Myelin water fraction in relation to fractional anisotropy and reading in 10-year-old children

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Received: 29 November 2021 / Accepted: 24 March 2022 / Published online: 11 April 2022
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Abstract
Diffusion-weighted imaging studies have repeatedly shown that white matter correlates with reading throughout development. However, the neurobiological interpretation of this relationship is constrained by the limited microstructural specificity of diffusion imaging. A critical component of white matter microstructure is myelin, which can be investigated noninvasively using MRI. Here, we examined the link between myelin water fraction (MWF) and reading ability in 10-year-old children (n = 69). To better understand this relationship, we additionally investigated how these two variables relate to fractional anisotropy (FA; a common index of diffusion-weighted imaging). Our analysis revealed that lower MWF coheres with better reading scores in left-hemispheric tracts relevant for reading. While we replicated previous reports on a positive relationship between FA and MWF, we did not find any evidence for an association between reading and FA. Together, these findings contrast previous research suggesting that poor reading abilities might be rooted in lower myelination and emphasize the need for further longitudinal research to understand how this relationship evolves throughout reading development. Altogether, this study contributes important insights into the role of myelin-related processes in the relationship between reading and white matter structure.

Keywords
White matter · Reading · Myelin · Children · Microstructure

Introduction
Skilled reading relies on coordinated processing across a widespread network of cortical areas. The white matter tracts connecting these areas play a key role in facilitating rapid signal transmission across the reading network. White matter properties can be studied noninvasively using diffusion-weighted imaging (DWI), which yields, among others, the fractional anisotropy (FA) index, a quantitative measure of the directionality of water diffusion used to characterize white matter organization.

There is ample evidence consolidating the involvement of white matter organization in relation to reading. Several DWI studies provide evidence of significant associations between white matter properties (as measured by FA) and reading ability in adults (for a review see Vandermosten et al. 2012b). Although a converging finding seems to be positive correlations in left frontal and temporoparietal white matter (Lebel et al. 2013), studies have also reported negative associations between FA and reading measures (Odegard et al. 2009; Yeatman et al. 2012; Christodoulou et al. 2016). In addition, evidence from studies on pre-reading children suggests that an early link between literacy-related skills and white matter is present before the onset of reading acquisition (Vanderauwera et al. 2018; Walton et al. 2018). There is also strong evidence to support a link between white matter and reading throughout development (Yeatman et al. 2016; Ford and Friedman 2006).
2012), as well as the predictive power of early white matter organization for later reading-related skills (Vanderauwera et al. 2017; Zuk et al. 2021). Together, the available findings, albeit from small samples, support a relationship between reading and white matter structure. However, general agreement on the direction of these effects and how consistent they are across studies is lacking (Moreau et al. 2018).

What factors or processes might be driving the observed relationships between diffusion properties such as FA and reading? One of the original hypotheses put forward is myelination (Klingberg et al. 2000), a critical component of human white matter with a known role in cognitive functions and plasticity (Kaller et al. 2017). While early experiments have shown that anisotropy is primarily influenced by axonal membranes, including the density (packing) and diameter of axons, there is evidence that it is to a certain extent also modulated by myelination (Beaulieu 2009). Indeed, FA is sensitive to both microscopic and macroscopic aspects of tissue properties but has demonstrably low specificity for any single neurobiological process (Jones et al. 2013), and therefore cannot inform us on the specific role of myelin-related processes. Myelin water imaging (MWI) is an approach that allows a more specific in vivo investigation of myelin, relying on the principles of varying T2 relaxation in the different cell compartments (reviewed in MacKay and Laule 2016). A quantitative index of MWI is myelin water fraction (MWF), which can be used as a proxy measure of cortical myelination. This measure represents a quantification of myelin water, based on the short relaxation rate of water trapped within the myelin bilayer (Whittall et al. 1997). Previous histology and imaging studies have validated the use of MWF as an indirect, yet specific measure of brain myelin using both qualitative and quantitative methods (Moore et al. 2000; Laule et al. 2006).

Few studies have investigated the link between MRI myelin measures and reading. Kraft et al. (2016) reported higher T1 intensities, interpreted as reduced myelin concentration, in the left anterior arcuate fasciculus of preliterate children at familial risk for developing dyslexia compared to children without risk. Notably, the opposite pattern was reported in adults, whereby an increased myelinated cortical thickness ratio is observed in the auditory cortex of dyslexic compared to typical readers (Skeide et al. 2018). To date, only one study has directly investigated the relationship between MWF and reading ability. In a sample of 20 participants aged 10–18 years old, Beaulieu et al. (2020) reported positive correlations between reading and MWF, as well as lower MWF in poor ($n = 7$) compared to good readers ($n = 11$) in several regions including bilateral thalamus, centrum semiovale, anterior and posterior limbs of the internal capsule and splenium of the corpus callosum. This study offers new insights into the relationship between myelin water and reading, however, replication of these findings is warranted given the small sample size, the wide age range and the selection of regions which are not typically considered part of the core reading circuitry.

An important factor in interpreting associations between white matter and cognitive measures is our understanding of how MWF relates to conventional DWI metrics such as FA. While some studies report an overall positive relationship between FA and MWF (Mädler et al. 2008; Friedrich et al. 2020), others find little evidence for shared variance between the two (Bells et al. 2011; Billiet et al. 2015). Notably, Mädler et al. (2008) reported that the relationship between FA and MWF differed across regions of interest. This finding is further corroborated by De Santis et al. (2014), who showed that the correlation between FA and MWF was only significant when regions with single fiber populations were considered, as compared to regions with multiple fiber populations. Altogether, these divergent findings suggest that a positive relationship between FA and MWF may exist, but is rather dependent on the underlying fiber architecture and potentially influenced by other microstructural factors as well. Importantly, most of the aforementioned studies were conducted using adult data. Given that white matter development is still ongoing throughout childhood and adolescence (Lebel and Beaulieu 2011), it is important to uncover whether the observed relations in adults also hold in children. In children, one study reported no significant correlation between MWF and FA (Morris et al. 2020), however, the literature here is very limited.

The goal of the present work is to elucidate the relationship between white matter microstructure and reading ability in children. First, we investigate the relationship between reading ability and the myelin water fraction (MWF) index in school-aged children. We focus on bilateral white matter tracts involved in reading processes, such as the dorsal direct temporoparietal segment of the AF (AF$_{direct}$), a dorsal anterior fronto-parietal segment of the AF (AF$_{anterior}$) and the ventral inferior fronto-occipital fasciculus (IFOF). In a second step, we dig deeper into this relationship by investigating how both reading and MWF relate to FA in our sample, given that it remains one of the most widely used measures in DWI studies of reading.

**Methods**

**Participants**

The participants of this study were 72 children aged 10–11 years old (mean age 10.6 years old). The data reported here were collected in the framework of a larger longitudinal project ($N=87$) investigating the neuroanatomical and neurophysiological correlates of dyslexia (for initial cohort description see Vandermoten et al. 2015). In
the original sample, children with and without a familial risk were matched based on school, sex, age, non-verbal intelligence and parental educational level. The following inclusion criteria were applied: non-verbal intelligence ≥ 80 based on Raven’s Coloured Progressive Matrices (Raven et al. 1984), normal hearing (pure tone average at 0.5, 1, 2 and 4 kHz below 20 dB HL), monolingual native Dutch speakers, no history of brain damage, vision or articulatory problems and no increased risk for developing ADHD. The current report focuses on data from 63 children from the initial cohort, who took part in an MRI examination during spring of fifth grade. An additional nine children who were not part of the longitudinal project were recruited to participate in the MRI measurements to reach a larger sample. Therefore, the sample of this study comprises 72 children, 36 of whom had a familial risk of dyslexia, defined by having at least one first-degree relative with dyslexia, while 36 children had no familial risk.

Cognitive assessment

The behavioral assessment took place at school during the spring of fifth grade of primary school. Standardized tests were used to assess word (Brus and Voeten 1979) and non-word (Van Den Bos et al. 1994) reading ability, during which words are presented and the participant is asked to read them as accurate and fast as possible. Scores were calculated based on the number of items correctly read in one minute (for the word reading test) or two minutes (for the non-word reading test). To assess spelling skills, a writing on dictation test was used (Dudal 1997). Raw scores were used in subsequent analyses.

MRI data acquisition

All participants underwent MRI scanning during spring of fifth grade. Images were acquired on a 3 T MRI Philips Achieva scanner using a 32-channel head coil. A DWI sequence was acquired using single-shot EPI with SENSE (parallel imaging). Following parameters were used: repetition time = 7600 ms, echo time = 65 ms, flip angle = 90°, voxel size = 2.5 × 2.5 × 2.5 mm, 60 non-collinear directions with b-value = 1300 s/mm², 6 nondiffusion-weighted images. In the same session, MWI data were acquired using a whole-cerebrum multi-echo 3D gradient spin echo (GRaSE) sequence (Prasloski et al. 2012). The acquisition scheme consisted of 48 slices for which 32 echoes were acquired with echo time = 10 ms, repetition time = 1000 ms, EPI factor = 3, flip angle = 90° and 2 mm isotropic voxel size.

MRI data processing

Pre-processing steps for DWI data are described in previous publications of the original cohort (Vandermosten et al. 2015; Vanderauwera et al. 2017). Briefly, the diffusion-weighted images were pre-processed using the software ExploreDTI (version 4.8.3) (Leemans et al. 2009). Images were corrected for subject motion and eddy current-induced distortions followed by fitting the diffusion tensor model and calculation of voxelwise FA maps. Additional parameter maps were computed for mean, axial and radial diffusivities (MD, AD and RD, respectively). Subsequently, whole-brain deterministic tractography was performed using the following parameters: minimum FA threshold = 0.20, step length between calculations = 1 mm, maximum turning angle = 40°. Individual white matter pathways (AFdirect, AFanterior, IFOF) were delineated in TrackVis (Wang et al. 2007) by an experienced rater (JV) by manual placement of anatomical regions of interest in native space (details outlined in Vandermosten et al. 2012a). The right AFdirect could not be identified in 13 participants, the left AFdirect could not be identified in 3 participants and the left AFanterior could not be identified in 1 participant. This inability to detect the right AFdirect in certain individuals is consistent with previous reports (Eluvathingal et al. 2007; Lebel and Beaulieu 2009) and has been attributed to methodological limitations rather than gross anatomical abnormalities (Yeatman et al. 2011). Head motion during DWI acquisition was quantified by calculating the absolute displacement of each volume relative to the first volume of the DWI series. Datasets from subjects whose average translational motion exceeded 2.5 mm (acquisition voxel size) were excluded from further analysis due to excessive motion. Note that no datasets were removed according to this criterion (median translational displacement = 0.43, range = 0.21–1.30).

MWI data were pre-processed in MATLAB 2016b using in-house scripts, following a protocol previously outlined in Billiet et al. (2015). Briefly, a multiexponential decay curve was fit in each voxel and then transformed into a continuous T2 distribution of mono-exponential T2 decay curves using a non-negative least squares algorithm (Whittall and MacKay 1989). The extended phase graph algorithm was used to account for possible stimulated echoes (Prasloski et al. 2012). To assure smooth T2 amplitude distributions, a 1.02 regularization factor was applied during the fitting procedure. From the T2 distributions, metrics were derived on a voxelwise basis. Maps of MWF were obtained for each participant, with MWF defined as the area fraction between 10 and 40 ms relative to the total T2 distribution area.

To achieve alignment between the two modalities, the first non-diffusion-weighted image (b0) of the DWI acquisition
was registered to the first echo (TE1) of the MWI acquisition using a rigid transformation with six degrees of freedom as implemented in Advanced Normalization Tools (ANTS; Avants et al. 2011). The inverse transformation was then applied on the MWI map to achieve alignment with the native DWI space. Binarized masks of all delineated white matter tracts were constructed and superimposed on FA and MWF parameter maps. For each subject, average FA and MWF measures were extracted from each corresponding tract by calculating the metric average across all mask voxels in the native space.

 Statistical analysis

All reported analyses were performed in R (version 4.0.0) (R Core Team 2020). All subjects were included in the analyses except one participant, for whom MWI data were not available and two datasets where the MWI pre-processing pipeline could not be successfully completed (analyzed N = 69). Significance was set at alpha = 0.05 for all analyses.

To investigate the association between word reading and MWF, linear models were constructed in each tract. Reading was modelled as the dependent variable with MWF and age as independent variables. Regression model estimates are reported as standardized beta coefficients (β), which enable their interpretation as effect sizes. The same procedure was followed to assess the effect of FA on word reading. To account for multiple comparisons a false discovery rate (FDR) correction was applied (q = 0.05).

Linear mixed-effects regression using the lme4 package (Bates et al. 2015) was employed to assess the relationship between metrics, with FA as the dependent variable and MWF and age as independent variables. Regression model estimates are reported as standardized beta coefficients (β), which enable their interpretation as effect sizes. The same procedure was followed to assess the effect of FA on word reading. To account for multiple comparisons a false discovery rate (FDR) correction was applied (q = 0.05).

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Table 1 Participant characteristics

| Variable                        | N = 69 a | Median (range) | Missing values (N) |
|--------------------------------|----------|----------------|-------------------|
| Sex (female/male)              | 24/45    |                |                   |
| Familial risk for dyslexia (yes/no) | 36/33    |                |                   |
| Age at MRI                     |          |                |                   |
| Months                         | 128 (3)  | 127 (122–134)  |                   |
| Years                          | 10.6 (0.3)| 10.6 (10.1–11.2)|                   |
| Socio-economic status b         | 5 (2)    | 6 (2–8)        | 8                 |
| Word reading                   |          |                |                   |
| Raw score                      | 59 (17)  | 59 (26–104)    |                   |
| Standardized score             | 85 (21)  | 85 (55–145)    |                   |

a Counts are reported for categorical variables while Mean (SD) is reported for continuous variables

b Assessed with the Family Affluence Scale (Boudreau and Poulin 2009)

measures derived from the diffusion tensor (for more details see Supplementary Information).

Results

Table 1 shows the participant demographic characteristics and cognitive skills.1 Reading scores were negatively associated with MWF in the left IFOF (β = −0.38, p FDR = 0.007) and left AF anterior (β = −0.32, p FDR = 0.022). No additional effects were observed in the other investigated pathways. The corresponding scatter plots and regression lines are shown in Fig. 1A. Complementary analyses showed that non-word reading is also negatively associated with MWF in the left IFOF, AF anterior and AF direct (p FDR < 0.05) but no relations between MWF and spelling were observed (data presented in Supplementary Information).

There were no significant associations between reading scores and FA in any of the investigated pathways in the present sample (Fig. 1B).

Figure 1C shows the results of the mixed-effects regression analysis. The best-fitting model, as indicated by the lowest AIC value, had a by-subject random intercept, a conditional R² of 72.9% and a marginal R² of 56.8%. The model revealed a significant positive relationship between MWF and FA, after accounting for the effect of age (F(1,
Fig. 1  A Scatter plots with regression lines for the relationship between myelin water fraction and raw reading scores. B Regression lines for the relationship between fractional anisotropy and raw reading scores. C The main effect of myelin water fraction on fractional anisotropy, across all tracts. The asterisk denotes significance at $p_{FDR} < 0.05$. Standardized coefficients and linear trend estimates are reported after accounting for age effects.
In this report, we investigated the relationship between reading ability and myelin water fraction (MWF) in 10-year-old children. First, we examined the relationship between reading scores and myelin index MWF in three bilateral white matter tracts relevant for reading. Our analysis revealed a negative association between reading and MWF in the left hemisphere, namely the inferior fronto-occipital fasciculus and anterior (fronto-parietal) segment of the arcuate fasciculus, indicating that poorer reading abilities cohered with more myelination. In the next step, we investigated how both reading and MWF relate to FA, a commonly used index of white matter organization in studies of reading and language. Here, we observed an overall positive relationship between FA and MWF across all tracts and no evidence of an association between reading and FA.

The present study contributes to fairly sparse literature on how reading relates to white matter structure beyond the classical DWI metrics. Here, our results support a negative correlation between reading skill and myelin measures in left-hemispheric tracts. This finding contradicts earlier hypotheses suggesting that lower or poor myelination might contribute to compromised conduction speed along axons and in turn to impaired reading (Klingberg et al. 2000). Moreover, this finding comes in contrast to previous DWI studies in which a positive association between reading and FA is found (Lebel et al. 2013; Zhang et al. 2014). The only direct comparison with the literature is the study by Beaulieu et al. (2020), where myelin water measures were linked to reading scores, revealing positive correlations in bilateral anterior, thalamic and callosal regions. Distinct methodological differences should be considered when comparing the conflicting results observed between our study and that of Beaulieu and colleagues, such as i) the choice and method of extracting regions-of-interest (tractography and manual delineations of white matter tracts vs ROI placement), ii) the sample size (n = 71 in our study, n = 20 in the study by Beaulieu et al.), and most importantly iii) the population characteristics (narrow vs wide age range).

With respect to the latter point, the influence of age effects on the correlation between reading and MWF is likely a major contributor. More specifically, Beaulieu et al. reported a positive correlation between MWF and reading in a relatively wide age range (10–18 years old) and did not find significant correlations between age and MWF within their sample. However, reading measures were standardized for age while MWF was not, which can be problematic for interpreting this correlation, given that previous studies have reported age-related increases in myelin volume fraction and thus ongoing myelination across a similar age span (Geeraert et al. 2019).

The present study also examined the link between FA and reading, revealing no associations between the two measures. Note here that in a previous study using a partially overlapping sample, a positive relationship between reading and FA was found (Van Der Auwera et al. 2021). However, the sample overlap (around 50%) is such that the study by Van Der Auwera et al. and the current study are not directly comparable. The lack of association between reading and FA in our study is unexpected in light of past research, however, is in line with very recent work by Meisler and Gabrieli (2022), who failed to find any associations between FA and word and non-word reading in a sample of 686 children and adolescents aged 5–18. This study supports the idea that the FA-reading relationship might not be as robust early studies with small sample sizes (and different methodologies) suggest and possibly varies more among individuals and across the lifespan than previously assumed. Note that in the aforementioned study, age was accounted for by standardizing reading scores but not FA, which can be problematic given the evidence for age-related FA changes throughout childhood and adolescence (Lebel and Beaulieu 2011; Lebel and Deoni 2018).

The findings of the present study should also be discussed in the context of developmental brain-behavior dynamics. For instance, it has been suggested that the nature of the associations between reading and white matter properties changes throughout development (Yeatman et al. 2012; Wandell and Yeatman 2013). This variability is presumably a result of individual differences in the rate and timing of processes such as pruning and myelination, and influenced by experience, genetic and environmental factors, among others. Hence, in line with this view, we can hypothesize that the direction of the association between reading and MWF does not remain stable throughout reading development and that the current cross-sectional investigation only captures a snapshot of this dynamic relationship. Similarly, this view could explain why the relationship between reading and FA seems to fluctuate throughout childhood and adolescence, as reflected in diverse (and sometimes null) findings across studies, including the present one (see Meisler and Gabrieli (2022) for an overview). A longitudinal design including two or more assessment points, in combination with a sample that is similar in age, would be better suited and is recommended for future studies aiming to disentangle these developmental influences (van Atteveldt et al. 2021).

Understanding how MWF relates to diffusion anisotropy measures enables a more comprehensive interpretation of
the associations reported between white matter properties and reading. Our analysis revealed a positive relationship between FA and MWF, a finding that is in agreement with some previous studies investigating associations among white matter microstructure metrics (Friedrich et al. 2020), but in contrast to others (Billiet et al. 2015; Morris et al. 2020). When interpreting the findings of the current study, it is important to acknowledge that previous research was almost solely conducted using adult data, whereas here we report on data from 10-year-old children. Given that most tracts are undergoing both myelination and increases of axonal packing during late childhood and adolescence (Geeraert et al. 2019), it is possible that the relationship between myelination and anisotropy is driven by different factors in children than it is in adults. Overall, our results provide evidence for shared variance between the two metrics in children.

A methodological consideration of the present study is the narrow focus on a specific set of white matter tracts. The choice of including the AF segments and IFOF is motivated mainly by earlier work conducted on the longitudinal project the present study is part of (see for example Vandermoten et al. 2015; Vanderauwera et al. 2018). These studies point specifically towards a role for the AF and IFOF in sustaining reading-related skills during the pre-reading and early-reading stages in a largely overlapping sample. However, associations with reading have also been shown elsewhere in the brain including white matter tracts that are not included in our analysis (see for example Ben-Shachar et al. 2007; Grotheer et al. 2019). We, therefore, acknowledge the importance of extending our analyses to a broader range of white matter pathways in future research, in order to better characterize the relationship between white matter and reading processes.

To conclude, this study used myelin water imaging in combination with diffusion imaging aiming to elucidate the relationship between white matter microstructure and reading ability. The study contributes new insights into the shared relationship between myelination and anisotropy in children. In addition, our findings support associations between reading scores and myelin water, but not fractional anisotropy, which were observed specifically in the left hemisphere.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00429-022-02486-x.

Acknowledgements We would like to thank Caroline Beelen, Thanh Van Phan and all the DYSCO colleagues and student assistants for participant selection and data collection. We are grateful to all families, children and schools for their participation in this research.

Author contributions ME and MV designed this study. ME conducted the data processing, data visualization, statistical analysis and drafted the manuscript. TB provided software for image processing, contributed to the methodology and revised the manuscript. JW, PG, JV and MV revised the manuscript, were involved in conceptualization, data curation, project administration, funding acquisition and supervised the project. All authors read and approved the final version of this manuscript.

Funding This work was supported by the European Union H2020 MSCA-ITN-2014-ETN Programme, Advancing brain research in children's developmental neurocognitive disorders-project (ChildBrain, #641652), and the Research Council of KU Leuven (C14/17/046). J.V. was a postdoctoral fellow of the Research Foundation Flanders (12T4818N).

Data availability The conditions of our ethics approval do not permit public archiving of anonymised study data, since consent had only been obtained for the participation in the study, and not to share data with third parties. Researchers seeking access to the study data should contact the last author (maaike.vandermosten@kuleuven.be) explaining the purpose of their request. In accordance with the EU general data protection regulation (GDPR), data will be released to requestors upon the following conditions: consent of the representative of the minor and a formal agreement between parties. Please note that the MRI data cannot be shared under any circumstance, as MRI data are person-specific and therefore cannot be considered anonymous.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Medical Ethical Committee of the Leuven University Hospital (B322201214607).

Consent to participate Written informed consent was obtained from the parents/guardians of the children participating in this study. Verbal assent was obtained from the children before participation.

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