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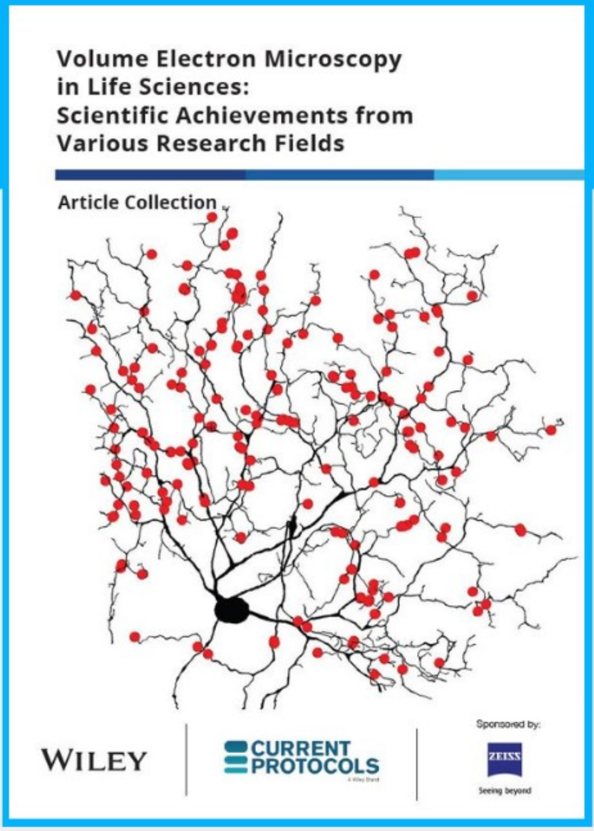

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RESEARCH REPORT

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Effect of number of diffusion encoding directions in neonatal diffusion tensor imaging using Tract-Based Spatial Statistical analysis

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Abstract

Diffusion tensor imaging (DTI) has been used to study the developing brain in early childhood, infants and in utero studies. In infants, number of used diffusion encoding directions has traditionally been smaller in earlier studies down to the minimum of 6 orthogonal directions. Whereas the more recent studies often involve more directions, number of used directions remain an issue when acquisition time is optimized without compromising on data quality and in retrospective studies. Variability in the number of used directions may introduce bias and uncertainties to the DTI scalar estimates that affect cross-sectional and longitudinal study of the brain. We analysed DTI images of 133 neonates, each data having 54 directions after quality control, to evaluate the effect of number of diffusion weighting directions from 6 to 54 with interval of 6 to the DTI scalars with Tract-Based Spatial Statistics (TBSS) analysis. The TBSS analysis was applied to DTI scalar maps, and the mean region of interest (ROI) values were extracted using JHU atlas. We found significant bias in ROI mean values when only 6 directions were used (positive in fractional anisotropy [FA] and negative in fractional anisotropy [MD], axial diffusivity [AD] and fractional anisotropy [RD]), while when using 24 directions and above, the difference to scalar values calculated from 54 direction DTI was

Abbreviations: AD, axial diffusivity; DTI, diffusion tensor imaging; FA, fractional anisotropy; ICC, intra-class correlation coefficient; MD, mean diffusivity; RD, radial diffusivity; ROI, region of interest; TBSS, Tract-Based Spatial Statistics tool that can be used for statistical analysis of white matter values.

negligible. In repeated measures voxel-wise analysis, notable differences to 54 direction DTI were observed with 6, 12 and 18 directions. DTI measurements from data with at least 24 directions may be used in comparisons with DTI measurements from data with higher numbers of directions.

KEYWORDS
diffusion tensor imaging, infant brain imaging, Tract-Based Spatial Statistics

1 | INTRODUCTION

Diffusion tensor imaging (DTI) is an established methodology for the study of the developing brain (Dubois et al., 2014; Lebel et al., 2019; Neil et al., 1998); it can be used to study typical brain development and in the study of the mechanisms of pathologies (Bui et al., 2006; Dubois et al., 2014; Dudink et al., 2007; McGraw et al., 2002; Miller et al., 2002; Neil et al., 2002; Partridge et al., 2004; Prayer & Prayer, 2003; Tanner et al., 2000). DTI has been used to study the developing brain in early childhood, in infancy (Forbes et al., 2002; Hüppi et al., 1996; Mukherjee et al., 2001; Neil et al., 1998) and in fetuses in utero (Bui et al., 2006; Jiang et al., 2009; Kasprian et al., 2008; Righini et al., 2003). Large changes occur in the first 24 months after birth in morphology and microstructure, which is tightly linked to rapid white matter myelination during this period of development (Mukherjee et al., 2001).

DTI studies have included pre-mature infant scans (Aeby et al., 2009, 2012; Miller et al., 2002; Neil et al., 1998; Partridge et al., 2004) and term born infants (Aeby et al., 2009; Forbes et al., 2002; Geng et al., 2012; Hermoye et al., 2006; McGraw et al., 2002; Mukherjee et al., 2001; Sadeghi et al., 2013). Commonly used DTI measures are axial diffusivity (AD, the magnitude of diffusion along the dominant axis), radial diffusivity (RD, the magnitude of diffusion perpendicular to the dominant axis), mean diffusivity (MD, the overall rate of diffusion) and fractional anisotropy (FA, the measure of how much diffusion occurs along single axis to the exclusion of the others). The first three (AD, RD, MD) are positive and have a physical unit (mm^2/s), whereas FA is unitless and ranges from 0 (no directional dependence of the diffusion) to 1 (diffusion along a single direction).

These DTI scalar measures (FA, MD, AD, RD) are expected to be in the same range when obtained from similar subjects in brain white matter, while naturally the scanner manufacturer and model, acquisition parameters, implemented quality control procedures, preprocessing and imaging data analysis method also surely affect them. The used b values (diffusion gradient strengths) have varied from 500 (Jiang et al., 2009) to 2600 s/mm^2 (Bastiani et al., 2019). Number of used diffusion encoding directions in acquisition has generally been smaller in earlier studies down to minimum of 6 orthogonal directions, while the more recent studies have involved more directions (Batalle et al., 2019; Lebenberg et al., 2019). DTI data collected as a part of the developing Human Connectome Project (dHCP) represent the state-of-the-art in the field, using 88 encoding directions acquisition with $b = 1000$ s/mm^2. For details on the whole multi shell sequence, please refer to Bastiani et al. (2019) and to Table S1 and Cachia et al. (2022) for literature review on infant brain DTI.

Earlier simulation study has suggested that as long as optimum encoding is used for six directions, no significant advantage is gained by adding more in terms of a quality measure normalized for imaging time (Hasan et al., 2001). Different acquisition protocols from 6 to 39 directions were simulated in (Landman et al., 2007), where bias in DTI scalars was noted. Additionally, in Landman et al. (2007), it was suggested that different protocols can be considered comparable in clinical studies, as long as sampling orientations are balanced. However, in Papadakis et al. (2000), directions from 18 to 21 were suggested as most efficient number of directions according to simulation experiments with various levels of signal-to-noise-ratio (SNR). In addition to simulations, five healthy subjects were used in Skare et al. (2000), demonstrating effectiveness of number of diffusion directions instead of signal averages for optimal FA measurement. Further, in Jones (2004), it was suggested that 20 directions are required for proper estimation of FA, whereas for tensor orientation and MD, 30 directions were suggested to avoid variations attributed to the orientation of the tissue. While the simulation studies provide good theoretical basis particularly for designing DTI acquisition protocol, they have not considered spatially heterogenous nature of brain in vivo, nor effects from different types of motion, occurring particularly during acquisition of infants.

For choosing between signal averaging and number of directions, it has been suggested to invest acquisition time into different diffusion directions (Correia et al., 2009). In tractography-based regions of interest

(ROI) analysis on DTI scalars evaluated in 13 healthy adults (Barrio-arranz et al., 2015), considerable changes were shown between 6, 21, 40 and 61 directions in the DTI scalars, with decrease in FA and AD and increase in RD when higher number of directions were used. Ten healthy adults were scanned with six acquisitions in two sessions for intra-session and inter-session variability in Wang et al. (2012). DTI acquisitions with 15 and 30 directions were compared, and differences were found between them in nine evaluated fibre tracts. In another study, DTI-derived brain connectivity was analysed in Vaessen et al. (2010) with six healthy adults scanned twice in six gradient sampling schemes with number of gradient directions as 6, 15 and 32, and significant differences were found in small world brain topology metrics.

In adult studies for effect of number of diffusion directions, investigators have reached widely different conclusions for the degree to which the amount of diffusion-encoding directions is acceptable. It may be tempting to use as few diffusion directions as possible (to the minimum of required 6 for DTI) as inclusion criteria for neurodevelopmental study for sake of shorter scanning time, and for allowing to keep as many cases as possible included the analysis, without needing them to be rejected due to too low number of directions after quality control procedures, for example, due to corrupted or interrupted data acquisition (Dubois et al., 2006). Short term repeatability of paediatric brain DTI was studied in Carlson et al. (2014) with two readers performing tractography on 47 subjects with age of 4–18 and in Merisaari et al. (2019) for 122 neonates, giving results in reliability of measurements with the applied number of diffusion directions. However, not only precision and repeatability, but also bias in the DTI scalars, may cause serious problems, especially in longitudinal and neurodevelopmental studies, when subjects have different number of diffusion directions within and between different age groups (Tamnes et al., 2018). To the best of our knowledge, effect of number of DTI gradient directions has not been assessed in infants, which is crucial time point in neurodevelopmental studies, and earlier studies have generally focused only on FA and MD. Also, evaluations of DTI are needed to verify that in statistically significant findings in larger brain studies, cohorts acquired with different settings are not due to differences in their acquisition parameters.

To summarize, the earlier DTI infant literature has used various numbers of diffusion encoding directions, without assessing the effect of the number of directions. Simulation studies have made efforts to demonstrate the effect, but the results may be hard to apply more widely to real life situations for brain DTI acquired at varying age, particularly in infants where brain tissue characteristics and acquisition circumstances largely differ from those of adults. Thus, in vivo validation of simulation results (Jones, 2004) and earlier results in adults are not warranted for their applicability in infant DTI.

In the current study, we evaluated effect of number of diffusion weighting directions to the DTI scalars in context of Tract-Based Spatial Statistics (TBSS) analysis, applied to all DTI scalars in 133 subjects. We used number of diffusion directions from 6 to 54 to systematically map the minimum amount of diffusion-encoding directions needed for reliable tensor estimates (FA, MD, AD and RD).

2 | MATERIALS AND METHODS

The study was conducted according to the Declaration of Helsinki and was reviewed and approved by the Ethics Committee of the Hospital District of Southwest Finland (ETMK:31/180/2011). The imaging data that support the findings of this study are available upon reasonable request from the corresponding author, whereas the imaging data are not publicly available due to privacy restrictions.

2.1 | Subjects

The participants were from the FinnBrain Birth Cohort study (Karlsson et al., 2018), which enrolled families to a neonatal magnetic resonance imaging (MRI) study (Lehtola et al., 2020). The families were recruited at three healthcare locations in Southwest Finland during their first trimester ultrasound visit at gestational week (GW) 12. From this broader participant pool, 189 newborn–mother dyads were recruited into this study. They were recruited based on willingness to participate, availability of the newborn to have an MRI 2–5 weeks after birth, childbirth being after GW 31, birthweight more than 1500 g and not having a previously diagnosed central nervous system (CNS) anomaly or abnormal findings in a previous MRI scan. After explaining the study's purpose and protocol, written informed consent was obtained from the parent(s). Of these 189 newborn participants, 54 had motion artefacts in the MR images. Additionally, two mothers had missing prenatal distress questionnaires. Therefore, 133 newborn–mother dyads were eligible for statistical analyses, where newborns had MRI at 2–5 weeks of age. For the current study, we included all neonates that had at least 54 successful unique diffusion encoding directions in their DTI data following rigorous quality control outlined below. See Table 1 for demographics of the study. The demographics are shown for reference and were not considered covariates of interest in the current study.

TABLE 1 Subject demographics.

Infant sex	N	Per cent
Male	69	51.8%
Female	64	48.2%
Infant age in weeks	Mean	SD
Gestational age (from estimated conception)	43.6	1.04
Age from term age (from GW 40)	3.5	1.04
Age from birth to scan	3.7	1.10
Birth characteristics	Mean	SD
Birth weight (g)	3487	436
Birth height (cm)	50.4	1.86
Mother characteristics	Mean	SD
Maternal age at birth (years)	29.6	4.4
Maternal body mass index (BMI)	24.2	4.0
Educational level (low/middle/high)	37/43/49 (4 missing)	
Maternal smoking during pregnancy (yes/no)	11/122	
SSRI/SNRI medication in use (yes/no)	12/121	

Abbreviations: GW, gestational week; SNRI, serotonin and norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor.

2.2 | Image acquisition

In the DTI scans, a 12-element Head Matrix coil was used in a twice-refocused Spin Echo-Echo Planar Imaging (SE-EPI) sequence with Siemens Magnetom Verio 3T scanner (Siemens Medical Solutions, Erlangen, Germany). The DTI acquisition time varied between 6 min and 12 s and 6 min and 48 s, in 40-min imaging protocol, total duration of the complete scanning protocol not exceeding 60 min. The imaging was performed during natural sleep of the infant. A diffusion weighting of $b = 1000$ s/mm^2 was used with $2 \times 2 \times 2$ mm^3 isotropic resolution (field of view [FOV] 208 mm, 64 slices, repetition time [TR]/echo time [TE] = 8500/90 ms). The data were acquired in three segments with 31, 32 and 33 diffusion encoding directions each containing 3 $b = 0$ images, composing of a total 96 uniformly distributed directions. The DTI acquisition scheme is provided in detail in (Merisaari et al., 2019).

2.3 | Image preprocessing and TBSS analysis

Data were pre-processed as described in Merisaari et al. (2019). In brief, good $b = 0$ images were selected, the images were quality controlled with DTIprep (Oguz et al., 2014) after which visual quality control was used to exclude any remaining motion corrupted volumes. Eddy/motion correction was applied with corresponding rotation of the B-matrix. Finally, the distribution of the encoding directions and the images were manually inspected for quality. Distortion correction was not applied here as we did not have opposing phase directions in the sequences, field maps were often corrupted with motion artefacts and distortions were considered to be minimal for the current data. FMRIB Software Library (FSL) tools were used to derive the tensor maps (FA, MD, AD and RD). For the purposes of the current study, we created a sub-set from the whole sample so that included cases had at least 54/96 acceptable directions after quality control. The dataset had 133/189 participants. We chose 54 directions as threshold limit to be included, as it was considered a suitable trade-off between number of subjects to be included, and number of directions for evaluations. From dataset with 54 directions, individual directions were dropped out while keeping diffusion direction distribution as uniform as possible by maximizing the angular resolution using algorithm described in Merisaari et al. (2019), to create simulated versions of increasing diffusion-encoding directions: 6, 12, 18, 24, 30, 36, 42, 48 and 54 directional datasets. The angular resolution was calculated in the algorithm as inverse of the largest empty region in spherical representation of the diffusion directions, which was measured using cap angles between triplets of directions calculated with spherical voronoi algorithm.

TBSS analysis (Smith et al., 2006) was first applied to each datasets containing 54 directions with FA values, and the mean FA image was then used as a template for all datasets. This generated spatially aligned images of DTI scalars for evaluations of number of diffusion encoding directions in voxels of DTI parameter maps aligned to the TBSS template. In addition to voxel-level analysis, to support the ROI analyses, John Hopkins University DTI-based white matter atlas for neonates (Oishi et al., 2011) (JHU atlas) was applied to the TBSS processed DTI scalar maps. The mean intensity values in 16 conventionally used ROI (see Figure S3 for list of ROIs) were then used bilaterally to analyse differences between number of used diffusion directions.

2.4 | Statistical analysis

We evaluated difference of using direction at voxel level using repeated measures analysis followed by paired t test as post hoc test (8 tests comparing 6, 12, 18, 24, 30, 36, 42, 48 directions against 54 directions, which was used as bronze standard), using fsl toolbox randomize tool (Winkler et al., 2014). As the scalar value distributions in ROIs were found deviating from normal distribution, we evaluated ROI mean value differences with Friedman test and Wilcoxon signed rank post hoc test. In DTI, smaller number of directions are expected to affect FA bias towards higher values (Pierpaoli & Basser, 1996). Thus, repeated measures Mann–Kendall test was used to test the existence of a non-zero trend in change of DTI scalar ROI values across number of directions. ICC(2,1) was applied between directions, measuring absolute agreement with scalar measures, to see their suitability for studies comparing patient groups with controls, or longitudinal studies. Repeated measures analysis was also applied to TBSS skeleton voxels, and we evaluated TBSS voxels for deviating more than 10% from the reference values with 54 directions data. We considered ICC(2,1) > .75 to be excellent, whereas <.75 to be poor or moderate (Koo & Li, 2016). All statistics were performed with R version 3.6.3 (Team, 2020). P values below .05 after false discovery rate (FDR) correction for multiple comparisons were considered statistically significant, unless otherwise noted.

3 | RESULTS

The DTI scalar values from images with 54 directions were used as benchmark, that is, the best possible estimate of the true tensor values in the analyses. The reference FA values across TBSS skeleton are shown in Figure 1 to visualize the typical values observed in our data (see Figure S1 for corresponding values in MD, AD and RD), where high FA values are found in the midsagittal callosum and central regions in superior fasciculus, and lower FA values are located in peripheral regions.

3.1 | Repeated measures voxel-wise analysis

When evaluating TBSS skeleton locations in repeated measures analysis, all of the skeleton voxels were found to differ in individuals statistically significantly in comparison with 54 direction scalar estimates ($p < .001$ after correction for multiple comparisons across TBSS voxels). We visualized the percentage of the subjects where a DTI scalar value was 10% or more different than corresponding value in 54 direction data, to see the scalar value differences. With number of 6, 12 and 18 directions, large regions across TBSS voxels (with 6: 100% of the skeleton; with 12: all corpus callosum (CC) and large portions across the skeleton; and with 18: middle of CC and large portions across the skeleton) demonstrated significant proportions (∼10% or more) of subjects were deviating more than 10% from the reference values. For more detailed results for FA from 6 to 48 directions, see Figure 2 (see Figure S2 for MD, AD and RD correspondingly).

3.2 | Repeated measures ROI-wise analysis

When comparing ROI mean values in TBSS skeleton, for MD, AD and RD, only 6 direction data were significantly different from 54 direction values ($p < .05$). The differences were within all of the 16 evaluated regions (see Figure S3 for list) at either side, apart from Cingulum Cingular Part (CGC). In FA, the 12 direction values were significantly different in 12 regions at either side ($p < .05$), the 18 direction values were different in 6 regions ($p < .05$) and 24 direction values in 3 regions ($p < .05$ for Cerebral peduncle, Corticospinal tract and CGC). Of those three, CGC (right) was also significantly different in all directions up to 42 directions.

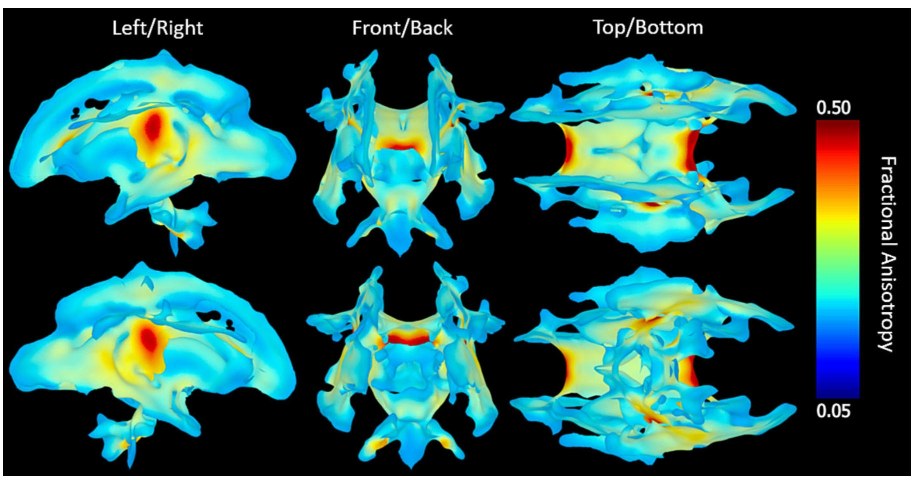

FIGURE 1 Rendering of diffusion tensor imaging (DTI) fractional anisotropy map of Tract-Based Spatial Statistics (TBSS) map in six orthogonal directions, calculated using 54 diffusion encoding directions in 133 neonates.

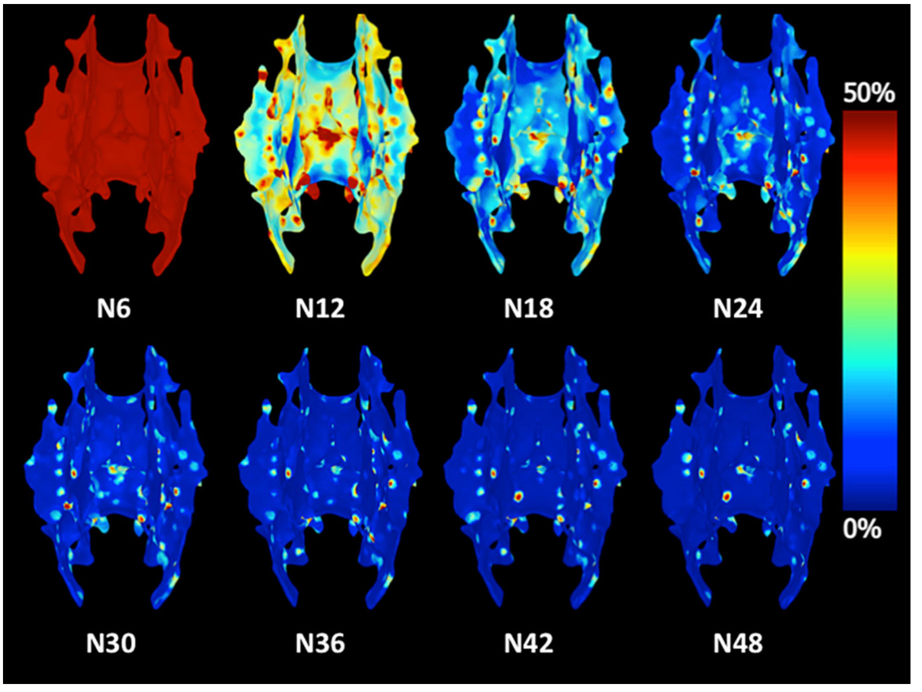

FIGURE 2 Rendering of proportions of neonatal subjects (%) from 133, where difference in fractional anisotropy was larger than 10%, when comparing values from 6(N6) to 48(N48) diffusion encoding directions to values with 54 directions. Directions 6, 12 and 18 contain larger regions where non-negligible proportion of subjects deviate more than 10% from the reference value with 54 directions.

3.3 | Effect of angular resolution to the ROI-level results

We also evaluated Spearman correlation of mean angular resolution and number of directions and evaluated interaction of directions and angular resolution in explaining standard deviation of scalar values across subjects. The angular resolution was found to interact with number of directions in all of the evaluated ROIs ($p < .05$). Spearman correlation between angular resolution and scalar value was found significant with 6 directions ($p < .05$), and no statistically significant correlation was found in other numbers of directions from 12 to 54. The variance of the scalar value was particularly high with 6 direction data (see Figure S3 for box plots with 6, 24, 54 directions). On the basis of found voxel-wise and ROI-wise differences, we excluded directions 6, 12 and 18 from subsequent analysis, to focus on examining if having larger number of directions 24 to 54 would be considered interchangeable.

3.4 | Mann–Kendall difference between diffusion encoding directions from 24 to 54 in ROI values

With Mann–Kendall test for monotonic trend in DTI scalars when number of diffusion directions varied from 24 to 54, we found statistically significant positive trend after FDR correction ($p < .05$) in FA of CC, Retrolenticular internal capsule (RLIC) and External capsule (EC). However, median FA difference between 24 and 54 directions was considered negligible (FA difference < .01). In AD, significant negative trend was found in CGC with median difference of $-.3 \times 10^{-3}$ mm^2/s (left), and $-.2 \times 10^{-3}$ mm^2/s (right), between 24 and 54 directions. In MD, significant negative trends were found in CGC ($-.2 \times 10^{-3}$ mm^2/s left and right) and Cerebral peduncle ($-.1 \times 10^{-3}$ mm^2/s). In RD, significant negative trend was found in CGC ($-.2 \times 10^{-3}$ mm^2/s left, $.1 \times 10^{-3}$ mm^2/s right) and Superior fronto-occipital fasciculus ($.1 \times 10^{-3}$ mm^2/s right). Other trends in AD, MD and RD were either not significant (FR corrected $p > .05$), or the scalar value difference between 24 and 54 was considered negligible (median difference $<.01 \times 10^{-3}$ mm^2/s).

3.5 | Comparison of 24 and 54 diffusion encoding directions in voxel-wise analysis

We calculated also voxel-wise differences across the TBSS skeleton are shown in Figure 3 for absolute differences in FA between 24 and 54 direction data (see Figure S4 for MD, AD and RD, correspondingly), as these measures can be considered as upper limit for scalar value differences for 24 directions and higher. Generally, there were individual voxels demonstrating higher dependence on the number of directions, while the majority of the TBSS skeleton voxels had negligible differences between using 24 and 54 diffusion directions.

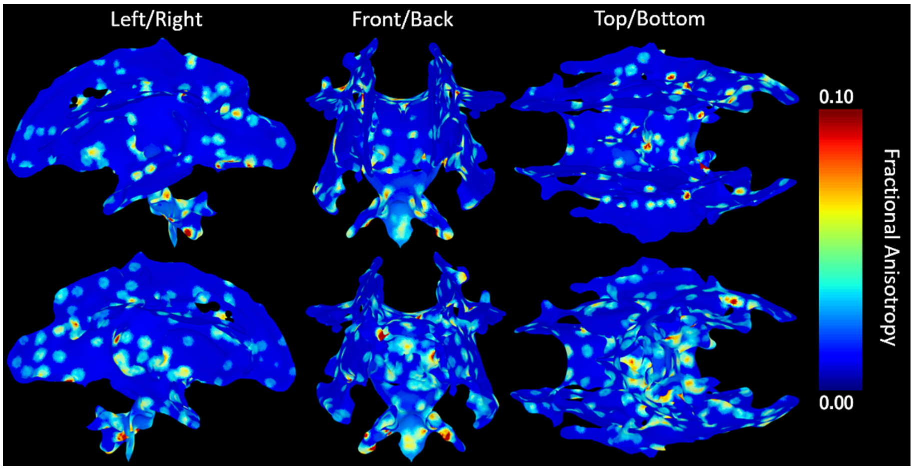

FIGURE 3 Rendering of fractional anisotropy (FA) difference, calculated using difference between using 24 and 54 encoding directions, in 133 neonates.

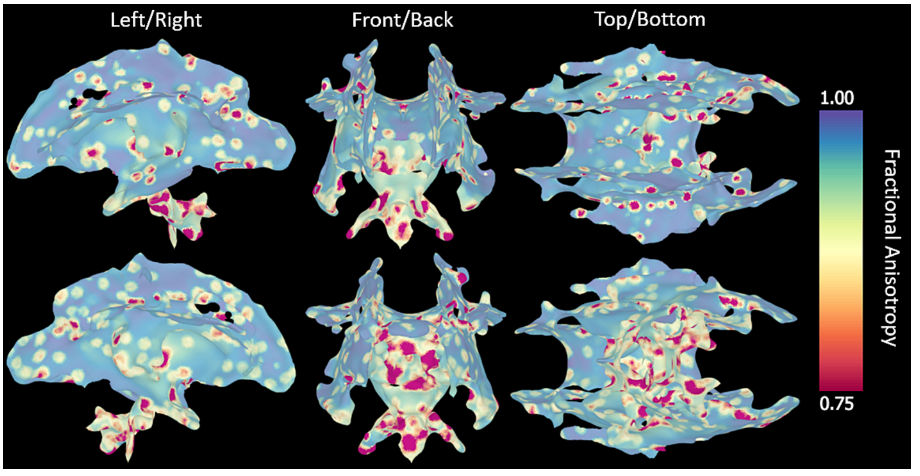

FIGURE 4 Rendering of intraclass correlation coefficient (ICC(2,1)) map of fractional anisotropy values, calculated using 24, 30, 36, 42, 48 and 54 encoding directions, in 133 neonates. Note that the colour coding starts from ICC 0.75, which is commonly considered as a sufficient threshold to pass reliability testing.

3.6 | Voxel-wise ICC(2,1) for 24, 30, 36, 42, 48 and 54 diffusion encoding directions

We used ICC(2,1) to reflect absolute agreement in the scalar values when the number of directions was considered as measurement method, high ICC(2,1) indicating that the measurements are reproducible when directions are varied from 24 to 54. Figure 4 demonstrates voxel-wise mapping of the ICC values across TBSS skeleton for FA (see Figure S5 for MD, AD and RD). In FA, ICC(2,1) (>.75) was good across TBSS skeleton. Correspondingly, in AD, RD and MD, ICC(2,1) was generally lower, while central regions still expressing generally good reproducibility (ICC > .75). Similar to measurements of absolute differences between 24 and 54 directions, individual locations with lower ICC(2,1) reproducibility were found to be present across TBSS skeleton, whereas majority of the skeleton had high reproducibility.

4 | DISCUSSION

Various diffusion encoding strategies have been applied in studies of neurodevelopment at the microstructural level in neonates, but effect of diffusion directions has not been evaluated in vivo in larger extent before. The DTI scalar values from acquisitions at early age form the basis for further longitudinal differential comparisons, and therefore, the accuracy of the scalar measurements is of high importance. In this study, we evaluated the effect of the number of diffusion encoding directions on the DTI scalar values in 2- to 5-week-old subjects, where our analysis pipeline included quality control, motion and eddy correction and final DTI scalar estimates extracted from TBSS scalar.

Even though the distribution of the angular resolution in the analysed data was minimized, our in vivo infant data had rotated b-vectors after motion correction.

In contrast to studies where motion artefacts and thus related motion induced deviations from uniform diffusion direction distribution are not assessed, we consider our approach to provide more realistic evaluations. While this may have partially affected deviance of 6 direction data (as angular resolution correlated significantly with the scalar values) to 54 direction reference values, in 12 and 18 direction data, we found no significant effect with angular resolution. Therefore, we consider that their difference to 54 data is majorly due to number of directions.

Our analysis was in both ROI-level and voxel-level. We used TBSS skeleton values, as this technique can be considered robust against degradations in image quality. In ROI-level analysis, as the sizes and shapes of ROIs make comparison between neurodevelopmental studies challenging (Dudink et al., 2007), we applied automatic ROI placement in TBSS parameter maps to provide results in an easily applicable and reproducible context.

When exclusion criteria for number of diffusion directions were increased, the available number of subjects reduced correspondingly. This trade-off between number of directions and included cases is to be interpreted in context where choice for required minimum number of directions may depend on number of factors, such as size of the smallest or smaller compared study group, general inter-subject variance in DTI measure of interest and general willingness to exclude obtained data in exchange of quality of included data.

The above in mind, we can generally consider that decision resulting in excluding more than 50% of subjects would have too low cost–benefit ratio for practical implementations. This would mean that requiring to have more than 70 directions with acceptable quality from original acquired 96 may be unrealistic with the type of cases used in this study. In theory, more acquisition time could be invested to obtain more original directions and, further, more acceptable quality data, given that challenges relating to neonatal image acquisition can be addressed for longer durations. In our experiments, for obtaining 24 quality-controlled directions, we estimate that at least close to 50 directions would be needed to be acquired during scan to compensate for loss of data due to expected artefacts. Naturally, the DTI acquisition success rate affects this estimate (in our study approximately 26% of cases were excluded), and imputation and other procedures may be used to replace corrupted or missing data.

We observed high systematic bias between using six directions and 54 directions confirming findings in theoretical and simulation studies (Jones, 2004) and studies with healthy adults relating to effect of the number of diffusion directions. In contrast to Jones (2004), we had motion induced effects in vivo, while considering also that the loss of directions may need to be compensated by acquiring extra directions to ensure desired amount in the final analysis after quality control. Our results are largely in alignment also with the earlier studies with adults (Barrio-arranz et al., 2015; Giannelli et al., 2010) for which our results can be considered as confirmatory with larger in vivo sample, although with children, being in contrast to Lebel et al. (2011).

The DTI scalar estimates were not largely found to change in higher number of directions from 24 up to 48 in comparison with 54-direction data (see Figures 2 and S2), with most of the subjects having less than 10% difference across the TBSS skeleton. This suggests that a dataset with varying number of diffusion directions between subjects could tentatively be combined into the same pooled neurodevelopmental sample, if number of diffusion directions is at least 24 in all of them, although more directions (Sakaie et al., 2012) and proper harmonization may be needed (Fortin et al., 2017). In addition to this, the difference to 54-direction data was varying depending on location in the skeleton. We speculate that this sensitivity to the number of encoding directions may be due to brain regions myelinating at different rates and underlying microstructure being different at different regions of the white matter. While larger numbers of diffusion directions are more challenging to acquire in infants/small children, our results indicate that composing together DTI data having less than 24 directions with other DTI data with more directions may be problematic. We suggest that interpreting scalar estimates from data where smaller than 18 directions are available after quality control steps is discouraged due to potential bias introduced from small number of directions. However, this bias could naturally be reduced with producing uniform data with the caveat of losing acquired diffusion direction images and thus accuracy of DTI measures.

We expect the results to remain the same if the actual image acquisition was performed completely separately for each of the number of directions from 6 to 54. Also, while we stress the importance of the studied age group due to potential to early neurodevelopmental differences between subjects and due to connection between the DTI angular resolution and scalar values, we expect the results to be generally applicable for older children as well. These remain to be verified in future studies. TBSS is frequently used in similar settings to what has been used in this study. We leave it to future studies to compare possible enhancements with alternative spatial co-registration tools. Finally, it is left for future research to study the effect of diffusion acquisition analysis schemes, for example, to tractography and after harmonization procedures.

5 | CONCLUSION

The number of diffusion directions leads to systematic bias in the DTI scalar measures, and using less than 24 diffusion directions is discouraged for neurodevelopmental studies. In evaluations of different numbers of diffusion encoding directions from 6 to 54, we observed that 24 quality-controlled directions in TBSS analysis provide negligible bias.

AUTHOR CONTRIBUTIONS

Harri Merisaari: Conceptualization; data curation; formal analysis; investigation; methodology; software; visualization; writing—original draft; writing—review and editing. **Linnea Karlsson:** Supervision; writing—review and editing. **Noora M. Scheinin:** Funding acquisition; investigation; writing—review and editing. **Satu J. Lehtola:** Formal analysis; writing—review and editing. **John David Lewis:** Methodology; writing—review and editing. **Hasse Karlsson:** Funding acquisition; project administration; resources; writing—review and editing. **Jetro J Tuulari:** Methodology; project administration; supervision; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

Nothing to declare.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ejn.16135.

DATA AVAILABILITY STATEMENT

The Finnish law and ethical permissions do not allow open sharing of the data used in this study, but data access is possible via formal material transfer agreements (MTA). Investigators that wish to access the data are encouraged to contact Principal Investigators of the FinnBrain Birth Cohort study (https://sites.utu.fi/finnbrain/en/contact/). For analysis codes, please contact the corresponding author.

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REFERENCES

Aeby, A., Liu, Y., David, P., Bale, D., Metens, T., & Van Bogaert, B. (2009). Maturation of thalamic radiations between 34 and 41 Weeks' gestation: A combined voxel- based study and probabilistic Tractography with diffusion tensor imaging. *American Journal of Neuroradiology*, *30*, 1780–1786. https://doi.org/10.3174/ajnr.A1660

Aeby, A., Van Bogaert, P., David, P., Balériaux, D., Vermeylen, D., Metens, T., & De Tiège, X. (2012). Nonlinear microstructural changes in the right superior temporal sulcus and lateral occipitotemporal gyrus between 35 and 43 weeks in the preterm brain. *NeuroImage*, *63*, 104–110. https://doi.org/10.1016/j.neuroimage.2012.06.013

Barrio-arranz, G., de Luis-garcía, R., Tristán-vega, A., & Martín-, M. (2015). Impact of MR acquisition parameters on DTI scalar indexes: A tractography based approach. *PLoS ONE*, *10*, 1, e0137905–19. https://doi.org/10.1371/journal.pone.0137905

Bastiani, M., Andersson, J. L. R., Cordero-Grande, L., Murgasova, M., Hutter, J., Price, A. N., Makropoulos, A., Fitzgibbon, S. P., Hughes, E., Rueckert, D., Victor, S., Rutherford, M., Edwards, A. D., Smith, S. M., Tournier, J. D., Hajnal, J. V., Jbabdi, S., & Sotiropoulos, S. N. (2019). Automated processing pipeline for neonatal diffusion MRI in the developing human connectome project. *NeuroImage*, *185*, 750–763. https://doi.org/10.1016/j.neuroimage.2018.05.064

Batalle, D., O'Muircheartaigh, J., Makropoulos, A., Kelly, C. J., Dimitrova, R., Hughes, E. J., Hajnal, J. V., Zhang, H., Alexander, D. C., Edwards, A. D., & Counsell, S. J. (2019). Different patterns of cortical maturation before and after 38 weeks gestational age demonstrated by diffusion MRI in vivo. *NeuroImage*, *185*, 764–775. https://doi.org/10.1016/j.neuroimage.2018.05.046

Bui, T., François, J. D., Zaccaria, I., Alberti, C., Elmaleh, M., Garel, C., Luton, D., Blanc, N., & Sebag, G. (2006). Microstructural development of human brain assessed in utero by diffusion tensor imaging. *Pediatric Radiology*, *36*, 1133–1140. https://doi.org/10.1007/s00247-006-0266-3

Cachia, A., Mangin, J., & Dubois, J. (2022). Mapping the human brain from the prenatal period to infancy using 3D magnetic reonance imaging. In O. Houdé & G. Borst (Eds.), *The Cambridge Handbook of Congitive Development* (p. 50). Cambridge University Press. https://doi.org/10.1017/9781108399838.005

Carlson, H. L., Laliberté, C., Brooks, B. L., Hodge, J., Kirton, A., Bello-espinosa, L., Hader, W., & Sherman, E. M. S. (2014). Epilepsy & Behavior Reliability and variability of diffusion tensor imaging (DTI) tractography in pediatric epilepsy. *Epilepsy & Behavior*, *37*, 116–122. https://doi.org/10.1016/j.yebeh.2014.06.020

Correia, M. M., Carpenter, T. A., & Williams, G. B. (2009). Looking for the optimal DTI acquisition scheme given a maximum scan time: Are more b -values a waste of time ? *Magnetic Resonance Imaging*, *27*, 163–175. https://doi.org/10.1016/j.mri.2008.06.011

Dubois, J., Kulikova, S., Poupon, C., & Hu, P. S. (2014). The early development of brain white matter: A review of imaging studies in fetuses, newborns and infants. *Neuroscience*, *276*, 48–71. https://doi.org/10.1016/j.neuroscience.2013.12.044

Dubois, J., Poupon, C., Lethimonnier, F., & Le Bihan, D. (2006). Optimized diffusion gradient orientation schemes for corrupted clinical DTI data sets. Magn. Reson. Mater. Physics. *Biologie et Médecine*, *19*, 134–143. https://doi.org/10.1007/s10334-006-0036-0

Dudink, J., Lequin, M., Van Pul, C., Buijs, J., Conneman, N., van Goudoever, J., & Govaert, P. (2007). Fractional anisotropy in white matter tracts of very-low-birth-weight infants. *Pediatric Radiology*, *37*, 1216–1223. https://doi.org/10.1007/s00247-007-0626-7

Forbes, K. P., Pipe, J. G., & Bird, C. R. (2002). Changes in brain water diffusion during the 1st year of life. *Radiology*, *222*, 405–409. https://doi.org/10.1148/radiol.2222010179

Fortin, J., Parker, D., Tunç, B., Watanabe, T., Elliott, M. A., Ruparel, K., Roalf, D. R., Satterthwaite, T. D., Gur, R. C., Gur, R. E., Schultz, R. T., Verma, R., & Shinohara, R. T. (2017). NeuroImage harmonization of multi-site diffusion tensor imaging data. *NeuroImage*, *161*, 149–170. https://doi.org/10.1016/j.neuroimage.2017.08.047

Geng, X., Gouttard, S., Sharma, A., Gu, H., Styner, M., Lin, W., Gerig, G., Gilmore, J. H., Gerig, G., & Gilmore, J. H. (2012). Quantitative tract-based white matter development from birth to age 2 years. *NeuroImage*, *61*, 542–557. https://doi.org/10.1016/j.neuroimage.2012.03.057

Giannelli, M., Cosottini, M., Michelassi, M. C., Lazzarotti, G., Belmonte, G., Bartolozzi, C., & Lazzeri, M. (2010). Dependence of brain DTI maps of fractional anisotropy and mean diffusivity on the number of diffusion weighting directions. *Journal of Applied Clinical Medical Physics*, *11*, 176, 2927–190. https://doi.org/10.1120/jacmp.v11i1.2927

Hasan, K. M., Parker, D. L., & Alexander, A. L. (2001). Comparison of gradient encoding schemes for diffusion-tensor MRI. *Journal of Magnetic Resonance Imaging*, *13*, 769–780. https://doi.org/10.1002/jmri.1107

Hermoye, L., Saint-martin, C., Cosnard, G., Lee, S., Kim, J., Nassogne, M., Menten, R., Clapuyt, P., Donohue, P. K., Hua, K., Wakana, S., Jiang, H., Van Zijl, P. C. M., & Mori, S. (2006). Pediatric diffusion tensor imaging: Normal database and observation of the white matter maturation in early childhood. *NeuroImage*, *29*, 493–504. https://doi.org/10.1016/j.neuroimage.2005.08.017

Hüppi, P. S., Schuknecht, B., Boesch, C., Bossi, E., Felblinger, J., Fusch, C., & Herschkowitz, N. (1996). Structural and neurobehavioral delay in postnatal brain development of preterm infants. *Pediatric Research*, *39*, 895–901. https://doi.org/10.1203/00006450-199605000-00026

Jiang, S., Xue, H., Counsell, S., Anjari, M., Allsop, J., Rutherford, M., Rueckert, D., & Hajnal, J. V. (2009). Diffusion tensor imaging (DTI) of the brain in moving subjects: Application to in-utero fetal and ex-utero studies. *Magnetic Resonance in Medicine*, *62*, 645–655. https://doi.org/10.1002/mrm.22032

Jones, D. K. (2004). The effect of gradient sampling schemes on measures derived from diffusion tensor MRI: A Monte Carlo study. *Magnetic Resonance in Medicine*, *51*, 807–815. https://doi.org/10.1002/mrm.20033

Karlsson, L., Tolvanen, M., Scheinin, N. M., Uusitupa, H. M., Korja, R., Ekholm, E., Tuulari, J. J., Pajulo, M., Huotilainen, M., Paunio, T., & Karlsson, H. (2018). Cohort profile: The FinnBrain birth cohort study (FinnBrain). *International Journal of Epidemiology*, *47*, 15–16j. https://doi.org/10.1093/ije/dyx173

Kasprian, G., Brugger, P. C., Weber, M., Krssák, M., Krampl, E., Herold, C., & Prayer, D. (2008). NeuroImage in utero tractography of fetal white matter development. *NeuroImage*, *43*, 213–224. https://doi.org/10.1016/j.neuroimage.2008.07.026

Koo, T. K., & Li, M. Y. (2016). A guideline of selecting and reporting Intraclass correlation coefficients for reliability research. *Journal of Chiropractic Medicine*, *15*, 155–163. https://doi.org/10.1016/j.jcm.2016.02.012

Landman, B. A., Farrell, J. A. D., Jones, C. K., Smith, S. A., Prince, J. L., & Mori, S. (2007). Effects of diffusion weighting schemes on the reproducibility of DTI-derived fractional anisotropy, mean diffusivity , and principal eigenvector measurements at 1.5T. *NeuroImage*, *36*, 1123–1138. https://doi.org/10.1016/j.neuroimage.2007.02.056

Lebel, C., Benner, T., & Beaulieu, C. (2011). Six is enough? Examining the controversy of 6 versus 30 diffusion encoding directions for deterministic tractography of human brain. *Proceedings of the International Society for Magnetic Resonance in Medicine*, *19*, 3895.

Lebel, C., Treit, S., & Beaulieu, C. (2019). A review of diffusion MRI of typical white matter development from early childhood to young adulthood. *NMR in Biomedicine*, *32*, e3778. https://doi.org/10.1002/nbm.3778

Lebenberg, J., Mangin, J. F., Thirion, B., Poupon, C., Hertz-Pannier, L., Leroy, F., Adibpour, P., Dehaene-Lambertz, G., & Dubois, J. (2019). Mapping the asynchrony of cortical maturation in the infant brain: A MRI multi-parametric clustering approach. *NeuroImage*, *185*, 641–653. https://doi.org/10.1016/j.neuroimage.2018.07.022

Lehtola, S. J., Tuulari, J. J., Scheinin, N. M., Karlsson, L., Parkkola, R., Merisaari, H., Lewis, J. D., Fonov, V. S., Louis Collins, D., Evans, A., Saunavaara, J., Hashempour, N., Lähdesmäki, T., Acosta, H., & Karlsson, H. (2020). Newborn amygdalar volumes are associated with maternal prenatal psychological distress in a sex-dependent way. *NeuroImage: Clinical*, *28*, 102380. https://doi.org/10.1016/j.nicl.2020.102380

McGraw, P., Liang, L., & Provenzale, J. M. (2002). Evaluation of normal age-related changes in anisotropy during infancy and childhood as shown by diffusion tensor imaging. *American Journal of Roentgenology*, *179*, 1515–1522. https://doi.org/10.2214/ajr.179.6.1791515

Merisaari, H., Tuulari, J. J., Karlsson, L., Scheinin, N. M., Parkkola, R., Saunavaara, J., Lähdesmäki, T., Lehtola, S. J., Keskinen, M., Lewis, J. D., Evans, A. C., & Karlsson, H. (2019). Test-retest reliability of diffusion tensor imaging metrics in neonates. *NeuroImage*, *197*, 598–607. https://doi.org/10.1016/j.neuroimage.2019.04.067

Miller, S. P., Vigneron, D. B., Henry, R. G., Bohland, M. A., Ceppi-cozzio, C., Hoffman, C., Newton, N., Partridge, J. C., Ferriero, D. M., & Barkovich, A. J. (2002). Serial quantitative diffusion tensor MRI of the premature brain: Development in newborns with and without injury. *Journal of Magnetic Resonance Imaging*, *16*, 621–632. https://doi.org/10.1002/jmri.10205

Mukherjee, P., Miller, J. H., Shimony, J. S., Conturo, T. E., Lee, B. C. P., Almli, C. R., & Mckinstry, R. C. (2001). Normal brain maturation during childhood: Developmental trends characterized with. *Radiology*, *221*, 349–358. https://doi.org/10.1148/radiol.2212001702

Neil, J., Miller, J., Mukherjee, P., & Hu, P. S. (2002). Diffusion tensor imaging of normal and injured developing human brain ± a technical review. *NMR in Biomedicine*, *15*, 543–552. https://doi.org/10.1002/nbm.784

Neil, J. J., Shiran, S. I., McKinstry, R. C., Schefft, G. L., Snyder, A. Z., Almli, C. R., Akbudak, E., Aronovitz, J. A., Miller, J. P., Lee, B. C., & Conturo, T. E. (1998). Normal brain in human newborns: Apparent diffusion coefficient and diffusion anisotropy measured by using diffusion tensor MR imaging. *Radiology*, *209*, 57–66. https://doi.org/10.1148/radiology.209.1.9769812

Oguz, I., Farzinfar, M., Matsui, J., Budin, F., Liu, Z., Gerig, G., Johnson, H. J., & Styner, M. (2014). DTIPrep: Quality control of diffusion-weighted images. *Frontiers in Neuroinformatics*, *8*, 1–11. https://doi.org/10.3389/fninf.2014.00004

Oishi, K., Mori, S., Donohue, P. K., Ernst, T., Anderson, L., Buchthal, S., Faria, A., Jiang, H., Li, X., Miller, M. I., van Zijl, P. C. M., & Chang, L. (2011). Multi-contrast human neonatal brain atlas: Application to normal neonate development analysis. *NeuroImage*, *56*, 8–20. https://doi.org/10.1016/j.neuroimage.2011.01.051

Papadakis, N. G., Murrills, C. D., Hall, L. D., Huang, C. L. H., & Adrian Carpenter, T. (2000). Minimal gradient encoding for robust estimation of diffusion anisotropy. *Magnetic Resonance Imaging*, *18*, 671–679. https://doi.org/10.1016/S0730-725X(00)00151-X

Partridge, S. C., Mukherjee, P., Henry, R. G., Miller, S. P., Berman, J. I., Jin, H., Lu, Y., Glenn, O. A., Ferriero, D. M., Barkovich, A. J., & Vigneron, D. B. (2004). Diffusion tensor imaging: Serial quantitation of white matter tract maturity in premature newborns. *NeuroImage*, *22*, 1302–1314. https://doi.org/10.1016/j.neuroimage.2004.02.038

Pierpaoli, C., & Basser, P. J. (1996). Toward a quantitative assessment of diffusion anisotropy. *Magnetic Resonance in Medicine*, *36*, 893–906. https://doi.org/10.1002/mrm.1910360612

Prayer, D., & Prayer, L. (2003). Diffusion-weighted magnetic resonance imaging of cerebral white matter de v elopment. *European Journal of Radiology*, *45*, 235–243. https://doi.org/10.1016/S0720-048X(02)00312-1

Righini, A., Bianchini, E., Parazzini, C., Gementi, P., Ramenghi, L., Baldoli, C., Nicolini, U., Mosca, F., & Triulzi, F. (2003). Apparent diffusion coefficient determination in Normal fetal brain: A prenatal MR imaging study. *AJNR. American Journal of Neuroradiology*, *24*, 799–804.

Sadeghi, N., Prastawa, M., Fletcher, P.T., Vachet, C., Wang, B., Gilmore, J., & Gerig, G. (2013). Multivariate modeling of longitudinal MRI in early brain development with confidence measures, in: IEEE 10th International Symposium on Biomedical Imaging (ISBI). IEEE, 1400–1403.

Sakaie, K., Lee, J., Debbins, J. P., Arnold, D. L., Melhem, E. R., Philips, M. D., & Lowe, M. (2012). A validation study of multicenter diffusion tensor imaging: Reliability of fractional anisotropy and diffusivity values. *American Journal of Neuroradiology*, *33*, 695–700. https://doi.org/10.3174/ajnr.A2844

Skare, S., Hedehus, M., Moseley, M. E., & Li, T. Q. (2000). Condition number as a measure of noise performance of diffusion tensor data acquisition schemes with MRI. *Journal of Magnetic Resonance*, *147*, 340–352. https://doi.org/10.1006/jmre.2000.2209

Smith, S. M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T. E., Mackay, C. E., Watkins, K. E., Ciccarelli, O., Cader, M. Z., Matthews, P. M., & Behrens, T. E. J. (2006). Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *NeuroImage*, *31*, 1487–1505. https://doi.org/10.1016/j.neuroimage.2006.02.024

Tamnes, C. K., Roalf, D. R., Goddings, A. L., & Lebel, C. (2018). Diffusion MRI of white matter microstructure development in childhood and adolescence: Methods, challenges and progress. *Developmental Cognitive Neuroscience*, *33*, 161–175. https://doi.org/10.1016/j.dcn.2017.12.002

Tanner, S. F., Ramenghi, L. A., Ridgway, J. P., Berry, E., Saysell, M. A., Martinez, D., Arthur, R. J., Smith, M. A., & Levene, M. I. (2000). Quantitative comparison of intrabrain diffusion in adults and preterm and term neonates and infants. *American Journal of Roentgenology*, *174*, 1643–1649. https://doi.org/10.2214/ajr.174.6.1741643

Team R.C. (2020). R: A Language and Environment for Statistical Computing [WWW Document]. https://www.r-project.org

Vaessen, M. J., Hofman, P. A. M., Tijssen, H. N., Aldenkamp, A. P., Jansen, J. F. A., & Backes, W. H. (2010). The effect and reproducibility of different clinical DTI gradient sets on small world brain connectivity measures. *NeuroImage*, *51*, 1106–1116. https://doi.org/10.1016/j.neuroimage.2010.03.011

Wang, Y. J., Abdi, H., Bakhadirov, K., Diaz-arrastia, R., & Devous, M. D. S. (2012). A comprehensive reliability assessment of quantitative diffusion tensor tractography. *NeuroImage*, *60*, 1127–1138. https://doi.org/10.1016/j.neuroimage.2011.12.062

Winkler, A. M., Ridgway, G. R., Webster, M. A., Smith, S. M., & Nichols, T. E. (2014). Permutation inference for the general linear model. *NeuroImage*, *92*, 381–397. https://doi.org/10.1016/j.neuroimage.2014.01.060

SUPPORTING INFORMATION

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