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Structural development of human brain white matter from mid-fetal to perinatal stage

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Abstract

The structures of developing human brain white matter (WM) tracts can be effectively quantified by DTI-derived metrics, including fractional anisotropy (FA), mean, axial and radial diffusivity (MD, AD and RD). However, dynamics of WM microstructure during very early developmental period from mid-fetal to perinatal stage is unknown. It is difficult to accurately measure microstructural properties of these WM tracts due to severe contamination from cerebrospinal fluid (CSF). In this study, high resolution DTI of fetal brains at mid-fetal stage (20 weeks of gestation or 20wg), 19 brains in the middle of 3rd trimester (35wg) and 17 brains around term (40wg) were acquired. We established first population-averaged DTI templates at these three time points and extracted WM skeleton. 16 major WM tracts in limbic, projection, commissural and association tract groups were traced with DTI tractography in native space. The WM skeleton in the template space was inversely transformed back to the native space for measuring core WM microstructures of each individual tract. Continuous microstructural enhancement and volumetric increase of WM tracts were found from 20wg to 40wg. The microstructural enhancement from FA measurement is decelerated in late 3rd trimester compared to mid-fetal to middle 3rd trimester, while volumetric increase of prefrontal WM tracts is accelerated. The microstructural enhancement from 35wg to 40wg is heterogeneous among different tract groups with microstructures of association tracts undergoing most dramatic change. Besides decreases of RD indicating active myelination, the decrease of AD for most WM tracts during late 3rd trimester suggests axonal packing process.

Keywords

white matter; tract; microstructure; template; development; fetal; DTI; skeleton

1. INTRODUCTION

Structural characterization of white matter (WM) maturational processes from mid-fetal stage (around 20 weeks of gestation (wg)) to normal time of birth (40wg) is critical for understanding not only the normal development of WM tracts but also the clinical implications associated with abnormal WM development such as periventricular leukomalacia (PVL) ^{e.g. 1}. Valuable insights into microstructural and macrostructural processes of the WM maturation in this period have been offered by DTI of fetal brain specimens ² and DTI of *in vivo* preterm and term brains ^{e.g. 3–5}. We hypothesized that spatiotemporally heterogeneous microstructural and macrostructural development takes place across different time periods of 20–35wg and 35–40wg and across different WM tracts. With high resolution DTI of early developing brains at 20wg, 35wg and 40wg, establishment of population-averaged age-specific template, WM skeleton extraction and DTI-based tractography of individual tracts, we aimed to understand the dynamics of structural changes of individual tracts, including myelination, axonal packing and volume growth, in 2nd half of fetal development.

2. METHODS AND MATERIALS

2.1 Ex vivo mid-fetal brain specimens at 20wg and in vivo neonates at 35wg and 40wg

46 normal subjects were involved in this study and divided into three groups according to the weeks of gestation (wg), including 10 *ex vivo* fetal brains in the middle of 2nd trimester (age range of 19wg to 20g with mean and standard deviation 19.5 ± 0.52 wg), 19 perinatal brains in the middle of 3rd trimester (age range of 34.3wg to 35.8wg with mean and standard deviation 35.1 ± 0.55 wg) and 17 perinatal brains around term (age range of 40wg to 41.7wg with mean and standard deviation 40.7 ± 0.55 wg). The *ex vivo* fetal brains were obtained from the University of Maryland Brain and Tissue Bank (BTB) for Developmental Disorder (NICHD contract no. N01-HD-9-0011). All the preterm and term neonates were recruited from Parkland Hospital at Dallas. All included neonates were part of the cohort for studying normal perinatal and prenatal development and were selected after rigorous screening procedures conducted by the neonatologist (LC). Neonates were well fed before scanning. All subjects gave informed written consent approved by the Institutional Review Board. Normality of the *ex vivo* fetal brain at mid-fetal stage was ensured by the BTB. Normality of *in vivo* perinatal brains at around 35wg and 40wg was ensured by screening procedures and confirmed by reading of their MR from a pediatric radiologist (NR).

2.2 DTI of ex vivo mid-fetal brain specimens at around 20wg

Fetal brain samples around 20 wg were kept immersed in the fixation solution until 48 hours before the MR experiments. The samples were then transferred to PBS to wash out the fixative. The samples were immersed in PBS in a custom-made MR compatible chamber throughout the MR scanning. 3D multiple spin echo diffusion tensor imaging was performed in a 4.7 T Bruker scanner with a 70mm inner diameter Bruker volume coil. A multiple echo (number of echoes = 8) sequence was adopted to improve the signal-to-noise ratio (SNR). A set of diffusion weighted images (DWI) were acquired in seven linearly independent directions with following parameters: effective TE=66ms, TR=0.8s, FOV=48–52mm/48–

52mm/48–52mm, imaging matrix=128×72×72 (zero filled to data matrix=128×128×128), b value=1000s/mm². The resultant image resolution was isotropic 280µm to 350µm. The total imaging time was approximately 20 hours per brain with two signal averages.

2.3 DTI of in vivo neonates at around 35wg and 40wg

All neonates were well fed before scanning and kept asleep during scan. Besides earplugs and earphones, extra foam padding was applied to reduce the sound of the scan while the neonates were asleep. DTI was acquired from 3T Philips Achieva MR system at Children's Medical Center. The dMRI imaging parameters were: TE=78ms, TR=6850ms, in-plane field of view = 168×168mm², in-plane imaging resolution=1.5×1.5mm², slice thickness=1.6mm, slice number=60, 30 independent diffusion-weighted directions⁶ uniformly distributed in space, b-value = 1000 sec/mm², repetition=2. The axial dMRI image dimension was 256×256 after reconstruction. For dMRI, the total acquisition time was 11 minutes. With 30 DWI volumes and 2 repetitions, we accepted those scanned dMRI datasets with less than 5 DWI volumes affected by motion more commonly seen in scanning of neonates and toddlers. The affected DWI volumes were replaced by the good DWI volumes of another DTI repetition during postprocessing.

2.4 Diffusion tensor fitting

DWI acquired from all the subjects was processed offline using DTIStudio⁷ DWI for each subject were corrected for motion and eddy current by registering all the DWIs to the b0 image using a 12-parameter (affine) linear image registration with automated image registration (AIR) algorithm⁸. FA, MD, AD and RD, derived from DTI, were obtained for all the subjects (e.g. ⁹).

2.5 Establishment of population-averaged FA templates at 20wg, 35wg and 40wg

As no age-specific template at 20wg, 35wg and 40wg could be found in the literature, we established population-averaged age-specific templates at these ages. A single subject brain with median brain size and symmetric hemispheres was used as a single-subject template for inter-subject registration at 20wg. Population-averaged FA maps and DTI color-encoded maps at 20wg were generated with affine and large diffeomorphic metric mapping (LDDMM) ¹⁰ transformation. For DTI at 35wg and 40wg, the six-step procedures used to generate the JHU-neonate-nonlinear atlas ¹¹ were adopted. The details of these 6 steps can be found in the literature ¹¹. Briefly, these 6 steps include one step of anterior commissure (AC)-posterior commissure (PC) alignment, one step of direct averaging of images, three consecutive steps of averaging of images after three affine transformations respectively and one last step of averaging after LDDMM transformation. For tensor transformation, the affine transformation matrix and LDDMM transformation matrix obtained from the scalar images were applied to the tensor field to create normalized tensor fields with *DiffeoMap* software (mrstudio.org).

2.6 Identification of core WM based on these averaged FA maps

In the template space, whole brain skeleton was extracted with “tbss_skeleton” function in TBSS of FSL (www.fmrib.ox.ac.uk/fsl). Note that with high cortical FA, the cortical

skeleton (red lines) was also extracted. WM skeleton was manually segmented as the green lines shown in Fig. 2.

2.7 Measurement of WM tract core microstructures and WM tract volumes

Fiber Assignment of Continuous Tractography (FACT)¹² method was used for DTI-based tractography of major white matter tracts in the native space. Turning angle threshold of principal eigenvector was 50°. Anisotropy threshold of 0.15 was used for 35wg and 40wg brains and Anisotropy threshold of 0.12 was used for 20wg brains. DTIStudio was used for conducting fiber tracking procedures with tractography region-of-interests (ROI) placement protocol described in the literature¹³. The following 16 major tracts were traced for white matter microstructural measurement of 35wg and 40wg brains, left and right corticospinal tract (cst-L/R), left and right inferior fronto-occipital fasciculus (ifo-L/R), left and right inferior longitudinal fasciculus (ilf-L/R), left and right superior longitudinal fasciculus (slf-L/R), left and right uncinated fasciculus (unc-L/R), left and right cingulate part of cingulum tract (cgc-L/R), left and right hippocampal part of cingulum tract (cgh-L/R), forceps major (fmajor) and forceps minor (fminor). These tracts could be reproducibly traced with DTI of 35wg and 40wg neonates and cover all four major tract groups, namely projection, limbic, commissural and association tract group. For 20wg brains, only cgc-L, cgc-R, cst-L, cst-R, fminor, ifo-L, ifo-R, unc-L and unc-R could be reproducibly traced. The details of tracking protocol of 20wg brain could be found in our previous publication². All tracts were reproducibly traced by three operators (QY, AO and VM). The binary masks of the individually traced tracts and skeletons from 2.6 inversely mapped back from the template space to the native space were used for measuring WM tract core microstructure, namely, tract-level FA, MD, AD and RD at WM skeleton. The binary masks of traced WM tract alone were used to compute the tract-level volumes. Coefficients of variation (CV) that quantifies inter-operator reproducibility were also computed. All CV values were less than 2%, indicating high quality of inter-operator reproducibility.

2.8 Statistical analysis

The following equation was used for fitting a linear model between y (FA, MD, AD, RD, and tract volume) and age t , $y = \beta_0 + \beta_1 t + \varepsilon$, where ε was an error term. Analysis across all time points was performed only on FA and tract volume and not for MD, AD and RD. The fixation of the tissue in the 20wg samples has been known to drastically reduce the diffusivity rendering comparison with in vivo subjects difficult to interpret. For FA and tract volume, the linear model was used to test 1) if the change of FA or tract volume over time were significant and 2) if the rate of change in the 20wg to 35wg period was significantly different than the rate of change in the 35wg to 40wg period. The null hypothesis for the period specific rates is that there is no significant trend of FA or tract volume over time. The null hypothesis for the two time period comparison is that the rate of change from 20wg to 35wg is the same as from 35wg to 40wg. An unpaired Student's t-test was performed on MD, AD and RD to test for significant differences between the 35wg and 40wg subjects.

3. RESULTS

3.1 WM skeleton based on population-averaged FA maps and WM tracts based on DTI tractography

Fig. 1 shows the age-specific population-averaged FA and DTI orientation-encoded colormaps at 20wg, 35wg, and 40wg. Major morphological changes of both WM and cerebral cortex are observed between 20wg and 35wg. For example, the cortical surface of 20wg is much smoother with less gyrification compared to 35wg or 40wg brains. The frontal area of 35wg brain has less gyrification compared to that of 40wg brain while gyrification of other cortical regions appears similar. Fig. 2 demonstrates the core WM based on age-specific population-averaged FA maps at 20wg, 35wg and 40wg. Both WM (green lines) and cortical (red lines) skeleton are overlaid on the population-averaged FA maps. The skeletonization alleviates partial volume effects (PVE) for DTI metric measurements of WM. The reconstructed traced WM tracts for a typical fetal brain at 20wg, 35wg and 40wg are shown in Fig. 3. To demonstrate the reproducibility of these traced WM tracts, Fig. 4 shows the probabilistic major WM tracts at each age. It is clear that all major tracts that can be traced with adult human brain appear at 35wg and 40wg, while many association tracts could not be identified or traced in 20wg fetal brain (Fig. 3 and Fig. 4).

3.2. Temporal changes of WM microstructure with FA, MD, AD and RD measurements and temporal changes of WM macrostructure with volumetric measurement

Fig. 5 shows significant increase of FA for all investigated tracts from 20wg to 35wg. From 35wg to 40wg, the significant increases of FA can be only found for several association tracts, namely ifo-L/R and slf-L/R. Decelerated FA increases were found in cgc-L/R, fminor, unc-L/R, while no significant change of FA increase rates were found for cst-L/R and ifo-L/R. Fig. 6–8 shows significant decrease of MD, AD and RD across all tracts with exception to ilf-L/R for MD and AD and except to ilf-L/R and cgc-L/R for RD. Fig. 9 shows the significant increase of tract volume for all investigated tracts from 20wg to 35wg. The significant increase of tract volume from 35wg to 40wg can be found in almost all tracts except fmajor and cgh-L/R. Accelerated increases of tract volumes were found in the tracts of cgc-L/R, fminor and unc-L/R.

4. DISCUSSION

Based on age-specific population-averaged DTI maps and DTI tractography of major WM tracts at 20wg, 35wg and 40wg, we have presented microstructural measurements in FA, MD, AD and RD of core WM tracts and characterized their temporal changes across these key developmental time points from mid-fetal to perinatal period. Heterogeneous WM microstructural enhancement was observed across different tracts. Volumes were also measured based on traced WM tracts. The significant FA increase takes place for all investigated tracts from 20wg to 35wg, while FA increase occurs only in association tracts from 35wg to 40wg (Fig. 5). The FA change rates are decelerated during 35wg to 40wg compared to 20wg to 35wg, suggesting temporal heterogeneity of microstructural development of WM (Fig. 5). From Fig. 5 and Fig. 9, faster microstructural WM maturation in terms of FA happens during 20wg to 35wg (Fig. 5), while faster macrostructural

prefrontal WM maturation in terms of tract volume takes place in later 3rd trimester from 35wg to 40wg (Fig. 9), suggesting distinguished WM maturational processes during the periods of 20–35wg and 35–40wg. Specifically, it appears that late half of 3rd trimester from 35wg to 40wg is more involved with fiber growth in amount rather than in microstructure such as myelination.

The heterogeneously significant decrease of MD, AD and RD also suggest both myelination and axonal packing take place during brain development in late 3rd trimester. It is noteworthy that the FA, MD, AD and RD results during this early development are in contrast to typical WM diseased states, where a decrease in FA is usually due to the increase in RD from disruption of the myelination in WM tracts in parallel with decrease of AD from axonal damage ^{e.g. 14–15}. This is not the case during development where FA increases (Fig. 5) while both AD and RD show significant decrease in specific tracts (Fig. 7–8). With FA considered approximately the ratio of AD over RD, our findings suggest that RD may be decreasing at a faster rate than AD. Decrease of RD is related to active myelination and decrease of AD is probably due to axonal packing ^{e.g. 16}. During the late 3rd trimester, it is possible that combined factors of faster myelination in parallel with axonal packing cause FA increases.

Previous studies comparing the FA of postmortem animal brain specimens with that of *in vivo* brain found that formalin fixation alters diffusivity magnitude but not anisotropy ^{17–18}. That is why only FA of 20wg *ex vivo* fetal brain was adopted for quantitative analysis. Ideally same MR imaging sequence and the same MR system should be used for consistency. However, the imaging resolution used for 35wg or 40wg brain is not sufficient to capture anatomical details for the much younger and smaller fetal brain at 20wg. High magnetic strength and long scan time were applied to DTI scan of 20wg fetal brain to increase SNR of high resolution DTI data.

5. CONCLUSION

A more accurate tract-level microstructural characterization at core WM was presented based on age-specific population-averaged DTI maps for human brain from mid-fetal to perinatal stage. The microstructural enhancement is heterogeneous among different tract groups with microstructures of association tracts undergoing most dramatic change and is heterogeneous across different developmental periods with 20–35wg undergoing faster microstructural enhancement. The FA increases while MD, AD and RD decrease for most major WM tracts during late 3rd trimester from 35wg to 40wg, indicating not only active myelination but also axonal packing process during this period.

Acknowledgments

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References

1. Huppi PS, Murphy B, Maier SE, Zientara GP, Inder TE, Barnes PD, Kikinis R, Jolesz FA, Volpe JJ. Microstructural brain development after perinatal cerebral white matter injury assessed by diffusion tensor magnetic resonance imaging. *Pediatrics*. 2001; 107(3):455–460. [PubMed: 11230582]

2. Huang H, Xue R, Zhang J, Ren T, Richards LJ, Yarowsky P, Miller MI, Mori S. Anatomical characterization of human fetal brain development with diffusion tensor MRI. *J Neurosci*. 2009; 29:4263–4273. [PubMed: 19339620]
3. Mukherjee P, Miller JH, Shimony JS, Philip JV, Nehra D, Snyder AZ, Conturo TE, Neill JJ, McKinstry RC. Diffusion-tensor MR imaging of gray and white matter development during normal human brain maturation. *Am J Neuroradiol*. 2002; 23(9):1445–1456. [PubMed: 12372731]
4. Huppi PS, Maier SE, Peled S, Zientara GP, Barnes PD, Jolesz FA, Volpe JJ. Microstructural development of human newborn cerebral white matter assessed in vivo by diffusion tensor magnetic resonance imaging. *Pediatr Res*. 1998; 44(4):584–590. [PubMed: 9773850]
5. Neil J, Miller J, Mukherjee P, Huppi PS. Diffusion tensor imaging of normal and injured developing human brain – a technical review. *NMR Biomed*. 2002; 15(7–8):543–552. [PubMed: 12489100]
6. Jones DK, Horsfield MA, Simmons A. Optimal strategies for measuring diffusion in anisotropic systems by magnetic resonance imaging. *Magn Reson Med*. 1999; 42(3):515–525. [PubMed: 10467296]
7. Jiang H, van Zijl PC, Kim J, Pearlson GD, Mori S. DtiStudio: resource program for diffusion tensor computation and fiber bundle tracking. *Comput Methods Programs Biomed*. 2006; 81(2):106–116. [PubMed: 16413083]
8. Woods RP, Grafton ST, Holmes CJ, Cherry SR, Mazziotta JC. Automated image registration: I. General methods and intrasubject, intramodality validation. *J Comput Assist Tomogr*. 1998; 22(1):139–152. [PubMed: 9448779]
9. Mishra V, Cheng H, Gong G, He Y, Dong Q, Huang H. Differences of inter-tract correlations between neonates and children around puberty: a study based on microstructural measurements with DTI. *Front Hum Neurosci*. 2013; 7:721. [PubMed: 24194711]
10. Miller MI, Troune A, Younes L. On the metrics and euler-lagrange equations of computational anatomy. *Annu Rev Biomed Eng*. 2002; 4:375–405. [PubMed: 12117763]
11. Oishi K, Mori S, Donohue PK, Ernst T, Anderson L, Buchthal S, Faria A, Jiang H, Li X, Miller MI, van Zijl PC, Chang L. Multi-contrast human neonatal brain atlas: application to normal neonate development analysis. *Neuroimage*. 2011; 56(1):8–20. [PubMed: 21276861]
12. Mori S, Crain BJ, Chacko VP, van Zijl PC. Three-dimensional tracking of axonal projections in the brain by magnetic resonance imaging. *Ann Neurol*. 1999; 45(2):265–269. [PubMed: 9989633]
13. Wakana S, Caprihan A, Panzenboeck MM, Fallon JH, Perry M, Gollub RL, Hua K, Zhang J, Jiang H, Dubey P, Blitz A, van Zijl P, Mori S. Reproducibility of quantitative tractography methods applied to cerebral white matter. *Neuroimage*. 2007; 36(3):630–644. [PubMed: 17481925]
14. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Demyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage*. 2002; 17:1429–1436. [PubMed: 12414282]
15. Huang H, Fan X, Weiner M, Martin-Cook K, Xiao G, Davis J, Devous M, Rosenberg R, Diaz-Arrastia R. Distinctive disruption patterns of white matter tracts in Alzheimer's disease with full diffusion tensor characterization. *Neurobiol Aging*. 2012; 33:2029–2045.
16. Dubois J, Dehaene-Lambertz G, Perrin M, Mangin JF, Cointepas Y, Duchesnay E, Le Bihan D, Hertz-Pannier L. Asynchrony of the early maturation of white matter bundles in healthy infants: Quantitative landmarks revealed noninvasively by diffusion tensor imaging. *Hum Brain Mapp*. 2008; 29:14–27. [PubMed: 17318834]
17. Sun SW, Neil JJ, Song SK. Relative indices of water diffusion anisotropy are equivalent to live and formalin-fixed mouse brains. *Magn Reson Med*. 2003; 50:743–748. [PubMed: 14523960]
18. Sun SW, Neil JJ, Liang HF, He YY, Schmidt RE, Hsu CY, Song SK. Formalin fixation alters water diffusion coefficient magnitude but not anisotropy in infarcted brain. *Magn Reson Med*. 2005; 53:1447–1451. [PubMed: 15906292]

Figure 1.

Axial (top row) and coronal (bottom row) slices of population-averaged age-specific FA (left columns) and DTI orientation-encoded colormaps (right columns) of 20wg, 35wg and 40wg brains from left to right.

Figure 2.

WM skeleton shown as green lines was extracted from underlying gray-scale population-averaged FA map at 20wg, 35wg and 40wg. The red lines are the cortical skeleton.

Figure 3.

3D reconstructed major WM tracts in a typical 20wg, 35wg and 40wg brain. Different tracts are encoded by different colors. See the text in Methods & Materials section for abbreviations of the WM tracts.

Figure 4.

Age-specific probabilistic major WM tracts with probabilities ranging from 0–1 overlaid on the gray scale population-averaged FA maps at 20wg, 35wg and 40wg.

Figure 5.

Boxplots of FA values for individual WM tracts. The lines connect the median values. Small gray dots showing averaged measurements across all tracts are displayed as references in all plots. Significance of the rate change across two periods: - no significance, $\uparrow/\downarrow$ $0.001 < p < 0.05$, $\uparrow\uparrow/\downarrow\downarrow$ $p < 0.001$; significance of the rate change compared to 0 in each period: - no significance, * $0.001 < p < 0.05$, ** $p < 0.001$.

Figure 6.

Boxplots of MD values for individual WM tracts. The lines connect the median values.

Small gray dots showing averaged measurements across all tracts are displayed as references in all plots. Significance of the rate change compared to 0 in each period: - no significance, * $0.001 < p < 0.05$, ** $p < 0.001$.

Figure 7.

Boxplots of AD values for individual WM tracts. The lines connect the median values.

Small gray dots showing averaged measurements across all tracts are displayed as references in all plots. Significance of the rate change compared to 0 in each period: - no significance, * $0.001 < p < 0.05$, ** $p < 0.001$.

Figure 8.

Boxplots of RD values for individual WM tracts. The lines connect the median values.

Small gray dots showing averaged measurements across all tracts are displayed as references in all plots. Significance of the rate change compared to 0 in each period: - no significance, * $0.001 < p < 0.05$, ** $p < 0.001$.

Figure 9.

Boxplots of tract volume values for individual WM tracts. The lines connect the median values. Small gray dots showing averaged measurements across all tracts are displayed as references in all plots. Significance of the rate change across two periods: - no significance, $\uparrow/\downarrow$ $0.001 < p < 0.05$, $\uparrow\uparrow/\downarrow\downarrow$ $p < 0.001$; significance of the rate change compared to 0 in each period: - no significance, * $0.001 < p < 0.05$, ** $p < 0.001$.