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Combined Diffusion Tensor and MR Spectroscopic Imaging Methodology for Automated Regional Brain Analysis: Application in a Normal Pediatric Population

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Abstract

During human brain development, anatomic regions mature at different rates. Quantitative anatomy-specific analysis of longitudinal diffusion tensor imaging (DTI) and magnetic resonance spectroscopic imaging (MRSI) data may improve our ability to quantify and categorize these maturational changes. Computational tools designed to quickly fuse and analyze imaging information from multiple, technically different data sets would facilitate research on changes during normal brain maturation and for comparison to disease states. In the current study, we developed a complete battery of computational tools to execute such data analyses that includes data preprocessing, tract-based statistical analysis from DTI data, automated brain-anatomy parsing from T1-weighted MR images, assignment of metabolite information from MRSI data, and co-alignment of these multimodality data streams for reporting of region-specific indices. We present statistical analyses of regional DTI and MRSI data in a cohort of normal pediatric subjects (n=72; range: 5–18 years; mean age 12.7±3.3 years) to establish normative data and evaluate maturational trends.

Several regions showed significant maturational changes for several DTI parameters and MRSI ratios, however, the percent change over the age range tended to be small. In the subcortical region (combined basal ganglia (BG), thalami (TH) and corpus callosum (CC)), the largest combined percent change was a 10% increase of fractional anisotropy (FA) primarily due to increases in the BG (12.7%) and TH (9%). The largest significant percent increase in N-acetylaspartate/creatine (NAA/Cr) was seen in the brain stem (BS) (18.8%) followed by the subcortical regions in the BG (11.9%), CC (8.9%) and the TH (6.0%). We found consistent, significant (p<0.01) but weak

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positive correlations ($r = 0.228\text{--}0.329$) between NAA ratios and mean FA in the BS, BG and CC regions.

Age- and region-specific normative MR diffusion and spectroscopic metabolite ranges show brain maturation changes and are requisite for detecting abnormalities in an injured or diseased population.

Keywords

human brain maturation; magnetic resonance spectroscopy; diffusion tensor imaging; pediatric; computational analysis; normal

Introduction

Diffusion tensor and magnetic resonance spectroscopic imaging (DTI, MRSI) have proven invaluable in non-invasively examining maturational changes during normal development as well as in children with neurometabolic, genetic and acquired disorders [1–14]. DTI of the pediatric population has been shown to provide quantitative measures of the spatial and temporal white matter development during the maturation process [3]. Numerous studies have shown that commonly measured DTI indices change rapidly from birth through infancy [1, 4, 5] followed by slower changes through adolescence [3, 6–8]. DTI studies have documented age-related regional variations, generally characterized overall by increasing fractional anisotropy (FA) and decreasing diffusivity with age. In contrast, MRSI measures specific brain metabolite concentrations that broadly reflect components of the neurovascular unit such as neuronal and glial cell metabolism [15]. Similar to DTI, MRSI shows regional maturational changes with rapid changes in metabolite levels from birth through 24 months [16, 17] and much slower changes through adolescence [18–20]. When using DTI or MRSI it is necessary to take these maturational changes into account when studying diseases or injury in the pediatric brain.

The complexity of neuroimaging data acquired with different MRI techniques warrants sophisticated post-processing computational tools [21–23]. In DTI studies, three broad approaches have been adopted, either individually or in some combination: (1) voxel-based analysis (VBA) where individual voxels are compared in a registered common 3D coordinate space [24]; (2) atlas based analysis (ABA) that globally registers the entire brain with an atlas to obtain information on individual entire white matter (WM) regions [8]; and (3) tract based statistical analysis (TBSS) where DTI tracts (skeletons of WM regions) are registered with one tract-atlas or template to quantify information for individual tracts [2, 25–28]. The requirement of registering each individual voxel in VBA has been shown to be too demanding in most cases [29] unless proper ground-truth templates are generated [30]. Hence VBA is often utilized after ABA or TBSS, at the final comparison stage [31].

As part of an NIH sponsored study on pediatric brain injury, we developed a computational method to anatomically fuse and directly compare data from whole brain DTI and 3D MRSI acquisitions. We used TBSS followed by whole-brain (not just white matter) registration and anatomy-based analysis (not voxel or tract-based) which may be more robust against eddy

current and bias field effects in DTI data, specifically as individual DTI data are not registered among themselves. In this paper we report age- and region-specific normative diffusion and metabolite ranges in a pediatric population that are requisite for detecting abnormalities in an injured or diseased population. Children in our study ranged from 5 to 18 years of age which, as described above, is a time when both DTI and MRSI brain maturational changes are subtle. Determining maturation profiles for commonly measured parameters in DTI and MRSI studies will allow us to determine whether age specific evaluations must be made. The purpose of this paper is to describe the steps of our multi-modality processing scheme (fig. 1) and report age and region specific diffusion and metabolite ranges from healthy (normal) pediatric subjects using the method.

Materials and Methods

Study Population

Control subjects (n=72; 36M/36F) between the ages of 4 and 18 years of age (Mean = 12.7 $\pm$ 3.3 years; range: 5.4 – 18.5 years) were recruited as part of an Institutional Review Board (IRB) approved, NIH sponsored study on pediatric traumatic brain injury (TBI). Written consent was obtained from the subject's parent or legal guardian and the subject if greater than 11 years old. Assent was obtained from subjects between 7–11 years old. Exclusion criteria included any MRI contraindications, neurological disorders, and previous brain trauma or alcohol or drug abuse. All subjects underwent neuropsychological testing per study protocol to determine eligibility. Subjects were scanned without sedation, initially and one year later to match the protocol for TBI subjects. Data from both time points, if obtained, were included in the data reported in this study. However, 12 subjects did not return for their follow-up study at one year because they did not want to have another MRI (n=3), or they moved out of the immediate area (n=8). We took time to familiarize the children within the scanner environment to make them more comfortable before scanning. Images and spectra were checked for motion and repeated if necessary. However, in 3 subjects, the data for both MRS and DTI were excluded due to excessive motion artifacts. These children did not return for follow-up studies. In an additional 2 studies, only the MRS data were excluded due to motion artifacts.

Magnetic Resonance Imaging Data Acquisition

Whole brain MRI and DTI were acquired on a 3T whole body imager (Trio/Tim, Siemens Medical Solutions, Erlangen, Germany) using a 12-channel receive-only head array coil. Imaging included an isotropic 3D sagittal T1-weighted inversion prepared fast spoiled gradient echo sequence (MPRAGE, TR/TE=1950/2.26 ms, NEX=1, voxel size 1.0 $\times$ 1.0 $\times$ 1.0 mm³) and a 3D sagittal T2-weighted fast spin echo sequence with variable flip angles (SPACE, TR/TE=3200/415 ms, NEX=1, voxel size 1.0 $\times$ 1.0 $\times$ 1.0 mm³). DTI was acquired with a spin echo single-shot echo planar sequence using diffusion encoding in 30 non-collinear directions, b-values of 0 and 1000 s/mm², TR/TE=5700/102 ms, resolution 1.2 $\times$ 1.2 mm², slice thickness = 3.0 mm with 0.9 mm gap; 35 axial slices were oriented along a line drawn between the anterior-posterior commissures. Prior to DTI imaging, 3D map shimming was performed.

Magnetic Resonance Spectroscopic Imaging Data Acquisition

3D proton MR spectroscopic imaging (MRSI) was acquired using a water-suppressed point resolved spectroscopy sequence (PRESS) with a TR/TE = 1700/144 ms, NEX = 1, 1024 data points sampled with a dwell time of 1ms. Images from three orthogonal planes acquired during the imaging portion of the study were used for slab placement for MRSI and matched DTI orientation to facilitate direct comparison. Four or five multi-voxel slabs, dependent on patient brain size, were acquired with the 3D MRSI (maximum of 160 mm field-of-view, 16×16 phase encodings, up to 64 voxels (8×8) volume-of-interest (VOI), nominal voxel volume of 1cc) covering from the level of the mid-corpus callosum (CC) through the superior brain stem (BS), including portions of the frontal, parietal, temporal and occipital white and gray matter, basal ganglia (BG) and thalami (TH).

DTI Data Processing

1.) DTI Pre-processing—DTI data were preprocessed before tract-based statistical analysis was performed. The pre-processing steps were written in-house and are detailed in the following subsections.

1a. 3D Interpolation: The manufacturer provided DTI tensor files were interpolated to fill the inter-slice gap for tract-based spatial statistics (TBSS) and multi-modality information fusion at a later stage. Due to time and clinical constraints, the DTI and MRI scans were not acquired at the same resolution. To produce a continuous volume of 3D data ($1.2 \times 1.2 \times 3$ mm), a 3D cubic interpolation routine developed in Matlab version R2014a (Mathworks, Natick, MA, USA) was used for better matching of the DTI volume to the continuous T1WI volume for each of the tensor images (D_{xx} , D_{xy} , D_{xz} , D_{yy} , D_{yz} , D_{zz}) and the b0 datasets.

1b. Skull-stripping based on (b0) diffusion scan: The unweighted DTI ($b=0$ mm²/sec or b0) images were used to extract brain area using a skull-stripping routine. All tensor and b0 images were co-registered by virtue of their acquisition and computation. Therefore, a binary mask of the skull-stripped brain from any of the 7 DTI volumes could be applied to others for stripping the skull. We found that the b0 images worked best for this purpose (fig. 2a–d). Each slice in the 3D dataset was rescaled so that voxel-values fell inside the [0, 1] range. A threshold of 0.005 was used to remove most of the background noise. For the remaining foreground (including the brain) regions, morphological cleaning (e.g., dilation, erosion, filling internal holes in the foreground), and area-based filtering (i.e., removing foreground regions < 5% of the slice area) operations were performed using Matlab routines to obtain a dataset of binary brain masks. The brain masks were then used to remove background noise and the skull in the DTI tensor images. All further DTI computations were performed only for the region defined by the binary brain mask. This process allowed for faster computation as the brain region consisted of only ~35% of the entire 3D matrix.

1c. DTI map computation: Skull-stripped tensor images were used to compute the following DTI parameter maps: fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and apparent diffusion coefficient (ADC) maps based on standard formulas [32, 33].

1d CSF-removal based on ADC maps: CSF masking was performed using ADC maps to identify high signal intensity voxels to produce a final binary brain mask which was applied to other DTI maps to improve tract-based analysis. ADC maps were used to detect CSF voxels using an ADC threshold $> 500 \times 10^{-5} \text{ mm}^2/\text{sec}$. After morphological cleaning (erosion, dilation, majority-filtering, and area-based filtering), voxels from CSF outside the brain were eliminated from the binary mask while the ventricular voxels were still included (fig. 2e–h). This provided the final brain mask used to clean the remaining DTI maps. If needed, additional skull stripping was performed during this step using an ADC threshold of $< 5 \times 10^{-5} \text{ mm}^2/\text{sec}$, followed by additional morphological cleaning.

2. Tract-based Spatial Statistics (TBSS) from DTI—In our TBSS method, the physical locations of the skeleton are the central voxels within a WM tract and the values assigned to each skeletal voxel change depending on which statistical parameter is being considered. TBSS processing steps were written in-house and detailed in the following subsections.

2a. Finding white matter (WM) masks and skeletons from FA maps: WM brain tracts were extracted (including peripheral WM tracts) and a skeleton was computed by using an FA threshold and iterative thinning of WM. Binary masks were obtained using a specific threshold of $\text{FA} > 0.2$ on our 2D FA maps (fig. 3a) because testing determined that this threshold best defined the WM of the brain. This process produced some outlier regions (fig. 3b) that were removed by morphological cleaning (fig. 3c). Finally, a region-based connected component analysis found small non-connected outlier regions less than 20 voxels in size which were then removed using area-based filtering. Using a Matlab routine for medial axis computation (Matlab function “*bwmorph (BinaryMask, ‘skel’, inf)*”) until the 2D skeletal connectivity was preserved, a skeleton of the WM mask was computed for every FA map by *iteratively* removing boundary pixels of the WM binary mask to produce a single connected skeleton for each WM region (fig. 3d). The rationale for using $\text{FA} > 0.2$ for WM is shown in fig. 3e–h illustrating that smaller FA thresholds overestimate WM regions while larger FA thresholds underestimate them.

2b. Finding perpendicular lines to the skeletal voxels: To find the perpendicular line for a particular skeletal voxel (V) with 2D location (x_v, y_v) , the neighborhood structure (10 skeletal voxels on each side of V along the skeleton) was fitted to a 5th degree polynomial curve. This fitted curve of the skeleton was then used to first calculate lines tangential to it and then to calculate the lines perpendicular to it at the location V . The voxels along the perpendicular line that were also located within the WM binary mask computed earlier were considered for computing the statistical measure for the skeletal voxel V in the next step. This method was followed for all skeleton voxels.

2c. Defining skeleton values: The skeletons represent the center of the WM tracts. However, we replaced the values in the skeleton voxels with several specific statistical measures (mean, median, maximum and mode) of the voxel values on the line perpendicular to the skeleton for *each* DTI index. The white matter binary mask and skeleton derived from the FA maps were superimposed onto other DTI maps (RD, AD and ADC). The

perpendicular line was computed for *every* skeletal voxel in *each* DTI map and the values along that line were collected. The mean, median, maximum and mode of these values were calculated and assigned to the corresponding skeletal voxel. The mean values are reported in this paper. To obtain a single volumetric statistic for each subject, we computed the mean and standard deviation of each statistical measure and DTI index for the entire interpolated 3D brain (total).

3. Brain anatomy detection—DTI is not the optimal modality for brain anatomy detection. For this, T1WI data were used and the following steps were followed and detailed in the following subsections.

3a. Segmentation: Segmentation routines were used to compute the percentage of each voxel in gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using T1WI data with the Statistical Parameter Mapping (SPM5) software (The Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, London, England) (<http://www.fil.ion.ucl.ac.uk/spm/>). Separate binary masks of these regions were saved for each brain T1WI slice. These data were used later for information fusion.

3b. Brain parsing/labeling: We determined brain anatomic regions from the T1WI data and co-registered DTI b0 scan volumes of each subject to their own T1WI volume and their corresponding anatomical regions obtained in step 2. To do this an established pediatric brain parsing pipeline with the Laboratory of Neuroimaging (LONI) brain Parser software from the University of Southern California, Los Angeles, USA (<http://www.pipeline.loni.usc.edu>) (namely “BrainParser (Pediatric Atlas)”) was modified to handle anisotropic pixel size of our T1WI data, as well as to optimize internal operational parameters, e.g., virtual-memory and stack-size parameters in “Syn” and “SSMA” modules for our specific purpose and used to label 83 T1 derived anatomic brain regions. Our modified LONI pediatric pipeline is shown in supplemental fig. 1.

3c. Co-registering DTI b0 and T1WI: Co-registration was performed between the b0 dataset from DTI (effectively T2 weighted images) and T1WI so that for each voxel, T1-based tissue classes and anatomic regions (from steps a and b above) could be subsequently utilized for fusion of DTI and MRSI data to directly compare measured parameters from both. Co-registration of the b0-volume and T1WI volume and subsequently translating T1-space anatomy to DTI-space was performed by another LONI workflow (supplemental fig. 2) developed from the available pipeline modules. Using Matlab, the 83 regions were then merged into 17 regions to directly compare developmental trends and comparisons between lower resolution MRSI data and DTI data (fig. 4a–c). Left and right regions were then merged into one region to finally produce 12 anatomic regions and 3 aggregate regions used for comparison between DTI and MRSI parameters (table 1).

MRSI Data Processing

MRSI spectra were post-processed using LCModel (Stephen Provencher Inc., Oakville, Ontario, Canada; LCModel Version 6.0) which allows quantification of the metabolites from the *in vivo* data. LCModel is a well-established, commercially available, automatic (user-

independent), time-domain fitting routine that employs a basis set of concentration calibrated model spectra of individual metabolites to estimate the absolute concentrations of similar brain metabolites from the in-vivo spectral data correcting for residual eddy current effects. For each MRSI voxel, individual metabolite concentrations are reported with a confidence measurement (SD%) reflecting maximum-likelihood estimates and their uncertainties (Cramer-Rao lower bounds). Metabolite concentrations with a SD% of 10 or less were included in this study. Concentrations for N-acetyl-aspartate (NAA; 2.02 ppm), total creatine (Cr; 3.02 ppm), total choline (Cho; 3.20 ppm), and Lactate (Lac), if present (identified as an inverted doublet at 1.33 ppm and 7-Hz splitting) were measured for each voxel that met the above criteria. Peak concentrations for each voxel were recorded in a spreadsheet for further analysis and used to calculate metabolite ratios (NAA/Cr, NAA/Cho, Cho/Cr and Lac/Cr).

Finding DTI Parameters, Tissue-types, and Anatomy for each MRSI Voxel

The 3D MRSI grid was manually oriented during scanning to cover relevant brain regions from above the CC through the top of the brain stem. The 3D location and orientation parameters in the MRSI data header were used to find anatomic correspondence between MRI and MRSI voxels. In-house software co-registered the 3D MRSI slabs with 3D T1WI/T2WI for assigning the anatomic location of the voxel and the segmented 3D T1WI to estimate the percent CSF, WM and GM for each voxel. To limit partial volume errors, only voxels with greater than 80 percent WM or GM were included in the analysis and voxels with greater than 50% CSF were excluded. This software was developed in our laboratory incorporating routines from Matlab and SPM5. MRSI voxels from the frontal, parietal and temporal regions were assigned as GM or WM resulting in data from a total of 12 anatomic regions listed in table 1.

The MRSI grid for each slab was also overlaid onto 3D DTI skeleton masks to obtain DTI parameters (mean FA, AD, RD, ADC) of each voxel for comparison to metabolite ratios (fig. 4d–e). The assigned anatomic regions and DTI results for each voxel were saved and mean values for each MRSI ratio and DTI parameter (WM and deep GM only) were then calculated for the spectroscopy defined individual regions. Mean values for measured parameters were also calculated for the following aggregate regions: Superficial Brain (SB); Subcortical (SC); Posterior Fossa (PF); Total (all regions) as defined in table 1.

Statistical Analysis

We performed multiple regression analyses (SPSS 22, Chicago, IL, USA) with age and gender as covariates to determine whether age-related changes in DTI parameters and MRSI metabolite ratios were present. These changes were considered to be statistically significant when the regression analyses yielded slopes that were significantly different from zero at a level of $P < 0.05$. To quantify possible outlier values, Studentized Deleted Residuals (SDR) from mean FA, ADC, AD, RD, NAA/Cr, NAA/Cho and Cho/Cr ratios were calculated. Regression analyses were performed again after outlier analysis, excluding individuals exceeding $SDR \pm 3$ to make sure that these subjects did not overly affect the results. A histogram with superimposed normal curve and a P-P probability plot were also used to assess normality for change in DTI parameters and MRSI metabolite ratios with age.

All variables were assessed for normality using the Kolmogorov-Smirnov and Shapiro-Wilks procedures. We found that all variables were normally distributed. We did not find significant left versus right differences between MRSI nor DTI parameters that were considered clinically significant (i.e. greater than 2 standard deviations). To determine whether DTI parameters were linearly related to metabolite ratios, Pearson correlation coefficients were calculated.

An outcome-based method was used to determine an optimal cut-point for age regarding DTI and MRSI parameters using SAS 9.3 (SAS Institute Inc., Cary, NC). Given the absence of *a priori* clinical information regarding the covariate relationship between age and MRSI values, a cut-point threshold of 12 years of age was determined empirically with graphical methods including grouped data plots and Martingale residuals used to investigate the lack of model fit. In addition, a two-fold cross-validation technique was used to determine the statistical significance of the cut-point threshold of 12 years of age that included the steps of randomly assigning all observations into either of two data sets of approximately equal size (n=24). Using similar methods, no reliable age-stratification threshold was observed for DTI parameters.

Results

Normal Developmental Patterns of DTI Parameters

DTI parameter changes with age are shown in graphs for individual and combined regions with linear fits, confidence intervals and R^2 values (fig. 5a–i). We found that significant increases of mean FA and decreases in mean diffusivity with age were regionally dependent. Results of multiple regression analyses to evaluate for significant changes with age for DTI and MRSI parameters are shown in table 2.

Several regions showed significant maturational changes for several DTI parameters, however, for this age range, the percent change tended to be small. In the posterior fossa which included only the brain stem /pons region (BS; fig. 5a), the largest change showed a 9% decrease of RD ($p=0.014$). In the subcortical region (fig. 5h) that included the combined basal ganglia (BG; fig. 5b), thalami (TH; fig. 5c) and corpus callosum (CC; fig. 5d), the largest combined percent change was an increased FA of 10% primarily due to increases in the BG (12.7%) and TH (9%). Although no change in FA was seen in the CC, a 15% decrease of RD was recorded. Maturational changes in the superior brain regions (fig. 5i) (i.e., temporal, TW-fig. 5e; frontal, FW-fig. 5f; and parietal, PW-fig. 5g) were observed in which, the largest combined percent change was a decreased ADC (−9.7%) due to a decrease in all three white matter regions. A 12.6% decrease of RD was also seen in the TW.

Normal Developmental Patterns of Metabolite Ratios

Metabolite changes with age are shown in graphs for individual and combined regions with linear fits, confidence intervals and R^2 values (fig. 6a–o). Several regional metabolite ratios showed significant change with age using multiple regression analyses (table 2). Overall, metabolite ratios showed greater percent maturational changes than the DTI parameters. The largest percent changes were seen with NAA/Cho ratios which may be expected since NAA

and Cho are changing in opposite directions. The largest significant percent increase in NAA/Cr was seen in the BS (18.8%; fig. 6b) followed by the subcortical regions in the BG (11.9%; fig. 6e), CC (8.9%; fig. 6g) and the TH (6.0%; fig. 6f). None of the superficial brain, white or gray matter regions showed a significant increase of NAA/Cr with age. The largest percent decreases in Cho/Cr were seen in the posterior superior brain regions of the OG (−14.6%; fig. 6j) and the PW (−12.5%; fig. 6k).

When subjects were stratified into two groups based on an age cut-point threshold of 12 years determined empirically with graphical methods, we found that the mean values of all ratios were within 1 standard deviation between the two groups even when statistical differences were highly significant (table 3). Overall, the mean NAA/Cr ratios were slightly higher and the mean Cho/Cr ratios were slightly lower for the older age group as would be expected with brain maturation.

Correlations between DTI Parameters and Metabolite Ratios

We found consistent, significant ($p < 0.01$) but weak positive correlations ($r = 0.228$ – 0.329) between NAA ratios and mean FA in the BS, BG and CC regions. No significant correlations between thalamic DTI and metabolite ratios were found. In white matter regions, no correlations between mean FA and NAA ratios were found, however, significant ($p < 0.01$), weak negative correlations ($r = -0.272$ to -0.357) were found between NAA ratios and WM ADC and AD measurements.

Discussion

Although we are reporting only normative data in this study, these methods were developed to ultimately compare data between healthy controls and TBI patients. We decided to take this approach since a key limitation of the TBSS method is the requirement of registering DTI tracts between subjects – for example, either healthy controls over time (for neurodevelopment) or controls to TBI patients (for neurodegeneration) – while handling tract-deformations robustly. Although TBSS skeletons are claimed to be alignment-invariant [25], skeletal voxel-wise comparison will suffer from the possibility of one-to-many mapping between two skeletons, accounting for fiber crossings, as well as branching – specifically between the control template and severely injured (deformed) brains [34, 35]. Also as pointed out in neurodevelopment studies, tract-based registration and comparison might suffer in cortical boundaries due to inherent WM variations, specifically in children [8, 26]. For large deformations in severe TBI [35, 36], such tract-wise registration might not work well, leading to considerable errors, and hence manual placement of bilateral ROI's on relevant tracts is often adopted as the last resort [35, 37]. The current method might be more robust in detecting inherent subtle variation in developing brains as its alignment is based on much larger regions (brain anatomy, compared to voxels or skeletons or WM tracts) including both WM and GM tissue.

To detect the WM regions, the current study utilized a mid-level FA threshold, $FA > 0.2$ that has been recently reported to be inclusive enough for robust statistical comparison [2, 28, 38–40] and also has been validated in our study (see fig.3). When FA constraints are too relaxed (e.g., $FA > 0.15$) [26, 41], small sub-tracts may be included leading to false positive

results. On the other hand, restrictive FA constraints, (e.g. $FA > 0.25$) [28, 31, 42] or $FA > 0.3$ [25] exclude WM regions and hence suffer from high false-negative rates. Although some research studies support a more inclusive FA threshold in analysis of neonatal white matter [43], demarcation between adult and neonates is uncertain as WM maturation is found to decelerate after age 2 years [8].

For anatomic parcellation of DTI data, previous studies have adopted a variety of methods—including (1) manual demarcation of selected DTI anatomy [38, 40], (2) utilization of T1 space atlas directly in DTI space [24], (3) estimation of indigenous DTI templates of the concerned age-group from TBSS [26] or tractography-based data [39], and (4) utilization of established adult DTI atlases even for pediatric data with an assumption of less structural difference after 2 years of age [1, 8, 31]. The current work adopts yet another option, by registering the pediatric subject's T1/T2-data with an established pediatric T1/T2-atlas and then registering the subject's T1/T2 with its own DTI-b0 data to map T1/T2 anatomy/tissue information into the subject's DTI coordinate space. This is probably a more logical approach because in the first stage of registration, ages are comparable and the MR techniques are the same; only the subjects are different. While in the second stage of registration, the subject and thus the age are the same, just the MR technique is different [2, 26].

MRS data complements DTI and other MRI data in multimodal studies [41, 44, 45]. Anatomic parcellation of MRS data has been performed by prior knowledge based manual bilateral ROI placement [35], ad-hoc heuristics [41] or an established (Harvard-Oxford) atlas [46]. One recent work reports explicit co-alignment of MRSI data, first to corresponding high-resolution T1 data and then to a T1-atlas space, using an additional low-resolution T1-overlay data with the same geometry as the MRSI data explicitly collected for this purpose [45]. Another study on 4 volunteers used a methodology based on linear displacement, angular rotation and pixel-based interpolation of DTI images to co-register MRSI voxels to DTI images [47]. In this study a similar approach was used with the image geometry of different acquisitions (MRSI, T1, T2, and DTI) being directly compared to determine image transformation parameters, map anatomical information from T1/T2 atlases to the MRSI coordinates, and fuse anatomy-specific MRS data with DTI. This was done to give us the capability to directly compare metabolite with DTI maturational changes in the same anatomic voxels or regions.

In agreement with other studies, we found evidence for white matter maturation throughout various brain regions from childhood through adolescence using DTI [3, 6, 48–50]. For children and adolescents included in this study (age range 5–18 years), most age-related changes in DTI parameters were below 10%, also in agreement with other authors showing subtle changes after age 5 years. Our methods did not include measurements of specific white matter tracts since we were trying to directly compare regions between MRSI sized voxels and DTI parameters within those voxels. We found that the largest significant increases of FA with age were in the subcortical regions of BG and TH (average of 10.9%; table 2) which may reflect increasing myelination, axonal density and diameter as well as overall maturation of the basal-ganglia-thalamo-cortical pathways [48]. We also observed small but significant decreases of AD, a measure of axonal volume, in the superior brain

WM regions (FW, PW, TW) and the CC. More significant decreases in RD, a measure of myelination, were seen in the BG, CC, TW, and FW with associated increases in FA suggesting that decreases in RD contributed more to increasing FA in brain maturation. Decreases in AD and RD may respectively reflect increasing compactness and myelination [51]. In agreement with other studies [8, 52, 53], we found that ADC, as an indicator of the total amount of diffusion, decreased with age in all regions except the TH. Although, we show that changes in DTI indices are relatively small in the 5-18 year age range, it may be necessary to take these small regional changes into consideration in order to detect subtle abnormalities especially in diseases where the brain may be mildly affected or when longitudinal studies (e.g. clinical trials) over extended time periods are performed.

Changes in metabolite ratios were found in normal subjects in the age range studied and are thought to be related to normal maturational changes. In general, the metabolite changes are a result of both neuronal differentiation seen as increasing NAA levels, and ongoing myelination seen as decreasing choline levels [18]. The NAA/Cr ratios showed the highest percent change in the BS followed by the subcortical regions and no change with age in the superior GM and WM regions. However, this study is reporting ratios rather than individual metabolite levels and we must take into consideration that the reason we are not seeing changes in the superior brain regions is not because maturation is not taking place but because Cr is also increasing. Particularly since the NAA/Cho ratios showed a greater significant increase in all regions (except the PG, TG and CBL) indicating that these changes appear to be related to both increasing NAA (as NAA/Cr and NAA/Cho) and declining Cho (as Cho/Cr). Other quantitative metabolite studies report a consistent subtle total Cr increase in the posteromedial cortex [54] and parietal/occipital GM regions [18] through adolescence. In our study, Cho/Cr ratio changes were greatest in the PW and OG regions which would support that changes were occurring due to decreasing Cho, as well as, increasing Cr. All other superior brain regions showed decreasing Cho/Cr ratios which may be associated with increasing myelination. It is interesting to note that frontal regions showed less change than posterior regions which follows prior brain maturation studies that emphasize a complex posterior to anterior gradient through adolescence and into adulthood [55].

Although we show significant spectroscopic changes with age, the rates of change are subtle compared to changes seen from birth through early childhood and agree with several other reports of normal metabolite changes in children and adolescents [19, 20]. In addition, we found that metabolite ratios varied slightly more from 5–12 years of age than from 12–18 years of age (table 3) which seems to be a natural division of age used as neurobehavioral landmarks in clinical neuropsychological studies [18]. However, when comparing mean values between the two age groups, no regions showed significant differences in Cho/Cr ratios and only a few regions showed significant differences with NAA ratios. In addition, means between groups were within one standard deviation demonstrating that metabolite ratio changes are subtle in the age range studied. Nevertheless, these means demonstrate slightly higher NAA ratios and lower Cho/Cr ratios for the older age group which is expected with brain maturation and can be used for comparison with other pediatric cohorts.

In this study, we did not observe any strong correlations between regional DTI parameters and corresponding regional metabolite ratios. This may be a result of comparing averaged

DTI measures from white matter contained within large brain regions rather than specific white matter tracks. One study in healthy adult volunteers using voxel based analysis and color coded correlation maps showed a strong positive correlation between quantitative NAA measurements and FA [45] only in the superior longitudinal fasciculus (SLF) while another tractography study showed strong correlation with the uncinate fasciculus [41]. These findings may be attributed to greater neuronal density and a greater structural organization in specific white matter tracts. Another study using similar methods to our own showed a strong negative correlation of ADC with NAA in an area containing the SLF in four adult volunteers, however, correlations between other DTI measures and metabolites showed trends only [47]. We also saw significant weak to moderate inverse correlations with ADC in WM regions. This may represent diffusion restriction of water in WM as a result of increased neuronal integrity. The thalamus was the only region in our study that showed no correlation between MRSI and DTI. A previous study [45] attributed lack of correlation in the thalamus to the presence of crossing fibers causing the net FA to equal nearly 0 in the thalamus.

Limitations

Both DTI and MRSI acquisitions are prone to various artifacts particularly in the pediatric population including patient motion and signal to noise sensitivity. Since subjects in this study were at least 5 years old, we were able to scan all subjects without sedation. We also took time to familiarize the children within the scanner environment to make them more comfortable before scanning. Images and spectra were checked for motion and repeated if necessary before the patient was released and studies were rejected if the subject was not able to hold still. Also, the SNR was similar in all patients since subjects were old enough that we did not need to change acquisition voxel size due to small head size. Partial volume effects can be pronounced in MRSI acquisitions. In this study, we used segmentation software to assign voxel tissue percentages in order to limit the partial volume effects. Rejection of outliers and cleaning of datasets with missing entries [17, 46, 56] were used in the current study. DTI images can also suffer from pulsation artifacts, hardware instability and susceptibility artifacts at tissue-air interfaces and particularly due to metal, therefore, children with braces were not included in the study.

Volumetric registration across multiple MRI and MRSI acquisition spaces of the subject and atlas data with different geometry and resolution is challenging. One limitation of the current method is suboptimal brain parsing in the subcortical region that is inherent in T1-based brain atlases. This can be improved by utilizing a T1-atlas for cortical regions and a T2-atlas for subcortical regions for brain parsing and subsequently merging them, at the cost of an additional registration step between subjects' T1WI and T2WI data. In the future when using this method for patients with significant brain injury (as in severe TBI), a modification of the current registration method may be needed to handle image distortion caused by deformities or large hemorrhages or contusions such as temporary removal of the distorted brain region before volumetric registration. The computed image transformation matrix can then be utilized to reinsert the injured region and then estimate its anatomy-specific statistics. In addition, methods to display subject specific abnormalities will be utilized for whole brain

DTI statistics based on Z-score distributions [57]. Such complexities would be handled in our future work.

Conclusions

We report on an automated computational method to anatomically fuse and directly compare data from whole brain DTI and 3D MRSI acquisitions from pediatric control subjects. We also provide extensive age- and region-specific normative MR diffusion and spectroscopic metabolite ranges that show brain maturation profiles and are requisite for detecting abnormalities in an injured or diseased population. Our particular interest is related to pediatric traumatic brain injury (TBI), however, the normative values presented in this paper can be applied for comparison to any injured or diseased pediatric population when similar acquisition techniques are used. Another unique aspect of this study is fusing spectroscopic and DTI data for direct regional comparisons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Overall flow diagram of the multi-modality information fusion methodology adopted in this work. The subject's high resolution T1 data was input into an atlas-based brain parcellation algorithm and segmented into tissue classes (white matter, gray matter, and cerebrospinal fluid) as well as 17 brain anatomic regions in T1/T2 space. The subject's diffusion and spectroscopy data was co-aligned with its own T1/T2 data to transfer tissue and anatomy information to DTI and MRSI spaces. Finally, tract-based statistics from DTI and metabolite

information from MRSI voxels were fused for individual brain anatomy and tissue regions to estimate normative developmental trends in a pediatric dataset.

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Fig. 2.

Representative skull-stripping results from the b0 diffusion scan and CSF-removal based on the ADC map. DTI b0 data (**a**) was first normalized within [0, 1] and binarized (**b**) using normalized intensity > 0.005 followed by morphological opening i.e., dilation followed by erosion, filling internal holes in foreground (white), and area-based filtering i.e. removing regions $< 5\%$ of DTI acquisition matrix size performed in sequence to obtain final skull-stripped DTI brain mask (**c**). This mask was used for other tensor images and for the rest of the methods. DTI b0 based skull-stripped ADC obtained from (**c**) was binarized by $\text{ADC} > 5 \times 10^{-5} \text{ mm}^2/\text{sec}$ threshold, morphologically cleaned, and small outlier regions were removed by area-based filtering (**d**). CSF was removed using $\text{ADC} < 500 \times 10^{-5} \text{ mm}^2/\text{sec}$ threshold (**e**), further morphologically cleaned (**f**), and the ventricle was reinstated into the DTI brain mask (**g**) to get the final skull/CSF-stripped ADC map (**h**). The final brain mask (**g**) was used to clean the rest of the DTI maps before further processing.

Fig. 3.

Illustration of FA based detection of white-matter region and corresponding skeleton. Skull- and CSF-stripped FA map (a) was binarized (b) with thresholding ($FA > 0.2$) and morphologically cleaned to obtain WM mask region (c), from which FA skeleton (blue), WM perimeter (green) and regional maxima (red) were computed (d). The WM regions and skeleton from the FA map were used for the remaining DTI maps for statistical analysis. Rationale for using $FA > 0.2$ for detecting WM is illustrated in the bottom row. Smaller FA thresholds of $FA > 0.1$ (e) or $FA > 0.15$ (f) over-estimated the WM regions, while larger thresholds of $FA > 0.25$ (g) or $FA > 0.3$ (h) under-estimated them compared to $FA > 0.2$ (d), which performed better and hence was utilized in the current study.

Fig. 4.

Examples of automated brain parsing and overlay of MRSI data on DTI space. **(a–c.)**

Regional boundaries of 17 different regions are color-coded and superimposed on corresponding DTI-b0 data for visualization. These regions were utilized for region specific statistics of different DTI parameters. **(d–e.)** A mean value for each DTI parameter was calculated for each MRSI voxel based on the DTI skeleton pixel values within the MRSI voxel. The skeleton pixels located within each MRSI voxel can represent different statistics (mean, median, maximum, mode) for different DTI metrics (FA, MD, AD, RD) calculated from pixels located along perpendicular lines to the skeleton.

Fig. 5. Measured DTI parameters for mean fractional anisotropy (FA), mean apparent diffusion coefficient (ADC), mean axial diffusivity (AD), and mean radial diffusivity (RD) for 7 brain regions and two combined regions; Subcortical (basal ganglia+thalamus+corpus callosum) and Superficial Brain (temporal+parietal+frontal white matter (WM)) versus age in years (yrs). The linear regression fits, R^2 value, and 95% confidence intervals are shown.

Fig. 6. Measured MRSI parameters for NAA/Cr (creatine), NAA/Cho (Cho) and Cho/Cr for 12 brain regions and three combined regions; Subcortical (basal ganglia+thalami+corpus callosum), Superficial Brain (temporal+parietal+frontal white matter (WM) and gray matter (GM)) and Posterior Fossa (brain stem+cerebellum) versus age in years (yrs). The linear regression fits, R^2 value, and 95% confidence intervals are shown.

Table 1

Individual and Aggregate Brain Regions used for Comparisons

Superficial Brain (SB)	Subcortical (SC)	Posterior Fossa (PF)
Frontal White Matter (FWM)	Basal Ganglia (BG)	Brain Stem/Pons (BS)
Frontal Gray Matter (FGM)	Corpus Callosum (CC)	Cerebellum (CB)
Occipital Gray Matter (OGM)	Thalami (TH)	
Parietal White Matter (PWM)		
Parietal Gray Matter (PGM)		
Temporal White Matter (TWM)		
Temporal White Matter (TWM)		

Table 2

Metabolite Ratio and DTI Parameter Regression Analyses with Age

Region	NAA/Cr	NAA/Cho	Cho/Cr	Mean FA	ADC	AD	RD
BS	F(2,79)=8.7	F(2,79)=12.9	F(2,80)=3.1	F(2,73)=0.4	F(2,75)=4.3	F(2,76)=3.7	F(2,76)=4.5
	p <0.001	<0.001	0.04	n.s.	0.02	0.03	0.014
	18.8%	23.4%	-7.3%	n.s.	-7.7%	-8.2%	-8.9%
BG	F(2,89)=9.4	F(2,87)=17.2	F(2,88)=4.5	F(2,89)=11.3	F(2,89)=3.6	F(2,89)=2.1	F(2,88)=8.3
	p <0.001	<0.001	0.014	<0.001	0.03	n.s.	0.001
	11.9%	18.1%	-9.3%	12.7%	-3.8%	n.s.	-9.5%
CC	F(2,87)=5.9	F(2,88)=8.8	F(2,87)=4.5	F(2,87)=1.03	F(2,88)=13.1	F(2,87)=7.3	F(2,87)=12.4
	p 0.004	<0.001	0.014	n.s.	<0.001	0.001	<0.001
	8.9%	15.5%	-9.3%	n.s.	-10.3%	-7.3%	-15.1%
TH	F(2,89)=4.2	F(2,89)=8.7	F(2,88)=5.5	F(2,89)=3.2	F(2,89)=0.2	F(2,88)=0.7	F(2,89)=0.3
	p 0.018	<0.001	0.006	0.047	n.s.	n.s.	n.s.
	6.0%	13.5%	-8.9%	9.2%	n.s.	n.s.	n.s.
FGM	F(2,88)=0	F(2,88)=2.2	F(2,86)=4.5				
	p n.s.	n.s.	0.013				
	n.s.	n.s.	-5.9%				
FWM	F(2,89)=0.9	F(2,89)=7.4	F(2,87)=6.8	F(2,89)=5.1	F(2,86)=21.1	F(2,87)=23.1	F(2,88)=11.7
	p n.s.	0.001	0.002	0.008	<0.001	<0.001	<0.001
	n.s.	19.2%	-10.8%	4.4%	-10.1%	-8.8%	-11.7%
OGM	F(2,74)=1.2	F(2,75)=3.5	F(2,76)=3.8				
	p n.s.	0.035	0.026				
	n.s.	13.6%	-14.6%				
PWM	F(2,86)=2.7	F(2,85)=9.7	F(2,85)=6.7	F(2,84)=1.3	F(2,85)=5.4	F(2,83)=8.5	F(2,86)=2.1
	p n.s.	<0.001	0.002	n.s.	0.006	<0.001	n.s.
	n.s.	19.2%	-10.8%	n.s.	-5.9%	-7.2%	n.s.

Region	NAA/Cr	NAA/Cho	Cho/Cr	Mean FA	ADC	AD	RD
PGM	F(2,85)=0.1	F(2,85)=2.7	F(2,86)=3.2				
	p n.s.	n.s.	0.046				
	n.s.	n.s.	-7.9%				
TWM	F(2,89)=0.3	F(2,88)=5.6	F(2,89)=7.1%	F(2,87)=11.4	F(2,89)=31.1	F(2,89)=21.2	F(2,88)=27.6
	p n.s.	0.005	0.001	<0.001	<0.001	<0.001	<0.001
	n.s.	11.6%	-9.9%	4.8%	8.8%	-6.1%	-12.6%
Post. Fossa	F(2,80)=8.7	F(2,80)=5.5	F(2,79)=1.4	F(2,89)=11.3	F(2,75)=4.3	F(2,76)=3.7	F(2,76)=4.5
	p <0.001	0.006	n.s.	<0.00	0.02	0.03	0.014
	19.1%	15.3%	n.s.	12.7%	-7.7%	-8.2%	-8.9%
Sub-Cortical	F(2,89)=14.2	F(2,88)=20.1	F(2,87)=8.8	F(2,89)=21.5	F(2,88)=1.5	F(2,87)=1.3	F(2,88)=4.4
	p <0.001	<0.001	<0.001	<0.001	n.s.	n.s.	0.015
	10.9%	17.7%	-10.4%	10.9%	n.s.	n.s.	-5.7%
Sup. Brain	F(2,89)=4.7	F(2,88)=14.8	F(2,89)=7.2	F(2,85)=4.1	F(2,88)=22.8	F(2,88)=18.9	F(2,87)=16.1
	p 0.011	<0.001	0.001	n.s.	<0.001	<0.001	<0.001
	9.7%	15.1%	-7.3%	n.s.	-9.7%	-8.3%	-10.8%

F-statistic and p value reported from multiple regression analyses. = % change from 5–18 years. Cerebellum (CBL) and Temporal Gray Matter (TGM) regions (not listed) showed no significant differences. n.s.= non-significant (p > 0.05). Post. Fossa=Posterior Fossa, Sup. Brain=Superficial Brain.

Table 3Mean Metabolite Ratios $\pm$ 1 Standard Deviation for Age Groups

	NAA/Cr		NAA/Cho		Cho/Cr	
	< 12 years	12 years	< 12 years	12 years	< 12 years	12 years
BG	2.01 $\pm$ 0.20	2.12 $\pm$ 0.21 [*]	1.91 $\pm$ 0.31	1.99 $\pm$ 0.26	1.1 $\pm$ 0.13	1.07 $\pm$ 0.08
BS	1.80 $\pm$ 0.20	1.99 $\pm$ 0.20 ^{***}	1.43 $\pm$ 0.20	1.61 $\pm$ 0.19 ^{***}	1.28 $\pm$ 0.14	1.25 $\pm$ 0.13
CBL	1.60 $\pm$ 0.34	1.66 $\pm$ 0.32	1.81 $\pm$ 0.28	1.92 $\pm$ 0.33	0.89 $\pm$ 0.13	0.86 $\pm$ 0.10
CC	2.73 $\pm$ 0.23	2.90 $\pm$ 0.21 ^{**}	2.28 $\pm$ 0.42	2.47 $\pm$ 0.29 [*]	1.27 $\pm$ 0.16	1.21 $\pm$ 0.11
FG	2.40 $\pm$ 0.33	2.43 $\pm$ 0.28	2.23 $\pm$ 0.38	2.23 $\pm$ 0.35	1.14 $\pm$ 0.12	1.13 $\pm$ 0.10
FW	2.63 $\pm$ 0.34	2.73 $\pm$ 0.31	2.40 $\pm$ 0.40	2.50 $\pm$ 0.35	1.17 $\pm$ 0.16	1.13 $\pm$ 0.10
OG	2.40 $\pm$ 0.40	2.50 $\pm$ 0.35 [*]	2.60 $\pm$ 0.56	2.87 $\pm$ 0.49 ^{**}	0.98 $\pm$ 0.11	0.89 $\pm$ 0.13
PG	2.45 $\pm$ 0.28	2.56 $\pm$ 0.30	2.58 $\pm$ 0.48	2.75 $\pm$ 0.51	1.00 $\pm$ 0.13	0.97 $\pm$ 0.09
PW	3.12 $\pm$ 0.29	3.26 $\pm$ 0.25 [*]	2.93 $\pm$ 0.44	3.23 $\pm$ 0.44 ^{**}	1.09 $\pm$ 0.16	1.04 $\pm$ 0.11
TG	1.99 $\pm$ 0.21	1.98 $\pm$ 0.22	1.93 $\pm$ 0.26	1.94 $\pm$ 0.22	1.05 $\pm$ 0.10	1.05 $\pm$ 0.09
TW	2.47 $\pm$ 0.22	2.47 $\pm$ 0.20	2.09 $\pm$ 0.19	2.19 $\pm$ 0.24	1.18 $\pm$ 0.12	1.15 $\pm$ 0.09
TH	2.12 $\pm$ 0.20	2.18 $\pm$ 0.20	1.80 $\pm$ 0.24	1.89 $\pm$ 0.24	1.21 $\pm$ 0.15	1.18 $\pm$ 0.09
Subcortical	2.22 $\pm$ 0.18	2.35 $\pm$ 0.15 ^{**}	1.89 $\pm$ 0.28	2.10 $\pm$ 0.21 [*]	1.18 $\pm$ 0.12	1.14 $\pm$ 0.08
Post. Fossa	1.74 $\pm$ 0.21	1.90 $\pm$ 0.18 ^{**}	1.53 $\pm$ 0.20	1.67 $\pm$ 0.20 ^{**}	1.19 $\pm$ 0.11	1.16 $\pm$ 0.11
Sup. Brain	2.47 $\pm$ 0.18	2.59 $\pm$ 0.17 ^{**}	2.33 $\pm$ 0.22	2.51 $\pm$ 0.22 ^{**}	1.09 $\pm$ 0.12	1.07 $\pm$ 0.07
TOTAL	2.33 $\pm$ 0.16	2.47 $\pm$ 0.13 ^{***}	2.15 $\pm$ 0.21	2.33 $\pm$ 0.19 ^{***}	1.13 $\pm$ 0.11	1.10 $\pm$ 0.07

Post. Fossa= Posterior Fossa, Sup. Brain = Superficial Brain;

^{*} p< 0.05,^{**} p<0.01,^{***} p<0.001