A Correlational Study between Microstructural White Matter Properties and Macrostructural Gray Matter Volume Across Normal Ageing: Conjoint DTI and VBM Analysis

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ABSTRACT: We investigate the relationship between Gray matter's volume vis-a-vis White matter's integrity indices, such Axial diffusivity, Radial diffusivity, Mean diffusivity, and Fractional anisotropy, in individuals undergoing healthy aging. We investigated MRI scans of 177 adults across 20 to 85 years. We used Voxel-based morphometry, and FDT-FSL analysis for estimation of Gray matter volume and White matter's diffusion indices respectively. Across the life span, we observed an inter-relationship between the Gray matter and White matter, namely that both Axial diffusivity and Mean Diffusivity show strong correlation with Gray matter volume, along the aging process. Furthermore, across all ages the Fractional anisotropy and Mean diffusivity are found to be significantly reduced in females when compared to males, but there are no significant gender differences in Axial Diffusivity and Radial diffusivity. We conclude that for both genders across all ages, the Gray matter's Volume is strongly correlated with White matter's Axial Diffusivity and Mean Diffusivity, while being weakly correlated with Fractional Anisotropy. Our study clarifies the multi-scale relationship in brain tissue, by elucidating how the White matter's micro-structural parameters influences the Gray matter's macro-structural characteristics, during healthy aging across the life-span.

KEYWORDS: White matter, gray matter volume, magnetic resonance imaging, diffusion tensor imaging, diffusivity, fiber tractography

Introduction

Gray matter (GM) and the white matter (WM) are the two major structural compartments of the human brain. GM primarily harbors the cell bodies of different neuronal cell types, whereas WM is majorly comprised of long-range myelinated axonal tracts. Conventional magnetic resonance imaging (MRI) studies have been long utilized in understanding the age-related changes in the brain's GM and WM.[1–3] Literature has reported GM tissue atrophy to be most prevalent in the cingulate cortex, orbital and inferior frontal cortex, insular region, inferior parietal cortex, and the mesial temporal regions, whereas the WM atrophy changes are quite global.[4] Though numerous studies have analyzed aging-induced alterations in the GM and the WM, the two entities are generally treated separately, and attempts to inquire on the relationship and interaction between WM and GM as aging progresses are not common. This is the aspect that we investigate in this paper using a population of a substantial number of normal subjects across aging (177 individuals) so that the inferences and implications are robust.

Regarding WM alteration, if one desires to analyze micro-structural modifications, then MRI-based diffusion tensor imaging (DTI) contributes an incisive picture regarding the complex alterations occurring in the brain across aging.[5] With respect to GM changes, the methodology of voxel-based morphometry (VBM) offers an insightful procedure to analyze macrostructural changes.[2] The reduction in the WM volume (WMV) of the human brain with aging is primarily related to the loss of thin-small diameter myelinated fibers.[6] In the older adults, the reduction of WMV is disproportionately more in quantum than the observed reduction of GM volume (GMV).[7,8] A cross-sectional aging study focussing on accessing regional volume changes with aging revealed a significant decrease in regional brain volumes globally, along with a concomitant increase in ventricular volume with aging.[9]

Longitudinal aging studies in healthy adults have identified the brain regions exhibiting volume changes across the different time points. Studies have shown changes in volume of the cortex, WM, and subcortical structures with aging.[10] There is also increased shrinkage of the caudate nuclei, the cerebellum, and the hippocampal structures with healthy aging.[11] Ageing results in atrophy of subcortical GM structures such as the thalamus, pallidum, nucleus accumbens, and the putamen.[12] Indeed, the strongest and most consistent impact of aging has been observed in the pallidum, cerebral cortex, accumbens volume, and the putamen,[13] on the contrary, the GM and the

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WM of the occipital area are with the least significant changes across aging.[14] Also, the limbic maturation leads to changes in GMV and WMV, cortical thickness, diffusion properties for the pericortical WM, and the fiber tracts in the association of limbic regions.[15]

The effect of age is not uniform in nature across the different regions of the brain. Age-related volumetric differences are strongly associated with the parietal and occipital regions.[16] In cerebrum, GMV has been shown to decrease linearly with age, whereas the WMV has been shown to increase until the mid-50s, but after this, there appears to be reduction in volume in an accelerated fashion. Hippocampal volumes follow a similar trend with increases up to 40 years during the normal course of aging, followed by rapid decline in volume after the age of 50 years.[17]

Numerous MRI morphometry–based studies on aging have indicated volume reduction in specific brain regions, especially the anterior cingulate gyrus, angular gyrus, superior parietal gyrus, pre- and postcentral gyrus, anterior insula bilaterally, posterior lobe of right cerebellum by preserving hippocampus, entorhinal cortex, amygdala, and thalamus bilaterally.[18,19] Another study reported the reduction in the cerebral cortex and basal ganglia GMV.[20] Also, it has been reported that reduction occurs in the volume of the middle frontal gyrus, pre- and postcentral gyrus, and insula bilaterally and right inferior frontal gyrus,[21] and frontal and temporal cortex bilaterally, including right cerebellum.[22] Another study showed volume reduction in the frontal, temporal, and parietal cortex, insula, and cerebellum bilaterally; left cingulate gyrus; and right posterior hippocampus.[23] Another study showed decrease in regional volumes of the cerebellum, frontal, parietal, and temporal cortex bilaterally along with left insula, left globus pallidus, left occipital cortex, and right thalamus.[24] A study reported an age-related reduction of frontal and temporal lobe structures in men only.[25] The reduction in the volume of bilateral superior temporal gyrus and insula, left medial frontal gyrus, left precentral and inferior frontal gyri are reported.[26] Another VBM- and DTI-based aging study reported no significant effects on either GMV asymmetry or WM asymmetry,[27] and the age-related FA decrease in selected striatal regions.[28]

The main challenge of establishing the relationship between the macrostructural (GMV) and microstructural (WM diffusivity measures) with the implication of neuroplasticity in humans needs to be understood. To date, none of the aging brain studies have reported the correlation between the T1-weighted GMV with various WM diffusion indices. Our study aims to investigate the relationship between global GMV and the WM diffusivity indices, latter being reflective of WM integrity measures, such as axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD), and fractional anisotropy (FA), among the young, middle, and old age individuals.

Since there is a logistic and ethical advantage of non-invasive imaging for probing age-related structural alterations in the human brain, we have sought to perform an investigation and draw interrelationship between macrostructural MRI characteristic (GMV) vis-à-vis microstructural MRI properties (WM diffusion indices). For this purpose, we use regional voxel-based analysis (for gauging GM) along with tract-based analysis (for gauging WM). We here consider the adult aging process from age 20 years onwards across the full lifespan divided into three groups, that is, the young age group (20-40), middle age adult group (41-60), and old age group (61-85 years).

Material and Methods

IXI dataset

The IXI dataset (http://www.brain-development.org/) comprises of MR images acquired from 181 normal, healthy subjects aged 20 to 85 years. The dataset provides the T1-weighted MRI images and the DTI images acquired using 15 gradient directions. The study assessed 181 subjects as a recruit for the study; the MRI and DTI images of all subjects were acquired at Hammersmith Hospital, age ranges from 20 to 85 years. With the gender ratio of 88:89 for males and females, we included 177 subjects for our analysis. Four subjects were excluded due to the non-availability of respective diffusion tensor images of these individuals. Age distribution bar-chart is given in Figure 1.

IXI MR image acquisition parameters

The structural and diffusion images were acquired on Philips Intera 3T scanner using Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence for T1-weighted structural MRI scans and single-shot echo-planar imaging (EPI) for DTI scans respectively. The T1-weighted MRI scans were acquired using the given parameters: repetitive time (TR) = 9.6 ms, echo time (TE) = 4.6 ms, flip angle = 8°, acquisition matrix = 208 x 208, reconstruction diameter = 240 cm, echo train length = 208 ms, and number of phase encoding steps = 208. The diffusion images were acquired in 15 diffusion weighted volumes with gradient encoding applied in 15 non-collinear directions (b = 1000 s/mm2) including one non-diffusion weighted reference image (nodif, b_0 = 0 s/mm²). The DTI acquisition parameters are as follows: TR = 11894.4 ms, TE = 51 ms, flip angle = 90°, reconstruction diameter = 224 cm, imaging matrix = 128 x 128, field of view = 224 x 224, number of PE steps is 110, slice thickness for the image acquisition = 2 mm, acquisition matrix = 112 x 110, pixel resolution = 1.75 x 1.75 mm², sense factor is set at value of 2 and the number of averages is equal to 2. MR Image acquisition parameters as given by IXI site http://brain-development.org/scanner-philips-medical-systems-intera-3t/

Image processing steps

Image processing was done in three sequential steps: first, T1-weighted MRI images were processed for volumetric analysis using VBM. Second, the voxel wise tensor values were assigned to all the voxels in DTI images using FDT-FSL.
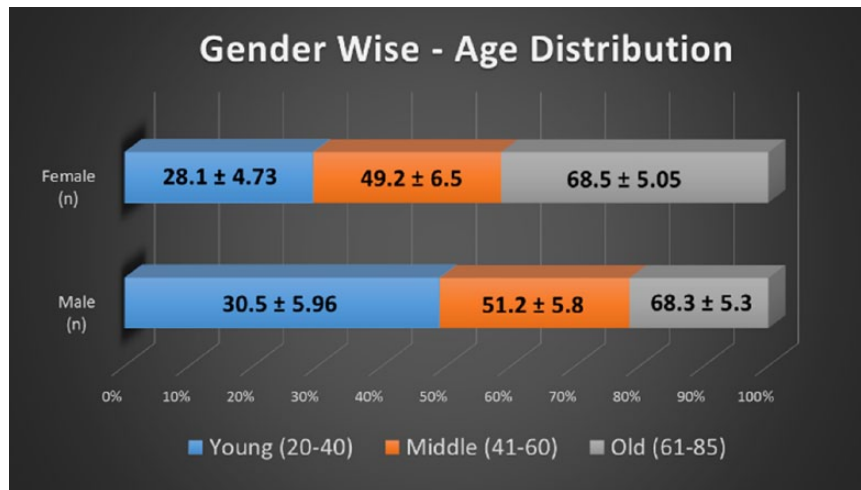
Figure 1. Bar chart representation of subjects age distribution. Mean age ± standard deviation is presented with in the blocks of individual sections of bar plot. (n) number of subjects, for females (n=89) and for (n=88).
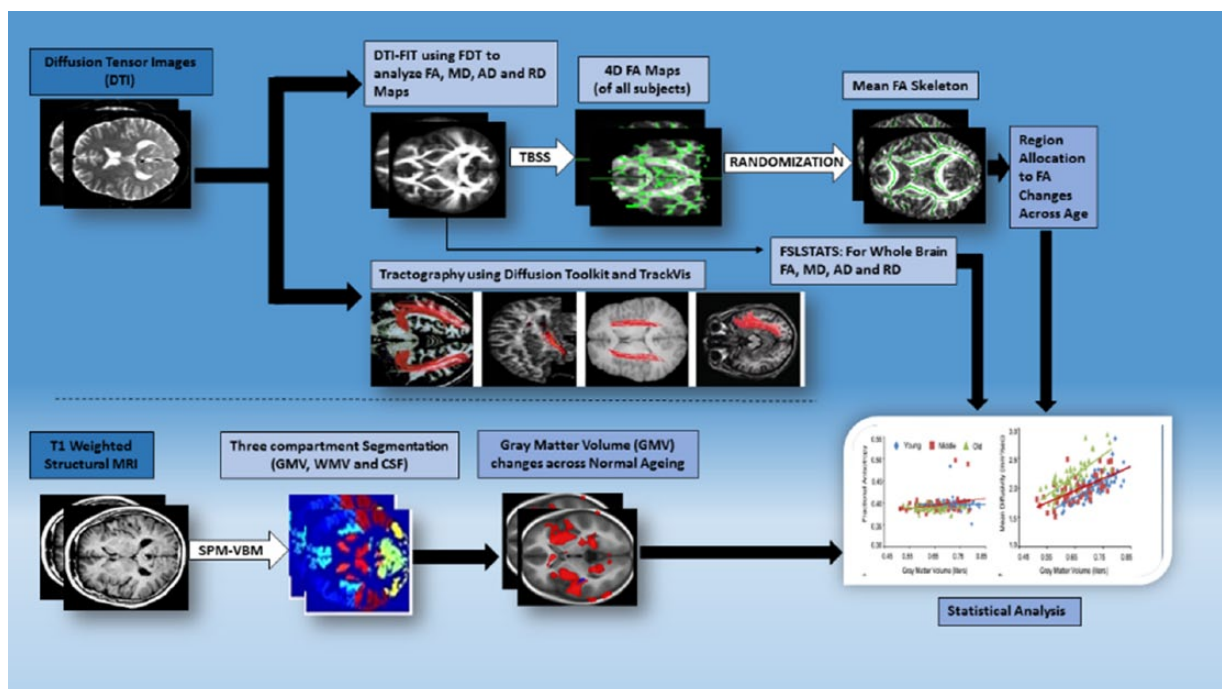
Figure 2. Work flow/ processing pipeline stating the methods involved in study.
AD: axial diffusivity; CSF: cerebrospinal fluid; FA: fractional anisotropy; MD: mean diffusivity; RD: radial diffusivity; SPM: statistical parametric mapping; TBSS: tract-based spatial statistics; VBM: voxel-based morphometry.
Diffusivity Indices, for white matter integrity fractional anisotropy (FA), mean diffusivity(MD), radial diffusivity (RD), and axial diffusivity (AD) are evaluated from the diffusion tensor images using FSL-FDT toolbox and the TBSS is used to perform all voxels statistical analysis on the DTIFIT generated maps. Further, the ROI based tractography is performed on the DTI images using Diffusion Toolkit and TrackVis software, ROI's are selected based on the areas with a significant decrease in FA across normal aging. T1- weighted Magnetic Resonance images were processed for three compartmental segmentations (white matter, gray matter, and CSF) and then the respective volumes of the parcelled compartments were evaluated using SPM-VBM, further the statistical analysis was done on the obtained data.

Third, the fiber tractography was done using Diffusion Toolkit and TrackVis. Work flow/processing pipeline stating the methodology used is given in Figure 2.

VBM analysis. VBM using Statistical Parametric Mapping (SPM8)[29] running on MATLAB platform was performed on T1-weighted structural MR images for tissue-specific segmentation. T1-weighted MRI images of all the subjects in their respective native space are segmented into three volumes based on the tissue types, that is, the GM, WM, and cerebrospinal fluid (CSF). Then, we estimated the global GMV and WMV using the *spm_get_volume* function; estimates have been further normalized by correction of intracranial volume.

DTI analysis. DTI data were processed using FMRIB software library, FSL.[30] Initially, each subject's DTI images (15 directions) in NIFTI format was merged to get a single 4D DTI image, and then, we applied head movement correction

and eddy current corrections using FDT's eddy command (FMRIB Diffusion Toolbox). In order to exclude the skull, scalp, and other non-brain tissues from images and extract the brain image, we used FSL brain extraction tool (BET) command on the eddy corrected image. Then, the DTIFIT command of FDT was used to fit the diffusion tensor model at each voxel; the BET extracted binary brain mask marks the boundary for brain volume restricting the fitting of tensors to only those voxels which are representing brain volume in the diffusion space. The output of DTIFIT processing was the tensor-fitted voxel-wise maps and the scalar magnitudes of tensors, that is, the three eigenvalues, the principal (L1), secondary (L2), and tertiary (L3). The further evaluations based on the three eigenvalues led to estimates of derived diffusion WM indices, fractional anisotropy (FA), radial diffusivity (RD) (average of L2 and L3), and mean diffusivity (MD: average of L1, L2, and L3).

Tract–based spatial statistics (TBSS). In between the three age groups, TBSS was performed in order to analyze differences based on skeleton-wise statistical analysis of the FA maps created in section *DTI Analysis*. First, the non-linear registration was applied on the FA image of each subject to register it over FMRIB58_FA standard template and then the affine transformation was applied over Montreal neurological institute based MNI152 standard space thus transforming all subjects FA images to the standard MNI space. Furthermore, the resultant transformed images were merged into a single 4D image called, "all FA" and the average of all transformed FA images taken to create the "mean FA" image. The generated mean of FA images "mean FA" was used to generate the WM skeleton identifying the common center of WM tracts in all the subjects. We applied the threshold value of 0.2 in FSL stats for computing FA values for each subject. To avoid the variation by inter-subject data and thus the partial volume and the regional effects of them, the "Mean FA skeleton" was analyzed by the 0.2 thresholds to track the prominent WM tracts present. The skeletonized FA map was generated for each subject's pre-aligned FA image and then it was registered to the mean FA skeleton. Furthermore, the estimation was done by voxel-wise statistics for group-wise FA comparisons between the young-middle-old age groups. Then, we performed between-group analysis utilizing the randomize tool in order to test significant *t*-value at each voxel against the null distribution estimated by the random permutation of 5000 steps. The JHU ICBM-DTI-81 atlas labels using FSL was utilized to specify the clusters which are significantly altered for the estimated values. The estimated diffusion indices were applied with TBSS for their FA maps in order to give non-linear transformation. Furthermore, the FA images of all the subjects were projected over mean FA skeleton one by one resulting in the projection vectors (output) respective to the individuals. The FLIRT command was used for the linear transformation of the derived seed masks to the diffusion space of the respective individuals. The

transformation matrix was formed based upon the inverse transformation of B0 image(nodif) to the T1 structural MRI image and the linear transformation of the latter to the standard brain MNI-152 template. The atlas for WM was used for pinpointing the ROI and thus assigning the seed masks over the subject's image.[31] The seed masks were allocated and confirmed under the presence of professional neuro-radiologist.

Fiber tractography. The ROI-based deterministic tractography was carried out using Diffusion Toolkit (http://trackvis.org/dtk/) and TrackVis to generate the three dimensional whole-brain fiber tracts; Orientation distribution function (ODF) model is used. The tractography parameters used were as follows: minimum tract length = 0.1 mm, threshold angle = 60°, a number of fibers estimated per voxel = 2, and the method of tractography was based on second-order Runge-Kutta method.

Statistical data analysis

Statistical analysis of all the diffusion indices was carried out in between the groups performing non-paired parametric group analysis of variance (ANOVA). Also, the post hoc analysis was done with pairwise Bonferroni-correction. The statistical analysis was performed using ANOVA for the intergroup and within group differences. Furthermore, regression analysis was performed to analyze the possible inter-relationship between GMV and quantified diffusion indices FA, MD, RD, and AD in the study.

Results
Differential pattern of age–associated alterations in regional brain volumes–evaluated using voxel–based morphometric analysis

We performed the VBM analysis on the T1-weighted MRI image derived GMV. The significant changes in regional GMV across normal aging are reported in Table 1. VBM analysis showed the significant reduction of GMV in the frontal and precentral regions bilaterally in middle-aged individuals when compared with the young aged individuals. There was a significant decrease in GMV in prefrontal, orbitofrontal, precentral gyrus, postcentral, temporo-parietal, and temporal regions namely inferior temporal gyrus, anterior and retrosplenial cingulate, posterior lobe of cerebellum, hippocampus, amygdala, parahippocampal gyrus, thalamus, entorhinal cortex, and the superior temporal gyrus in old-age subjects when compared to middle-aged individuals as shown in Figure 3. However, we did not notice any significant age-related changes for the auditory, primary motor, and the primary visual (Area V1) cortex (parietal and occipital regions).

Plotting the ratio of the GMV: WMV (Table 2, Figure 4), across age groups revealed a quadratic curve, with a U-shaped form, having minimization during middle age. This finding demonstrates minimization during middle age, illustrating the modest decrease in the WMV in comparison to GMV. Old age

Table 1. Age-related changes in gray matter volume (GMV) observed across age (young to old) using regional VBM analysis.

AREA/REGION	AGE-RELATED CHANGES	MNI COORDINATES (X, Y, Z) (RIGHT) (LEFT)	Z-STATISTICS
Dorsolateral prefrontal cortex	Decrease	(−6, 38, 58) (5, 26, 42)	7.92
Anterior prefrontal cortex (rostral superior middle frontal gyri)	Decrease	(−28, 52, 19) (27, 50, 28)	7.86
Orbitofrontal cortex (orbital rectus gyri, rostral superior frontal gyrus)	Decrease	(−24, 17, −21) (22, 41, −16)	6.51
Precentral gyrus	Decrease	(−54, −9, 50) (56, −12, 30)	7.92
Inferior/middle temporal gyrus	Decrease	(−56, 10, −24) (50, 12, −28)	6.62
Entorhinal cortex (parahippocampal gyrus)	Decrease	(−27,−32,−12) (18, −17, −16)	7.06
Anterior cingulate cortex	Decrease	(−6, 10, 45) (4, 14, 32)	6.91
Retrosplenial cingulate cortex	Decrease	(−10, −46, 12) (9, −51, 18)	6.52
Cerebellum (posterior lobe)	Decrease	(−32, −84, −38) (31, −90, −34)	6.28
Temporo-parietal cortex	Decrease	(−52, −40, 28) (54, −42, 25)	7.22
Amygdala/hippocampus	Decrease	(−27,−4,−21) (26,−3,−22)	7.43
Anterior thalamus	Decrease	(−2, 1, 3) (5, 2, 7)	6.98
Posterior thalamus	Decrease	(−14, 18, 8) (15,−17, 9)	6.89
Fusiform gyrus	No significant change	(−30, −53, −9) (32, −43, −11)	6.21
Angular gyrus	No significant change	(−35, −65, 40) (42, −54, 32)	7.97
Supramarginal gyrus	No significant change	(−50, −21, 35) (54, −13, 26)	6.02
Frontoparietal cortex	No significant change	(−41, 11, 39) (46, 21, 32)	7.37
Superior temporal gyrus	No significant change	(−48, −34, 14) (52, −38, 12)	6.18
Auditory cortex	No significant change	(−42, −30, 13) (45, −27, 19)	6.54
Postcentral gyrus	No significant change	(−57, −28, 45) (62, −20, 40)	7.87
Primary somatosensory cortex	No significant change	(−8 −54 62) (12,−52, 68)	6.84
Posterior primary motor cortex	No significant change	(−28,−28, 70) (32, −28, 70)	6.92
Premotor/ Supplementary cortex	No significant change	(−3, −6, 53) (8, −2, 40)	6.16
Parieto-occipital cortex	No significant change	(−5, −58, 42) (6, −52, 53)	7.08
Primary visual cortex (V1)	No significant change	(−8,−76, 3) (6, 72, 4)	7.28
Inferior occipital gyrus	No significant change	(9, −70, −6) (−8, −68, −4)	6.99
Superior occipital gyrus	No significant change	(−16, −77, 17) (18, −75, 20)	7.11
Striatum	No significant change	(−32, 20, 0) (30, 24, 6)	6.8

subjects in comparison with the middle age individuals have the more prominent decline in WMV than the GMV.

Age–related WM degeneration assessed using DTI tract–based analysis

The age-related WM integrity changes based on FA values are estimated using TBSS analysis, the results are given in Table 3 and Figure 5 depicting changes seen across normal aging. From TBSS analysis, we noticed region-specific changes of FA estimates, and hence, we explored the alterations in fiber tracts related to these regions across the lifespan. The ROI-based tractography was performed, the results are shown in Figure 6. We noticed a significant loss of fiber tracts due to age-related processes. Loss of fibers was seen bilaterally in major fiber tracts namely genu, body, and the splenium of corpus callosum
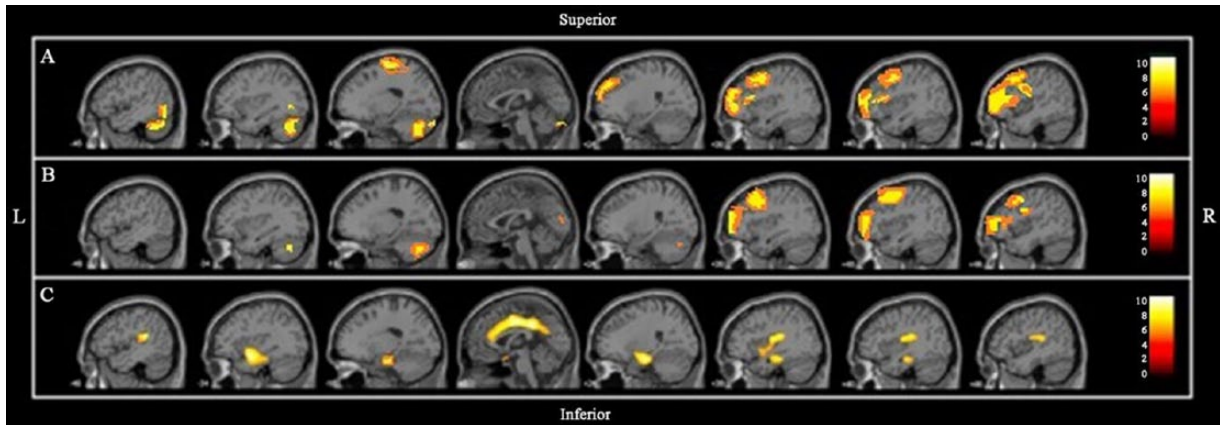
Figure 3. Regional gray matter volume using voxel-based morphometry analysis. A. young>old; B. young>middle; C. middle>old.

Table 2. Alteration of the relative gray matter ratio as aging transition occurs.

BRAIN TISSUE PARAMETERS	YOUNG AGE	MID AGE	OLD AGE
Gray matter volume (cc.)	715	668	636
White matter volume (cc.)	534	523	472
Gray matter ratio	1.339	1.277	1.347

Gray matter ratio = gray matter volume/White matter volume; ratio indicates the relative amount of gray matter when normalized with respect to the White matter size.
Significant decrease in gray matter volume ($P < .001$) in the old aged individuals compared to the middle age subjects.
Significant decrease in gray matter volume ($P < .05$) in the old aged individuals compared to the middle age subjects.
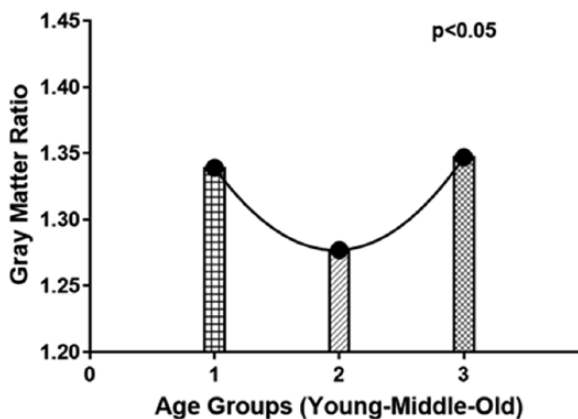
Figure 4. As the aging process ensues, there is a U-shaped alteration in the gray matter ratio, which indicates the amount of gray matter (in cc.) corresponding to unit amount (1 cc.) of White matter size. The curve of quadratic fit is shown. The second-order polynomial (quadratic) fit: $P < .05$ (perfect fit), 0.87; -0.2; 0.06, for B0, B1 and B2 respectively.

(CC), corticospinal tracts, superior and anterior corona radiata, inferior fronto-occipital fasciculus, internal capsule, external capsule, cingulum, superior longitudinal fasciculus, and optic thalamic radiation ($P < .05$) as shown in Figure 6.

We noticed the following changes at the two aging transitions: (a) anterior aging transition (around 40 years' age): there was a decrease in fiber tract count bilaterally specifically in corona radiata (both superior and anterior regions) and CC (genu and body). (b) Posterior aging transition (around 60 years of age): we observed prominent loss of fiber tracts bilaterally in the following regions: cortical association fibers (superior and inferior longitudinal fasciculus), internal capsule (anterior and posterior limb), external capsule, fornix, projective peduncles (cerebral peduncle, and cerebellar peduncle superior).

Global relationship between gray matter volume and white matter diffusion indices

In middle-aged groups, significant decrease of global GMV ($P < .05$) was noticed when compared with the young age group. On the other hand, there was no change in the diffusivity indices, FA ($P = .93$), MD ($P = .5$), RD ($P = .66$), and AD ($P = .38$) between the two groups. In the old age group, while Global GMV ($P < .001$) and FA ($P < .05$) were found to be significantly reduced compared with the middle age adults, remarkably, MD, AD, and RD were found to be significantly increased ($P < .05$) in the same comparison for old age people. The age-related changes in global GMV along with WM integrity measures, the AD, RD, MD, and the FA are summarized in Table 4.

For the comparison between the young and old age groups, the significant decrease in global GMV and FA values ($P < .05$) is observed, we also found the significant increase in the values of MD, AD, and RD ($P < .05$). We also observed an impact of gender on GMV and WM diffusivity indices. Global GMV was significantly reduced for females in comparison to

Table 3. List of brain regions which show significant FA changes ($P < .05$) as aging occurs from young (20 years) to old (85 years). For each brain region the MNI coordinates and the number of voxels (cluster size) exhibiting that change in the brain region is shown. R & L implies that the particular brain region shows the change bilaterally.

WHITE MATTER LOCATION (FA)	CLUSTER SIZE (VOXEL)	MNI COORDINATES (X Y Z (MM))
Posterior radiations of thalamus (geniculocalcarine tract) R	435	(34 -42 8)
Anterior thalamic radiations of thalamus (geniculocalcarine tract) R	566	(-39 -44 3)
Body – corpus callosum	581	(18 -17 35)
Genus of corpus callosum	622	(12 32 3)
Fornix (Cres)/Stria Terminalis(R & L)	1030	(-31 -18 -12) (35 -15 -12)
Cerebral peduncle R & L	1032	(19 -17 -7) (-14 -19 -12)
Stratum sagittale (inclusive of inf. fronto-occipital fasciculus) (R & L)	1042	(38 -17 -12) (-37 -13 -12)
Retrolentricular part of internal capsule R & L	1090	(29 -21 8) (-32 -24 3)
External capsule (R & L)	1110	(31 -17 10) (-32 -18 3)
Posterior limb of internal capsule (R & L)	1114	(28 -17 17) (-10 -4 3)
Superior longitudinal fasciculus (R & L)	1166	(40 -17 32) (-34 -17 36)
Cingulate cortex (R & L)	1220	(9 -17 34) (-9 -16 34)
Superior corona radiata (R & L)	1224	(20 -19 37) (-18 -17 37)
Internal capsule (Anterior Limb-R)	1236	(12 3 3) (-22 23 3)
Anterior corona radiata (R & L)	1312	(17 38 3) (-24 29 3)
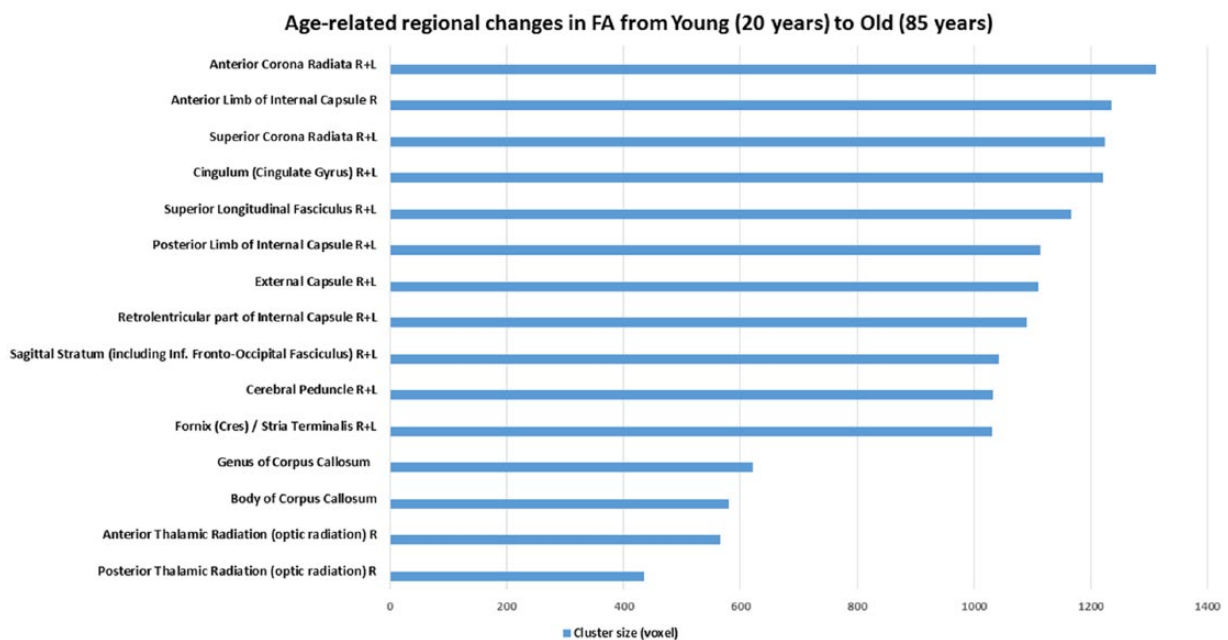
Figure 5. Normal aging based regions with significant FA changes from young (20 years) to old (85 years) ages and their respective cluster sizes. R+L, for the same regions showing significant differences in both hemispheres.

males ($P < .05$). Also, FA and MD were found to be significantly reduced in the female candidates ($P < .05$). However, we did not find any significant differences for the values of AD and RD in females vs. males; data are given in Figure 7. The one-way ANOVA analysis for GMV and diffusion indices between groups for age-sex interactions $F_{2,267} = 17.7$ and age
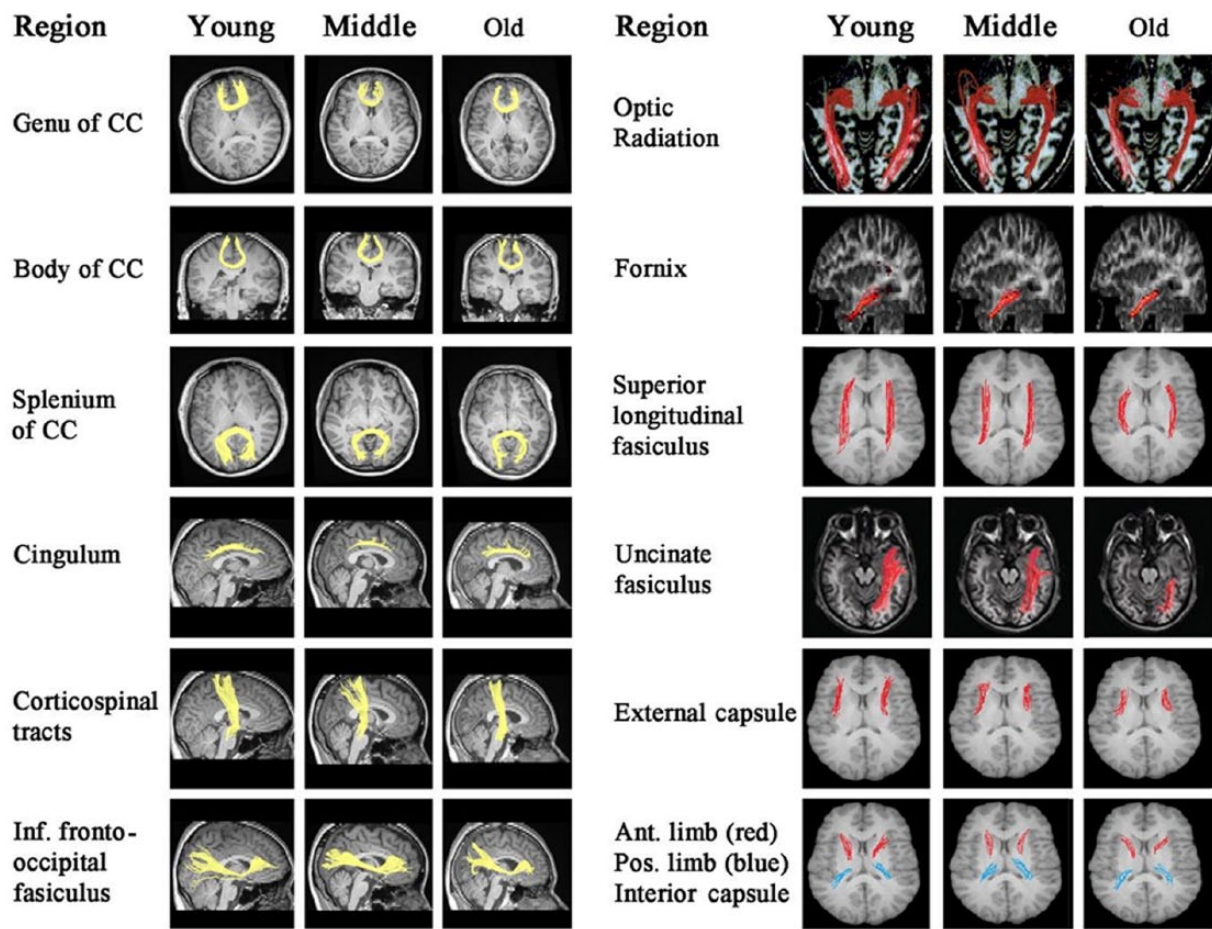
Figure 6. Region specific changes of fibertractography in all the three age groups. ROI based tractography using Diffusion Toolkit and TrackVis.

Table 4. Global mean gray matter volume and various diffusion indices in three age groups (Young-middle-old).

PARAMETER	YOUNG (MEAN ± SD)	MIDDLE (MEAN ± SD)	OLD (MEAN ± SD)
Gray matter volume	0.715 ± 0.061	0.668 ± 0.076	0.636 ± 0.062***
Fractional anisotropy	0.397 ± 0.0133	0.396 ± 0.0210	0.388 ± 0.007*
Mean diffusivity	0.000202 ± 2.248e-05	0.000198 ± 2.281e-05	0.000217 ± 2.69e-05*
Radial diffusivity	0.000176 ± 1.98e-05	0.000174 ± 2.0e-05	0.000193 ± 2.46e-05*
Axial diffusivity	0.000253 ± 2.71e-05	0.000248 ± 2.95e-05	0.000264± 3.172e-05*

$*P < .05. ***P < .001$
The significant decrease in gray matter volume ($P < .001$) and the fractional anisotropy values ($P < .05$) is observed in the old age subjects compared to the middle age subjects. The significant increase in the mean diffusivity, radial diffusivity, and axial diffusivity ($P < .05$) is observed in the old age subjects compared to the Young and the middle age subjects. In Young to Old comparison the significant (p<0.05) decrease in global GMV and FA values is observed.

interaction $F_{3.048} = 18.0$ were found to be statistically significant ($P < .05$).

Furthermore, we assessed correlation between GMV and WM diffusion indices (FA, RD, MD, and AD) in relationship with normal aging. The linear regression analysis for whole brain GMV and various diffusion indices (FA, RD, MD, and AD) in young, middle, and old-aged individuals are shown in Figure 8. The MD displayed the significant correlation with the GMV ($P < .05$) in all age groups: young, middle, and old, the correlation r = 0.72, 0.67, and 0.77 for the respective age groups. Likewise, the AD also exhibited significant correlation with the GMV ($P < .05$) for all the three age groups, the respective correlation r being 0.72, 0.71, and 0.81. On the other hand, we did not observe any significant correlation between GMV and RD or between, GMV and FA, ($P > .05$) (Table 5). We notice that the GMV showed higher values for correlation with the AD than with MD for the three age groups.
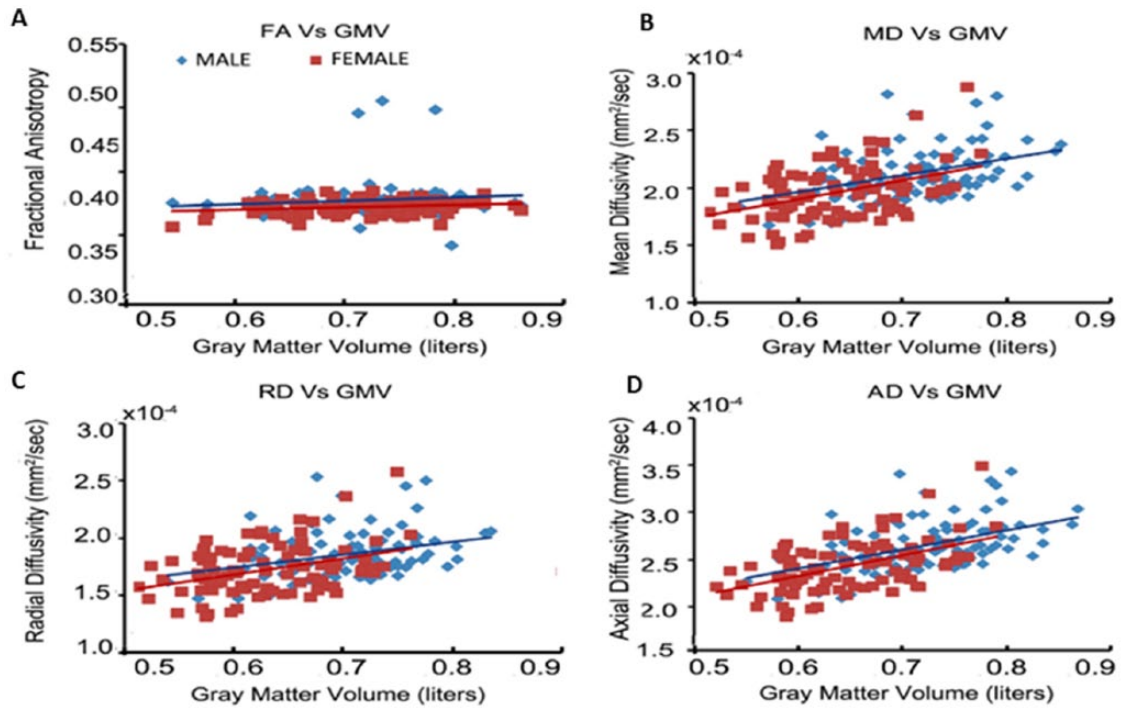
Figure 7. Regression analysis, for the relationship between diffusion indices (FA, MD, AD and RD) with GMV [A-D] and the subjective differences between male and female and the respective relationship of the young, middle and old age groups.
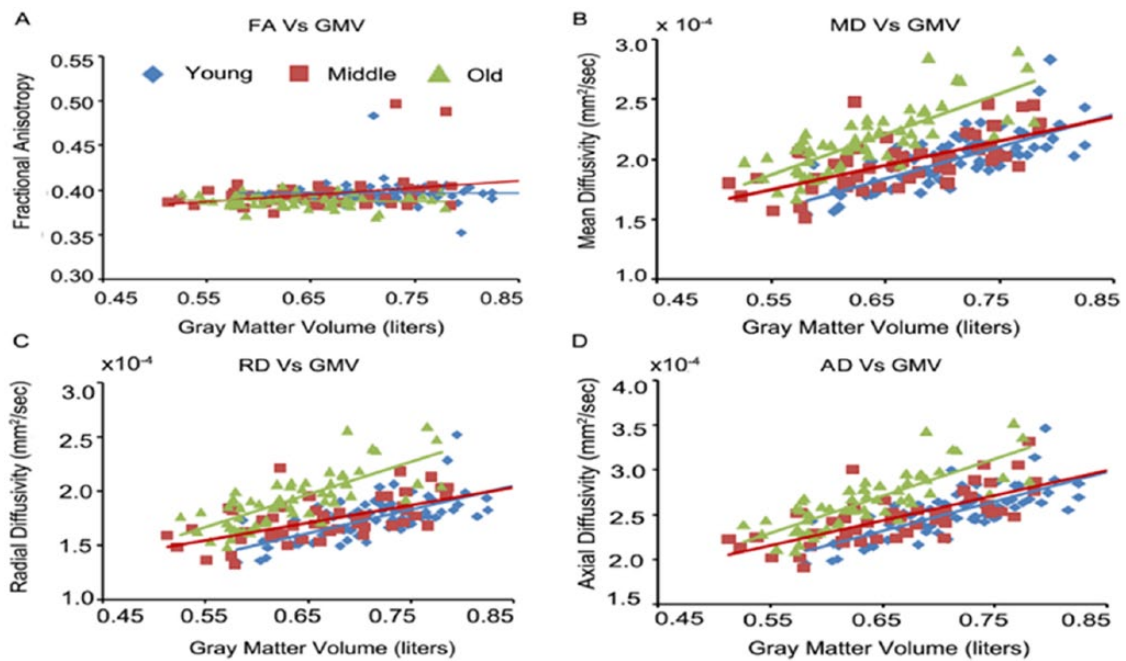
Figure 8. Regression analysis, for the relationship between Diffusion Indices (FA, MD, AD and RD) with GMV [A-D] and the subjective differences between the relationship of the young, middle and old age groups.

Figure 9 illustrates the age-related changes in AD. One can note that the curve shows a quadratic U-shaped profile, with minimization at mid-age. The regression analysis mentioned above has been done using linear regression approach, as informed before. It may also be commented that we have also used nonlinear regression approach (as a regression model from a nonlinear quadratic fit), but we noticed that the correlation value reduced when compared to the earlier linear regression fit.

Discussion

The present work is a novel approach of utilizing different MRI modalities to explore the association and interrelationship between the two major complimentary functional compartments of the brain, the WM, and GM in healthy aging. We arrive at a noteworthy finding showing a significant association between the micro level WM property (as AD) and the macro level GM characteristic (the gross volume); there is a higher level of

Table 5. Value of correlation between white matter indices and gray matter volume while aging progresses.

WHITE MATTER INDICES	GRAY MATTER VOLUME		
	YOUNG AGE	*MID AGE*	*OLD AGE*
Axial diffusivity	0.73	0.71	0.81
Mean diffusivity	0.72	0.67	0.77
Radial diffusivity	Not significant	Not significant	Not significant
Fractional anisotropy	Not significant	Not significant	Not significant

"Not significant" implies that the correlation value is not statistically significant, with the *P* value exceeding .05 (see text).
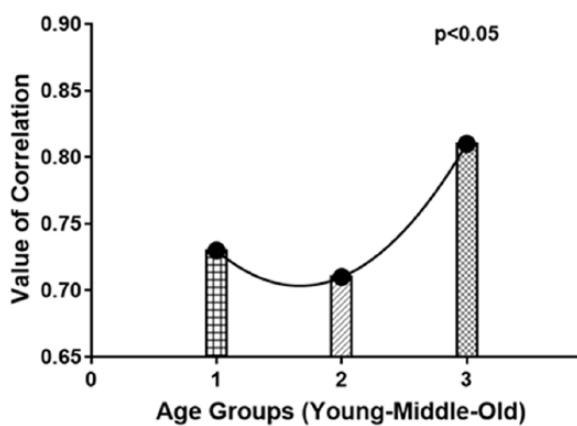
Figure 9. While the aging transition takes place, there occurs a U-shaped change in the value of correlation between the white matter axial diffusivity and the gray matter volume. The data is fitted to a quadratic curve. The second-order polynomial (quadratic) fit: $P < .05$ (perfect fit); 1.533; -0.26; 0.066 for B0, B1 and B2 respectively.

correlation between the two entities across the life-span. GM and WM are the main functional compartments of brain tissue where GM is for neuronal cell bodies, and the WM belongs to myelinated axonal tracts. One of the important issues in human life-span studies is to understand the relationship between the WM and GM changes during the normal aging process.

Regression analysis between white matter diffusivity indices and gray matter volume

Age-related and gender-related changes in DTI indices of deep-lying GM nuclei in different regions of the normal human brain have been observed with FA values increasing up to adulthood, and the rise up to the third decade of life being followed by a subsequent decrease.[32] Whole brain FA and cortical GM thickness values have been shown to follow quadratic trajectories with age, but with a linear relationship between them, indicating that a putative biological mechanism may explain the non-linearity of their age trajectories.[33] No significant correlation has been observed between the FA and GMV which demarcates that relative anisotropy (RA) or FA has no appreciable correlation with axonal functioning. Since augmented axonal functioning enhances GMV, one can expect that RA or FA would have a weak or no correlation with GMV.

In our study, we find a strong correlation between AD and GMV; on the contrary, we did not find any significant correlation between the RD and the GMV. The result indicates that increase of RD may correlate with rising myelin dysfunction, but not with axonal functioning.[42] The MD is defined as a third of the sum of the axial and radial diffusivities. Hence, we can predict that the MD should show correlation with the GMV, but the correlation value should be less than the value of correlation of AD with GMV. Our results are consistent with a recent study where the authors have explored the association of age with GM anatomical network changes in a large group of middle- to old-aged adults between 45 and 85 years of age.[34] The regression analysis by us between the MD and GMV showed good correlation suggesting that MD is an inverse measure of membrane density, signifying cellular changes in GM which consist of cell bodies such as neurons and glial cells distributed across the regions of brain: MD is a direction independent measure. On the other hand, the direction-dependent measure, FA showed poor correlation with GMV because GM contains neuronal cell bodies and unmyelinated axons. Myelinated axons in brain pathways are tightly packed so that even small changes with age in myelin and fiber tract density resulting in a higher signal change for both FA and WM density.

The VBM analysis study showed a significant positive correlation between GMV and information and digit span subtests of the Wechsler Adult Intelligent Scale-Revised, but no significant correlation was seen between WMV and performance on the cognitive tests of semantic and short-term memory, which is relatively well preserved with aging.[35] Further, the global and regional effects of aging on brain volume, MD, and FA using voxel-based analysis showed that GMV and FA were negatively correlated, whereas MD was positively correlated with age. In addition, brain volume and FA were negatively correlated predominantly in anterior structures, and MD was positively correlated in the cortical GM and periventricular WM indicating that diffusion properties and brain volume are complementary markers to the effects of aging.[24] An earlier study reported changes in MD and FA in the central nervous system during normal aging and results showed a significant increase in MD especially in frontal WM and lentiform

nucleus and significant FA decline in the genu of the CC with aging.[36] A recent study showed the age-related decline in FA and fiber numbers in both anterior and superior cingulum during old age (60s-70s) compared to young age (20s-30s) individuals.[37] Also, it has been shown that age-related increase of iron deposits in the brain may also influence the DTI metrics.[38] The voxel-wise analysis also revealed the substantial effect of body mass index on GMV along with the significant effect of age, gender, and age × gender interaction.[39] The voxel-wise analyses showed no significant effects of aging on either GMV asymmetry or WM-FA asymmetry in each gender.[27] The study using VBM analysis showed that periventricular WM hyperintensity was correlated with GMV in elderly aged subjects between 60 and 64 years.[40] As it is known that the proliferation and migration of the progenitor cells from subventricular zone to GM, via WM tracts, are hampered in the mid age,[1] it can be inferred that GMV's increase, is comparatively diminished in mid-life. Our earlier study has shown that during middle age, there is minimization in the WM structural network parameters (e.g. betweenness-centrality, normalized to tract density);[41] this indicates that structural efficiency of the white matter tracts minimizes during that age, implying that the correlation between White matter functioning and its afferent effect (Gray matter volume), would also show a minimization in mid age (Figure 9). A combined MRI-DTI study in multiple sclerosis subjects has shown that, in comparison to controls, there is no alteration in the AD (that pertains to the axonal direction), but there is increase in RD (that pertains to the centrifugal orthogonal direction toward the myelin sheath); the damage and breakage of the myelin sheath enables water molecules to diffuse out prominently in the radial direction.[42] Our results corroborate with our earlier study, showing the regional specific changes in FA across the normal aging.[43] However, MRI and DTI studies are unable to reveal the underlying neurobiological processes directly. Nevertheless, the MRI-DTI study does provides the basis for the multifactorial changes in the structural GM volume and WM diffusion properties during healthy aging.

Strengths and limitations of the approach

TBSS for spatial statistics of generated WM tracts offers advantages over local VBM analysis, particularly as TBSS does not require spatial smoothing, while also minimizing methodological limitations, such as misalignment and misregistration. This, therefore, enhances the sensitivity and interpretation of our findings. The automated TBSS process is able to process the whole brain as a data point and is advantageous compared with the traditional ROI based standard deterministic tractography approach. Moreover, we observed how microstructural manifestations of neuroplasticity using MRI-DTI diffusivity measure could be well substantiated with differential neurogenic activity, functioning at different ages and different regions

of the brain. A future scope of our work would be to perform a longitudinal study on individual subjects, taking multiple scans at different time points over a number of decades, as each individual ages across the lifeline.

Implications for human aging

Our findings, along with collateral observations, imply the constructive interrelationship between the WM and GM compartments, namely the WM axonal connection and afference (as gauged by the AD) have a high correlation with GMV, across the normal aging process. Indeed, the interrelationship is causational and generative, WM axonal afferent functioning stimulates the GMV. The normal aging process is associated with some cognitive decline and neurodegenerative changes; it is imperative that we explore how the WM-GM relationship can be probed from a neuroprotective perspective. Of considerable importance is to augment the AD and axonal functioning in WM, which may act as an enhancing agent for increasing GMV and functionality.

In summary, we noticed a strong correlation between direction independent WM measure, MD with GMV but weak correlation of GMV with direction-dependent WMI measure, FA. The regression analysis showed differential relationship for both age and gender differences between GMV and various diffusivity indices. Thus, this study helps to understand the cross-structural relationship between structural and diffusion properties of brain tissue underlying the age-related changes including neuropsychological functioning and age-dependent atrophy across the lifespan. Furthermore, a longitudinal study will help in delineating this relationship between GMV and WM diffusivity indices during lifespan changes of brain atrophy.

Conclusion

In this article, we have elucidated the relationship between structural changes in GM (using VBM) and the WM diffusivity indices (using TBSS and fiber tractography analysis) in healthy aging. The VBM analysis showed a significant age-related reduction of GMV in prefrontal and temporal lobe, precentral, and postcentral structures, with no significant changes observed in the parietal lobe and the occipital lobe regions of both cerebral hemispheres. On the other hand, the TBSS analysis showed a significant decrease in FA, while ROI-based tractography showed loss of fiber tracts mainly in body, genu, and splenium of CC, fornix, superior longitudinal fasciculus, cingulum, anterior and superior corona radiata, external capsule, the anterior and posterior limb of the internal capsule in both left and right hemispheres of brain. The regression analysis showed good correlation for WM-AD and MD measures with GMV across all age groups, thus concluding that the GMV is strongly correlated with direction independent and unidirectional WM

diffusivity measures, but weakly correlated with direction-dependent WM diffusivity measures. The differential spatiotemporal alteration pattern of GM and WM in specific brain regions across the two aging transitions (i.e., young to the middle, and then, middle to old) is also observed in our study. The quantitative relationship of the WM diffusivity indices and the GM volume in the healthy aging individuals is explored in the current study.

Author Contributions

VP, VPSR, and PKR conceived and designed the experiments. VP and VPSR analyzed the data. VP, VPSR, and PKR wrote the first draft of the manuscript. VP, VPSR, and PKR contributed to the writing of the manuscript. VP, VPSR, and PKR agree with manuscript results and conclusions. VP, VPSR, and PKR jointly developed the structure and arguments for the paper. VP, VPSR, and PKR made critical revisions and approved final version. All authors reviewed and approved of the final manuscript. VP and VPSR contributed equally to this work.

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