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Non-Gaussian water diffusion in aging white matter

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Abstract

Age-associated white matter degeneration has been well-documented and is likely an important mechanism contributing to cognitive decline in older adults. Recent work has explored a range of noninvasive neuroimaging procedures to differentially highlight alterations in the tissue microenvironment. Diffusion kurtosis imaging (DKI) is an extension of diffusion tensor imaging (DTI) that accounts for non-Gaussian water diffusion and can reflect alterations in the distribution and diffusion properties of tissue compartments. We used DKI to produce whole-brain voxel-based maps of mean, axial and radial diffusional kurtoses (MK, AK and RK, respectively), quantitative indices of the tissue microstructure's diffusional heterogeneity, in 111 participants ranging from 33 to 91 years of age. As suggested from prior DTI studies, greater age was associated with alterations in white-matter tissue microstructure, which was reflected by a reduction in all three DKI metrics. Prominent effects were found in prefrontal and association white matter compared to relatively preserved primary motor and visual areas. Although DKI metrics co-varied with DTI metrics on a global level, DKI provided unique regional sensitivity to the effects of age not available with DTI. DKI metrics were additionally useful in combination with DTI metrics for the classification of regions according to their multivariate 'diffusion footprint', or pattern of relative age effect sizes. It is possible that the specific multivariate patterns of age-associated changes measured are representative of different types of microstructural pathology. These results suggest that DKI provides important complementary indices of brain microstructure for the study of brain aging and neurological disease.

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Keywords

MRI; diffusional kurtosis imaging; diffusion tensor imaging; aging; white matter integrity; region-of-interest analysis; tract-based spatial statistics; k-means clustering; multivariate analysis

1 Introduction

Diffusion-weighted magnetic resonance imaging has been applied across several studies as a means to investigate the microstructural properties of white matter as well as changes due to age and disease (Abe et al. 2002; Basser et al. 1994; Basser and Pierpaoli 1996; Le Bihan et al. 2001; Pfefferbaum et al. 2000; Salat et al. 2005). The utility of techniques such as diffusion tensor imaging (DTI) comes from the fact that a range of microstructural properties can be obtained from a standard acquisition and that different metrics show differential sensitivity to effects in group comparisons. Fractional anisotropy (FA) and mean diffusivity (MD) are two common metrics calculated in DTI studies that are sensitive to changes with development and disease (Dong et al. 2004; Le Bihan 2003; Sundgren et al. 2004). FA is a measure of the directional dominance of water diffusion in tissue and has been loosely interpreted as an indirect quantitative metric of the density of nerve fibers and their myelin sheaths (Beaulieu 2002; Moseley 2002). In contrast, MD is a measure of the overall (direction-independent) degree of water diffusion within the tissue, and has been utilized as an important marker of ischemia, edema, and cell death (Chenevert et al. 2000; Sotak 2002). In the context of healthy aging, decreases in FA and increases in MD have been reported throughout much of the cerebral white matter (Pfefferbaum et al. 2000; Salat et al. 2005). More recently, the directional components of diffusivity, such as axial (AD) and radial (RD) diffusivity have been shown to have spatially specific and differential sensitivities to the effects of aging (Bennett et al. 2010; Madden et al. 2009). The idea that different pathologies affect specific diffusional properties preferentially (Song et al. 2003; Song et al. 2002) has potential value in the diagnosis and tracking of specific disease processes as opposed to more generic tracking of cumulative white matter damage without specific etiology. To date, however, little if any work has attempted to differentiate between various types of age-associated white matter changes based on multivariate diffusion properties.

Although DTI provides multiple indices of diffusional behavior, it is possible that composite information across a wider range of diffusional processes beyond what DTI provides would enable better classification of differing patterns of white matter damage with aging and disease. The physical model widely used to extract DTI parameters assumes that water molecules diffuse according to a Gaussian distribution, which corresponds to free, unrestricted diffusion in a homogenous environment. However, given the structural complexity of neural tissue, this assumption can only be an approximation, and appreciable differences in diffusivity are expected among tissue compartments (e.g. intra- and extra-cellular) within a same volume element. In theory, these differences are better characterized using higher-order diffusion statistics. By acquiring images with multiple diffusion weightings (b-values), thus allowing for a better estimation of the water molecules' displacement distribution, the excess kurtosis of the distribution can be calculated, which is a unitless index of its non-Gaussianity (Liu et al. 2004). Diffusional kurtosis imaging (DKI) was developed recently with the goal of characterizing the diffusional heterogeneity arising from multiple tissue compartments with different diffusivities (Jensen et al. 2005). Diffusional heterogeneity can be described by how variable the diffusivity index varies across different cellular compartments within a voxel, and cannot be measured with diffusion tensor imaging which only provides the average diffusivity over a given voxel. DKI therefore provides a novel set of *in vivo* microstructural properties that describe tissue

microstructure beyond the scope of traditional DTI (De Santis et al. 2011b); these properties are quantified through the mean, axial and radial diffusional kurtoses (MK, AK and RK, respectively). MK corresponds to the mean of the excess kurtosis for all diffusion directions, and represents a direction-independent index of diffusional heterogeneity. Analogous to diffusivity, diffusional heterogeneity or kurtosis also varies depending on the direction of diffusion weighting. AK and RK represent respectively the diffusional kurtosis in the principal diffusion direction and averaged over its perpendicular directions, based on the diffusion tensor orientation. Several multi-compartment models have been proposed to describe the biophysical and biological nature of diffusional kurtosis (Jensen and Helpert 2010; Jensen et al. 2005), particularly in the white matter (De Santis et al. 2011a; Fieremans et al. 2011). Quantitative measures from DKI may be sensitive to developmental or disease-associated conditions in which there is a differential alteration in diffusion and permeability properties across cellular compartments. For instance, MK is known to vary with developmental stage in the rat (Blockx et al. 2011; Cheung et al. 2009) and human brain (Falangola et al. 2008; Helpert et al. 2011; Latt et al. 2013), suggesting a maturational increase and subsequent decline in white matter integrity during aging. These prior studies demonstrated coarse changes in MK with development and aging and suggested that DKI metrics may be sensitive to subtle microstructural changes related to age.

The major goals of this study were twofold: first, to examine the regional age trajectories of white-matter microstructural alterations observed through DKI metrics in a large cross-sectional sample of generally healthy adults, and second, to determine whether DKI provides additional, unique information compared to DTI for studying healthy aging. The results demonstrate that although both DKI and DTI metrics show substantial age-associated patterns of change throughout the cerebral white matter, DTI and DKI measures demonstrate differential age effects and complement one another in the identification of different types of microstructural changes. This work suggests a novel framework for understanding changes in microstructural properties with aging and disease.

2 Materials and methods

2.1 Participants

A total of 111 healthy adults between 33 and 91 years of age (62 women, 49 men) were recruited through the Massachusetts General Hospital (MGH) and local community. The sample included healthy individuals as well as older adults with some mild forms of vascular risk, including hypertension, hyperlipidemia, hypercholesterolemia and diabetes. Individuals were excluded for signs of major neurologic or psychiatric illness including dementia, high cerebrovascular disease risk or overt disease (large vessel stroke or hemorrhage), cancer of the central nervous system, major head trauma, and/or other neurological or psychiatric, or therapeutic conditions that may influence cognition or imaging measures. Participants were non-demented, as assessed by a minimum score of 24 on the Mini Mental State Exam (MMSE) (Folstein et al. 1975). Twelve participants in the sample had some degree of depression according to the BDI-II score (Beck and Beamesderfer 1974). Fourteen participants were either left-handed or ambidextrous. However, the exclusion of all depressed and left-handed or ambidextrous participants did not alter our findings (see Supplementary Figure 1). One-hundred four participants were Caucasian and seven participants were African-American. Characteristics of the study group are provided in Table 1. All participants gave informed consent and the study protocol was approved by the Massachusetts General Hospital/Partners Healthcare Institutional Review Board.

2.2 MRI data acquisition

All participants were imaged on a Siemens 3T Trio system (Erlangen, Germany) with a 32-channel head coil. Whole-brain diffusion-weighted scans were acquired (TR = 9250 ms, TE = 103 ms, slice thickness = 2 mm isotropic, 64 slices total, acquisition matrix 128×128 (FOV = 256 mm $\times$ 256 mm), 6/8 partial Fourier, bandwidth = 1396 Hz/pixel, 24 non-collinear directions with b-values of 700, 1400 and 2100 s/mm², single average, and 10 T₂-weighted (b0) images with b-value = 0 s/mm²). The b-values were chosen to be optimal for both DTI and DKI analysis (DKI requires b-value < 2500 mm/s²) and the number of directions to accommodate scan time while ensuring proper estimation of the model. The DKI acquisition sequence used a twice-refocused balanced spin echo to reduce eddy current distortions (Reese et al. 2003). Head motion was minimized using tightly padded clamps attached to the head coil. Oblique axial slices were acquired with total scan duration of 13 min 8 s. The mean signal-to-noise ratio (SNR) at the highest b-value (2100 mm/s²) across individuals was 37, obtained in the middle-brain slice by dividing the global-mean brain signal by the standard deviation of the background noise.

2.3 Preprocessing

Data were processed using a combination of in-house image processing tools developed in MATLAB (Mathworks, Natick, USA) and tools available as part of Freesurfer (<http://surfer.nmr.mgh.harvard.edu>) and FSL (<http://www.fmrib.ox.ac.uk/fsl>). The diffusion dataset was corrected for any potential 3D head motion and eddy current distortion using FSL `eddy_correct`.

The diffusion and diffusional kurtosis tensors were fitted using the FanDTasia toolbox implemented in MATLAB (Barmpoutis and Vemuri 2010; Barmpoutis and Zhuo 2011). Coefficients from both tensors were robustly estimated for each voxel using a previously described homogenous polynomials approach, which enforces a positive diffusivity function, a positive-definite estimated diffusion tensor and a constrained estimated apparent kurtosis (Barmpoutis and Vemuri 2010; Barmpoutis and Zhuo 2011).

The estimated tensor coefficients were then used to interpolate the apparent diffusional kurtosis in any desired direction. Mean kurtosis (MK) maps were created by interpolating the apparent diffusional kurtosis in 1000 directions based on the original 24-direction data and averaging them for every voxel. Axial kurtosis (AK) maps were obtained by estimating the apparent diffusional kurtosis in the principal diffusion direction. Radial kurtosis (RK) maps were obtained by averaging the apparent diffusional kurtosis interpolated in 1000 directions perpendicular to the principal diffusion direction, again based on the original 24-direction data. Interpolation was performed in 1000 directions for MK and RK to obtain an approximation of these metrics using the tensor coefficients while having a low directional bias (less than 0.1%, chosen directions were kept constant across individuals). In order to account for noise resulting from the lower SNR at high b-value, each resulting DKI metric map was median-filtered with a 3x3x3 kernel to remove potential outlying values. Maps of DTI metrics were additionally calculated based on the first b-value of the DKI dataset (i.e. b = 700 mm²/s) since a b-value of 700 mm/s² has been used in ours and other prior work showing sensitive associations with age (Damoiseaux et al. 2009; Kochunov et al. 2007; Salat et al. 2005; Westlye et al. 2010). We also examined DTI maps calculated from data at the highest b-value (i.e. b = 2100 mm²/s) and found similar associations with age (Supplementary Figure 2). A comparison of age effects on DTI metrics obtained at both b-values is provided in Supplementary Figure 2, along with a comparison with DKI metrics, confirming that a higher b-value does not bias DTI results towards DKI results.

2.4 Per-voxel statistical analyses

Parametric maps of each diffusion metric were registered to a common FA template in MNI152 space using FSL registration tools and the Tract-Based Spatial Statistics procedure (TBSS) (Smith et al. 2006). As a result, regression analyses were limited to voxels with mean FA values higher than 0.2 on a standard tract skeleton, as described previously (Smith et al. 2006), minimizing partial volume and registration confounds. We used general linear model (GLM) regression analyses with a simple linear age regressor and quadratic age regressors to determine which model better fit the data across each voxel. Unless otherwise noted, age was used as a continuous variable in all analyses. We tested models including sex as a categorical covariate, but this did not affect the results. Furthermore, no age by sex interaction was found. Thus, we only present herein data not using the sex covariate. An additional set of analyses examined DTI metrics as voxelwise explanatory demeaned covariates in the model to demonstrate unique age effects in DKI metrics that are unexplained by DTI metrics. Significance testing and correction for multiple comparisons in the per-voxel analyses were achieved with 5000 permutations using FSL randomise with threshold-free cluster enhancement (TFCE), a combination of cluster-based and voxelwise thresholding proven to be more sensitive to both spatially extended and sharply focused signals (Smith and Nichols 2009).

2.5 Region-of-interest analyses

Region-of-interest (ROI) analyses were used to examine spatial patterns associated with the multiple diffusion metrics, providing more stable values for each individual than voxelwise analyses. White matter tracts and subcortical regions on the TBSS skeleton were segmented using a combination of the FreeSurfer T1-based white-matter parcellation (Salat et al. 2009) and FSL's Johns Hopkins University white matter labels (Mori and Crain 2005). These atlases were co-registered with FSL's standard MNI152 space, and the segmented white-matter regions were deprojected to the native space of each individual participant as in our prior work using previously described TBSS techniques (Salat et al. 2012; Smith et al. 2007). This deprojection allowed visual confirmation of correct translation between atlas to native space, and the use of the native space data in the analysis minimized registration- and interpolation-related biases. Furthermore, native voxels with FA values lower than 0.2 were excluded to further prevent partial-volume effects, and white-matter regions with a mean native-space skeleton volume lower than 0.95 cm³ across both hemispheres were excluded. This resulted in 82 white matter ROIs across the left and right hemispheres, and 5 commissural (intrinsically bilateral) regions for a total of 87 regions (complete list provided in Supplementary Table 1). The mean DKI/DTI measure of every ROI was extracted in the native space, and fed into the GLM to analyze the effect of age. We corrected for multiple comparisons in the ROI analyses using the Holms-Bonferroni correction for 87 tests (same as the number of ROIs).

2.5.1 Relative-effect maps—We implemented a novel procedure to demonstrate ‘focal points’ of age effects as regions demonstrating the strongest statistical associations with age relative to all other regions tested, as opposed to an absolute effect at each ROI. While a p-value can be useful in rejecting the null hypothesis that there is no age effect, it does not demonstrate the relative strength of age effects across regions. On the other hand, the Pearson product-moment correlation coefficient is a quantitative metric that can be used to demonstrate the *relative* effect size. Thus, the two-tailed Steiger's Z-test, based on Pearson's correlation, was used to make pairwise statistical comparisons of the regional age effect size on diffusion metrics. For each diffusion metric, a quantitative ‘relative-effect score’ was attributed for each region based on how strong the age effect was relative to other regions. For a given region, comparisons of age effect size were made with every other region and the number of comparisons that resulted in a greater correlation with age with $p < 0.05$ was

summed to provide the relative-effect score (example calculation below in the Results section). This procedure does not attribute different scores to regions with similar age effect sizes, and takes into account the correlation between the diffusion metrics associated with each region. Unlike ranking, this procedure can quantitatively distinguish groups of regions with similar age effects, not artificially creating a continuum of scores across groups that prevents the observation of the regional patterns that we seek to uncover. Furthermore, it allows a quantitative comparison of regional patterns across different metrics, by providing a metric-specific score that is proportional to how much more affected by age a given region is relative to other regions. Using this method, novel maps were generated demonstrating a relative-effect score for each region for each diffusion metric. Secondary maps demonstrate the mean and standard deviation of this relative-effect score across diffusion metrics, as well as the metric that demonstrated the strongest relative-effect score compared to all other metrics for each region.

2.5.2 Clustering of unique age-effects based on multivariate diffusion patterns

—K-means clustering was used to group regions according to their multivariate pattern of age effect sizes across all diffusion parameters, determined by the Pearson's product-moment correlation coefficient between age and each DTI/DKI metric. The 87 regions were combined into 46 bilateral regions (ROI values of the non-commissural regions were averaged pairwise across hemispheres into 41 bilateral regions and the values for the 5 remaining commissural regions were considered as is) in order to equalize the weighting of commissural and non-commissural regions in the definition of the clusters. This combination was justified by the general non-laterality of the age effects observed in this study and the confirmation that there are no significant differences in the correlation coefficients with age between hemispheres for any diffusion measure (as demonstrated in Supplementary Table 1). For clustering purposes, the effect size of FA, MK, AK and RK, which are metrics that decline with age, was inverted to be made positive and comparable to the effect size of MD, AD and RD, which generally increase with age. The 46 regions were then clustered using MATLAB's k-means clustering procedure based on their resulting effect size profile, or 'diffusion footprint', using two different similarity measures, namely squared Euclidean distance and correlation. Here, we define the concept of 'diffusion footprint' of a region by the visual pattern left by the effect size profile, formed by the relative differences in age effect size across diffusion metrics. This notion is important for the correlation measure, as two identical diffusion footprints will produce a correlation coefficient of 1, even though they might not have the same average effect size across metrics (i.e. one region may have an overall strong effect whereas the other has an overall weak effect, but these regions still cluster together because of their effect pattern across metrics as opposed to their effect size). The k-means clustering procedure was replicated with each similarity measure using an effect size profile or diffusion footprint including only the DTI metrics in order to assess potential clustering differences with and without DKI metrics. Repeating the procedure including only the DKI metrics was considered not practically meaningful as DTI metrics will always be available with the diffusion-weighting images required for the DKI model. This resulted in 4 different k-means procedures. All 4 procedures were used to generate 2 or 3 clusters; data are presented in detail from the procedures generating 3 clusters and summary results are provided in Supplementary Table 2 for the procedures generating 2 clusters.

3 Results

3.1 Per-voxel statistical analyses

The results of the TBSS voxel-based age regression analyses on mean, axial and radial kurtoses (respectively MK, AK and RK) are shown in Figure 1. All results displayed are

significant at $p < 0.01$ after TFCE correction for multiple comparisons. Linear age effects of MK and RK were similar across almost the entire white-matter skeleton. Notable exceptions included the posterior limb of the internal capsule, the junction between the superior and posterior corona radiata and small portions of the body of the corpus callosum and the external capsule, which have either a significant age association in AK only, or an overlap between significant MK and AK age-associations. In general, AK showed a weaker but still broad association with age across the skeleton, and was the only DKI metric to display significant quadratic age dependence unexplained by linear effects in our sample. However, the linear age effects remained significant across the white matter at $p < 0.001$ unlike the quadratic age effects (not shown), suggesting that the age effects were essentially linear.

Figure 2a shows a matrix of the squared pairwise correlation coefficients amongst individual DTI/DKI metrics, averaged across the entire white-matter skeleton. A high degree of shared variance within DTI and within DKI metrics was observed, especially between mean and radial metrics. That is, RK was similarly related to MK as RD was to MD. A high degree of shared variance was also observed between DTI and DKI metrics (36%-78%), but was generally lower than within modality (within DTI or within DKI metrics) shared variance, and also notably always lower than the variance shared between the commonly used MD and FA (84%).

Given the shared variance across these measures, we investigated the residual age effects in DKI metrics unexplained by DTI metrics. Shown in Figure 2b are the residual age effects in RK when accounting for RD. Although RK and RD are the DKI and DTI metrics most correlated with each other (78%), Figure 2b provides evidence for a difference in age effect between them. Similarly, the DTI metrics underwent the same procedure and the results for AD, which is the DTI metric least correlated with RD (69%), are displayed in Figure 2c. The residual age effects in MD and FA accounting for RD were respectively spatially limited to the same areas and part of the same areas as AD (data not shown), suggesting RD accounted for the majority of age effects in all of the other DTI metrics. While complementary aging information was still obtained from multiple DTI metrics, RK showed broader differences from RD spatially than any DTI metric. This was apparent even though the brain slices displayed in Figure 2b and 2c were chosen to show as much residual AD-age effect as possible. Displayed in Figures 2d and 2e are the scatter plots of the mean RK, AD and RD values of the most significant clusters from Figure 2b and 2c, respectively. In Figure 2d, RK shows a stronger association with age than RD, demonstrating the results obtained in Figure 2b, while AD (and other DTI metrics) displayed similar aging trends as RD. In Figure 2e, AD shows a stronger association with age than RD, demonstrating the results from Figure 2c, while RK and RD showed similar age effects. In summary, the results showed unique variance in DKI measures broadly across the white matter skeleton when accounting for the most highly correlated DTI measure. In comparison, the areas where different DTI metrics show unique variance were limited.

3.2 Region-of-interest analyses

A table detailing the results of the region-of-interest age regression analyses for all DTI and DKI metrics can be found in Supplementary Table 1, including the statistical significance of the age effect, the age slope, the standard deviation of the residuals and the correlation coefficient with age. These results mirror the voxel-based findings with regard to regional effects.

3.2.1 Relative-effect maps—Relative-effect scores for each region and each DTI/DKI metric can be found in Supplementary Table 1. A detailed example calculation of the RK

relative-effect score of the right superior frontal white matter (RSFWM) is demonstrated in Figure 3a and was calculated as follow:

1. A region to be compared is chosen: e.g. left inferior parietal white matter (LIPWM);
2. The Steiger Z-test is used to determine whether the correlation between RK_{RSFWM} and age is significantly stronger than the correlation between RK_{LIPWM} and age, taking into account the correlation between RK_{RSFWM} and RK_{LIPWM} ;
3. If $p < 0.05$, the RK relative-effect score of the right superior frontal white matter is increased by 1;
4. This is repeated for every other region and the sum is the RK relative-effect score of the RSFWM.

This process is shown in Figure 3a, where the right superior frontal white matter had a greater RK-age effect size than 40 other regions and therefore was attributed a relative-effect score of 40 on the RK map. The mean and standard deviation of the relative-effect scores across all metrics within each region are represented in Figures 3b and 3c and demonstrate, respectively, areas most affected by age across all diffusional measures and areas where diffusion metrics vary widely in their sensitivity to age. Here, we dilated the white-matter area to include voxels neighboring the TBSS skeleton for better visual representation of the results. As shown in Figure 3b, prefrontal regions demonstrated the highest correlation with age (averaged across all diffusion metrics), followed by parietal and temporal areas. The occipital, brainstem and pre- and post-central gyral white matter had overall a lower age effect size across all diffusion metrics, along with the posterior limb of the internal capsule and the splenium and body of the corpus callosum. These observations were especially true for FA, MK and RK. As shown in Figure 3c, regions with high relative-effect scores also had greater variability in their scores across diffusion metrics, and this variation in age-associations with different diffusion metrics underlies the notion that the metrics provide differential information. For example, the body of the corpus callosum had especially strong variability in its age effects across diffusion metrics, and this was due mainly to a very strong relative age effect in axial and mean diffusivities and a very weak relative age effect in other metrics (Supplementary Table 1). This notion is reinforced in Figure 3d, in which the diffusion metric with the highest relative-effect score is shown for each region. For instance, the highest score across metrics for periventricular fiber regions was mostly achieved by AD, MD and RD, while the highest score for cortically subjacent white matter regions was mostly achieved by FA and DKI metrics. Overall, all of the metrics were informative and sensitive markers of aging, and showed superior sensitivity for at least one region compared to the other metrics, demonstrating that no one metric can be replaced by another.

3.2.2 Clustering of unique age-effects based on multivariate diffusion patterns

—We implemented a novel procedure to examine the regional patterns of age-associations across all diffusion metrics. K-means clustering was used on both DTI metrics alone and on DTI+DKI metrics to group together regions with similar age effect sizes across metrics, based on two different measures of similarity: (1) squared Euclidean distance, which is the typical distance measure in metric space normally used in k-means clustering, with similar regions separated by a short distance; this measure clustered regions according to their *overall* degree of age effect size across metrics (e.g. clustered regions showing strong effects together and regions showing weak effects together), and was unaffected by the addition of DKI metrics (described in greater detail below); (2) correlation, which clustered together regions with a similar pattern of relative metric-age effect sizes ('diffusion footprint') as

opposed to the overall effect size. This procedure was affected by the addition of DKI metrics, suggesting that DKI metrics provide unique information not present in DTI metrics.

We first clustered regions using the squared Euclidean distance measure, and each cluster center was computed as the average age effect size for each metric across all regions belonging to that cluster. The three cluster centers corresponding to DTI metrics alone and DTI+DKI metrics are shown in Figure 4a and 4b, respectively. The regions belonging to each cluster are colored to match their respective cluster center. As can be observed, the addition of DKI metrics had only a negligible effect on the clustering using this similarity measure. Indeed, each center cluster based on DTI metrics alone had DTI-age effect sizes very similar to that of a matched center cluster based on DTI+DKI metrics. Interestingly, clusters 1, 2 and 3 respectively resemble regions with low, medium and high relative-effect scores from Figure 3b, and the average age effect size in each cluster confirmed this partition. This suggests that the greatest distinction between clusters seems to be their average age effect size across all diffusion metrics, and indicates that DKI metrics do not add any significant information in that respect.

We next clustered regions based on their diffusion footprints rather than their overall age effect size across metrics, using correlation as the similarity measure. This second clustering approach is based on the pattern of relative age effect sizes across metrics. Shown in Figure 4c and 4d are the results of this clustering procedure, based on DTI metrics alone and DTI +DKI metrics respectively. Arbitrary units are used to identify this pattern as only the relative differences between metric-age effect sizes matters, not their absolute levels. The patterns of each center cluster in Figure 4c and 4d were therefore scaled to be similar in range to patterns shown in Figure 4a and 4b, and any absolute value in isolation is arbitrary and therefore meaningless. The clusters now differ from those based purely on the overall strength of the metric-age associations, as shown in Figure 4a and 4b. Indeed, in Figure 4a, the center clusters all shared a similar diffusion footprint, characterized with a relatively high RD-age effect size and a low AD-age effect size as well as medium to high MD- and FA-age effect sizes. Most of these regions were therefore clustered together (cluster 1) in Figure 4c. Cluster 2 had few regions and was similar to cluster 1 except for a lower relative FA-age effect size, and cluster 3 represented regions driven mainly by a high increase in MD with age, with milder effects in FA. A key observation is that the addition of DKI metrics split cluster 1 into two distinct clusters, shown by clusters 1 and 2 in Figure 4d. Indeed, the pairwise correlation coefficients between the DTI footprint of cluster 1 in Figure 4c and clusters 1 and 2 in Figure 4d were all above 0.99. The main difference therefore lied in the DKI metrics, with both DTI and DKI metrics having similar age effects in cluster 1, and DKI metrics having higher age effects in cluster 2.

Regions from cluster 3 however were already properly clustered using DTI metrics only, and these regions exhibited mostly isotropic changes in diffusivity and diffusional kurtosis with a high degree of axial kurtosis changes with age.

Complete descriptions of clusters based on both similarity measures, with DTI and DTI +DKI metrics are provided in Supplementary Table 2. In summary, the addition of DKI metrics to clustering using the correlation similarity measure contributed unique information that distinguished regions belonging to a single cluster when using DTI metrics alone.

4 Discussion

The current data demonstrate that mean, axial and radial diffusional kurtoses (MK, AK and RK, respectively), recently described metrics computed using the DKI framework, are sensitive markers of age-associated tissue alterations and allow a novel classification of

microstructural changes with age that could not be achieved with DTI measures in isolation. In this cross-sectional study, an aging-associated decrease in the complexity of the diffusion microenvironment was found in most white matter structures, suggesting a global trend towards tissue homogeneity. This was primarily explained by a global decrease in RK, with prefrontal, parietal and temporal white matter showing stronger age-associations than primary motor and sensory areas. AK displayed a less pronounced but relatively uniform age-association throughout the white matter and there was evidence of a weak quadratic age effect in our sample. Importantly, DKI provided unique and complementary regional markers of microstructural changes relative to DTI. While DTI metrics were sufficient in identifying white matter regions most strongly affected by age, DKI metrics enabled a novel mapping of aging processes based on a multivariate diffusion footprint that is distinct from the spatial patterns based on the overall age effects in DTI/DKI metrics. These data additionally suggested that DKI and the multivariate combination of all diffusion metrics may reveal early microstructural changes in neurodegenerative diseases.

The first goal of the study was achieved through the findings that, although much of the white matter showed age-associated reduction in DKI metrics, signifying a diffusivity homogenization across its various cellular compartments, this effect is not spatially uniform. These more affected regions identified by DKI tended also to exhibit greater increases in diffusivity in aging, and coincided with regions previously implicated in aging (Barrick et al. 2010). Interestingly, these regions tended also to exhibit greater variability of age effects across the various diffusion metrics. The spatial variation in age-associations across all diffusion metrics within white matter seems to be in accord with previously hypothesized models of cortical aging where prefrontal and association areas are greatly affected while primary motor and sensory areas such as the occipital and central areas are relatively preserved (Driscoll et al. 2009; Raz et al. 1997). While regional patterns of aging obtained with DKI metrics were similar to those obtained with DTI metrics, different regions were often characterized by optimal age-sensitivity in different metrics.

The second goal of the study was to determine whether DKI metrics provide any additional information on microstructural changes during aging that is unaccounted for by DTI metrics. DKI metrics co-varied with DTI metrics to a lower extent on a global level than DTI metrics or DKI metrics did among themselves, suggesting that the use of both DTI and DKI metrics might enable better characterization of age effects. Furthermore, even the most correlated DKI and DTI metrics, RK and RD, were differentially affected by aging over large areas of the white matter, while RD accounted for the majority of age effects in all of the other DTI metrics. Although initial clustering experiments were driven by the overall average age effects across all diffusion metrics, to which the addition of DKI metrics did not have a noticeable effect, our multivariate diffusion footprint approach suggested otherwise. The diffusion footprint-based clustering approach provided a multiparameter view of the effect of age on the diffusion microenvironment and could be applied in the future to differentiate between effects using a different condition of interest than age (e.g. disease status). It is interesting to note that the diffusion footprint approach leads to a quite different spatial pattern than that produced from the average age effect across diffusion metrics. For example, while the cerebellum and superior frontal white matter seem to undergo a similar type of microstructural changes, the superior frontal white matter undergoes a greater degree of these same changes than the cerebellum. Furthermore, DKI metrics provided additional information that allowed a clustering of regions that was not possible with DTI metrics alone. This is consistent with the previous finding that RK provides differential information to RD. Therefore, we conclude that DKI provides additional, unique complementary information about types of microstructural changes in the context of healthy aging when used in combination with DTI metrics.

The histological basis of the diffusional kurtosis cross-sectional changes reported here is currently unknown. Several biological processes may underlie the global MK and RK decline in white matter with age, such as myelin breakdown, increases in axonal membrane permeability, edema, fiber loss and shortening as well as decrease in density of myelinated axons (Bartzokis, 2004; Fazekas et al. 1993; Peters, 2002), which may all increase the tissue homogeneity and decrease the variability in diffusivity among tissue compartments. The age-effect in AK is likely to be associated with the same biological processes in crossing fibers perpendicular to the principal diffusion direction. However, as cross-sectional changes are also observed in predominantly non-crossing fibers, the age-effect in AK might additionally be linked to an increased homogeneity along the principal diffusion direction, either due to an aging-related diffusion homogenization process or to an increased extracellular space presumed to have lower heterogeneity than axons and glia (Fieremans et al. 2010). Ischemia and chronic damage may also result in a change in DKI metrics following tissue infarction and cell death, as recent evidence points to an increase in AK in the acute ischemic period (Hui et al. 2012a; Hui et al. 2012b; Jensen et al. 2011). In this study, a multivariate classification of white matter regions based on the relative differences between age effects of DTI and DKI metrics was introduced. For instance, the first of the three clusters obtained using all diffusion metrics represents regions with similar age-associations in diffusional kurtosis and diffusivity, and with stronger effects in radial than axial metrics, potentially attributable to changes in the density of myelinated axons, myelin integrity and axonal membrane permeability. The second cluster also had a strong radial component but diffusional kurtosis changes were stronger than diffusivity changes, indicating stronger tissue-compartment changes perpendicular to the fibers, resulting, for instance, from an increasing similarity between the diffusivities of intra- and extra-axonal spaces. The third cluster had a strong isotropic age effect in both DTI and DKI metrics, potentially related to fiber loss and shortening, edema and gliosis. These interpretations are speculative and based on limited prior work. Future studies will address the histological validation and neuropathological assessment of DKI properties and of the potential types of microstructural changes suggested in this study.

This study has limitations that will be addressed in future work. First, it is important to emphasize that the underlying tissue microstructure measured by DKI remains poorly understood and that animal studies will be required to confirm the physiological and histological underpinnings associated with the diffusional kurtosis changes observed in this study. Second, this is a cross-sectional study providing limited information about what may be expected longitudinally. Third, the low image SNR at higher b-values could have resulted in less-than-optimal fitting of the DKI parameters on which a median filter was used. To examine the influence of the median filter, MK was fitted using an in-house unconstrained nonlinear estimation algorithm (Jensen et al. 2005) and interpolation was used to replace improbable fitted kurtosis values, which resulted in the same spatial distribution of significant age effects. Head motion is also a major potential confound in diffusion imaging studies; however linearly regressing out motion parameters for each participant did not qualitatively change the results (see Supplementary Figure 1). Finally, it is important to note that the same type of change in diffusion property might be more sensitively detected by one diffusion metric than another simply due to the specific anatomy of a given region. For example, the interpretation of axial and radial metrics relies on the assumption of a single population of aligned fibers, while crossing fibers have been shown to predominate in the cerebral white matter (Jeurissen et al. 2012; Wedeen et al. 2012). Indeed, factors such as the underlying white matter structure affect diffusion measures (Wheeler-Kingshott and Cercignani 2009) and therefore their biological interpretation is not simple. Future studies will address this issue by using the potential of DKI to resolve crossing fibers and enable characterization of different fiber populations with aging and neurodegenerative disease. Despite these limitations, the current results suggest that DKI metrics provide important

complementary indices of brain microstructure and should be used in combination with DTI metrics for the study of brain aging and neurological disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Significant associations between voxelwise mean, axial and radial kurtoses (MK, AK and RK) on the TBSS skeleton and a) a simple linear age regressor as well as a b) quadratic age regressor testing for additional quadratic effects not explained by a simple linear age regressor. There was a broad coinciding negative linear correlation between age and all DKI metrics as shown in purple. MK and RK age-associations overlapped throughout most of the skeleton as additionally shown in blue. There was a slight additional quadratic age effect for AK that appeared bilateral when the statistical threshold was lowered. The significant results ($p < 0.01$ for linear age effects, $p < 0.01$, $p < 0.05$ and $p < 0.10$ for quadratic effects, all corrected for multiple comparisons) have been dilated out of the skeleton for better visualization. No part of the skeleton had a positive linear correlation with age.

Figure 2.

Differential age effects between DTI and DKI metrics. a) Symmetrical matrix of the variance explained by each individual metric for each other metric. The mean value of each metric across the entire skeleton of each individual was correlated with the mean value of each other metric and the resulting coefficients were squared to obtain the explained variance. The correlations between DKI and DTI metrics were emphasized by a box and were generally lower than correlations within DTI or DKI metrics. Voxelwise results of general linear models for b) RK and c) AD including age and voxelwise RD as covariates. RK still had significant negative associations with age over broad areas of the white matter skeleton while controlling for RD, despite the two metrics sharing the highest variance explained between DTI and DKI metrics. AD, which is the DTI metric least correlated with RD, had remaining age effects that were spatially limited when controlling for RD. The significant results ($p < 0.001$, corrected for multiple comparisons) have been dilated out of the skeleton for better visualization. The mean RK, AD and RD of the most significant clusters from b) and c) are plotted for each participant in d) and e) respectively, demonstrating that the RK-age association is stronger than the RD-age association in the former, while the AD-age association is stronger than the RD-age association in the latter, as formerly tested in b) and c). (MD: mean diffusivity; AD: axial diffusivity; RD: radial diffusivity; FA: fractional anisotropy; MK: mean kurtosis; AK: axial kurtosis; RK: radial kurtosis).

Figure 3.

Statistical ranking of regional effects using a relative-effect score obtained from comparing each metric-age effect size pairwise between all regions. a) For a given DTI or DKI metric (e.g. RK), the age effect size of each region is sequentially compared to all other regions, and the relative-effect score is obtained from the number of comparisons showing a greater RK-age correlation for the region being scored, using the Steiger's Z-test with $p < 0.05$. This was obtained for every DTI and DKI metric. b) Mean and c) standard deviation of the relative-effect score across all DTI and DKI metrics. d) Diffusion metric with highest relative-effect score for every region. Blank regions indicate where more than one diffusion metric had the same highest relative-effect score. ROI results have been dilated into neighboring voxels of the TBSS skeleton for easier visual representation. Abrupt transitions represent boundaries between two different ROIs.

Figure 4.

Results of the k-means clustering procedure using a squared Euclidean distance as similarity measure based on DTI metrics alone (a) and with DTI+DKI metrics (b). The age effect size of every diffusion metric is shown for each cluster center. The sign of the age effect size, as determined by the Pearson product-moment correlation coefficient of each metric with age, was inverted for FA, MK, AK and RK for clustering purposes. Results of the k-means clustering procedure using correlation as similarity measure based on DTI metrics alone (c) and with DTI+DKI metrics (d). In this case, regions in each cluster shared a diffusion footprint that was most highly correlated with the diffusion footprint of their cluster center, regardless of their overall age-association strength for all metrics. When using correlation as the similarity measure, arbitrary units are used to represent each cluster center as the diffusion footprint can be demeaned and scaled across metrics without changing its correlation coefficient with the diffusion footprint of each region. In every case, a representation of each region colored according to its classification is shown on the dilated TBSS skeleton for easier visualization (analyses were solely performed on the white matter skeleton). Complete lists of each set of regions are also provided in Supplementary Table 2. (MD: mean diffusivity; AD: axial diffusivity; RD: radial diffusivity; FA: fractional anisotropy; MK: mean kurtosis; AK: axial kurtosis; RK: radial kurtosis; WM: white matter).

Table 1

Demographics for all participants (MMSE: Mini-Mental State Exam; BP: Blood Pressure).

Group	Age (years)	Education (years)	MMSE ^a	Systolic BP ^b (mmHg)	Diastolic BP ^b (mmHg)
All (111)	60.5±11.5	16.4±2.7	28.7±1.5	123.3±13.3	76.4±8.7
Men (49)	59.1±12.6	16.3±2.8	28.7±1.5	125.6±9.6	76.1±8.0
Women (62)	61.7±10.4	16.5±2.7	28.6±1.5	121.8±15.2	76.6±9.2

^a Information not available for the youngest participant.^b Information not available for 19 participants.