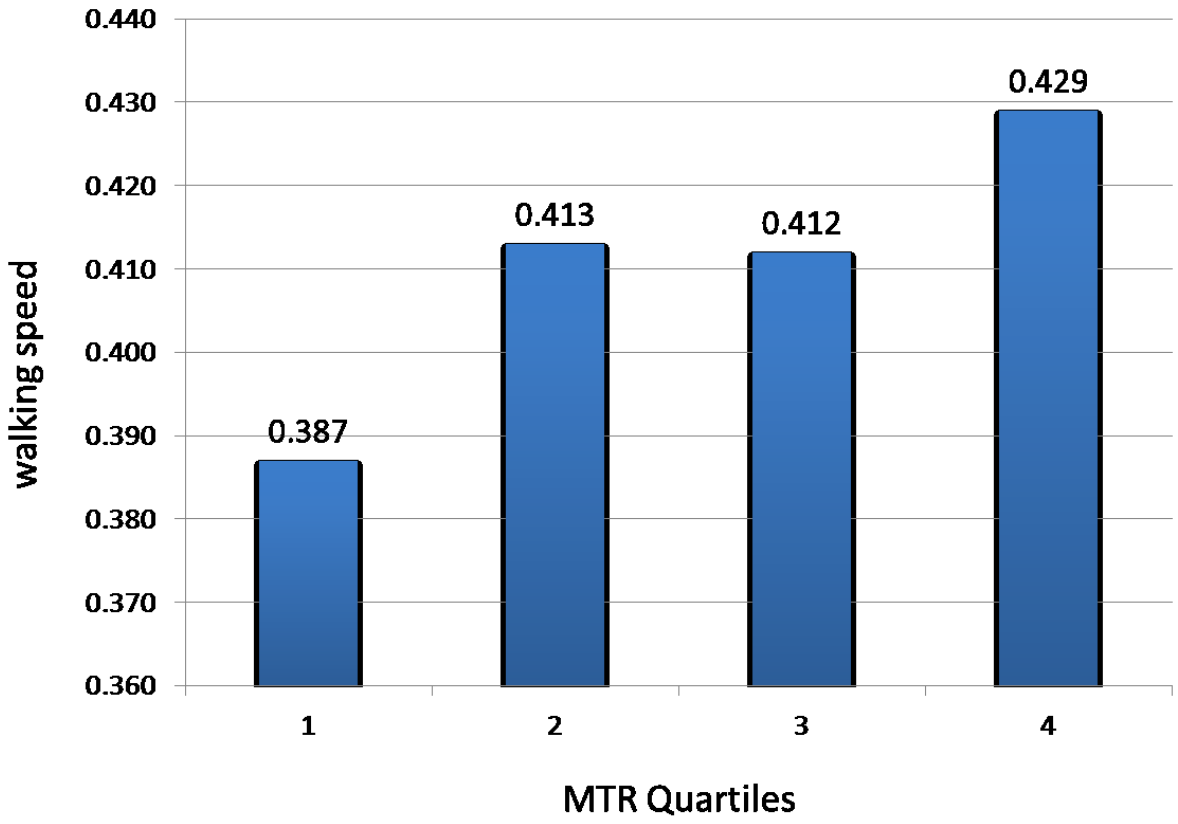

Figure 10 Analysis of covariance (ANCOVA) results. The mean MTR of the forceps minor was divided in quartiles (x-axis), the first quartile (1) being the lowest. Values on the y-axis represent the estimated mean walking speed of subjects within each quartile, adjusted for age, sex, height, diabetes, hyperuricemia and brain volume. Increasing MTR values within the forceps minor related to higher gait velocity in a dose-dependent manner (p for linear trend = 0.016). MTR=Magnetization Transfer Ratio

Discussion

The discussion covers each study separately.

1st study: MTR and cognition

This section resembles in large the "discussion" of the paper by Seiler S, Pirpamer L, Hofer E, Duering M, Jouvent E, Fazekas F, Mangin JF, Chabriat H, Dichgans M, Ropele S, Schmidt R **"Magnetization transfer ratio relates to cognitive impairment in normal elderly"** published in *Frontiers in Aging Neuroscience* 2014 Sep 25;6:263. doi: 10.3389/fnagi.2014.00263. eCollection 2014 (1)

In study#1, we assessed the association between MTR-determined microstructural tissue changes in the cortex, NAWM and deep gray matter structures and cognitive performance in a well-defined cohort of 355 older adults. The most striking result was that lower cortical MTR related to performance in all cognitive domains examined. Regional analysis revealed that associations were present across all lobes. Fascinatingly, we found an almost linear dose-effect relationship between quartile distribution of cortical MTR and scores of memory, executive function and motor skills. Within the deep gray matter nuclei, lowering of MTR related mostly to executive dysfunction.

This is in line with our hypothesis that microstructural brain tissue alterations affect cognitive performance according to their occurrence in distinct, well-defined brain regions. It is well established that subcortical circuits spreading through the frontal lobes are important for the maintenance of executive function. These networks interconnect the prefrontal cortex, the striatum, the globus pallidus, the substantia nigra and thalamus (190,191).
Lesions within these circuits and interconnected structures are therefore important for the pathogenesis of dysexecutive syndromes (66,192,193).

Intriguingly, the association between MTR in the NAWM and cognitive performance was much weaker than that observed between MTR in the cortex and neuropsychological test results. The consistent inverse relationship between cortex MTR and cognition in this study in middle aged and elderly persons which was independent of the presence and extent of

structural brain changes and normalized cortex volume suggests that widespread microstructural tissue changes in the cortex are an important early substrate of age-related cognitive decline. The cause for diffuse cortical MTR decrease during brain aging remains to be determined. We realize that MTR calculations within the cortex are to be interpreted with caution as diffuse cortical MTR reductions might simply reflect partial volume effects from CSF as a consequence of age-related loss of brain parenchyma. CSF has MTR values approaching zero and thus increased widening of sulci could result in a decrease of MTR in voxels with mixed contributions from cortex and CSF. Several points argue against this explanation. While the slice thickness of the MT sequence used in the current study was only moderately thin, it had an in-plane resolution higher than the hires T1 scan that was used for segmentation of the cortex. All analyses were corrected for cortical volume (194-196). Moreover, all MTR masks in our study have been eroded by one voxel to avoid inclusion of "edge" values, which should make CSF contamination of cortex MTR unlikely. It is also of note, that an MTR decrease was associated with cognitive impairment not only in neocortical but also in deep gray matter structures. Other than surface gray matter, which is surrounded by CSF, deep gray matter structures are surrounded by white matter, which in case of partial volume effects would have rather increased MTR values.

The histopathological tissue alterations that may lead to MTR lowering in the aging brain are not yet determined. MTR post mortem comparisons were only done in patients with multiple sclerosis (112,197). In these studies, MTR was considered to primarily reflect the amount of myelin (148,198) in white matter structures, but also axonal count (148). A recent study also reported MTR lowering with focal demyelination in the MS cortex (112).
In the aging brain demyelination often occurs as a consequence of cerebral small vessel disease (5,199,200). If demyelination in the wake of cerebral small vessel disease was indeed the leading cause for the MTR changes seen in our study we would have expected to find an inverse relationship between MTR values in different tissue compartments and WMH volume. This was not the case, neither for cortical MTR, nor for NAWM MTR. Thus, other yet unknown microstructural changes might have been responsible. A recent study scanned unfixed post-mortem brain slices of 12 multiple sclerosis patients by MTI at 1.5 Tesla, assessed blocks containing non-lesional brain tissue microscopically, and microdissected adjacent tissue to quantify specific protein levels (197). The authors reported that post-translational modifications of axonal proteins such as phosphorylation of neurofilaments occurred in non-lesional brain tissue and suggested that the resulting lowering of MTR was

caused by a hyperphosphorylation-related change in proton mobility. Several mechanisms driving neurodegeneration such as glutamate excitotoxicity or mitochondrial failure relate to Ca^{2+} influx, activation of kinases and subsequent protein phosphorylation (201,202). Altered cortical MTR in the aging brain may thus reflect hyper-phosphorylation of proteins and pathologic accumulation of soluble and non-soluble proteins, a process that is known to precede cell death in many primary degenerative diseases (203). In this context, a recent MTI study in Alzheimer dementia and mild cognitive impairment is of interest (138). The authors applied a model-based multiparameter approach that allowed to separately quantify the presence and amount of macromolecules in pre-specified regions of interest and to investigate the coupling characteristics of protons by modeling deposition and interactions between macromolecules (138). MTI metrics indicating increased coupling to the environment which can be expected under conditions of pathologic protein accumulation differentiated patients with AD and MCI from controls with high accuracy. This suggests that MTI metrics including MTR can depict changes in the macromolecular tissue composition caused by neurodegenerative disease. This could be an explanation for our finding that a drop in MTR of gray matter had much stronger effects on cognitive functioning than MTR lowering in the white matter compartment.

Cortical and NAWM MTR mediated the association between age and executive function in our analysis, but not between age and memory or motor skills. DTI studies found similar mediating effects between age and executive function in NAWM of older adults (204,205). Given the influence of higher age on both, MTR metrics and executive function, it is of special interest that these mediating effects were present independent of atrophy. The brain's microstructure might change interindividually variable with aging and play an important part in mediating age-related effects on cognitive performance, largely independent of cortical- or subcortical atrophy.

Our study has several strengths. It is the largest cohort study using MTI to date. The study is community-based with prospectively planned radiological and clinical protocols. Using MTI, images with a much higher spatial resolution and signal-to-noise ratio can be produced than is possible with DTI acquisitions. There are also no echo planar imaging-induced artefacts. The high scan resolution allowed accurate registration of scans and segmentation of tissue types and WMH. A thorough post-processing procedure and rigorous quality control of

segmentations reduced the effects of coregistration errors and CSF contamination to a minimum.

A limitation of our investigation is its cross sectional design.

The variability of MTR occurring in asymptomatic individuals is relatively low, but despite that the associations between cortical MTR and cognitive performance were substantial with little regional variability. At this point the importance of MTI in a clinical setting is unknown. If a decrease in MTR indeed reflects alterations of the macromolecular tissue composition resulting from age-related neurodegenerative processes, MTI metrics may turn out to be very early markers of neurodegenerative processes during brain aging. There has been only a single small longitudinal MTI study in Alzheimer patients, which showed a progressive decrease of MTR paralleling cognitive decline over a one-year follow-up period (141). Longitudinal data in cognitively healthy subjects or in cases with prodromal dementia are not yet available. Such studies are warranted to determine the rate of MTR change over time during normal and pathologic brain aging and to assess the role of MTR as possible imaging biomarkers in dementia research.

2nd study: MTR and gait disturbances

In study#2 of the PhD project, we assessed a large number of older adults free of stroke, dementia and other neurological diseases with two observer-independent methods to identify macro- and microstructural determinants of gait velocity: Voxel based lesion- symptom mapping (VLSM) showed no voxel clusters in which WMH were significantly related to a slower gait velocity. However, there was a dose-dependent association between MTR values in the forceps minor and gait velocity, independent of age, sex, height, diabetes, hyperuricemia, brain volume and WMH volume. Lower MTR, which is associated with decreased myelin content (105), related to slower gait velocity.

Our results are in line with DTI findings in normal aging and in patients with cSVD. De Laat et al. (206) studied 429 individuals aged 50 to 85 years with cerebral small vessel disease. These authors found loss of white matter integrity in the corpus callosum, particularly the genu where the forceps minor crosses. Similar results were reported by Della Nave et al. (59) and by Bhadelia et al. (104) who also showed that participants with abnormal gait had lower FA in the genu of the corpus callosum. Even though DTI probes brain tissue organization rather than brain tissue composition as MTI does, it is striking that previous DTI results in large resemble our MTR finding. Microstructural brain tissue changes in the forceps minor were consistently linked with gait disturbances both in normal aging but also in patients with cerebral small vessel disease.

The forceps minor is a large fiber bundle that connects the bilateral prefrontal cortices of the hemispheres (163,207), which play an important role in motor control, especially in older adults (208). Intact interhemispheric connections may be important for maintaining motor control at a high level (209). We observed no significant relationship between WMH and gait performance in our community-dwelling sample. Moreover, the inclusion of WMH in the regression analysis did not significantly change the association between microstructural integrity of the forceps minor and gait velocity. The two previous DTI studies (59,104) also found that DTI measures in the genu of the corpus callosum remained significantly associated with poorer gait performance after adding WMH volume to the analysis. Under consideration that more widespread WMH seen in elderly people are a marker of co-existing cSVD, previous results and the results of current study indicate that factors other than cSVD may also play an important role in the development of gait disturbances during aging.

Post-mortem studies, that correlate DTI measures and MTR with brain tissue alterations, are thus likely to improve our pathophysiological understanding of age-related gait abnormalities.

The current study has several strengths. It is the largest cohort study on gait performance using voxel-based MTR mapping to date. The study is community-based with prospectively planned radiological and clinical protocols. Using MTI, images with a much higher spatial resolution and signal-to-noise ratio can be produced than is possible with DTI acquisitions. There are also no echo planar imaging-induced artefacts. The high scan resolution allowed accurate registration of scans and segmentation of tissue types and WMH. A thorough post-

processing procedure and rigorous quality control of segmentations reduced the effects of coregistration errors and CSF contamination to a minimum.

There are also limitations. The study has a cross sectional design. Neurophysiological examination to exclude peripheral neuromuscular disease was not performed. However, participants were normal on standard neurological examination.

We realize that we tested the association between voxel values and walking speed in a far larger number of voxels in MTR analysis that comprises the whole white matter, than in the lesion based analysis. Consequently, the risk to find associations due to chance alone is probably higher in the MTR analyses and by contrast, the risk to miss associations that actually exist is probably higher in the lesion-based analysis. In this study, besides FWE-control, the symmetry of our findings in both hemispheres is a strong argument against findings due to chance alone.

Some subjects originate from the same families. This might lead to correlated errors in the statistical models. FSL includes no regular option to correct voxel-wise analyses for family structure. Including for instance random effects would be very difficult to interpret. However, we consider this a minor issue here, given the small number of subjects per family.

In conclusion, our study provides new evidence for the importance of microstructural brain tissue changes in gait disturbances at older age, particularly in the forceps minor. Identification of the pathophysiological origin of these MR-detected tissue alterations is of clinical importance as therapeutic measures may be derived.

Summary/general conclusion

In the course of this PhD project, we found evidence for the importance of microstructural brain tissue changes in aging. MTR lowering was associated with cognitive decline and poorer gait performance, independent of visible atrophy. Study #1 found associations between lower MTR and poorer cognitive performance mainly in the neocortex. Although there is some preliminary concept on the pathophysiological correlate of MTR in the cortex, no histopathological studies have yet been performed to relate MTR to specific cortical

macromolecules. Therefore, we hope that our results may stimulate future research in this field, as it would be highly warranted to know the correlates of MTR lowering on the tissue level.

In study #2, we evidenced an association between MTR in the forceps minor and gait velocity. This is important, because the location of MTR lowering in the forceps minor was found using an observer-independent method and the location of MTR lowering seems to determine the clinical phenotype. In the white matter, MTR corresponds most likely to myelin content. Importantly, the associations found in our study were independent of visible WMH. This strengthens the theory that visible WMH, caused by chronic hypoperfusion or cerebral small vessel disease are only the tip of the iceberg while widespread, strategically relevant microstructural tissue damage is present, maybe years before visible changes occur. As described in the former chapters, MTR is an attractive method to study the microstructure of the brain because it has several advantages as compared to DTI that is much more known and used. It produces fewer artifacts, a higher resolution is possible and the acquisition only takes a few minutes. Although these advantages, MTR is currently underrepresented in the study of age-related brain changes. The scarce literature on MTI in normal aging, cSVD and dementia shows that lower MTR relates to worse cognitive performance in every single entity. Little is known about the histopathological basis that underlies the MTI results and their relationship with cognitive functioning. Oedema and demyelination give influence MTR values, and in the multiple sclerosis literature, good correlation was found between the grade of demyelination and axonal loss and MTR (210). Lowering of MTR due to demyelination as a consequence of incomplete tissue destruction due to small vessel disease is the most likely substrate of MTR alterations in NAWM during normal brain aging and in patients with various types of small vessel disease-related brain lesions including WMH, lacunes, and microbleeds. The origin of cortical MTR changes in MCI and AD and normal aging is also not known but maybe resembles changes in the cortical macromolecular tissue composition caused by neurodegenerative disease (138). The second pertinent question regarding MTI besides its histopathological basis is the prognostic significance of MTR changes. Longitudinal studies are still scarce and are needed to determine if MTR in NAWM and gray matter will allow the prediction of cognitive decline beyond focal and diffuse brain abnormalities such as atrophy in normal aging and AD and if it can determine the conversion from MCI to dementia (105).

Biomarker research using neuroimaging is currently a rapidly evolving field of science. We hope that our results may motivate researchers to apply MTR with more confidence in their work, as MTR may serve as a helpful outcome measure in treatment studies.

References

(1) Seiler S, Pirpamer L, Hofer E, Duering M, Jouvent E, Fazekas F, et al. Magnetization transfer ratio relates to cognitive impairment in normal elderly. Front Aging Neurosci 2014 Sep 25;6:263.

(2) Granados JA. Health at advanced age: social inequality and other factors potentially impacting longevity in nine high-income countries. Maturitas 2013 Feb;74(2):137-147.

(3) Hofmarcher MM, Quentin W. Austria: health system review. Health Syst Transit 2013;15(7):1-292.

(4) DeCarli C, Massaro J, Harvey D, Hald J, Tullberg M, Au R, et al. Measures of brain morphology and infarction in the framingham heart study: establishing what is normal. Neurobiol Aging 2005 Apr;26(4):491-510.

(5) Schmidt R, Schmidt H, Haybaeck J, Loitfelder M, Weis S, Cavalieri M, et al. Heterogeneity in age-related white matter changes. Acta Neuropathol 2011 Aug;122(2):171-185.

(6) Henkelman RM, Stanisz GJ, Graham SJ. Magnetization transfer in MRI: a review. NMR Biomed 2001 Apr;14(2):57-64.

(7) Mori S, Zhang J. Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 2006 Sep 7;51(5):527-539.

(8) Bushong S, Clarke G. Magnetic Resonance Imaging: Physical and Biological Principles. 4th ed.; 2014.

(9) Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM. Fsl. Neuroimage 2012 Aug 15;62(2):782-790.

(10) Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 2002 Jan 31;33(3):341-355.

(11) Lockhart SN, DeCarli C. Structural imaging measures of brain aging. Neuropsychol Rev 2014 Sep;24(3):271-289.

(12) Jernigan TL, Archibald SL, Fennema-Notestine C, Gamst AC, Stout JC, Bonner J, et al. Effects of age on tissues and regions of the cerebrum and cerebellum. Neurobiol Aging 2001 Jul-Aug;22(4):581-594.

(13) Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, et al. Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. Cereb Cortex 2005 Nov;15(11):1676-1689.

(14) Sullivan EV, Pfefferbaum A. Neuroradiological characterization of normal adult ageing. Br J Radiol 2007 Dec;80 Spec No 2:S99-108.

(15) Crivello F, Tzourio-Mazoyer N, Tzourio C, Mazoyer B. Longitudinal assessment of global and regional rate of gray matter atrophy in 1,172 healthy older adults: modulation by sex and age. PLoS One 2014 Dec 3;9(12):e114478.

(16) Fotenos AF, Snyder AZ, Girton LE, Morris JC, Buckner RL. Normative estimates of cross-sectional and longitudinal brain volume decline in aging and AD. Neurology 2005 Mar 22;64(6):1032-1039.

(17) Lim KO, Zipursky RB, Murphy GM,Jr, Pfefferbaum A. In vivo quantification of the limbic system using MRI: effects of normal aging. Psychiatry Res 1990 Apr;35(1):15-26.

(18) Seab JP, Jagust WJ, Wong ST, Roos MS, Reed BR, Budinger TF. Quantitative NMR measurements of hippocampal atrophy in Alzheimer's disease. Magn Reson Med 1988 Oct;8(2):200-208.

(19) Taylor JL, Scanlon BK, Farrell M, Hernandez B, Adamson MM, Ashford JW, et al. APOE-epsilon4 and aging of medial temporal lobe gray matter in healthy adults older than 50 years. Neurobiol Aging 2014 Nov;35(11):2479-2485.

(20) Du AT, Schuff N, Chao LL, Kornak J, Jagust WJ, Kramer JH, et al. Age effects on atrophy rates of entorhinal cortex and hippocampus. Neurobiol Aging 2006 May;27(5):733-740.

(21) Pfefferbaum A, Rohlfing T, Rosenbloom MJ, Chu W, Colrain IM, Sullivan EV. Variation in longitudinal trajectories of regional brain volumes of healthy men and women (ages 10 to 85 years) measured with atlas-based parcellation of MRI. Neuroimage 2013 Jan 15;65:176-193.

(22) Mak E, Su L, Williams GB, Watson R, Firbank MJ, Blamire AM, et al. Progressive cortical thinning and subcortical atrophy in dementia with Lewy bodies and Alzheimer's disease. Neurobiol Aging 2015 Apr;36(4):1743-1750.

(23) Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RS, Busa E, et al. Thinning of the cerebral cortex in aging. Cereb Cortex 2004 Jul;14(7):721-730.

(24) Lemaitre H, Goldman AL, Sambataro F, Verchinski BA, Meyer-Lindenberg A, Weinberger DR, et al. Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? Neurobiol Aging 2012 Mar;33(3):617.e1-617.e9.

(25) van Velsen EF, Vernooij MW, Vrooman HA, van der Lugt A, Breteler MM, Hofman A, et al. Brain cortical thickness in the general elderly population: the Rotterdam Scan Study. Neurosci Lett 2013 Aug 29;550:189-194.

(26) Storsve AB, Fjell AM, Tamnes CK, Westlye LT, Overbye K, Aasland HW, et al. Differential longitudinal changes in cortical thickness, surface area and volume across the adult life span: regions of accelerating and decelerating change. J Neurosci 2014 Jun 18;34(25):8488-8498.

(27) Dickerson BC, Fenstermacher E, Salat DH, Wolk DA, Maguire RP, Desikan R, et al. Detection of cortical thickness correlates of cognitive performance: Reliability across MRI scan sessions, scanners, and field strengths. Neuroimage 2008 Jan 1;39(1):10-18.

(28) Murphy EA, Holland D, Donohue M, McEvoy LK, Hagler DJ,Jr, Dale AM, et al. Six-month atrophy in MTL structures is associated with subsequent memory decline in elderly controls. Neuroimage 2010 Dec;53(4):1310-1317.

(29) Smith CD, Andersen AH, Gold BT, Alzheimer's Disease Neuroimaging Initiative. Structural brain alterations before mild cognitive impairment in ADNI: validation of volume loss in a predefined antero-temporal region. J Alzheimers Dis 2012;31 Suppl 3:S49-58.

(30) Evans MC, Barnes J, Nielsen C, Kim LG, Clegg SL, Blair M, et al. Volume changes in Alzheimer's disease and mild cognitive impairment: cognitive associations. Eur Radiol 2010 Mar;20(3):674-682.

(31) Stonnington CM, Chu C, Kloppel S, Jack CR,Jr, Ashburner J, Frackowiak RS, et al. Predicting clinical scores from magnetic resonance scans in Alzheimer's disease. Neuroimage 2010 Jul 15;51(4):1405-1413.

(32) Nho K, Risacher SL, Crane PK, DeCarli C, Glymour MM, Habeck C, et al. Voxel and surface-based topography of memory and executive deficits in mild cognitive impairment and Alzheimer's disease. Brain Imaging Behav 2012 Dec;6(4):551-567.

(33) Ryberg C, Rostrup E, Paulson OB, Barkhof F, Scheltens P, van Straaten EC, et al. Corpus callosum atrophy as a predictor of age-related cognitive and motor impairment: a 3-year follow-up of the LADIS study cohort. J Neurol Sci 2011 Aug 15;307(1-2):100-105.

(34) Rosano C, Bennett DA, Newman AB, Venkatraman V, Yaffe K, Harris T, et al. Patterns of focal gray matter atrophy are associated with bradykinesia and gait disturbances in older adults. J Gerontol A Biol Sci Med Sci 2012 Sep;67(9):957-962.

(35) Fazekas F, Niederkorn K, Schmidt R, Offenbacher H, Horner S, Bertha G, et al. White matter signal abnormalities in normal individuals: correlation with carotid ultrasonography, cerebral blood flow measurements, and cerebrovascular risk factors. Stroke 1988 Oct;19(10):1285-1288.

(36) van Dijk EJ, Prins ND, Vermeer SE, Koudstaal PJ, Breteler MM. Frequency of white matter lesions and silent lacunar infarcts. J Neural Transm Suppl 2002;(62)(62):25-39.

(37) Mantyla R, Erkinjuntti T, Salonen O, Aronen HJ, Peltonen T, Pohjasvaara T, et al. Variable agreement between visual rating scales for white matter hyperintensities on MRI. Comparison of 13 rating scales in a poststroke cohort. Stroke 1997 Aug;28(8):1614-1623.

(38) Chimowitz MI, Estes ML, Furlan AJ, Awad IA. Further observations on the pathology of subcortical lesions identified on magnetic resonance imaging. Arch Neurol 1992 Jul;49(7):747-752.

(39) Fazekas F, Kleinert R, Offenbacher H, Payer F, Schmidt R, Kleinert G, et al. The morphologic correlate of incidental punctate white matter hyperintensities on MR images. AJNR Am J Neuroradiol 1991 Sep-Oct;12(5):915-921.

(40) Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. Neurology 1993 Sep;43(9):1683-1689.

(41) Munoz DG, Hastak SM, Harper B, Lee D, Hachinski VC. Pathologic correlates of increased signals of the centrum ovale on magnetic resonance imaging. Arch Neurol 1993 May;50(5):492-497.

(42) Schmidt R, Petrovic K, Ropele S, Enzinger C, Fazekas F. Progression of leukoaraiosis and cognition. Stroke 2007 Sep;38(9):2619-2625.

(43) Longstreth WT,Jr, Arnold AM, Beauchamp NJ,Jr, Manolio TA, Lefkowitz D, Jungreis C, et al. Incidence, manifestations, and predictors of worsening white matter on serial cranial magnetic resonance imaging in the elderly: the Cardiovascular Health Study. Stroke 2005 Jan;36(1):56-61.

(44) Garde E, Mortensen EL, Krabbe K, Rostrup E, Larsson HB. Relation between age-related decline in intelligence and cerebral white-matter hyperintensities in healthy octogenarians: a longitudinal study. Lancet 2000 Aug 19;356(9230):628-634.

(45) van den Heuvel DM, ten Dam VH, de Craen AJ, Admiraal-Behloul F, Olofsen H, Bollen EL, et al. Increase in periventricular white matter hyperintensities parallels decline in mental processing speed in a non-demented elderly population. J Neurol Neurosurg Psychiatry 2006 Feb;77(2):149-153.

(46) Kramer JH, Mungas D, Reed BR, Wetzel ME, Burnett MM, Miller BL, et al. Longitudinal MRI and cognitive change in healthy elderly. Neuropsychology 2007 Jul;21(4):412-418.

(47) Silbert LC, Nelson C, Howieson DB, Moore MM, Kaye JA. Impact of white matter hyperintensity volume progression on rate of cognitive and motor decline. Neurology 2008 Jul 8;71(2):108-113.

(48) Schmidt R, Ropele S, Enzinger C, Petrovic K, Smith S, Schmidt H, et al. White matter lesion progression, brain atrophy, and cognitive decline: the Austrian stroke prevention study. Ann Neurol 2005 Oct;58(4):610-616.

(49) van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM. Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. Stroke 2008 Oct;39(10):2712-2719.

(50) Prins ND, van Dijk EJ, den Heijer T, Vermeer SE, Koudstaal PJ, Oudkerk M, et al. Cerebral white matter lesions and the risk of dementia. Arch Neurol 2004 Oct;61(10):1531-1534.

(51) Prasad K, Wiryasaputra L, Ng A, Kandiah N. White matter disease independently predicts progression from mild cognitive impairment to Alzheimer's disease in a clinic cohort. Dement Geriatr Cogn Disord 2011;31(6):431-434.

(52) Seiler S, Cavalieri M, Schmidt R. Vascular cognitive impairment - an ill-defined concept with the need to define its vascular component. J Neurol Sci 2012 Nov 15;322(1-2):11-16.

(53) Soumare A, Elbaz A, Zhu Y, Maillard P, Crivello F, Tavernier B, et al. White matter lesions volume and motor performances in the elderly. Ann Neurol 2009 Jun;65(6):706-715.

(54) Murray ME, Senjem ML, Petersen RC, Hollman JH, Preboske GM, Weigand SD, et al. Functional impact of white matter hyperintensities in cognitively normal elderly subjects. Arch Neurol 2010 Nov;67(11):1379-1385.

(55) Poggesi A, Gouw A, van der Flier W, Pracucci G, Chabriat H, Erkinjuntti T, et al. Cerebral white matter changes are associated with abnormalities on neurological examination in non-disabled elderly: the LADIS study. J Neurol 2013 Apr;260(4):1014-1021.

(56) Baezner H, Blahak C, Poggesi A, Pantoni L, Inzitari D, Chabriat H, et al. Association of gait and balance disorders with age-related white matter changes: the LADIS study. Neurology 2008 Mar 18;70(12):935-942.

(57) Novak V, Haertle M, Zhao P, Hu K, Munshi M, Novak P, et al. White matter hyperintensities and dynamics of postural control. Magn Reson Imaging 2009 Jul;27(6):752-759.

(58) Guttmann CR, Benson R, Warfield SK, Wei X, Anderson MC, Hall CB, et al. White matter abnormalities in mobility-impaired older persons. Neurology 2000 Mar 28;54(6):1277-1283.

(59) Della Nave R, Foresti S, Pratesi A, Ginestroni A, Inzitari M, Salvadori E, et al. Whole-brain histogram and voxel-based analyses of diffusion tensor imaging in patients with leukoaraiosis: correlation with motor and cognitive impairment. AJNR Am J Neuroradiol 2007 Aug;28(7):1313-1319.

(60) Wardlaw JM. What is a lacune? Stroke 2008 Nov;39(11):2921-2922.

(61) FISHER CM. Lacunes: Small, Deep Cerebral Infarcts. Neurology 1965 Aug;15:774-784.

(62) Chen X, Wen W, Anstey KJ, Sachdev PS. Prevalence, incidence, and risk factors of lacunar infarcts in a community sample. Neurology 2009 Jul 28;73(4):266-272.

(63) Benisty S, Gouw AA, Porcher R, Madureira S, Hernandez K, Poggesi A, et al. Location of lacunar infarcts correlates with cognition in a sample of non-disabled subjects with age-related white-matter changes: the LADIS study. J Neurol Neurosurg Psychiatry 2009 May;80(5):478-483.

(64) Gouw AA, van der Flier WM, Fazekas F, van Straaten EC, Pantoni L, Poggesi A, et al. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. Stroke 2008 May;39(5):1414-1420.

(65) Duering M, Gonik M, Malik R, Zieren N, Reyes S, Jouvent E, et al. Identification of a strategic brain network underlying processing speed deficits in vascular cognitive impairment. Neuroimage 2013 Feb 1;66:177-183.

(66) Duering M, Zieren N, Herve D, Jouvent E, Reyes S, Peters N, et al. Strategic role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL. Brain 2011 Aug;134(Pt 8):2366-2375.

(67) Jokinen H, Gouw AA, Madureira S, Ylikoski R, van Straaten EC, van der Flier WM, et al. Incident lacunes influence cognitive decline: the LADIS study. Neurology 2011 May 31;76(22):1872-1878.

(68) de Laat KF, van Norden AG, Gons RA, van Oudheusden LJ, van Uden IW, Bloem BR, et al. Gait in elderly with cerebral small vessel disease. Stroke 2010 Aug;41(8):1652-1658.

(69) Okroglic S, Widmann CN, Urbach H, Scheltens P, Heneka MT. Clinical symptoms and risk factors in cerebral microangiopathy patients. PLoS One 2013;8(2):e53455.

(70) Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, et al. Cerebral microbleeds: a guide to detection and interpretation. Lancet Neurol 2009 Feb;8(2):165-174.

(71) Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, et al. Histopathologic analysis of foci of signal loss on gradient-echo T2*-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. AJNR Am J Neuroradiol 1999 Apr;20(4):637-642.

(72) Cordonnier C, van der Flier WM, Sluimer JD, Leys D, Barkhof F, Scheltens P. Prevalence and severity of microbleeds in a memory clinic setting. Neurology 2006 May 9;66(9):1356-1360.

(73) Poels MM, Ikram MA, van der Lugt A, Hofman A, Krestin GP, Breteler MM, et al. Incidence of cerebral microbleeds in the general population: the Rotterdam Scan Study. Stroke 2011 Mar;42(3):656-661.

(74) Werring DJ, Frazer DW, Coward LJ, Losseff NA, Watt H, Cipolotti L, et al. Cognitive dysfunction in patients with cerebral microbleeds on T2*-weighted gradient-echo MRI. Brain 2004 Oct;127(Pt 10):2265-2275.

(75) Viswanathan A, Godin O, Jouvent E, O'Sullivan M, Gschwendtner A, Peters N, et al. Impact of MRI markers in subcortical vascular dementia: a multi-modal analysis in CADASIL. Neurobiol Aging 2010 Sep;31(9):1629-1636.

(76) Yakushiji Y, Nishiyama M, Yakushiji S, Hirotsu T, Uchino A, Nakajima J, et al. Brain microbleeds and global cognitive function in adults without neurological disorder. Stroke 2008 Dec;39(12):3323-3328.

(77) Pettersen JA, Sathiyamoorthy G, Gao FQ, Szilagyi G, Nadkarni NK, St George-Hyslop P, et al. Microbleed topography, leukoaraiosis, and cognition in probable Alzheimer disease from the Sunnybrook dementia study. Arch Neurol 2008 Jun;65(6):790-795.

(78) Qiu C, Cotch MF, Sigurdsson S, Jonsson PV, Jonsdottir MK, Sveinbjrnsdottir S, et al. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. Neurology 2010 Dec 14;75(24):2221-2228.

(79) Liem MK, Lesnik Oberstein SA, Haan J, van der Neut IL, Ferrari MD, van Buchem MA, et al. MRI correlates of cognitive decline in CADASIL: a 7-year follow-up study. Neurology 2009 Jan 13;72(2):143-148.

(80) van Norden AG, van den Berg HA, de Laat KF, Gons RA, van Dijk EJ, de Leeuw FE. Frontal and temporal microbleeds are related to cognitive function: the Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort (RUN DMC) Study. Stroke 2011 Dec;42(12):3382-3386.

(81) Gregoire SM, Smith K, Jager HR, Benjamin M, Kallis C, Brown MM, et al. Cerebral microbleeds and long-term cognitive outcome: longitudinal cohort study of stroke clinic patients. Cerebrovasc Dis 2012;33(5):430-435.

(82) Meier IB, Gu Y, Guzaman VA, Wiegman AF, Schupf N, Manly JJ, et al. Lobar microbleeds are associated with a decline in executive functioning in older adults. Cerebrovasc Dis 2014;38(5):377-383.

(83) Orken DN, Uysal E, Timer E, Kuloglu-Pazarci N, Mumcu S, Forta H. New cerebral microbleeds in ischemic stroke patients on warfarin treatment: two-year follow-up. Clin Neurol Neurosurg 2013 Sep;115(9):1682-1685.

(84) Kleinig TJ. Associations and implications of cerebral microbleeds. J Clin Neurosci 2013 Jul;20(7):919-927.

(85) Tournier JD, Mori S, Leemans A. Diffusion tensor imaging and beyond. Magn Reson Med 2011 Jun;65(6):1532-1556.

(86) Conturo TE, Lori NF, Cull TS, Akbudak E, Snyder AZ, Shimony JS, et al. Tracking neuronal fiber pathways in the living human brain. Proc Natl Acad Sci U S A 1999 Aug 31;96(18):10422-10427.

(87) Hagmann P, Thiran JP, Jonasson L, Vandergheynst P, Clarke S, Maeder P, et al. DTI mapping of human brain connectivity: statistical fibre tracking and virtual dissection. Neuroimage 2003 Jul;19(3):545-554.

(88) Le Bihan D. Looking into the functional architecture of the brain with diffusion MRI. Nat Rev Neurosci 2003 Jun;4(6):469-480.

(89) Behrens TE, Johansen-Berg H. Relating connectional architecture to gray matter function using diffusion imaging. Philos Trans R Soc Lond B Biol Sci 2005 May 29;360(1457):903-911.

(90) Tuch DS, Reese TG, Wiegell MR, Wedeen VJ. Diffusion MRI of complex neural architecture. Neuron 2003 Dec 4;40(5):885-895.

(91) Shereen A, Nemkul N, Yang D, Adhami F, Dunn RS, Hazen ML, et al. Ex vivo diffusion tensor imaging and neuropathological correlation in a murine model of hypoxia-ischemia-induced thrombotic stroke. J Cereb Blood Flow Metab 2011 Apr;31(4):1155-1169.

(92) Sexton CE, Walhovd KB, Storsve AB, Tamnes CK, Westlye LT, Johansen-Berg H, et al. Accelerated changes in white matter microstructure during aging: a longitudinal diffusion tensor imaging study. J Neurosci 2014 Nov 12;34(46):15425-15436.

(93) Bender AR, Raz N. Normal-appearing cerebral white matter in healthy adults: mean change over 2 years and individual differences in change. Neurobiol Aging 2015 May;36(5):1834-1848.

(94) Maillard P, Seshadri S, Beiser A, Himali JJ, Au R, Fletcher E, et al. Effects of systolic blood pressure on white-matter integrity in young adults in the Framingham Heart Study: a cross-sectional study. Lancet Neurol 2012 Dec;11(12):1039-1047.

(95) Davis SW, Dennis NA, Buchler NG, White LE, Madden DJ, Cabeza R. Assessing the effects of age on long white matter tracts using diffusion tensor tractography. Neuroimage 2009 Jun;46(2):530-541.

(96) West RL. An application of prefrontal cortex function theory to cognitive aging. Psychol Bull 1996 Sep;120(2):272-292.

(97) Charlton RA, Barrick TR, McIntyre DJ, Shen Y, O'Sullivan M, Howe FA, et al. White matter damage on diffusion tensor imaging correlates with age-related cognitive decline. Neurology 2006 Jan 24;66(2):217-222.

(98) Bastin ME, Clayden JD, Pattie A, Gerrish IF, Wardlaw JM, Deary IJ. Diffusion tensor and magnetization transfer MRI measurements of periventricular white matter hyperintensities in old age. Neurobiol Aging 2009 Jan;30(1):125-136.

(99) Schmidt R, Ropele S, Ferro J, Madureira S, Verdelho A, Petrovic K, et al. Diffusion-weighted imaging and cognition in the leukoariosis and disability in the elderly study. Stroke 2010 May;41(5):e402-8.

(100) Gunning-Dixon FM, Brickman AM, Cheng JC, Alexopoulos GS. Aging of cerebral white matter: a review of MRI findings. Int J Geriatr Psychiatry 2009 Feb;24(2):109-117.

(101) O'Sullivan M, Morris RG, Huckstep B, Jones DK, Williams SC, Markus HS. Diffusion tensor MRI correlates with executive dysfunction in patients with ischaemic leukoaraiosis. J Neurol Neurosurg Psychiatry 2004 Mar;75(3):441-447.

(102) Viana-Baptista M, Bugalho P, Jordao C, Ferreira N, Ferreira A, Forjaz Secca M, et al. Cognitive function correlates with frontal white matter apparent diffusion coefficients in patients with leukoaraiosis. J Neurol 2008 Mar;255(3):360-366.

(103) Chabriat H, Pappata S, Poupon C, Clark CA, Vahedi K, Poupon F, et al. Clinical severity in CADASIL related to ultrastructural damage in white matter: in vivo study with diffusion tensor MRI. Stroke 1999 Dec;30(12):2637-2643.

(104) Bhadelia RA, Price LL, Tedesco KL, Scott T, Qiu WQ, Patz S, et al. Diffusion tensor imaging, white matter lesions, the corpus callosum, and gait in the elderly. Stroke 2009 Dec;40(12):3816-3820.

(105) Seiler S, Ropele S, Schmidt R. Magnetization transfer imaging for in vivo detection of microstructural tissue changes in aging and dementia: a short literature review. J Alzheimers Dis 2014;42 Suppl 3:S229-37.

(106) Draganski B, Ashburner J, Hutton C, Kherif F, Frackowiak RS, Helms G, et al. Regional specificity of MRI contrast parameter changes in normal ageing revealed by voxel-based quantification (VBQ). Neuroimage 2011 Apr 15;55(4):1423-1434.

(107) Giulietti G, Bozzali M, Figura V, Spano B, Perri R, Marra C, et al. Quantitative magnetization transfer provides information complementary to gray matter atrophy in Alzheimer's disease brains. Neuroimage 2012 Jan 16;59(2):1114-1122.

(108) Graham SJ, Henkelman RM. Understanding pulsed magnetization transfer. J Magn Reson Imaging 1997 Sep-Oct;7(5):903-912.

(109) Weishaupt D, Köchli V, Marincek B. Wie funktioniert MRI? 6th Edition ed. Heidelberg: Springer Medizin Verlag; 2009.

(110) Fazekas F, Ropele S, Enzinger C, Gorani F, Seewann A, Petrovic K, et al. MTI of white matter hyperintensities. Brain 2005 Dec;128(Pt 12):2926-2932.

(111) Schmierer K, Scaravilli F, Altmann DR, Barker GJ, Miller DH. Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. Ann Neurol 2004 Sep;56(3):407-415.

(112) Chen JT, Easley K, Schneider C, Nakamura K, Kidd GJ, Chang A, et al. Clinically feasible MTR is sensitive to cortical demyelination in MS. Neurology 2013 Jan 15;80(3):246-252.

(113) Ropele S, Enzinger C, Sollinger M, Langkammer C, Wallner-Blazek M, Schmidt R, et al. The impact of sex and vascular risk factors on brain tissue changes with aging: magnetization transfer imaging results of the Austrian stroke prevention study. AJNR Am J Neuroradiol 2010 Aug;31(7):1297-1301.

(114) Schiavone F, Charlton RA, Barrick TR, Morris RG, Markus HS. Imaging age-related cognitive decline: A comparison of diffusion tensor and magnetization transfer MRI. J Magn Reson Imaging 2009 Jan;29(1):23-30.

(115) Spilt A, Goekoop R, Westendorp RG, Blauw GJ, de Craen AJ, van Buchem MA. Not all age-related white matter hyperintensities are the same: a magnetization transfer imaging study. AJNR Am J Neuroradiol 2006 Oct;27(9):1964-1968.

(116) Benedetti B, Charil A, Rovaris M, Judica E, Valsasina P, Sormani MP, et al. Influence of aging on brain gray and white matter changes assessed by conventional, MT, and DT MRI. Neurology 2006 Feb 28;66(4):535-539.

(117) Ge Y, Grossman RI, Babb JS, Rabin ML, Mannon LJ, Kolson DL. Age-related total gray matter and white matter changes in normal adult brain. Part II: quantitative magnetization transfer ratio histogram analysis. AJNR Am J Neuroradiol 2002 Sep;23(8):1334-1341.

(118) Venkatraman VK, Aizenstein HJ, Newman AB, Yaffe K, Harris T, Kritchevsky S, et al. Lower Digit Symbol Substitution Score in the Oldest Old is Related to Magnetization Transfer and Diffusion Tensor Imaging of the White Matter. Front Aging Neurosci 2011;3:11.

(119) Lee KY, Kim TK, Park M, Ko S, Song IC, Cho IH. Age-related changes in conventional and magnetization transfer MR imaging in elderly people: comparison with neurocognitive performance. Korean J Radiol 2004 Apr-Jun;5(2):96-101.

(120) Duzel S, Munte TF, Lindenberger U, Bunzeck N, Schutze H, Heinze HJ, et al. Basal forebrain integrity and cognitive memory profile in healthy aging. Brain Res 2010 Jan 13;1308:124-136.

(121) Seiler CB, Jones KE, Shera D, Armstrong CL. Brain region white matter associations with visual selective attention. Brain Imaging Behav 2011 Dec;5(4):262-273.

(122) De Groot JC, De Leeuw FE, Oudkerk M, Van Gijn J, Hofman A, Jolles J, et al. Periventricular cerebral white matter lesions predict rate of cognitive decline. Ann Neurol 2002 Sep;52(3):335-341.

(123) Debette S, Bombois S, Bruandet A, Delbeuck X, Lepoittevin S, Delmaire C, et al. Subcortical hyperintensities are associated with cognitive decline in patients with mild cognitive impairment. Stroke 2007 Nov;38(11):2924-2930.

(124) Kee Hyung P, Lee JY, Na DL, Kim SY, Cheong HK, Moon SY, et al. Different associations of periventricular and deep white matter lesions with cognition, neuropsychiatric symptoms, and daily activities in dementia. J Geriatr Psychiatry Neurol 2011 Jun;24(2):84-90.

(125) Seo SW, Lee JM, Im K, Park JS, Kim SH, Kim ST, et al. Cortical thinning related to periventricular and deep white matter hyperintensities. Neurobiol Aging 2012 Jul;33(7):1156-1167.

(126) Bennett DA, Wilson RS, Gilley DW, Fox JH. Clinical diagnosis of Binswanger's disease. J Neurol Neurosurg Psychiatry 1990 Nov;53(11):961-965.

(127) Hanyu H, Asano T, Sakurai H, Iwamoto T, Takasaki M, Shindo H, et al. Magnetization transfer ratio in cerebral white matter lesions of Binswanger's disease. J Neurol Sci 1999 Jul 1;166(2):85-90.

(128) Tanabe JL, Ezekiel F, Jagust WJ, Reed BR, Norman D, Schuff N, et al. Magnetization transfer ratio of white matter hyperintensities in subcortical ischemic vascular dementia. AJNR Am J Neuroradiol 1999 May;20(5):839-844.

(129) Iannucci G, Dichgans M, Rovaris M, Bruning R, Gasser T, Giacomotti L, et al. Correlations between clinical findings and magnetization transfer imaging metrics of tissue damage in individuals with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Stroke 2001 Mar;32(3):643-648.

(130) Ridha BH, Symms MR, Tozer DJ, Stockton KC, Frost C, Siddique MM, et al. Magnetization transfer ratio in Alzheimer disease: comparison with volumetric measurements. AJNR Am J Neuroradiol 2007 May;28(5):965-970.

(131) Hanyu H, Asano T, Iwamoto T, Takasaki M, Shindo H, Abe K. Magnetization transfer measurements of the hippocampus in patients with Alzheimer's disease, vascular dementia, and other types of dementia. AJNR Am J Neuroradiol 2000 Aug;21(7):1235-1242.

(132) Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999 Mar;56(3):303-308.

(133) Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. Acta Psychiatr Scand 2009 Apr;119(4):252-265.

(134) Perri R, Carlesimo GA, Serra L, Caltagirone C, Early Diagnosis Group of the Italian Interdisciplinary Network on Alzheimer's Disease. When the amnestic mild cognitive impairment disappears: characterisation of the memory profile. Cogn Behav Neurol 2009 Jun;22(2):109-116.

(135) Visser PJ, Verhey FR, Ponds RW, Cruts M, Van Broeckhoven CL, Jolles J. Course of objective memory impairment in non-demented subjects attending a memory clinic and predictors of outcome. Int J Geriatr Psychiatry 2000 Apr;15(4):363-372.

(136) van Es AC, van der Flier WM, Admiraal-Behloul F, Olofsen H, Bollen EL, Middelkoop HA, et al. Lobar distribution of changes in gray matter and white matter in memory clinic patients: detected using magnetization transfer imaging. AJNR Am J Neuroradiol 2007 Nov-Dec;28(10):1938-1942.

(137) van der Flier WM, van den Heuvel DM, Weverling-Rijnsburger AW, Bollen EL, Westendorp RG, van Buchem MA, et al. Magnetization transfer imaging in normal aging, mild cognitive impairment, and Alzheimer's disease. Ann Neurol 2002 Jul;52(1):62-67.

(138) Wiest R, Burren Y, Hauf M, Schroth G, Pruessner J, Zbinden M, et al. Classification of Mild Cognitive Impairment and Alzheimer Disease Using Model-Based MR and Magnetization Transfer Imaging. AJNR Am J Neuroradiol 2012 Oct 11.

(139) Kiefer C, Brockhaus L, Cattapan-Ludewig K, Ballinari P, Burren Y, Schroth G, et al. Multi-parametric classification of Alzheimer's disease and mild cognitive impairment: the impact of quantitative magnetization transfer MR imaging. Neuroimage 2009 Dec;48(4):657-667.

(140) Carmeli C, Donati A, Antille V, Viceic D, Ghika J, von Gunten A, et al. Demyelination in mild cognitive impairment suggests progression path to Alzheimer's disease. PLoS One 2013 Aug 30;8(8):e72759.

(141) Ropele S, Schmidt R, Enzinger C, Windisch M, Martinez NP, Fazekas F. Longitudinal magnetization transfer imaging in mild to severe Alzheimer disease. AJNR Am J Neuroradiol 2012 Mar;33(3):570-575.

(142) Fornari E, Maeder P, Meuli R, Ghika J, Knyazeva MG. Demyelination of superficial white matter in early Alzheimer's disease: a magnetization transfer imaging study. Neurobiol Aging 2012 Feb;33(2):428.e7-428.19.

(143) Van Der Flier WM, Van Den Heuvel DM, Weverling-Rijnsburger AW, Spilt A, Bollen EL, Westendorp RG, et al. Cognitive decline in AD and mild cognitive impairment is associated with global brain damage. Neurology 2002 Sep 24;59(6):874-879.

(144) Jokinen H, Lipsanen J, Schmidt R, Fazekas F, Gouw AA, van der Flier WM, et al. Brain atrophy accelerates cognitive decline in cerebral small vessel disease: the LADIS study. Neurology 2012 May 29;78(22):1785-1792.

(145) Camicioli R, Moore MM, Sexton G, Howieson DB, Kaye JA. Age-related brain changes associated with motor function in healthy older people. J Am Geriatr Soc 1999 Mar;47(3):330-334.

(146) Vernooij MW, Ikram MA, Vrooman HA, Wielopolski PA, Krestin GP, Hofman A, et al. White matter microstructural integrity and cognitive function in a general elderly population. Arch Gen Psychiatry 2009 May;66(5):545-553.

(147) Xu Q, Zhou Y, Li YS, Cao WW, Lin Y, Pan YM, et al. Diffusion tensor imaging changes correlate with cognition better than conventional MRI findings in patients with subcortical ischemic vascular disease. Dement Geriatr Cogn Disord 2010;30(4):317-326.

(148) Schmierer K, Tozer DJ, Scaravilli F, Altmann DR, Barker GJ, Tofts PS, et al. Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. J Magn Reson Imaging 2007 Jul;26(1):41-51.

(149) Chen TF, Chen YF, Cheng TW, Hua MS, Liu HM, Chiu MJ. Executive dysfunction and periventricular diffusion tensor changes in amnesic mild cognitive impairment and early Alzheimer's disease. Hum Brain Mapp 2009 Nov;30(11):3826-3836.

(150) Huang J, Auchus AP. Diffusion tensor imaging of normal appearing white matter and its correlation with cognitive functioning in mild cognitive impairment and Alzheimer's disease. Ann N Y Acad Sci 2007 Feb;1097:259-264.

(151) Zhou Y, Qun-Xu, Qin LD, Qian LJ, Cao WW, Xu JR. A primary study of diffusion tensor imaging-based histogram analysis in vascular cognitive impairment with no dementia. Clin Neurol Neurosurg 2011 Feb;113(2):92-97.

(152) Deary IJ, Bastin ME, Pattie A, Clayden JD, Whalley LJ, Starr JM, et al. White matter integrity and cognition in childhood and old age. Neurology 2006 Feb 28;66(4):505-512.

(153) Verghese J, LeValley A, Hall CB, Katz MJ, Ambrose AF, Lipton RB. Epidemiology of gait disorders in community-residing older adults. J Am Geriatr Soc 2006 Feb;54(2):255-261.

(154) Mertz KJ, Lee DC, Sui X, Powell KE, Blair SN. Falls among adults: the association of cardiorespiratory fitness and physical activity with walking-related falls. Am J Prev Med 2010 Jul;39(1):15-24.

(155) Weiss A, Brozgol M, Dorfman M, Herman T, Shema S, Giladi N, et al. Does the evaluation of gait quality during daily life provide insight into fall risk? A novel approach using 3-day accelerometer recordings. Neurorehabil Neural Repair 2013 Oct;27(8):742-752.

(156) Mignardot JB, Deschamps T, Barrey E, Auvinet B, Berrut G, Cornu C, et al. Gait disturbances as specific predictive markers of the first fall onset in elderly people: a two-year prospective observational study. Front Aging Neurosci 2014 Feb 25;6:22.

(157) Mirelman A, Herman T, Brozgol M, Dorfman M, Sprecher E, Schweiger A, et al. Executive function and falls in older adults: new findings from a five-year prospective study link fall risk to cognition. PLoS One 2012;7(6):e40297.

(158) Verghese J, Buschke H, Viola L, Katz M, Hall C, Kuslansky G, et al. Validity of divided attention tasks in predicting falls in older individuals: a preliminary study. J Am Geriatr Soc 2002 Sep;50(9):1572-1576.

(159) Kang HG, Dingwell JB. Effects of walking speed, strength and range of motion on gait stability in healthy older adults. J Biomech 2008 Oct 20;41(14):2899-2905.

(160) Mahlknecht P, Kiechl S, Bloem BR, Willeit J, Scherfler C, Gasperi A, et al. Prevalence and burden of gait disorders in elderly men and women aged 60-97 years: a population-based study. PLoS One 2013 Jul 24;8(7):e69627.

(161) la Fougere C, Zwergal A, Rominger A, Forster S, Fesl G, Dieterich M, et al. Real versus imagined locomotion: a [18F]-FDG PET-fMRI comparison. Neuroimage 2010 May 1;50(4):1589-1598.

(162) Jones DK, Lythgoe D, Horsfield MA, Simmons A, Williams SC, Markus HS. Characterization of white matter damage in ischemic leukoaraiosis with diffusion tensor MRI. Stroke 1999 Feb;30(2):393-397.

(163) Catani M, ffytche DH. The rises and falls of disconnection syndromes. Brain 2005 Oct;128(Pt 10):2224-2239.

(164) Rosano C, Sigurdsson S, Siggeirsdottir K, Phillips CL, Garcia M, Jonsson PV, et al. Magnetization transfer imaging, white matter hyperintensities, brain atrophy and slower gait in older men and women. Neurobiol Aging 2010 Jul;31(7):1197-1204.

(165) Schmidt R, Fazekas F, Kapeller P, Schmidt H, Hartung HP. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. Neurology 1999 Jul 13;53(1):132-139.

(166) Schmidt R, Lechner H, Fazekas F, Niederkorn K, Reinhart B, Grieshofer P, et al. Assessment of cerebrovascular risk profiles in healthy persons: definition of research goals and the Austrian Stroke Prevention Study (ASPS). Neuroepidemiology 1994;13(6):308-313.

(167) Bäumler G. Lern und Gedächtnistest (LGT 3). Göttingen, Germany: Verlag Psychologie, 1974.

(168) Heaton R editor. Wisconsin Card Sorting Test manual. . Odessa, FL: Psychological Assessment Resources,; 1981.

(169) United States War Department. editor. Army individual test battery manual of directions and scoring. Washington, DC: United States War Department, Adjutant General's Office,; 1944.

(170) Tewes U. Hamburg-Wechsler Intelligenztest für Erwachsene, revision 1991. Manual. Bern, Switzerland; 1991.

(171) Milner B. Effects of different brain lesion on card sorting test: the role of the frontal lobes. Arch Neurol 1963;9:100-110.

(172) Tiffin J, Asher E. The Purdue pegboard; norms and studies of reliability and validity. J Appl Psychol 1948 Jun;32(3):234-247.

(173) Plummer D. DispImage: a display and analysis tool for medical images. Revista di Neuroradiologica 1992;5:489-495.

(174) Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. Med Image Anal 2001 Jun;5(2):143-156.

(175) Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 2001 Jan;20(1):45-57.

(176) Patenaude B, Smith SM, Kennedy DN, Jenkinson M. A Bayesian model of shape and appearance for subcortical brain segmentation. Neuroimage 2011 Jun 1;56(3):907-922.

(177) van der Flier WM, van Straaten EC, Barkhof F, Verdelho A, Madureira S, Pantoni L, et al. Small vessel disease and general cognitive function in nondisabled elderly: the LADIS study. Stroke 2005 Oct;36(10):2116-2120.

(178) Verdelho A, Madureira S, Moleiro C, Ferro JM, Santos CO, Erkinjuntti T, et al. White matter changes and diabetes predict cognitive decline in the elderly: the LADIS study. Neurology 2010 Jul 13;75(2):160-167.

(179) Suchindran S, Vana AM, Shaffer RA, Alcaraz JE, McCarthy JJ. Racial differences in the interaction between family history and risk factors associated with diabetes in the National Health and Nutritional Examination Survey, 1999-2004. Genet Med 2009 Jul;11(7):542-547.

(180) Newton-Cheh C, Eijgelsheim M, Rice KM, de Bakker PI, Yin X, Estrada K, et al. Common variants at ten loci influence QT interval duration in the QTGEN Study. Nat Genet 2009 Apr;41(4):399-406.

(181) Hayes AF, Preacher KJ. Statistical mediation analysis with a multicategorical independent variable. Br J Math Stat Psychol in press 2003.

(182) Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav Res Methods 2008 Aug;40(3):879-891.

(183) Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological) 1995;57(1):289-300.

(184) Hua K, Zhang J, Wakana S, Jiang H, Li X, Reich DS, et al. Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. Neuroimage 2008 Jan 1;39(1):336-347.

(185) Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002 Nov;17(3):143-155.

(186) Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000 Jun;11(6 Pt 1):805-821.

(187) Rorden C, Karnath HO, Bonilha L. Improving lesion-symptom mapping. J Cogn Neurosci 2007 Jul;19(7):1081-1088.

(188) Kimberg DY, Coslett HB, Schwartz MF. Power in Voxel-based lesion-symptom mapping. J Cogn Neurosci 2007 Jul;19(7):1067-1080.

(189) Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. Neuroimage 2014 May 15;92:381-397.

(190) Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 1986;9:357-381.

(191) Purves, D., Augustine, G., Fitzpatrick, D., et al. Circuits within the Basal Ganglia System. Neuroscience. 2nd edition. Sunderland (MA). : Sinauer Associates; 2001.

(192) Krause M, Mahant N, Kotschet K, Fung VS, Vagg D, Wong CH, et al. Dysexecutive behaviour following deep brain lesions--a different type of disconnection syndrome? Cortex 2012 Jan;48(1):97-119.

(193) Bonelli RM, Cummings JL. Frontal-subcortical circuitry and behavior. Dialogues Clin Neurosci 2007;9(2):141-151.

(194) Rashid W, Hadjiprocopis A, Griffin CM, Chard DT, Davies GR, Barker GJ, et al. Diffusion tensor imaging of early relapsing-remitting multiple sclerosis with histogram analysis using automated segmentation and brain volume correction. Mult Scler 2004 Feb;10(1):9-15.

(195) Takao H, Hayashi N, Inano S, Ohtomo K. Effect of head size on diffusion tensor imaging. Neuroimage 2011 Aug 1;57(3):958-967.

(196) Metzler-Baddeley C, O'Sullivan MJ, Bells S, Pasternak O, Jones DK. How and how not to correct for CSF-contamination in diffusion MRI. Neuroimage 2012 Jan 16;59(2):1394-1403.

(197) Petzold A, Tozer DJ, Schmierer K. Axonal damage in the making: neurofilament phosphorylation, proton mobility and magnetisation transfer in multiple sclerosis normal appearing white matter. Exp Neurol 2011 Dec;232(2):234-239.

(198) Barkhof F, Bruck W, De Groot CJ, Bergers E, Hulshof S, Geurts J, et al. Remyelinated lesions in multiple sclerosis: magnetic resonance image appearance. Arch Neurol 2003 Aug;60(8):1073-1081.

(199) Grinberg LT, Thal DR. Vascular pathology in the aged human brain. Acta Neuropathol 2010 Mar;119(3):277-290.

(200) Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol 2010 Jul;9(7):689-701.

(201) Akassoglou K, Strickland S. Nervous system pathology: the fibrin perspective. Biol Chem 2002 Jan;383(1):37-45.

(202) Brownlees J, Yates A, Bajaj NP, Davis D, Anderton BH, Leigh PN, et al. Phosphorylation of neurofilament heavy chain side-arms by stress activated protein kinase-1b/Jun N-terminal kinase-3. J Cell Sci 2000 Feb;113 (Pt 3)(Pt 3):401-407.

(203) Nakamura T, Lipton SA. Cell death: protein misfolding and neurodegenerative diseases. Apoptosis 2009 Apr;14(4):455-468.

(204) Kerchner GA, Racine CA, Hale S, Wilheim R, Laluz V, Miller BL, et al. Cognitive processing speed in older adults: relationship with white matter integrity. PLoS One 2012;7(11):e50425.

(205) Salami A, Eriksson J, Nilsson LG, Nyberg L. Age-related white matter microstructural differences partly mediate age-related decline in processing speed but not cognition. Biochim Biophys Acta 2012 Mar;1822(3):408-415.

(206) de Laat KF, Tuladhar AM, van Norden AG, Norris DG, Zwiers MP, de Leeuw FE. Loss of white matter integrity is associated with gait disorders in cerebral small vessel disease. Brain 2011 Jan;134(Pt 1):73-83.

(207) Montero-Odasso M, Verghese J, Beauchet O, Hausdorff JM. Gait and cognition: a complementary approach to understanding brain function and the risk of falling. J Am Geriatr Soc 2012 Nov;60(11):2127-2136.

(208) Chao YP, Cho KH, Yeh CH, Chou KH, Chen JH, Lin CP. Probabilistic topography of human corpus callosum using cytoarchitectural parcellation and high angular resolution diffusion imaging tractography. Hum Brain Mapp 2009 Oct;30(10):3172-3187.

(209) Seidler RD, Bernard JA, Burutolu TB, Fling BW, Gordon MT, Gwin JT, et al. Motor control and aging: links to age-related brain structural, functional, and biochemical effects. Neurosci Biobehav Rev 2010 Apr;34(5):721-733.

(210) Mehta RC, Pike GB, Enzmann DR. Measure of magnetization transfer in multiple sclerosis demyelinating plaques, white matter ischemic lesions, and edema. AJNR Am J Neuroradiol 1996 Jun-Jul;17(6):1051-1055.