Heterogeneity in age-related white matter changes

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Abstract White matter changes occur endemically in routine magnetic resonance imaging (MRI) scans of elderly persons. MRI appearance and histopathological correlates of white matter changes are heterogeneous. Smooth periventricular hyperintensities, including caps around the ventricular horns, periventricular lining and halos are likely to be of non-vascular origin. They relate to a disruption of the ependymal lining with subependymal widening of the extracellular space and have to be differentiated from subcortical and deep white matter abnormalities. For the latter a distinction needs to be made between punctate, early confluent and confluent types. Although punctate white matter lesions often represent widened perivascular spaces without substantial ischemic tissue damage, early confluent and confluent lesions correspond to incomplete ischemic destruction. Punctate abnormalities on MRI show a low tendency for progression, while early confluent and confluent changes progress rapidly. The causative and

modifying pathways involved in the occurrence of sporadic age-related white matter changes are still incompletely understood, but recent microarray and genome-wide association approaches increased the notion of pathways that might be considered as targets for therapeutic intervention. The majority of differentially regulated transcripts in white matter lesions encode genes associated with immune function, cell cycle, proteolysis, and ion transport. Genome-wide association studies identified six SNPs mapping to a locus on chromosome 17q25 to be related to white matter lesion load in the general population. We also report first and preliminary data that demonstrate apolipoprotein E (ApoE) immunoreactivity in white matter lesions and support epidemiological findings indicating that ApoE is another factor possibly related to white matter lesion occurrence. Further insights come from modern MRI techniques, such as diffusion tensor and magnetization transfer imaging, as they provide tools for the characterization of

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normal-appearing brain tissue beyond what can be expected from standard MRI scans. There is a need for additional pre- and postmortem studies in humans, including these new imaging techniques.

Keywords White matter lesions · Leukoaraiosis · White matter change · MRI

Introduction

Incidental white matter changes are a common finding on magnetic resonance imaging (MRI) of the brain. They relate to advancing age and always occur on the basis of specific pathological and biological processes. Different terms such as, white matter lesions, white matter hyperintensities or leukoaraiosis are interchangeably used to define these abnormalities. Hachinski [33] introduced the term leukoaraiosis (from the Greek *leuko* [white] and *araiosis* [rarefaction]) to designate both periventricular or subcortical (centrum semiovale) areas of hypodensity on CT or hyperintensity on T₂-weighted MRI. It is of note that the term leukoaraiosis refers to both the CT- and MRI-detectable alterations, although the lesions are not completely superimposable as to number, site and extension

[54]. This review will focus on the heterogeneity of age-related white matter changes regarding their MRI appearance, clinical consequences, rate of progression and pathohistological correlates.

White matter changes on in vivo and ex vivo MRI

MRI classifications of white matter changes usually separate (a) periventricular and (b) deep/subcortical signal abnormalities [45]. Periventricular changes typically include caps around the frontal horns of the lateral ventricles and pencil-thin lining or a smooth halo along the side of the lateral ventricles. Deep/subcortical signal changes can occur as punctate changes or beginning confluent or confluent abnormalities [18] (Fig. 1). Numerous studies examined the histopathological correlates of age-related white matter changes using postmortem MRIs as the references [2, 3, 10, 11, 15, 17, 20, 21, 23, 24, 30, 46, 50, 58, 59, 63, 77–80, 91, 95]. It is important to realize that fixation, but also autolysis, and changes in temperature may significantly change relaxation times, water content, and tissue structure, which may significantly impact on the reliability of postmortem MRI for white matter lesion detection. The most significant changes typically come

Fig. 1 White matter changes in T₂-weighted FLAIR MRI scans. The *first column* shows periventricular white matter changes: **a** caps, **b** pencil-thin lining and **c** halo. The *second column* shows deep or subcortical white matter changes: **d** punctate, **e** early confluent, and **f** confluent lesions

Table 1 Prevalence of different types of white matter changes in 993 participants (age range 45–87 years) from the Austrian Stroke Prevention Study

Age (years)	Periventricular white matter lesions ^a		Deep/subcortical white matter lesions ^a		
	Caps/lining (<i>N</i> = 632) <i>N</i> (%)	Halo (<i>N</i> = 61) <i>N</i> (%)	Punctate (<i>N</i> = 586) <i>N</i> (%)	Early confluent (<i>N</i> = 150) <i>N</i> (%)	Confluent (<i>N</i> = 76) <i>N</i> (%)
<55 (<i>N</i> = 128)	66 (51.5)	0 (0)	76 (59.3)	3 (2.3)	0 (0)
55–64 (<i>N</i> = 344)	203 (59.0)	5 (1.4)	236 (68.6)	17 (4.9)	9 (2.6)
65–74 (<i>N</i> = 374)	254 (67.9)	27 (7.2)	209 (55.8)	88 (23.5)	32 (8.5)
≥75 (<i>N</i> = 147)	109 (74.1)	29 (19.7)	65 (44.2)	42 (28.5)	35 (23.5)

^a Several individuals had more than one lesion type

from the fixation process, which dehydrates the tissue and consequently shortens all relaxation times, T_2 much more than T_1 . The relaxation times are usually stabilized after approximately 3–4 weeks of fixation [55, 75]. Early studies claimed that postmortem MRI reliably detects white matter lesions with some problems occurring with small punctate foci [20]. Fernando et al. [23] compared postmortem MRI of white matter lesions in fixed brain slices with pathology in 33 brains and described that MRI detection of lesions was less sensitive than pathology. A sensitivity of 95% and a specificity of 71% were reported for periventricular abnormalities, for deep/subcortical white matter changes the sensitivity was 86% and the specificity was 80%. In general, postmortem MRI of formalin-fixed brain slices was considered a reliable method to obtain data on both the severity and also distribution of white matter changes.

Frequency of age-related white matter changes

In population-based studies, the prevalence of any white matter lesions varies between 45 and 95%. Depending on the composition of the investigated cohorts, 12–33% of subjects have severe abnormalities [89]. Table 1 displays the frequency of the different types of MRI white matter lesions in the community-dwelling Austrian Stroke Prevention Study [67]. Caps/lining and punctate deep/subcortical white matter lesions are by far the most common signal changes in elderly subjects and occur in more than half of asymptomatic persons even in age groups below 55 years. All other white matter abnormalities are substantially less frequent, but their frequency increases with age, and in subjects above 75 years they affect at least one-fifth of individuals free of any overt signs of neurologic disease. In symptomatic patients, the frequency of white matter abnormalities is often higher. This is particularly seen in dementia where periventricular changes have been reported in all patients irrespective of the dementia type, and deep/subcortical lesions occurred in 85% of patients

with Lewy body dementia, 89% with Alzheimer's disease, and 96% with vascular dementia [5].

Risk factors and key clinical findings

The heterogeneous appearance of white matter changes on MRI implies differences in risk factors and clinical consequences. The Leukoaraiosis and Disability (LADIS) Study provides evidence for such differences. The study investigated risk factor profile effects on heterogeneous white matter lesion types and is the only study that systematically compared equally sized groups of individuals with punctate, early confluent, and confluent white matter changes [53]. Individuals with coalescent white matter lesions were significantly older and more frequently had hypertension and a history of stroke as compared to subjects with punctate abnormalities. The amount of cigarettes smoked, and the interaction between hypercholesterolemia and smoking were associated with confluent white matter changes only [6]. Consequences on cognitive function represent a key clinical finding related to age-related white matter changes. White matter lesions affect executive function often severely enough to impair activities of daily life resulting from erroneous goal formation, planning, and organization [39–41, 57]. Abstract reasoning is also commonly affected, but patients only suffer from mild memory deficits [38] with slight recognition and cueing difficulties [16]. Motor disturbances affect gait, postural control, and urinary continence [4, 35, 36, 56]. There exists a large inter-individual variability of the clinical presentation in subjects with white matter changes. Clinical consequences are likely to be influenced by lesion distribution in strategically relevant brain regions and by volume. Nonetheless, different lesion types have different clinical impact. LADIS consistently demonstrated significant differences between the performance of patients with punctate changes when compared with those with early confluent and confluent changes with the latter always being most impaired. Such

relationships were seen for all clinical correlates, including cognition [87], depression [86], gait and balance [4], as well as urinary incontinence [56]. Most importantly, early confluent and to a greater extent confluent white matter abnormalities predicted functional decline in activities of daily living during a relatively short observational period of 3 years [37].

Progression of white matter changes

White matter lesion heterogeneity is not only reflected by differences in clinical consequences, but also by the rate of lesion progression. All investigations agree that the extent of lesions at baseline is a major predictor of subsequent progression [62, 65, 85, 90].

In the Austrian Stroke Prevention Study [65], there was almost no increase in lesion volume in subjects with punctate abnormalities, whereas participants with a baseline finding of early confluent or confluent changes showed a remarkably more rapid increase in lesion volume. After 3 and 6 years of follow-up, subjects with confluent white matter abnormalities at baseline experienced a 5 and

13 cm³ increase in lesion volume, respectively. Figure 2 shows examples of different lesion progression depending on the baseline lesion grade.

Table 2 demonstrates the frequency of progression of the various white matter lesion types in our own community-based cohort (unpublished data). The lowest rate of progression is seen for punctate abnormalities, while the majority of subjects with early confluent and almost all subjects with confluent white matter lesions show progression. These data together with the pathological findings support the view that punctate white matter lesions are of mixed origin and relatively benign, whereas early confluent and confluent white matter abnormalities are ischemic, progressive, and thus malignant [68]. According to our data, smooth periventricular halos also show a considerable tendency to progress even though they are assumed to be of non-ischemic origin [21]. The reason for the relatively high progression rate of this lesion type might be seen in its association with ventricular enlargement which in the elderly is known to increase over time and probably leads to enhanced disruption of the ventricular ependymal lining with subsequent leakage of cerebrospinal fluid into the periventricular white matter [19].

Fig. 2 Flair images of three clinically normal subjects at baseline and 3-year follow-up. Upper row gives examples of punctate (a), early confluent (b) and confluent (c) white matter lesions. At follow-up, lesion progression is noted depending on the baseline grade (arrows).

It is absent or only minimal as in the displayed case with punctate (d), moderately severe in subjects with early confluent and confluent abnormalities (e, f)

Table 2 Number and percentage of participants of the Austrian Stroke Prevention Study who showed progression of specific white matter lesion types at 3-year and 6-year follow-up

	Periventricular white matter lesions ^a		Deep white matter lesions ^a		
	Caps/lining Progression <i>N/N^b</i> (%)	Halo Progression <i>N/N^b</i> (%)	Punctate Progression <i>N/N^b</i> (%)	Early confluent Progression <i>N/N^b</i> (%)	Confluent Progression <i>N/N^b</i> (%)
3-year follow-up	55/241 (22.8)	9/38 (23.6)	9/233 (3.8)	17/59 (28.8)	19/35 (54.3)
6-year follow-up	54/132 (40.9)	6/8 (75.0)	19/128 (14.8)	10/16 (62.5)	10/11 (90.9)

The sample of subjects with 3-year and 6-year follow-up scans comprised of 468 and 232 community-dwelling individuals aged 47–87 years

^a Lesion type refers to baseline MRI readings. Several subjects had more than one lesion type. For periventricular white matter lesions progression was assessed as present or absent based on visual side by side comparison, for deep white matter lesions we defined presence of progression as an increase in lesion volume beyond 1.81 cm³. This measure relates to the upper 95% limit of agreement from the most pronounced difference between repeated measurements and is considered to represent the range of possible measurement error [60]

^b Progression *N/N* = number of subjects with progression of specific lesion type/total number of subjects with this type of lesion at baseline

Neuropathologic correlates of different types of age-related white matter changes

In general, white matter lesions have been described to reflect partial loss of myelin, axons, and astrogliosis in the vicinity of small vessel changes. Tissue changes are thought to relate to incomplete infarction, although small deep white matter infarcts have also been described [28]. Importantly, only few histopathologic correlative investigations accounted for the heterogeneity of MRI-detected white matter changes. Typical examples of MRI to histopathology correlations are displayed in Fig. 3.

Histopathological studies assessing the pathological correlates of different white matter lesion types are summarized in Table 3. As can be seen from this table, non-ischemic and ischemic brain changes have been described.

Periventricular WMC

Smooth periventricular white matter hyperintensities appear to be of non-vascular origin. The smooth caps around the frontal horns were consistently found to correspond to a spongiotic zone of finely textured myelin [15, 21] (Fig. 3a, b), and are considered to represent normal anatomical structures in the fasciculus subcallosus. Some authors suggest increased periventricular water content, which converges from the surrounding white matter in this area or comes from the intense venous network of this region [21, 83]. Disruption of the ependymal lining of the lateral ventricle and subependymal gliosis, so-called ependymitis granularis, is another histologic finding almost invariably noted with periventricular smooth caps. It is not yet clear, if the ependymal alterations truly represent a pathologic phenomenon and contribute to the occurrence of periventricular changes [44, 83]. A significant correlation between denudation of the ventricular lining and the extent of periventricular hyperintensities is in favor of such a mechanism [64]. Smooth periventricular caps extending

posteriorly along the body of the lateral ventricles is called “lining” or when broader “smooth halo” (Fig. 1c). The tissue changes resemble those seen in the region of hyperintense caps [21]. Frequently, the ependymal lining of the ventricular walls is irregular or partly disrupted and a rim of gliotic tissue borders the ventricular lumen. Next to the ependymal and subependymal changes, there follows a zone with markedly reduced or absent myelin staining. Although myelin loss may be more pronounced around veins, there is no evidence for associated arteriolosclerosis or periarteriolar tissue damage.

Deep/subcortical WMC

The histopathological correlates of deep/subcortical white matter changes are non-uniform. As can be derived from Table 3, it is important to distinguish between the punctate lesion type, and more widespread early confluent and confluent changes. Punctate hyperintensities are commonly of non-ischemic origin as their most common correlate is widening of periarteriolar spaces [15, 20, 21, 50] accompanied by reduced myelination with atrophy of the neuropil around fibrohyalinotic arteries (Fig. 3c, d). Early confluent and confluent white matter hyperintensities are true ischemic lesions and represent a continuum of increasing tissue damage, including widespread perivascular rarefaction of myelin, mild to moderate fiber loss, and varying extents of gliosis. Confluent changes are irregular, mostly relatively well-demarcated areas of incomplete parenchymal destruction with focal transitions to true infarcts.

Molecular pathology

In line with the previous reports [15, 20, 21, 50], the MRC-CFAS also showed that periventricular lesion severity was also associated with loss of ventricular ependyma, while for deep/subcortical white matter lesions arteriolar

Fig. 3 Correlation of in vivo magnetic resonance imaging (MRI) and histomorphological features of affected fixed brains in representative cases with white matter lesions. **a** Caps abutting upon the frontal horns of the lateral ventricles, **b** Gross overview of ventricle, subventricular zone and partly pale white matter (stain Luxol fast blue), **c** multiple punctate white matter hyperintensities in the centrum semiovale bilaterally, **d** enlarged cell-free perivascular spaces in the presence of vessel wall hyalinosis. Luxol fast blue-periodic acid–Schiff (PAS)-stained whole mount section, **e** early confluent and confluent white matter lesions in the deep and periventricular white matter affecting both hemispheres, **f** overview picture of severe white matter changes with many large and small white matter lesions of variable shape characterized by patchy myelin loss with some cystic lacunar lesions representing areas of more severe tissue damage

Table 3 Studies describing the histopathological correlates of different types of white matter lesions

Study	Sample	Histochemistry/ immunohistochemistry	MRI [Tesla (T)]	
Fazekas [20]	2 subjects without and 4 with neurologic disease	HE, Masson's trichrome, KB	1.5	Punctate lesions relate to perivascular damage with fibrohyalinosi, atrophic neuropil and rarefaction of myelinated fibers, 2 patients had cortical heterotopia
Chimowitz [15]	7 subjects with neurologic disease	KB, Congo red, desmine	1.5	Periventricular rims relate to ependymal loss and subependymal gliosis Periventricular caps relate to myelin pallor Punctate DWMH are widened perivascular spaces
Fazekas [21]	11 subjects partly with neurologic disease	HE, Masson's trichrome, KB	1.5	Periventricular rims: smooth myelin pallor, loose fibers, tortuous venules, no arteriosclerosis, discontinuity of ependym with mild-moderate gliosis Irregular periventricular lesions have varying fiber loss, gliosis and cavitation with fibrohyalinosi Deep white matter lesions: Punctate: no ischemic changes; demyelination, atrophic neuropil around fibrohyalintotic arterioles and perivenous damage Early confluent: perivascular rarefaction of myelin, mild to moderate fiber loss, varying gliosis Confluent: irregular areas of incomplete parenchymal destruction with focal transitions to true infarcts
Munoz [50]	2 Alzheimer cases and 13 controls	HE, chromoxane cyanine, Bielschowsky, Congo red	1.5	Punctate relates to widened perivascular spaces Extensive white matter lesions are broad areas of loss of myelin, axons, and gliosis. No infarction or vascular wall changes
Fernando [24]	99 demented subjects and 108 controls	CD68, Col IV, ICAM1, HIF1alpha/2alpha, MMP7, Ngb, NMBR, VEGFR2, betaA4	1.0	Vessel wall thickening, dilated perivascular spaces in WMH, ependymal denudation in PVH. In WMH microglial activation and upregulation of hypoxia factors
Simpson [77]	Unselected tissue blocks: 12 PVH, 12 DWMH, 15 NAWM	HE, LFB, MBP, GFAP, CD68, PDGFalphaR, MAP-2(+13), fibrinogen	1.0	More microglial activation in DWMH than in PVH. Clasmatodendritic astrocytosis, positive for serum proteins suggesting blood–brain barrier dysfunction. Indicators for regeneration/remyelination in PVH
Simpson [78]	Unselected tissue blocks: 12 PVH, 12 DWMH, 15 NAWM	HLA-DR, B7-2, CD40, Mcm2, PCNA, Ki67	1.0	PVH have more MHCII positive microglia and costimulatory B7-2 and CD40, suggesting a more proliferative/immune reactive environment. Amoeboid microglia for phagocytosis of myelin breakdown products in DWMH

B7-2 CD86, provides costimulatory signals necessary for T cell activation and survival, *CD40* and *CD40 ligand* immune costimulatory molecules, *CD68* immunocytochemical marker for staining of monocytes/macrophages, *Col IV* collagen-IV, *DWMH* deep white matter hyperintensities, *GFAP* glial fibrillary acidic protein, *HE* hematoxylin–eosin, *HIF* hypoxia inducible factor, *HLA-DR* human leucocyte antigen-DR, *ICAM1* intercellular adhesion molecule, *KB* Kluver–Barrera, *LFB* Luxol fast blue, *MAP-2 +13* microtubule associated protein 2 expressing exon 13, *MBP* myelin basic protein, *Mcm2* DNA replication licensing factor MCM2, *MMP7* matrix metalloproteinase 7, *NAWM* normal-appearing white matter, *Ngb* neuroglobin, *NMBR* neuromedin B receptor, *PCNA* and *Ki67* molecules related to cell proliferation, *PDGFalphaR* platelet-derived growth factor alpha receptor, *PVH* periventricular white matter hyperintensities, *VEGFR2* vascular endothelial growth factor receptor 2, *WMH* white matter hyperintensities

sclerosis was observed [24]. Importantly, the authors reported that factors induced by hypoxia were elevated in deep/subcortical lesions, but not in periventricular abnormalities. [24]. This supports a hypoxic etiology for deep lesions and lends further evidence for periventricular white matter changes being non-ischemic in etiology probably caused by increased fluid accumulation related to the proximity of the ventricles. Moreover, MRC-CFAS also

studied the astrocytic, microglial and oligodendroglial responses in white matter abnormalities, and described that both periventricular and deep/subcortical lesions are associated with severe myelin loss and increased microglia compared with non-lesional brain tissue [77]. Clasmato-dendritic astroglia immunoreactive for fibrinogen were more commonly present in periventricular lesions which also showed more MAP-2 +13 positive remyelinating

oligodendrocytes and platelet-derived growth factor α receptor-positive reactive astrocytes. The authors interpreted these findings as an indication for blood–brain barrier dysfunction in a proportion of white matter lesions and emphasize that attempts of remyelination appear to be solely associated with periventricular white matter abnormalities [77]. The important role of the blood–brain barrier in the pathogenesis of periventricular white matter changes is also supported by the study's finding that this lesion type contains significantly higher levels of ramified activated microglia which may result from blood–brain barrier disruption [77]. In contrast, activated microglia seen in deep/subcortical abnormalities may rather reflect an innate amoeboid phenotype, which is involved in phagocytosis of myelin breakdown products [78].

Genetic aspects

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is the most common heritable cause of white matter lesions caused by pathogenetic mutations that alter the number of cysteine residues in the extracellular domain of NOTCH3. NOTCH3 accumulates in small arteries of affected individuals [13]. These data and the estimated heritability index of white matter lesions in the range of 55–73% [1, 12] led to the conduct of genetic association studies to determine the role of genetic factors in the etiology of age-related white matter changes. Previous epidemiologic studies reported positive associations between the presence of the apolipoprotein E (ApoE) $\epsilon 4$ allele, and the extent and progression of MRI lesions in the deep/subcortical white matter [27, 88]. Our own group conducted a pilot study in which we performed postmortem MRIs of unfixed brains in 13 patients who had died of various causes and used immunohistochemistry to assess the presence of apoE immunoreactivity in brain areas including white matter lesions. ApoE immunoreactivity was observed in white matter lesions, it appeared in astrocytes, in microglia and also in neurons (Fig. 4). Quantification of ApoE expression levels will be needed to better define the association between ApoE and age-related white matter abnormalities. Another important step will be to assess the influence of the ApoE genotype on the expression level of the protein in brain regions with and without white matter lesions.

Production or deposition of ApoE is hardly the cause of but appears to associate with white matter lesions. Possibly, ApoE plays a role in repair mechanisms for myelin. The MRC-CFAS Study Group also provides important information on pathways that may be involved in the occurrence of age-related white matter changes [79]. These authors extracted RNA to compare white matter lesions

with non-lesional white matter samples from cases with white matter lesions and from cases with no lesions using RNA microarray and pathway analysis. They identified 8 major pathways in which multiple genes showed altered RNA transcription among 502 genes that were differentially expressed in white matter lesions as compared to non-lesional control tissue. The majority of differentially regulated transcripts in white matter lesions coded for genes associated with immune function, cell cycle, proteolysis, and ion transport. Changes were also observed in pathways associated with electron transport, metabolism and cell structure. The authors emphasize that the microarray approach is not able to discriminate the underlying fundamental pathogenic insult from secondary changes in cell function and pathophysiology [79]. The results reported by this investigation are therefore compatible with a primary ischemic origin of white matter changes, but increase the range of pathways in which target identification for possible therapeutic intervention might be considered. Genome-wide association studies are another way to identify pathways involved in white matter lesion development. The CHARGE Consortium [25] provided the first meta-analysis of genome-wide association data from 9,361 individuals of European descent and identified novel genetic associations with white matter hyperintensity burden. Six SNPs mapping to a locus on chromosome 17q25 reached genome-wide significance, of which five were in strong linkage disequilibrium ($r^2 > 0.8$). These SNPs belong to a large cluster of associated SNPs spanning a ~ 100 -kb region, which contains several genes: Two tripartite motif-containing genes, *TRIM65* and *TRIM47*; the WW domain binding protein 2 gene (*WBP2*); the mitochondrial ribosomal protein L38 gene (*MRPL38*); the Fas-binding factor 1 gene (*FBF1*); and the acyl-coenzyme A oxidase 1 gene (*ACOX1*). It is important that several of these genes are related to the apoptosis superfamily and apoptosis-related transcripts have also been shown to be differentially expressed between white matter lesions and normal white matter of brains in the MRC-CFAS [79]. Another mechanism that has been implicated in the etiology of white matter lesions is increased production of reactive oxygen species and again the MRC-CFAS provides some evidence for such a relationship by showing that the hemochromatosis gene appears to be a genetic risk factor for severe age-related white matter lesions independently of the APOE $\epsilon 4$ genotype. [26] It is of note in this context that our own group has described evidence of an association between low plasma concentrations of vitamin E and a higher risk of cerebral white matter disease in elderly subjects [66]. The relative importance of these pathways for white matter lesion development and propagation is yet unclear as is their relationship to different white matter lesion types.

Fig. 4 Postmortem MRI from non-fixed brains and immunohistochemical staining for APO-E. **a** postmortem MRI shows only mild white matter hyperintensity in the vicinity of the posterior horn of the lateral ventricle (*arrow*), **b** punctate white matter changes in the subcortical white matter (*arrow*), **c** severe patchy confluent white matter damage in deep/subcortical white matter at supraventricular level (*arrow*), **d–i** Micrographs showing an overview of apoE expression in representative cases in vessel walls, astrocytes and

oligodendrocytes in white matter containing MRI white matter hyperintensities and in micrographs highlighting blood vessels with different expression intensities, **d** Immunohistochemical staining at low magnification showing ApoE-positive staining in multiple astrocytes and in the area of a blood vessel (*arrow head*), **e, f** Apo-E positively stained vessel wall (*arrow heads*) and astrocytes (*arrows*), **g** Apo-E weakly **h** moderately, and **i** strongly stained vessel walls

Age-related white matter changes and normal-appearing brain tissue

Recent advances in imaging techniques have highlighted the importance of normal-appearing brain tissue in the context of age-related white matter abnormalities. There is more widespread tissue damage associated white matter lesions which is not visible in conventional MR images, but can be detected by new quantitative MR techniques, such as diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI) [31, 47]. DTI provides information about the microstructural integrity of brain tissue by measuring the direction and magnitude of water diffusion in the

intracellular cytoplasm of the axons. Owing to the restriction by lipid bilayers and other cell components, water diffusion in brain tissue is anisotropic with the highest diffusivity along the directions of the axons.

MTI is an approach to explore the composition of brain tissue. Protons bound to larger molecules, such as the myelin lipids and proteins have relaxation properties that make them invisible for conventional MRI. However, the pool of bound protons can be indirectly quantified by utilizing magnetization transfer. MTI for mapping the magnetization transfer ratio is readily available on most clinical scanners and allows semi-quantitative assessment of tissue changes with a higher image resolution than

Table 4 Composite of studies on histopathological and clinical correlates of diffusion tensor and magnetization transfer imaging (DTI and MTI) metrics in ex vivo and in vivo models

Study	Tissue type	Cohort (N)	Brain region	Measure (parameters)	Findings
Animal model					
Holland et al. [36]	WM	Mice	4 WM tracts	DTI (FA)/MIT (MTR)	FA and MTR correlate with markers of myelin integrity/degradation
Shereen et al. [76]	WM	Mice	Fimbria, internal + external capsule	DTI (FA)	DTI parameters are sensitive to axonal and dendritic injury in acute ischemic stroke
Postmortem studies					
Schmierer et al. [73]	Postmortem—WM	MS (16)	One slice	DTI (FA, MD)	Increase in MD and decrease in FA of WMLs and/or NAWM indicate the presence of axonal loss
Schmierer et al. [72]	Postmortem—NAWM	MS (20)	Slices (thickness 1 cm)	MTI (MTR)	MTR parameter as predictors of myelin content; MTR differs between WML and NAWM
Schmierer et al. [71]	Postmortem—WM	MS (20)	Slices (thickness 1 cm)	MTI (MTR)	Neg. correlation between myelin content and MTR; MTR is affected by myelin content in MS WM
Gouw et al. [29]	Postmortem—NAWM and WMH	AD (33) versus HC (7)	104 ROIs in WMH and NAWM	DTI (ADC, FA), FLAIR (T ₁ -relax.)	Quantitative MR techniques reveal differences in WMH between AD and non-demented elderly and are able to reflect the severity of neuropathological changes
In vivo studies					
Chabriet et al. [14]	WMH versus WM	CADASIL (16) versus HC (10)	Four slices at the level of centrum semiovale	DTI (FA, MD)	Microstructural changes are related to severity of the clinical status; DTI parameters are sensitive to changes in WML and NAWM
Tanabe et al. [84]	WMH	SIVD (15) versus HC (16)	Whole brain (global)	MTI (MTR)	MTR of PVWMH was lower in SIVD than in controls; pathologic changes in VD are most severe in PVWM
Schmierer et al. [70]	NABT	PP-MS (15) versus HC (6)	10 ROIs (NA-thalamus and NAWM)	DTI (ADCav)	Increase of ADC in PPMS over 1 year in NAWM; this correlates with increases in lesion volume; NAWM measured with DTI is clinically relevant
O'Sullivan et al. [52]	WM damage	IL (36) versus HC (19)	Whole brain (global)	DTI/cMRI	Increased diffusivity in WM lesions and NAWM; MD of NAWM correlated with IQ and executive function
Fazekas et al. [22]	NABT	Healthy elderly (198)	Whole brain (global)	MTI (MTR)	MTR decreased significantly with increasing scores of WMH severity and WMH volume; MTR can serve to quantify WMH-associated tissue damage
Spitt et al. [81]	NAWM, WM(H)	Young (11), minimal WMH (51), abundant WMH (41)	Whole brain (global)	MTI (MTR)	MTR decreases with age and WMH increase; age gives rise to changes in NABT; these changes seem to have a relationship with age-related WMH and might have different etiology
Spitt et al. [82]	Periventricular WMH versus deep WMH	WMH (56) versus mild/no WMH (19)	Periventricular WMH and deep WMH	MTI (MTR)	Mean MTR of PVWMH was lower than DWMH; age-related WMH are heterogeneous despite similar appearance on T ₂ -weighted images
Benedetti et al. [8]	WM, GM	HC (89)	Whole brain (global)	DTI (MD)/MTI (MTR)	Normalized brain, GM and WM and the number of T ₂ -hyperintensities correlate with age; GM average MD and GM volume are independent predictors of subject's age; DTI correlated with NAA (neg.)—depending on severity of disease; NAA is a marker for axonal loss/dysfunction
Nitkunan et al. [51]	Asymptotic WMH to symptomatic cerebral SVD	SVD (29) versus hi-subjects (63) versus HC (42)	Centrum semiovale WM	DTI (MD)/MRS	

Table 4 continued

Study	Tissue type	Cohort (N)	Brain region	Measure (parameters)	Findings
Bastin et al. [7]	NAWM versus PVWMH	Elderly (42)	NAWM versus PVWMH	DTI (FA, MD)/MTI (MTR)	PVWMH had greater MD than NAWM; FA and MTR were significantly reduced in PVWMH compared to NAWM; PVWMH MTR correlated with MD and FA
Lee et al. [43]	NAWM and WMH in AD, MCI and HC	AD (47)/MCI (73)/HC (95)	Whole brain (global)	DTI (FA)	AD had lower FA than HC or MCI in regions with higher baseline anisotropy; FA in WMH was lower than in NAWM
Bhadelia et al. [9]	WM in cc	(gait-impaired) elderly (173)	cc (FA in genu and splenium)	DTI (FA)	Patients with abnormal gait had low FA in the genu, but not in splenium; WM integrity of the genu is an important marker of gait in the elderly
Ropele et al. [60]	NABT and within WMH	Elderly (340 DWI/177 MTR)	Whole brain (global)	DWI (ADC)/MIT (MTR)	WMH severity and age are significant predictors of global ADC and MTR changes; ADC and MTR are sensitive for age and WMH-related changes in NABT
Schmidt et al. [69]	NABT	Elderly (340)	Whole brain (global)	DWI (ADC)	Increased WMH correlated with higher frequency of hypertension, a greater WMH volume, more brain atrophy, worse overall cognitive performance and changes in ADC
Wang et al. [93]	WMHs and WM changes	AD with mild WMH (15), severe WMH (12), HC with mild WMH (17)	ROI-based	DTI (FA, MD), D(R), D(A)	FA and D(R) were more sensitive than MD and D(A) in differentiating AD from controls, MD was more sensitive in distinguishing AD with severe WMHs from those with mild WMHs.
Rosano et al. [61]	WM, WMH	(gait-impaired) elderly (795)	Whole brain (global)	MTI (MTR)	Women had lower MTR, higher WMH and brain atrophy (BA), but not brain infarcts (BI), remained associated with slower gait; in men WMH and BA, but not MTR or BI, remained associated with slower gait

AD Alzheimer's disease, ADC apparent diffusion coefficient, CADASIL cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, cc corpus callosum, D(A) axial diffusivity, D(R) radial diffusivity, DTI diffusion tensor imaging, DWI diffusion weighted imaging, FA fractional anisotropy, GM gray matter, HC healthy controls, IL ischemic leukoaraiosis, MCI mild cognitive impairment, MD mean diffusivity, MRS magnetic resonance spectroscopy, MS multiple sclerosis, MTI magnetization transfer imaging, MTR magnetization transfer ratio, NAA N-acetyl aspartate, NABT normal-appearing brain tissue, NAWM normal-appearing white matter, PVWMH periventricular white matter hyperintensities, ROI region of interest, SIVD subcortical ischemic vascular dementia, SVD small vessel disease, WM(L) white matter (lesion), WMH white matter hyperintensities

provided by DTI [34, 48, 49]. Table 4 describes the results of DTI and MTI in various settings and disease entities. Animal models and postmortem studies indicate that increased fractional anisotropy and mean diffusivity derived from DTI and magnetization transfer ratio from MTI correlate with axonal loss, demyelination and dentritic injury [29, 36, 70, 76]. A recent study in hypoperfused mice is of particular interest as it examined the complementary value of DTI and MTI measures for delineation of white matter damage [36]. The authors showed that an experimental comparison between quantitative MRI and pathology is possible even in small animals like mice. Moreover, they reported that a reduction in fractional anisotropy is paralleled by a decrease in the magnetization transfer ratio in several tissue compartments after continuous moderate hypoperfusion over 1 month. They concluded that both techniques are sufficiently suited to detect microstructural tissue alterations at least in the white matter of the murine brain. Postmortem histological analyses assessing myelin content, axonal density, and gliosis revealed similar correlations in brains of patients with Alzheimer's disease [29] or multiple sclerosis [71–73].

As can also be seen from Table 4 in vivo studies in humans consistently reported that mean diffusivity and fractional anisotropy, and in most investigations also magnetization transfer ratios, are more affected in age-related white matter lesions than in normal-appearing white matter [7, 22, 43, 59, 69, 72, 81]. There existed a significant association between white matter lesion load and severity of DTI and MTR measures in the normal-appearing white matter with the microstructural changes in normal brain tissue being more closely related to the patients' clinical presentation than the volume of visible white matter abnormalities [9, 32, 52, 61, 69, 70, 74, 92, 93]. These observations clearly indicate that age-related small vessel disease of the brain is a diffuse process affecting the entire brain and that white matter lesions are probably only the tip of the iceberg. Similar data come from the application of DTI in CADASIL. These studies also show an increase in diffusivity, which is better correlated with severity of executive dysfunction and clinical disability than are T2 hyperintensities [14].

So far, there exists only little information as to whether different types of age-related white matter changes are accompanied by different microstructural tissue changes. One study reported the mean magnetization ratio in periventricular white matter hyperintensities to be lower than in deep white matter abnormalities, and the mean magnetization ratio in frontal periventricular changes to be lower than in occipital periventricular changes [82]. Further studies on possible differences in accompanying tissue destruction between different white matter lesion types are needed, as their results are likely to improve our

understanding of the differential clinical effects of white matter abnormalities occurring in aging individuals. Metrics derived from these novel quantitative MRI techniques will not only help in understanding the microstructural tissue changes associated with cerebral small vessel disease in white matter, but also in other affected tissue compartments, such as the cortex. Based on the association between white matter alterations and neurodegenerative diseases at least in patients with neurodegenerative disorders one possible explanation for white matter changes could be Wallerian degeneration as a consequence of cortical neuron loss [94]. Moreover, one very recent study has shown that postmortem high-resolution MRI is able to detect intracortical infarcts in CADASIL and possibly also in other small vessel diseases of the brain [42]. In this study, MRI was used to guide the neuropathological examination of the cortex. Just as in this case, future studies will have to be supplemented by postmortem correlations in samples of normal-appearing brain tissue on standard MRI that have also been scanned by modern imaging techniques like DTI and MTI prior to the histopathological examination. However, it is important to realize that pathohistological correlations with new quantitative MRI techniques have to be done preferentially by the use of fresh brain tissue as formalin fixation influences tissue proton relaxation characteristics.

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