# Longitudinal white matter development in children is associated with puberty, attentional difficulties, and mental health.

Sila Genc[1,2,3], Charles B Malpas[2,4], Alisha Gulenc[5], Emma Sciberras[3,5,6], Daryl Efron[5,7], Timothy J Silk[2,6]*, Marc L Seal[2,3]*.

[1]Cardiff University Brain Research Imaging Centre (CUBRIC), Cardiff University, Cardiff, UK;
[2]Developmental Imaging, Murdoch Children's Research Institute, Parkville, Australia;
[3]Department of Paediatrics, University of Melbourne, Parkville, Australia;
[4]Clinical Outcomes Research Unit (CORe), Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Parkville, Australia;
[5]Population Health, Murdoch Children's Research Institute, Parkville, Australia;
[6]School of Psychology, Deakin University, Geelong, Australia.
[7]The Royal Children's Hospital, Parkville, Australia;
*Equal senior author

**Corresponding author:**
Sila Genc
Cardiff University Brain Research Imaging Centre (CUBRIC)
Cardiff University, Cardiff, CF24 4HQ, UK
Email: sila.genc@mcri.edu.au

**Abbreviations:**
CCG=cingulum cingulate gyrus; CCH=cingulum hippocampus; CSD=Constrained Spherical Deconvolution; CST=Cortico-spinal tract; FA=Fractional anisotropy; FBA=Fixel-based analysis; FC=Fibre cross-section; FD=Fibre density; FDC=Fibre density and cross-section; FOD=Fibre orientation distribution; Fmajor=Forceps major; Fminor=Forceps minor; Fx=fornix; IFOF=inferior fronto-occipital fasciculus; ILF=inferior longitudinal fasciculus; MRI=Magnetic resonance imaging; NICAP=Neuroimaging of the Children's Attention Project; PDS=Pubertal development scale; SEIFA=Socio-Economic Indexes for Areas; SES=Socio-economic Status; SLF=Superior longitudinal fasciculus; UF=uncinate fasciculus.

**Keywords:** White matter; Longitudinal; Fixel-based analysis; Puberty; Attention; Internalising

## ABSTRACT

**Purpose:**
The pubertal period involves dynamic white matter development. This period also corresponds with rapid gains in higher cognitive functions including attention, as well as increased risk of developing mental health difficulties. We performed a longitudinal investigation of the relationship between white matter fibre properties and pubertal stage, attentional difficulties, and internalising problems.

**Methods:**
This study reports on a community-based sample of children aged 9-13 years (n=130, 47 female). Diffusion magnetic resonance imaging (dMRI) data were acquired on a 3.0T Siemens Tim Trio (b=2800 s/mm$^2$, 60 directions) at two time-points approximately 16 months apart: time-point 1 (age: M = 10.4, SD = .44 years old), time-point 2 (age: M = 11.7, SD = .51 years old). We leverage the fixel-based analysis (FBA) framework, to derive measures of: fibre density (FD), fibre cross-section (FC), and fibre density and cross-section (FDC), in 17 manually delineated white matter tracts. We apply a longitudinal mixed-effects modelling analysis: to understand how specific fibre properties vary with age, sex, and pubertal stage; and as a function of the development of internalising behaviours and attentional difficulties.

**Results:**
We observed significant increases in FD, FC, and FDC across a large number of white matter pathways, with protracted development of frontal pathways such as the inferior fronto-occipital fasciculus (IFOF) and uncinate fasciculus (UF). We observed a linear relationship between FBA metrics and pubertal stage, in the right superior longitudinal fasciculus (SLF). Increases in FD were associated with greater attentional dysfunction, particularly in older males. Lastly, we found evidence for an association between lower FD and greater internalising problems in the right UF, a region known to be implicated in psychiatric disorders.

**Discussion:**
These longitudinal results shed light on regional fibre developmental profiles in early puberty. The development of fibre density and morphology across ages 9-13 years involved the expansion of key white matter tracts, excluding regions known to have protracted development over adolescence. The associations between mental health and attentional problems with fibre density in the UF suggests that this region may be sensitive to adopting a different neurodevelopmental course in the presence of such symptoms. Overall, our findings highlight the interrelated nature of fibre development with puberty, mental health problems, and attentional difficulties.

## 1. Introduction

Puberty is a critical period of development, marking the transition from childhood to reproductive maturity (Dorn et al., 2006). White matter is particularly sensitive to remodelling with exposure to pubertal hormones (Juraska and Willing, 2017). Evidence suggests that individual variability in pubertal timing is a strong predictor of mental health problems (Kaltiala-Heino et al., 2003). World-wide, the peak age of onset of psychiatric disorders is age 14 years (Kessler et al., 2005). The presence of internalising problems, including anxiety and depression, during this sensitive period of development pose risk for later case-level disorder (Shankman et al., 2009). Whether disrupted pubertal timing influences the onset of mental health problems and neurodevelopmental pathways, or vice versa, is not well understood. What is clear, however, is that these interrelated factors can alter the structural and functional reorganisation of the brain (Paus et al., 2008).

Alongside internalising problems, externalising disorders can co-exist with commonly occurring disorders in childhood, such as Attention-Deficit/Hyperactivity Disorder (ADHD) (Polanczyk et al., 2007). Whilst ADHD symptoms often present before pubertal onset, these symptoms can become more severe during the transition to adolescence, whereby increases in attentional difficulties and externalising symptoms can alter neuropsychological function (Langberg et al., 2008). Mapping these apparent links between puberty, mental health, and neurodevelopmental anomalies, is imperative.

Previous studies have shown relationships between white matter microstructure, and attentional difficulties (Cooper et al. 2015), pubertal stage (Ladouceur et al. 2012), and internalising problems (Hettema et al. 2012), using measures derived from diffusion tensor imaging (DTI). Whilst DTI metrics are sensitive to local differences in white matter organisation, attributing a specific microstructural property to this group difference is not possible due to technical limitations of the model (Jones 2013). Recent advances in diffusion magnetic resonance imaging (dMRI) models facilitate the investigation of links between pubertal processes and behaviour with brain development on a magnified neurobiological scale (Tamnes et al., 2017).

The recently introduced fixel-based analysis (FBA) framework (Raffelt et al., 2017) and subsequent longitudinal modifications to this (Genc et al., 2018b), allows the quantification of white matter fibre properties such as fibre density and morphology. The measure of apparent fibre density (FD) reflects the total intra-axonal volume fraction per fibre pathway, where increases in FD can be reflective of increasing axon diameter and/or local axon count. The measure of fibre morphology encompasses both fibre cross-section (FC) and fibre-density and cross-section (FDC). FC is sensitive to macroscopic changes in the cross-sectional area perpendicular to a fibre bundle experienced during registration to a template image. FDC incorporates features of FD and FC, and acts as a surrogate marker of alterations to the capacity for information transfer across a fibre bundle, whereby high FDC values signify this greater capacity.

With the use of robust longitudinal neuroimaging studies, it is possible to unravel the specific neurobiological links between brain structure and developmental factors during this sensitive period of growth. To the best of our knowledge, this is the first longitudinal study to examine neurodevelopmental profiles with puberty, attention, and mental health, using advanced dMRI metrics.

The first aim of this study was to specifically quantify the development of fibre density and morphology in a variety of white matter tracts, in attempt to replicate previous developmental findings, and generate new insights using FBA metrics. The second aim was to understand longitudinal relationships between pubertal and behavioural factors on white matter fibre properties. In order to unravel these complex associations, we combine a tract-based approach with mixed-effects modelling upon a cohort of children with and without attentional difficulties. Rather than dichotomise groups at the extreme ends of pubertal stage, or mental health and attentional difficulties, we aimed to perform a dimensional analysis to shed light on how continuous changes in these variables relate to the longitudinal development of fibre properties. It was hypothesised that greater internalising symptoms and attentional difficulties would be negatively associated with fibre density and morphology.

## 2. Methods

*Participants*

This study reports on a sample of children aged 9-13 years recruited as part of the Neuroimaging of the Children's Attention Project study (see Silk et al. (2016) for a detailed protocol). This longitudinal study was approved by The Royal Children's Hospital Melbourne Human Research Ethics Committee (HREC #34071). Briefly, children were initially recruited at 7 years of age from 43 socio-economically diverse primary schools distributed across the Melbourne metropolitan area, Victoria, Australia. Children underwent comprehensive assessment for ADHD at age 7, and at age 10 (imaging time-point 1) via the Diagnostic Interview Schedule for Children (DISC-IV) completed with parents face-to-face (Sciberras et al. 2013). Children were categorised as either meeting a negative or positive diagnosis for ADHD.

At 10 years of age, children and their primary caregiver were invited for a 3.5-hour appointment at The Melbourne Children's campus, which included a child assessment, parent questionnaire, mock scan, and MRI scan. Direct assessments and MRI scans were performed by a trained research assistant which was blind to the child's diagnostic status. Children were invited for a follow-up appointment approximately 16 months following their initial visit (M = 16.14, SD = 2.37 months). Overall, only data from the two imaging time-points: time-point 1 (age: M = 10.4, SD = .44 years old) and time-point 2 (age: M = 11.7, SD = .51 years old); were included for analysis in the current study.

Written informed consent was obtained from the parent/guardian of all children enrolled in the study. Children were excluded from the study if they had a neurological disorder, intellectual disability, or serious medical condition (e.g. diabetes, kidney disease).

*Measures*

The following measures were obtained at both imaging time-points. General intellectual ability was estimated using the Wechsler Abbreviated Scale of Intelligence (WASI) matrix reasoning sub-test (Wechsler, 1999). The Connors 3 ADHD Index (10-items) was administered via parent survey (Conners et al. 2011), in order to capture the variation in ADHD symptom severity across time-

points. The Strengths and Difficulties Questionnaire (SDQ) was administered in the form of a parent survey as a measure of emotional/behavioural difficulties (Goodman, 1997). Using the responses from this questionnaire, the scores derived from the peer problems and emotional problems scales were added to generate a combined internalising difficulties score. The Pubertal Development Scale (Petersen et al. 1988) was administered to parents, and a combined score reflecting pubertal stage was constructed for each imaging time-point (PDSS; Shirtcliff et al., 2009). Additional information on the psychometric properties of these measures are summarised in Supplementary Information.

Child height and weight were measured using the average of two consecutive measurements to calculate a Body-Mass index (BMI) (kg/m$^2$). Socio-economic status (SES) was determined using the Socio-Economic Indexes for Areas (SEIFA), based on Australian Census data (Mean = 1000, SD = 100).

*Image acquisition and pre-processing*

Diffusion MRI data were acquired at two distinct time-points on a 3.0 T Siemens Tim Trio, at The Melbourne Children's Campus, Parkville, Australia. Data were acquired using the following protocol: b = 2800 s/mm$^2$, 60 directions, 4 volumes without diffusion weighting, 2.4 x 2.4 x 2.4 mm voxel size, echo-time / repetition time (TE/TR) = 110/3200 ms, multi-band acceleration factor of 3, acquisition matrix = 110 x 100, bandwidth = 1758 Hz. A total of 152 participants had longitudinal MRI data. Of those, 130 participants had useable diffusion MRI data, therefore the subsequent image processing and analysis was performed on these 130 participants with imaging data at two time-points.

All dMRI data were processed using MRtrix3 (v3.0RC3; Tournier et al., 2019) using pre-processing steps from a recommended fixel-based analysis (FBA) pipeline (Raffelt et al., 2017). For each scan, these pre-processing steps were: denoising (Veraart et al., 2016); eddy, motion, and susceptibility induced distortion correction (Andersson and Sotiropoulos, 2016); bias field correction; and group-wise intensity normalisation. Data were then upsampled by a factor of 2, and a fibre-orientation distribution (FOD) was estimated in each voxel. Intra-cranial volume for each T1-weighted image at each time-point was calculated using FreeSurfer (version 6) (Reuter et al., 2012). Images were visually inspected for motion artefact (assessed by the presence of Venetian blinding artefact), and whole datasets were excluded if excessive motion was present. In addition, we calculated mean frame-wise displacement using the FSL software library (v5.0.10) (Smith et al. 2004).

*Longitudinal template generation*

In order to build an unbiased longitudinal template, we selected 40 individuals, with equal numbers of males and females, to first generate intra- subject templates. For each of these individuals, the time-point 1 and time-point 2 FOD maps were transformed to their midway space and subsequently averaged to generate an unbiased intra-subject template. The 40 intra-subject FOD templates were used as input for the population template generation step.

Following generation of the population template, each individual's FOD image was registered to this longitudinal template (Raffelt et al., 2011), and the resulting transformed FOD within each template space voxel segmented to produce a set of discrete fixels (Smith et al., 2013). Reorientation of fixel directions due to spatial transformation, correspondence of these fixels with the template image,

and derivation of FBA metrics, was performed as described previously (Genc et al., 2018b). The output metrics fibre density (FD), fibre cross-section (FC), and fibre density and cross-section (FDC) (in template space) were then subjected to further statistical analysis.

*Tractography*

We chose to delineate 7 key bilateral white matter fibre pathways, alongside 3 commissural bundles, which make up the John's Hopkins University (JHU) white matter tractography atlas available in FSL. These pathways were delineated to segment fixels from the whole-brain fixel template which corresponded with our tracts of interest. To aid in the spatial identification of specific fibre bundles, we used a three-step process to improve specificity, summarised as follows:

- *Atlas registration:* The FA image derived from the JHU-ICBM atlas (Smith et al., 2004) was non-linearly transformed to our population template, and the resulting transformations were applied to warp the JHU tractography atlas to population template space.
- *Whole-brain tractogram overlay:* The whole-brain population-based tractogram was visualised in order to identify specific bundles. This tractogram was visualised as colour-coded directions, to further enable the identification of specific bundles (i.e. corticospinal tract runs inferior to superior, therefore is coloured blue).
- *Region of interest (ROI) selection:* We used a protocol defined in Wakana et al. (2007) to aid in the placement of inclusion ROIs for each major fibre bundle. We placed two separate inclusion ROIs in regions identified in the protocol, making sure they overlapped the JHU tractography atlas and whole brain tractogram. For bilateral tracts, the opposite hemisphere was used as an exclusion ROI. The whole brain tractography map was then edited using *tckedit*, and visualised, to check the anatomical correctness of the tract. In cases where spurious streamlines were present, these were rerun with an additional manually drawn exclusion ROI.

The subsequently generated 17 white matter tracts, also referred to as regions, were then converted to fixel maps using the whole-brain fixel template (*tck2fixel*), and binarised for statistical sampling of output maps (*mrthreshold*). For each participant at each time-point, we calculated mean FD, FC, and FDC values in each of the fixel masks derived from the 17 white matter tracts for subsequent statistical analyses. An example of these steps for one representative white matter tract can be visualised in Figure 1.1.

*Statistical analyses*

All statistical analyses were performed within R (version 1.1.423). We tested the relationship between each FBA metric and the variables of interest (age, sex, socio-economic status, pubertal stage, ADHD symptoms, and internalising problems) using a repeated measures ANOVA.

For the mixed-effects model, we set the time interval between scans and subject ID as random effects as per (Morrell et al., 2009), in order to assess the longitudinal change in fibre metrics whilst accounting for variation in the time interval between scans. We also included age at first scan (FAge), in order to model variation in fibre development associated with age at entry into the imaging study. These models were repeated for FC and FDC.

We computed bootstrapped 95% confidence intervals (n=5000 simulations) and report these for each mixed-model coefficient as β [95% CI]. Evidence for an association is represented when confidence intervals do not cross zero. As multiple regressions were run, p-values were adjusted using False Discovery Rate (FDR) correction (Benjamini and Hochberg, 1995). Where evidence for a main effect was identified (95% CI not crossing zero) across similar tracts, we subjected those regions to further statistical analysis for exploratory model interactions.

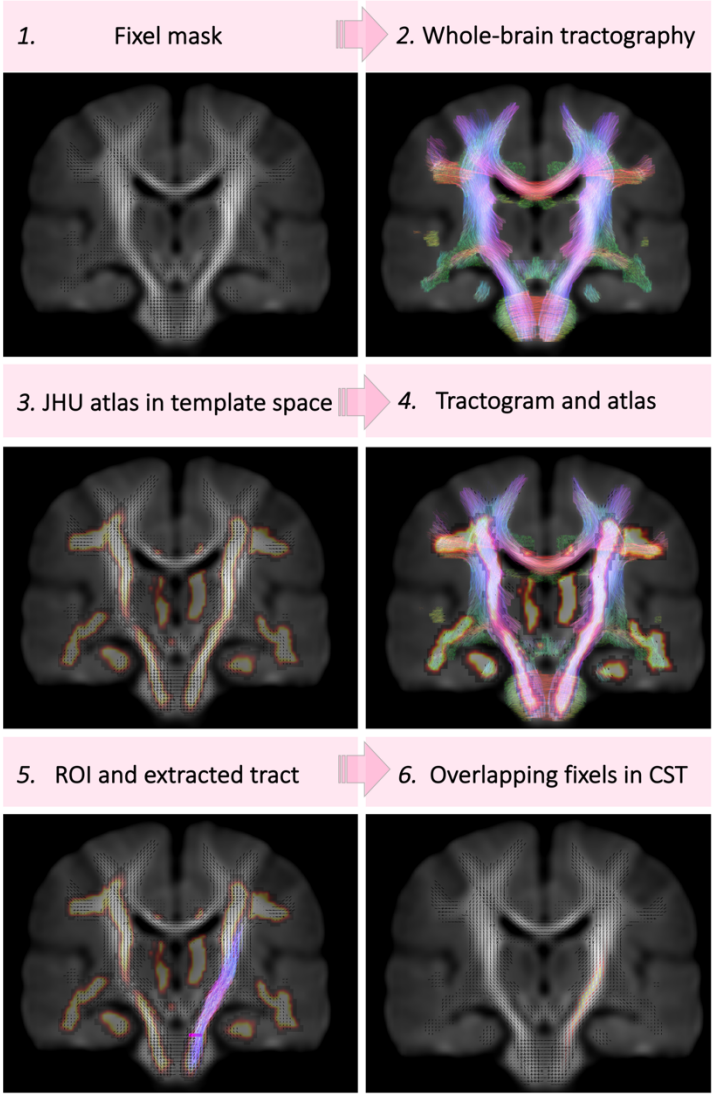


**Figure 1.1:** Protocol for defining fixels overlapping tracts of interest. Example shown is for a single ROI to delineate the left corticospinal tract

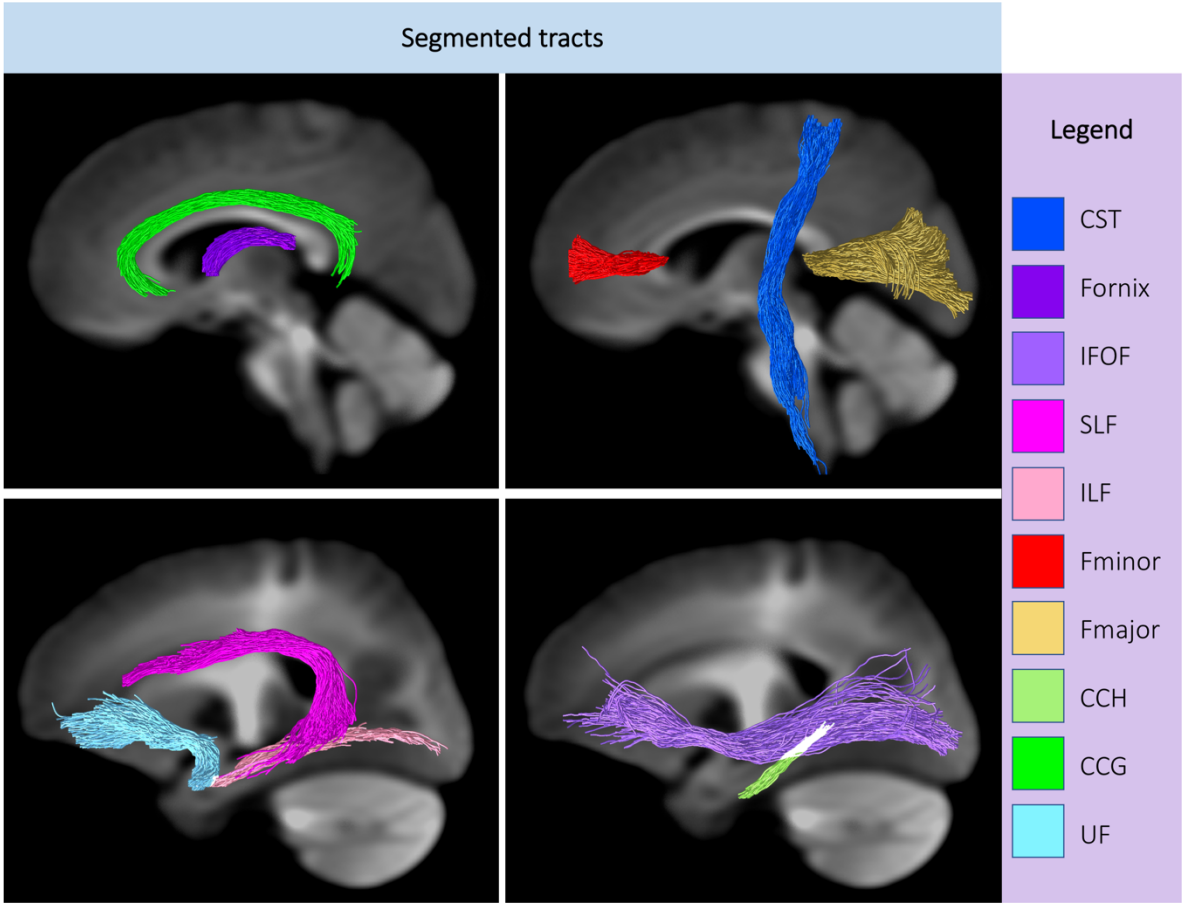


**Figure 1.2:** Visualisation of the 17 white matter tracts derived for analysis, overlaid on the population template. Tracts are 3D representations overlaid on a single representative slice for visualisation. Tracts were derived in FOD template space, using the protocol defined in Figure 1.1

**Table 1.1:** Change in participant characteristics over the follow-up interval

| Variable | Time-point 1 | | Time-point 2 | | Difference |
|---|---|---|---|---|---|
| | M | SD | M | SD | p-value |
| Age, years | 10.38 | 0.44 | 11.72 | 0.51 | < .001 |
| Socio-economic status (SES) | 1017 | 45 | 1016 | 45 | 0.32 |
| Body Mass Index, kg/m$^2$ | 19.10 | 3.76 | 20.25 | 4.28 | < .001 |
| Pubertal stage, PDSS | 1.48 | 0.73 | 2.05 | 1.05 | < .001 |
| SDQ internalising | 4.62 | 4.00 | 7.68 | 3.48 | < .001 |
| ADHD symptoms | 6.17 | 6.78 | 5.59 | 6.58 | 0.04 |
| Matrix reasoning, raw score | 22.48 | 4.86 | 24.79 | 4.25 | < .001 |

Difference calculated using a paired samples t-test. M = mean, SD = standard deviation

## 3. Results

*Participant characteristics*

Differences in participant characteristics over the 16-month follow-up period are reported in Table 1.1. We observed developmental increases in physical characteristics such as BMI and PDSS ($p < .001$), and in WASI matrix reasoning raw score ($p < .001$). Behaviourally, we observed an increase in internalising symptoms assessed by the SDQ ($p < .001$). There was some weaker evidence for a decrease in ADHD symptoms over time ($p = .04$).

*Developmental patterns*

We assessed whether there was evidence for regional differences in fibre development; differential pathways of fibre development; and associations between our variables of interest, and regional fibre development over time. We observed a significant region by time-point interaction, suggesting that specific regions are developing differently over time. To investigate these region-specific relationships, we computed multiple linear mixed-effects models for each white matter tract. Results from the mixed-effects modelling analysis are represented for each variable studied for FD (Figure 1.3), FC (Figure S3), and FDC (Figure S4).

Developmental increases in fibre density (FD) over time were localised to the bilateral CCG, bilateral CST, forceps major and minor, left IFOF, left ILF, and bilateral SLF. Significant increases in fibre cross-section (FC) were observed in: bilateral CCG, bilateral CST, forceps major, left IFOF, and bilateral SLF. Increases in fibre density and cross-section were observed in bilateral CCG, bilateral CST, forceps major and minor, fornix, bilateral IFOF, and bilateral SLF. All regions listed significantly increased in fibre density and morphology over time, and no regions decreased. The longitudinal changes in common fibre pathways changing over time are visualised for FD (Figure 1.4). We observed no association between age at first scan, with fibre properties in any of the tracts investigated.

Sex differences were only observed in forceps minor, whereby males had lower FDC compared with females. We observed no significant relationship between age of first scan with the development of fibre properties in any of the white matter tracts studied.

We observed a significantly positive relationship between intra-cranial volume (ICV) and fibre morphology in the majority of white matter regions studied. ICV significantly predicted FC values across all regions, and FDC values in all regions except the left CCH. Overall, ICV explained little variance in FD, as this association was only apparent in the right uncinate fasciculus.

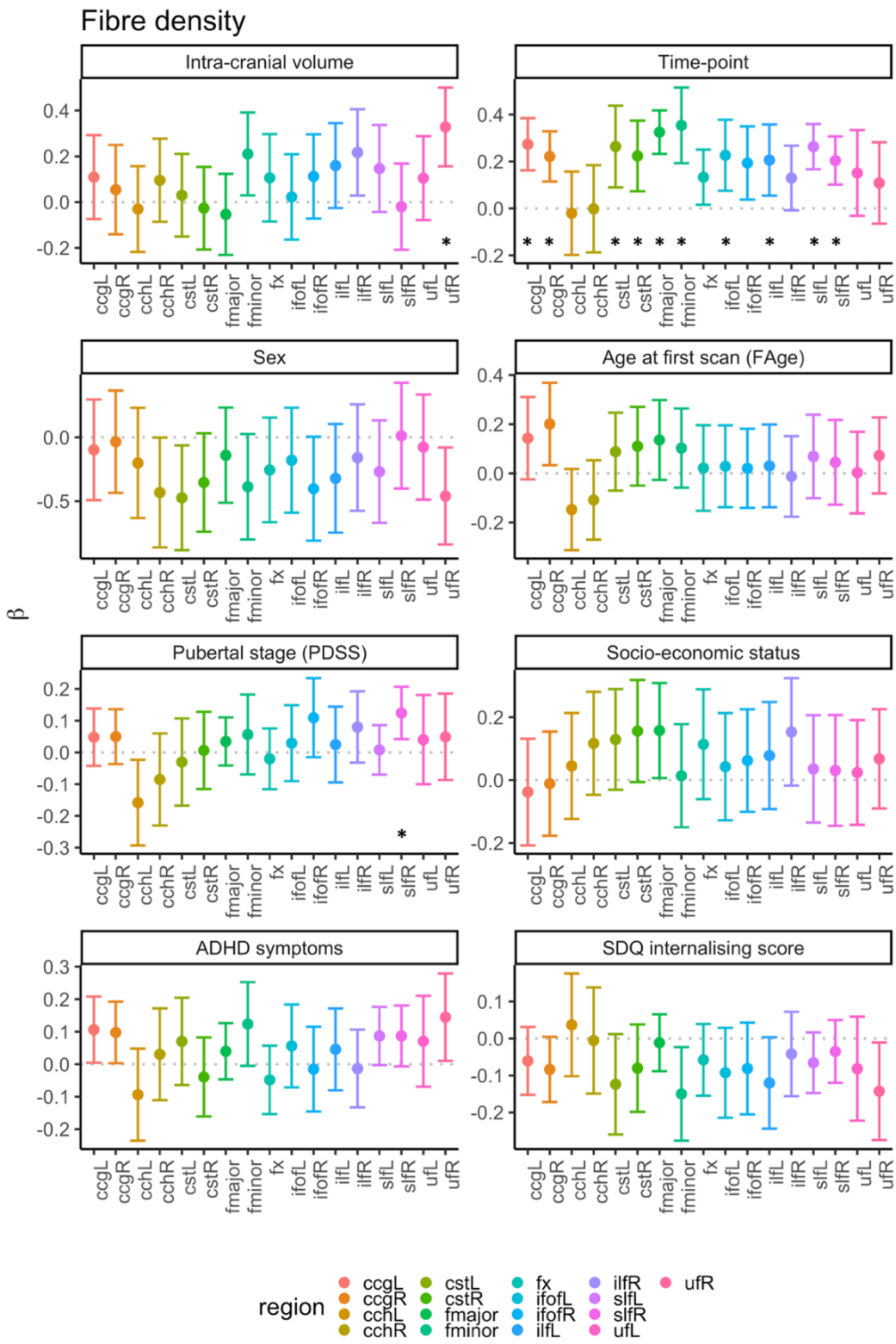


**Figure 1.3:** Relationships between participant characteristics and fibre density across all white matter tracts. 95% confidence intervals which do not cross zero suggest a relationship between that variable and the metric of interest. Regions that additionally reach statistical significance at $p_{FDR} < .05$ are annotated with an asterisk (*)

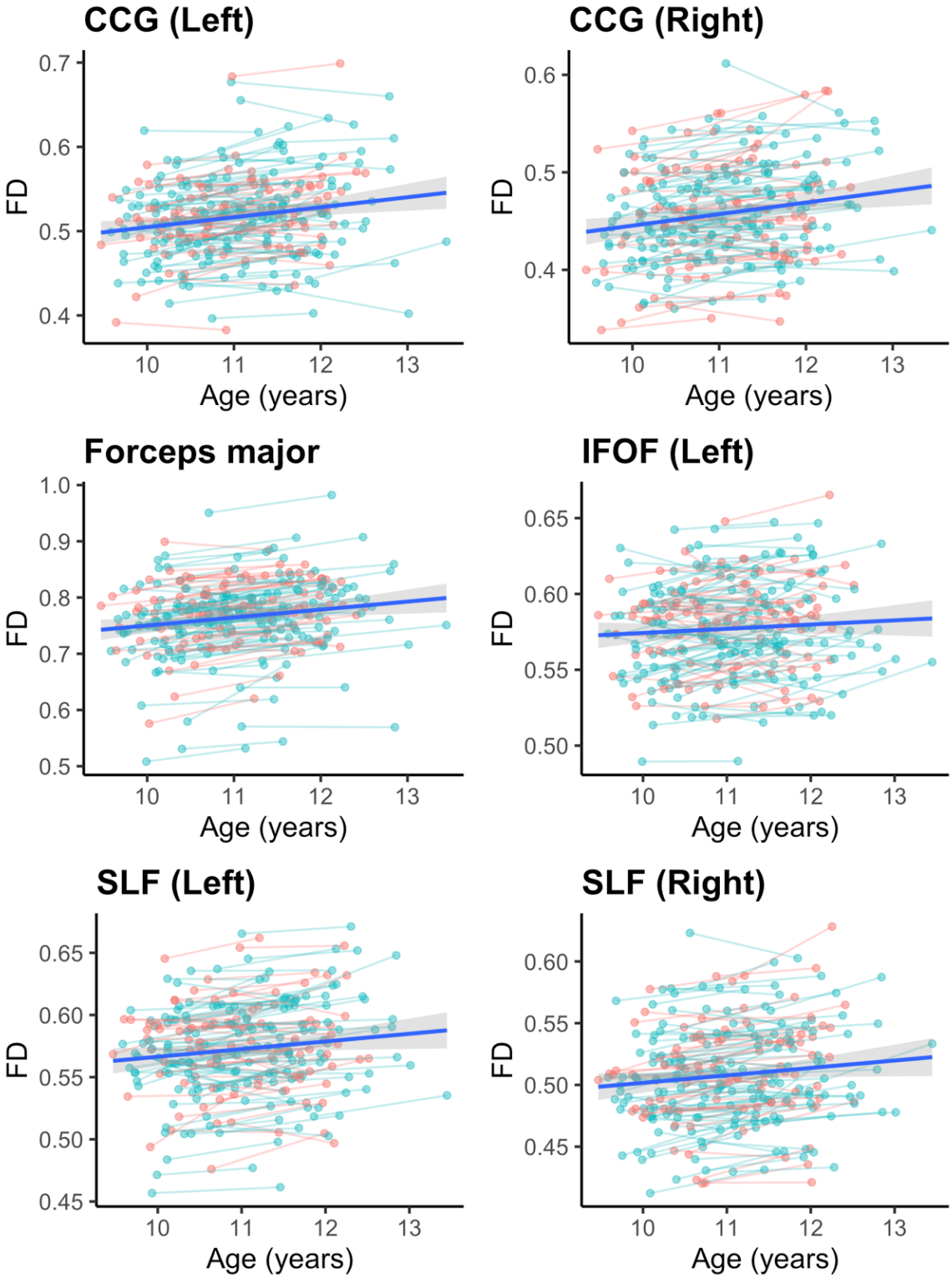


**Figure 1.4:** Longitudinal change in FD for regions with significant changes in fibre properties over time. Blue = boys, Red = girls

*Impact of puberty, attentional difficulties, and mental health*

There was a significantly positive relationship between pubertal stage (PDSS) and FD, β [95% CI] = .12 [.04, .20], FC, β [95% CI] = .15 [.06, .23], and FDC, β [95% CI] = .16 [.08, .24]. We observed evidence for a positive association between FD and ADHD symptoms in the left CCG, β [95% CI] = .10 [.01, .21], and the right UF, β [95% CI] = .15 [.01, .28]. There was also evidence for a positive association between ADHD symptoms and FDC in the right superior longitudinal fasciculus, β [95% CI] = .12 [.03, .21]. There was evidence for a negative association between internalising symptoms and FD in the forceps minor, β [95% CI] = -.15 [-.27, -.02], and right UF, β [95% CI] = -.14 [-.27, -.01].

*Impact of motion*

Analyses were rerun with frame-wise displacement included to mitigate any impact of motion on our findings, particularly given the nature of the sample and potential for greater motion artefact in children with attention/hyperactivity phenotypes. The results for the linear mixed-effects models remained unchanged, and we observed no main effect of motion on the models tested for fibre density in each tract ($p_{FDR} > .05$). We did observe an effect of motion on the morphological measures (FC and FDC) for some tracts. However, the regions exhibiting significant increase in fibre morphology over time were consistent with and without motion included in the model, and the only other significant positive associations were between FC/FDC and PDSS in the right SLF, which remained significant after adjusting for frame-wise displacement ($p_{FDR} < .05$). Therefore, although motion appeared to influence the computation of morphological measures, this had no bearing on the final results.

*Interactions between participant characteristics*

Based on the results from the mixed-effects modelling analysis, we identified two regions which may exhibit time, sex, and puberty dependent reorganisation, justifying the investigation of interactions. Of the interaction models tested, across the forceps minor and right superior longitudinal fasciculus, we identified one model with significant time interactions. Fibre density and cross-section (FDC) exhibited a significant time-point by sex by pubertal stage interaction in the forceps minor, β [95% CI] = .31 [.12, .52]. It is apparent that females exhibit a weak negative, and males a weak positive, relationship between FDC and pubertal stage over time in the forceps minor (Figure 1.5a).

We identified three regions associated with attentional and internalising phenotypes, the forceps minor, right SLF, and right UF. We observed a time-point by sex by ADHD symptoms interaction with FDC in the right superior longitudinal fasciculus, β [95% CI] = -.42 [-.73, -.13], suggesting that boys with ADHD symptoms are developing faster than girls, in this region. We additionally observed evidence for an age by ADHD symptoms interaction with FD in the right UF, β [95% CI] = .16 [.03, .29]. Younger children with high ADHD symptoms have relatively lower fibre density, whereas older children with high ADHD symptoms have relatively higher fibre density.

We observed evidence for a sex by internalising symptoms interaction for FD in the right UF, β [95% CI] = .25 [.04, .46]. This was reflected as a negative relationship between FD and internalising symptoms in females, and absence of this relationship in males (Figure 1.5b). This suggests that

females with a greater presence of internalising symptoms have lower fibre density in the uncinate fasciculus.

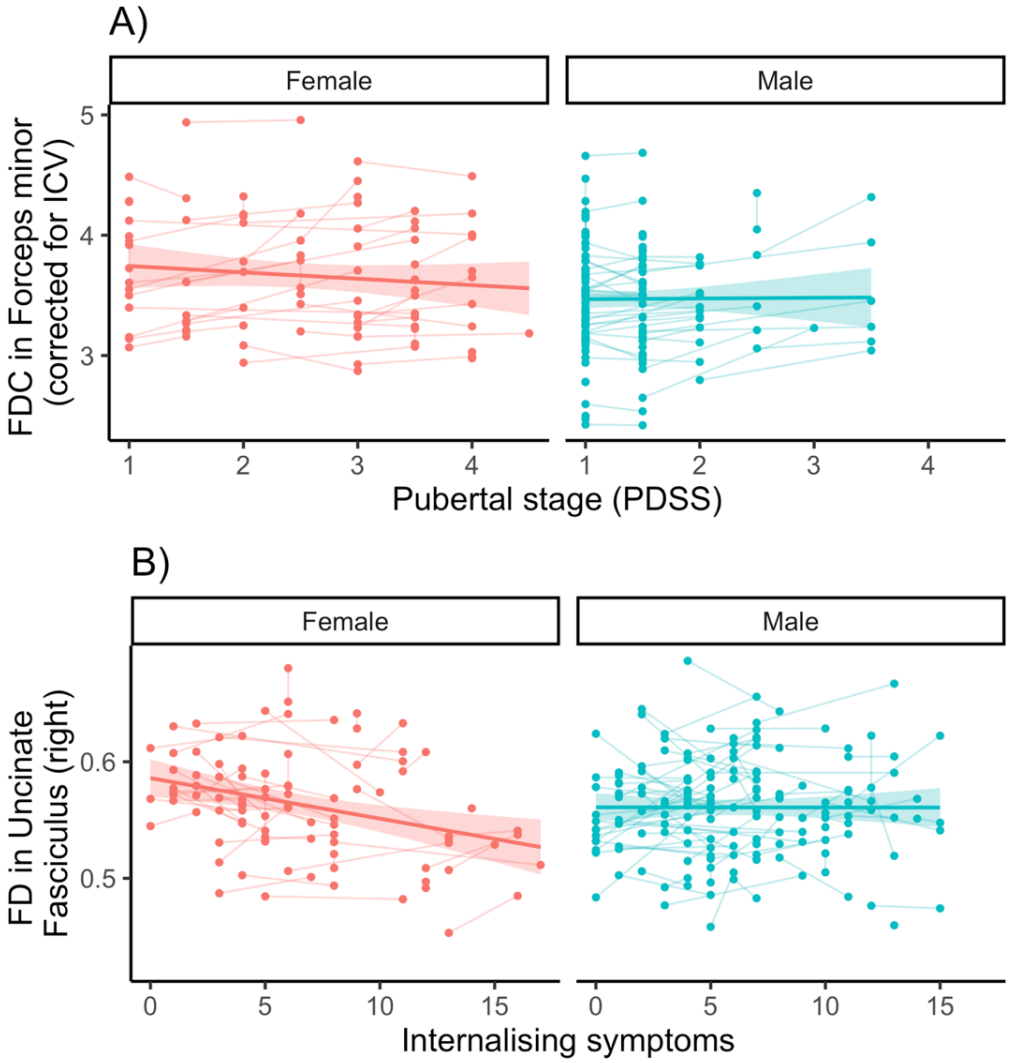


**Figure 1.5:** Model interactions. A) Interaction between time, sex, and pubertal stage with FDC in the forceps minor. FDC was modulated by ICV to account for global variation in brain morphology. B) Interaction between internalising symptoms and sex for fibre density in the right uncinate fasciculus. Longitudinal data is represented by a line connecting the two time-points

## 4. Discussion

In a longitudinal study of children with and without attentional difficulties, we have identified regional patterns of white matter fibre density and morphology development. Our results highlight specific alterations to this microstructural development with pubertal stage, attentional difficulties, and mental health. These findings open up new avenues for investigating the interaction between developmental factors, to unravel multiple contributions to altered neurodevelopmental pathways.

*Developmental patterns*

The region-specific development we observed is consistent with findings from Genc et al. (2018b), which was derived from a smaller subset of typically developing children. This may suggest that regional fibre development is consistent in this age range (9 - 13 years), regardless of variations due to pubertal stage, socio-economic status, attentional difficulties, and mental health. The regions that did not significantly increase in fibre density or morphology may provide better clues as to which regions are delayed in their development, or alternatively are already matured. The bilateral cingulum hippocampus, right inferior fronto-occipital fasciculus, right inferior longitudinal fasciculus, and bilateral uncinate fasciculus, all did not exhibit significant increases in fibre density over time. The IFOF and UF are association tracts which connect frontal pathways with the occipital cortex and temporal lobes respectively. It is known that frontal pathways have delayed maturation, and complete maturation closer to the completion of adolescence and early adulthood (Lebel and Beaulieu, 2011), which may explain why we did not observe changes in fibre density or morphology in these regions over time.

The sex differences in FDC observed in the forceps minor suggest that females have greater capacity for information transfer across this white matter tract. We have previously shown that females have greater fibre density in the mid and posterior segments of the genu of the corpus callosum (Genc et al., 2018a). We postulated, based on these findings and accompanied previously reported histological studies, that females have a greater proportion of small diameter axons compared with males in these regions (Highley et al., 1999). The current longitudinal results suggest two important findings. First, the projections of the genu (forceps minor) have similar properties to the genu properties in the mid-sagittal slice, suggesting that the axons along this fibre have similar properties and that this finding is not isolated to a region with no crossing fibres. Second, this association is upheld in the longitudinal context, suggesting that these cross-sectional differences are sustained and that females are consistently ahead in their capacity for information transfer across this sub-region of the corpus callosum.

These differences could plausibly be a signature of anatomical sex differences between males and females, potentially induced by early perinatal exposure to testosterone (Sisk and Foster, 2004). Additionally (or alternatively) these differences could be perpetuated by the onset of pubertal processes and rising hormone levels in females, compared with males. Either scenario appears to accelerate the maturation of these fibres crossing the genu. Importantly, these findings were not isolated to either fibre density or fibre cross-section metrics, but the combined fibre density and cross-section metric, suggesting that a coupled response in fibre density and morphology are both responsible for increasing the capacity for information transfer across this region in females.

*Pubertal progression*

A positive association between pubertal stage and FBA metrics was observed in the right superior longitudinal fasciculus. This suggests a linear relationship between pubertal development and fibre maturation, likely driven by the expansion of fibre bundles by virtue of axonal growth and myelination. The superior longitudinal fasciculus is a white matter fibre most associated with language ability, semantic memory, and executive function, in developmental and adult populations. It anatomically connects two regions important for language - Broca's and Wernicke's area, and the relationship between language ability and microstructure in the SLF has been extensively reported (Catani et al., 2005). This unique tract, unlike other white matter regions, has a continuous maturation period over childhood into adolescence, rapidly developing during pubertal onset (Genc et al., 2018b) and does not reach full maturation until the post-pubertal stage (Ladouceur et al., 2012). Therefore, it is not clear whether the coupling of SLF development with pubertal stage is a direct relationship (i.e. hormonal influence on fibre maturation), or purely developmental whereby maturation of this region happens to coincide with maturation of physical characteristics associated with puberty.

In order to accurately assess longitudinal trajectories, typically more than 2 time-points are required. A recent longitudinal study of non-human primate (marmoset) brain development investigated grey matter and white matter changes over 3 - 7 time-points across the pre-pubertal, pubertal, and post-pubertal periods (Sawiak et al., 2018). Their results showed that the most dynamically developing white matter tracts in the early-pubertal period are the splenium of the corpus callosum, and ILF. These results are in line with our previous cross-sectional results, whereby fibre density appears to be increasing in the splenium in response to pubertal onset (Genc et al., 2017b). During the pubertal period, regions experiencing most marked white matter tract thickening were localised to the SLF (Sawiak et al., 2018), in line with the findings of the current study. Overall, we have replicable evidence that the splenium of the corpus callosum is sensitive to pubertal timing, and the SLF is sensitive to pubertal progression. These findings additionally highlight the sensitivity of advanced white matter fibre metrics such as fibre density, as these metrics appear to be sensitive to region-specific puberty-mediated white matter changes.

*Attention and mental health*

We observed two key patterns of fibre density in the uncinate fasciculus with respect to internalising symptoms and attentional difficulties. Greater presence of internalising behaviours was linked with lower FD in the right uncinate fasciculus. Similar findings have been reported in the context of anxiety disorders (Hettema et al., 2012; Phan et al., 2009), reflected by lower fractional anisotropy (FA) in the uncinate fasciculus. The uncinate fasciculus has been implicated in many developmental and psychiatric disorders and has protracted development into adulthood (Von Der Heide et al., 2013). Our interaction model revealed that this negative correlation between internalising symptoms and fibre density was only present in females. Females could potentially be at more risk due to the earlier influx of adrenal and gonadal hormones.

Conversely, greater ADHD symptoms was associated with higher fibre density in the right uncinate

fasciculus. Our interaction model revealed that age differences in symptom severity appear to interact and influence the magnitude of fibre density in this region. Relatively younger children with greater ADHD symptoms had lower fibre density, compared with relatively older children with the same level of symptom count which had higher fibre density. Interpreting these findings in the wider context of previous findings is difficult as the ADHD literature surrounding macro- or micro-structural development in this population is consistently inconsistent (van Ewijk et al., 2012). Some studies report that microstructural organisation is greater in ADHD cases compared with controls (Peterson et al., 2011; Silk et al., 2009), whereas other studies report the exact opposite (Ashtari et al., 2005; Nagel 2011).

The underlying factors causing these differences in results are unknown. They could be neurobiological, whereby children with attentional difficulties have altered patterns of microstructure. These differences could also be induced by motion-artefact causing local increase/decrease in FA (Aoki et al., 2018), or by other limitations of analysis techniques (Bach et al., 2014). In the context of the current study, our findings are concordant with previous reports of higher FA with greater ADHD symptom severity (Cooper et al., 2015), but have the added level of novelty with the longitudinal study design. Given the transient nature of ADHD symptomatology, where children can transition in and out of diagnostic categories, longitudinal studies are imperative to tease apart robust relationships between symptomatology and microstructure - not just static associations.

*Relationship with brain volume*

Across all of the white matter regions studied, fibre cross-section had a significantly positive relationship with intra-cranial volume (ICV). Fibre cross-section is inherently a morphological measure of the macroscopic change in cross-sectional area perpendicular to a fibre bundle experienced during registration to a template image (Raffelt et al., 2017). This finding is of special interest to users of this analysis framework and derived metrics, as any variable associated with ICV can potentially conflate interpretations on changes in fibre cross-section. For example, it is known that on average (for a given age), males have greater ICV than females. Without adjusting for ICV in fibre cross-section estimates, sex differences could be attributed to biologically relevant differences, when in fact the differences may purely due to large-scale anatomical differences in head size. Similar patterns are observed in the analyses of fibre density and cross-section, given that this metric is calculated from fibre cross-section.

Fibre density was not associated with ICV in the majority of white matter tracts, which is expected as this is a microscopic measure of density which should be unaffected by macroscopic morphology. However, the positive association between ICV and fibre density in the uncinate fasciculus could potentially be due to the shape of this fronto-temporal association tract. Given its unique ‘C’ shape, proximity to the frontal lobe, and protracted development in childhood - it is plausible that variation in size could induce partial volume effects, affecting tract shape and subsequent computation of the fibre density metric. Therefore, the main message of these results, are that these metrics must be interpreted with caution, and that morphological measures may be better analysed whilst adjusting for ICV.

*Limitations and future directions*

Operating within the recommended fixel-based analysis framework may not necessarily be optimal for the tract segmentation method described in the current study. The fixel mask generation step is optimised to ensure the core or 'deep' white matter is included for subsequent fixel segmentation and reducing the number of multiple comparisons. However, in the context of a tract-based analysis, these steps may result in a smaller area of tract being delineated, and therefore we may potentially miss subtle variations of fibre density and morphology in the more peripheral aspects of a tract. Future studies might consider adopting a looser threshold for fixel segmentation to capture peripheral white matter more effectively.

*Conclusion*

We summarise our longitudinal findings with four important conclusions: (1) white matter development over the ages of 9 – 13 involves dynamic increases in fibre density in a variety of white matter regions, with protracted development of fronto-temporal and fronto-parietal pathways, (2) pubertal stage is positively associated with fibre density and morphology in the right superior longitudinal fasciculus, (3) intra-cranial volume is highly predictive of fibre morphology and should be modelled appropriately, and (4) attentional dysfunction and internalising behaviours are associated with fibre density in the uncinate fasciculus. These results, in conjunction with our previous work, shed light on key fibre developmental properties occurring around the time of pubertal onset, and how these may relate to attention and mental health.

## 5. References


Andersson, J. L. R. and Sotiropoulos, S. N. (2016). An integrated approach to cor- rection for off-resonance effects and subject movement in diffusion mr imaging. Neuroimage, 125:1063–1078.

Aoki, Y., Cortese, S., and Castellanos, F. X. (2018). Research review: Diffusion tensor imaging studies of attention-deficit/hyperactivity disorder: meta-analyses and reflections on head motion. J Child Psychol Psychiatry, 59(3):193–202.

Ashtari, M., Kumra, S., Bhaskar, S. L., Clarke, T., Thaden, E., Cervellione, K. L., Rhinewine, J., Kane, J. M., Adesman, A., Milanaik, R., Maytal, J., Diamond, A., Szeszko, P., and Ardekani, B. A. (2005). Attention-deficit/hyperactivity disorder: a preliminary diffusion tensor imaging study. Biol Psychiatry, 57(5):448–55.

Bach, M., Laun, F. B., Leemans, A., Tax, C. M., Biessels, G. J., Stieltjes, B., and Maier-Hein, K. H. (2014). Methodological considerations on tract-based spatial statistics (tbss). Neuroimage, 100:358–69.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological), 57(1):289–300.

Catani, M., Jones, D. K., and ffytche, D. H. (2005). Perisylvian language networks of the human brain. Ann Neurol, 57(1):8–16.

Conners, C. K., Pitkanen, J., and Rzepa, S. R. (2011). Conners 3rd Edition (Conners 3; Conners 2008),

pages 675–678. Springer New York, New York, NY.
Cooper, M., Thapar, A., and Jones, D. K. (2015). Adhd severity is associated with white matter microstructure in the subgenual cingulum. NeuroImage. Clinical, 7:653–660.
Dorn, L. D., Dahl, R. E., Woodward, H. R., and Biro, F. (2006). Defining the bound- aries of early adolescence: A user's guide to assessing pubertal status and pubertal timing in research with adolescents. Applied Developmental Science, 10(1):30–56.
Genc, S., Malpas, C. B., Ball, G., Silk, T. J., and Seal, M. L. (2018a). Age, sex, and puberty related development of the corpus callosum: a multi-technique diffusion mri study. Brain Struct Funct.
Genc, S., Seal, M. L., Dhollander, T., Malpas, C. B., Hazell, P., and Silk, T. J. (2017).
White matter alterations at pubertal onset. Neuroimage, 156:286–292.
Genc, S., Smith, R. E., Malpas, C. B., Anderson, V., Nicholson, J. M., Efron, D., Sciberras, E., Seal, M. L., and Silk, T. J. (2018b). Development of white matter fibre density and morphology over childhood: A longitudinal fixel-based analysis. NeuroImage, 183:666–676.
Goodman, R. (1997). The strengths and difficulties questionnaire: a research note. J Child Psychol Psychiatry, 38(5):581–6.
Goodman, R. (2001). Psychometric properties of the strengths and difficulties questionnaire. J Am Acad Child Adolesc Psychiatry 40(11): 1337-1345.
Hettema, J. M., Kettenmann, B., Ahluwalia, V., McCarthy, C., Kates, W. R., Schmitt, J. E., Silberg, J. L., Neale, M. C., Kendler, K. S., and Fatouros, P. (2012). Pilot multimodal twin imaging study of generalized anxiety disorder. De- press Anxiety, 29(3):202–9.
Highley, J. R., Esiri, M. M., McDonald, B., Cortina-Borja, M., Herron, B. M., and Crow, T. J. (1999). The size and fibre composition of the corpus callosum with respect to gender and schizophrenia: a post-mortem study. Brain, 122 ( Pt 1):99– 110.
Jones, D. K., Knosche, T. R., and Turner, R. (2013). White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion mri. Neuroimage, 73:239– 54.
Juraska, J. M. and Willing, J. (2017). Pubertal onset as a critical transition for neural development and cognition. Brain Res, 1654(Pt B):87–94.
Kaltiala-Heino, R., Marttunen, M., Rantanen, P., and Rimpela, M. (2003). Early puberty is associated with mental health problems in middle adolescence. Soc Sci Med, 57(6):1055–64.
Kao GS, Thomas HM. Test review: C. Keith, Conners, Conners 3rd edition Toronto, Ontario, Canada: Multi-Health Systems, 2008. J Psychoeduc Assess. 2010;28:598–602.
Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., and Walters,
E. E. (2005). Lifetime prevalence and age-of-onset distributions of dsm-iv disorders in the national comorbidity survey replication. Arch Gen Psychiatry, 62(6):593– 602.
Kim, J. H. Y. and Juraska, J. M. (1997). Sex differences in the development of axon number in the splenium of the rat corpus callosum from postnatal day 15 through
60. Developmental Brain Research, 102(1):77–85.
Ladouceur, C. D., Peper, J. S., Crone, E. A., and Dahl, R. E. (2012). White matter development in adolescence: the influence of puberty and implications for affective disorders. Dev Cogn Neurosci, 2(1):36–54.
Langberg, J. M., Epstein, J. N., Altaye, M., Molina, B. S. G., Arnold, L. E., and Vitiello, B. (2008). The transition to middle school is associated with changes in the developmental trajectory of adhd symptomatology in young adolescents with adhd. Journal of clinical child and adolescent psychology : the official journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53, 37(3):651–663.

Lebel, C. and Beaulieu, C. (2011). Longitudinal development of human brain wiring continues from childhood into adulthood. J Neurosci, 31(30):10937–47.
Morrell, C. H., Brant, L. J., and Ferrucci, L. (2009). Model choice can obscure results in longitudinal studies. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 64A(2):215–222.
Nagel, B. J., Bathula, D., Herting, M., Schmitt, C., Kroenke, C. D., Fair, D., & Nigg, J. T. (2011). Altered white matter microstructure in children with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry, 50(3), 283-292. doi:10.1016/j.jaac.2010.12.003.
Paus, T., Keshavan, M., and Giedd, J. N. (2008). Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci, 9(12):947–57.
Petersen AC, Crockett L, Richards M, Boxer A (1988) A self-report measure of pubertal status: Reliability, validity, and initial norms. J Youth Adolesc 17 (2):117-133. doi:10.1007/BF01537962
Peterson, D. J., Ryan, M., Rimrodt, S. L., Cutting, L. E., Denckla, M. B., Kaufmann,
W. E., and Mahone, E. M. (2011). Increased regional fractional anisotropy in highly screened attention-deficit hyperactivity disorder (adhd). Journal of child neurology, 26(10):1296–1302.
Phan, K. L., Orlichenko, A., Boyd, E., Angstadt, M., Coccaro, E. F., Liberzon, I., and Arfanakis, K. (2009). Preliminary evidence of white matter abnormality in the uncinate fasciculus in generalized social anxiety disorder. Biol Psychiatry, 66(7):691–4.
Polanczyk, G., de Lima, M. S., Horta, B. L., Biederman, J., and Rohde, L. A. (2007). The worldwide prevalence of adhd: A systematic review and metaregression anal- ysis. American Journal of Psychiatry, 164(6):942–948.
Raffelt, D., Tournier, J. D., Fripp, J., Crozier, S., Connelly, A., and Salvado, O. (2011). Symmetric diffeomorphic registration of fibre orientation distributions. Neuroimage, 56(3):1171–80.
Raffelt, D. A., Tournier, J. D., Smith, R. E., Vaughan, D. N., Jackson, G., Ridg- way, G. R., and Connelly, A. (2017). Investigating white matter fibre density and morphology using fixel-based analysis. Neuroimage, 144(Pt A):58–73.
Reuter, M., Schmansky, N. J., Rosas, H. D., and Fischl, B. (2012). Within- subject template estimation for unbiased longitudinal image analysis. Neuroimage, 61(4):1402–18.
Sawiak, S. J., Shiba, Y., Oikonomidis, L., Windle, C. P., Santangelo, A. M., Gryde- land, H., Cockcroft, G., Bullmore, E. T., and Roberts, A. C. (2018). Trajectories and milestones of cortical and subcortical development of the marmoset brain from infancy to adulthood. Cerebral Cortex, pages bhy256–bhy256.
Shankman, S. A., Lewinsohn, P. M., Klein, D. N., Small, J. W., Seeley, J. R., and Altman, S. E. (2009). Subthreshold conditions as precursors for full syndrome disorders: a 15-year longitudinal study of multiple diagnostic classes. J Child Psychol Psychiatry, 50(12):1485–94.
Shirtcliff, E. A., R. E. Dahl and S. D. Pollak (2009). "Pubertal Development: Correspondence Between Hormonal and Physical Development." Child Development 80(2): 327-337.
Silk, T. J., Genc, S., Anderson, V., Efron, D., Hazell, P., Nicholson, J. M., Kean, M., Malpas, C. B., and Sciberras, E. (2016). Developmental brain trajectories in children with adhd and controls: a longitudinal neuroimaging study. BMC Psychiatry, 16:59.
Silk, T. J., Vance, A., Rinehart, N., Bradshaw, J. L., and Cunnington, R. (2009). White-matter abnormalities in attention deficit hyperactivity disorder: a diffusion tensor imaging study. Hum Brain Mapp, 30(9):2757–65.
Sisk, C. L. and D. L. Foster (2004). "The neural basis of puberty and adolescence." Nat Neurosci 7(10): 1040-1047.

Smith, R. E., Tournier, J. D., Calamante, F., and Connelly, A. (2013). Sift: Spherical- deconvolution informed filtering of tractograms. Neuroimage, 67:298–312.
Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., and Matthews, P. M. (2004). Advances in functional and structural mr image analysis and implementation as fsl. Neuroimage, 23 Suppl 1:S208–19.
Tamnes, C. K., Roalf, D. R., Goddings, A. L., and Lebel, C. (2017). Diffusion mri of white matter microstructure development in childhood and adolescence: Methods, challenges and progress. Dev Cogn Neurosci.
Tournier, J. D., R. E. Smith, D. A. Raffelt, R. Tabbara, T. Dhollander, M. Pietsch, D. Christiaens, B. Jeurissen, C.-H. Yeh and A. Connelly (2019). "MRtrix3: A fast, flexible and open software framework for medical image processing and visualisation." bioRxiv: 551739.
van Ewijk, H., Heslenfeld, D. J., Zwiers, M. P., Buitelaar, J. K., and Oosterlaan, J. (2012). Diffusion tensor imaging in attention deficit/hyperactivity disorder: A systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews, 36(4):1093–1106.
Veraart, J., Fieremans, E., and Novikov, D. S. (2016). Diffusion mri noise mapping using random matrix theory. Magn Reson Med, 76(5):1582–1593.
Von Der Heide, R. J., Skipper, L. M., Klobusicky, E., and Olson, I. R. (2013). Dis- secting the uncinate fasciculus: disorders, controversies and a hypothesis. Brain : a journal of neurology, 136(Pt 6):1692–1707.
Wakana, S., Caprihan, A., Panzenboeck, M. M., Fallon, J. H., Perry, M., Gollub, R. L., Hua, K., Zhang, J., Jiang, H., Dubey, P., Blitz, A., van Zijl, P., and Mori, S. (2007). Reproducibility of quantitative tractography methods applied to cerebral white matter. Neuroimage, 36(3):630–44.
Wechsler, D. (1999). Wechsler Abbreviated Scale of Intelligence. The Psychological Corporation: Harcourt Brace & Company. The Psychological Corporation: Har- court Brace & Company, New York, NY.

## 6. Supplementary section

### 6.1. Supplementary Information

Conners 3 Attention-Deficit/Hyperactivity Disorder Index

Parent report of the Conners 3 Attention Deficit/Hyperactivity Disorder (ADHD) index was used to assess ADHD symptom severity (10 items; e.g., restless or overactive). This measure has been shown to have moderate-to-strong reliability and validity estimates (Kao et al 2010). Items were rated on frequency of occurrence from 1 (never/seldom) to 3 (very often/very frequent), with higher scores indicating greater severity.

Strengths and Difficulties Questionnaire

The Strengths and Difficulties Questionnaire (SDQ) has strong psychometric properties including good reliability and structural validity (Goodman 2001). Three subscales from the parent-reported SDQ were used to assess emotional problems (5 items; e.g., nervous or clingy in new situations) and peer problems (5 items; e.g., rather solitary, prefers to play alone). All items were rated from 0 (not true) to 2 (certainly true). These two scales were added to generate a total internalising problems score, whereby greater values indicated greater internalising problems.

Pubertal Development Scale

Pubertal development was assessed using the Pubertal Development Scale (PDS) (Petersen et al., 1988). The PDS is a measure that assesses pubertal stage and is highly correlated with hormonal measures and physical exam by a trained physician, rendering it a reliable measure of pubertal maturation (Shirtcliff et al., 2009). For the current study, the primary caregiver was asked to rate their child's physical development on a four-point scale. This included questions assessing the presence of characteristics phenotypical of pubertal onset such as deepening of voice and presence of facial hair in boys, and breast development and menarche for females. A combined PDS-Shirtcliff (PDSS) score was calculated (Shirtcliff et al., 2009) by taking the mean of the adrenarche and gonadarche scores generated from the syntax described in the aforementioned study. The PDSS scores in our sample ranged from 1 – 3.5, where children with a score of 1 had no physical signs of pubertal onset, and children with a score of 1.5 - 3.5 had some phenotypic characteristics of pubertal onset.

### 6.2. Supplementary Figures

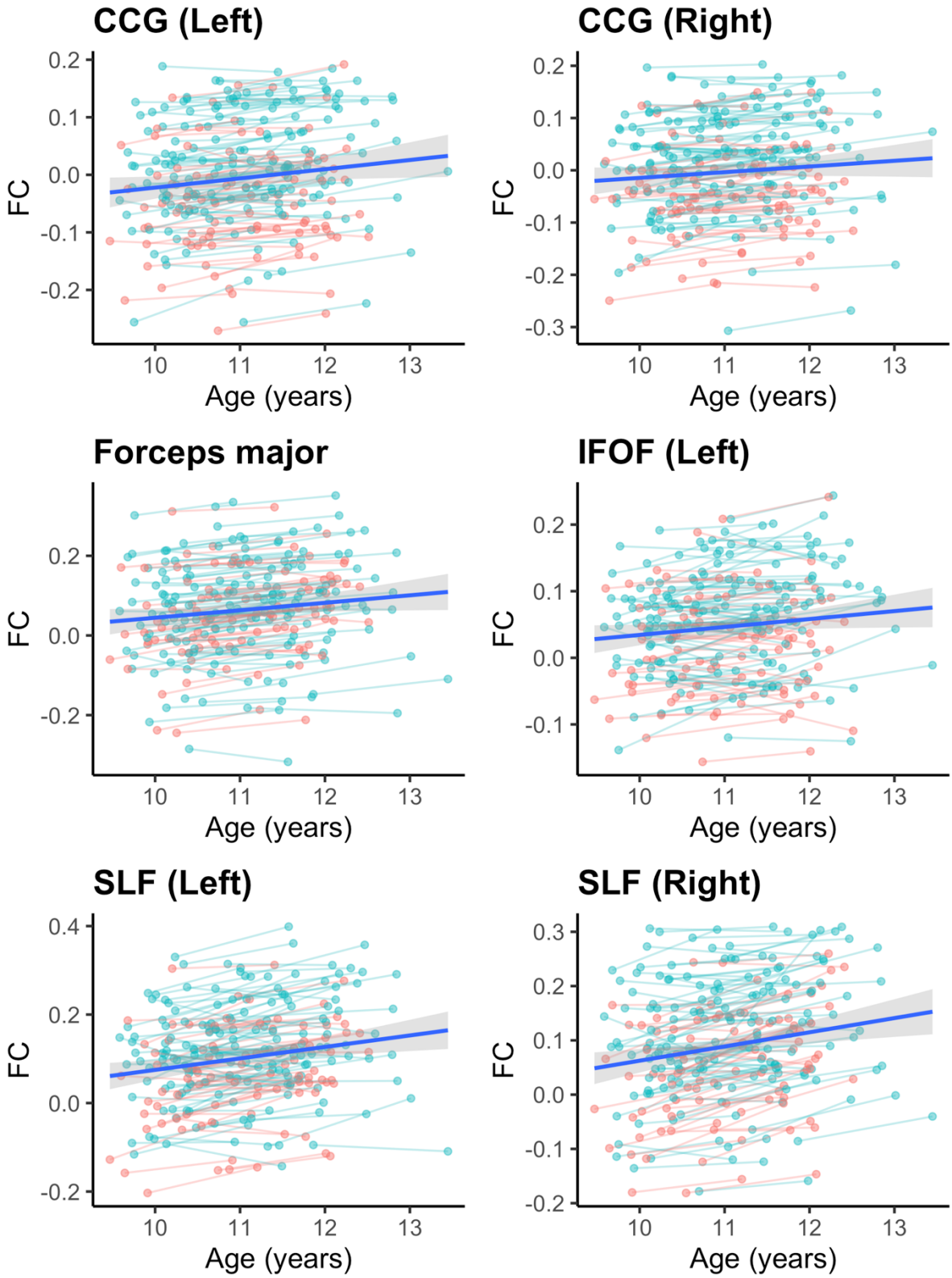


**Figure S1**: Longitudinal change in FC for regions with significant changes in fibre properties over time. Blue = boys, Red = girls

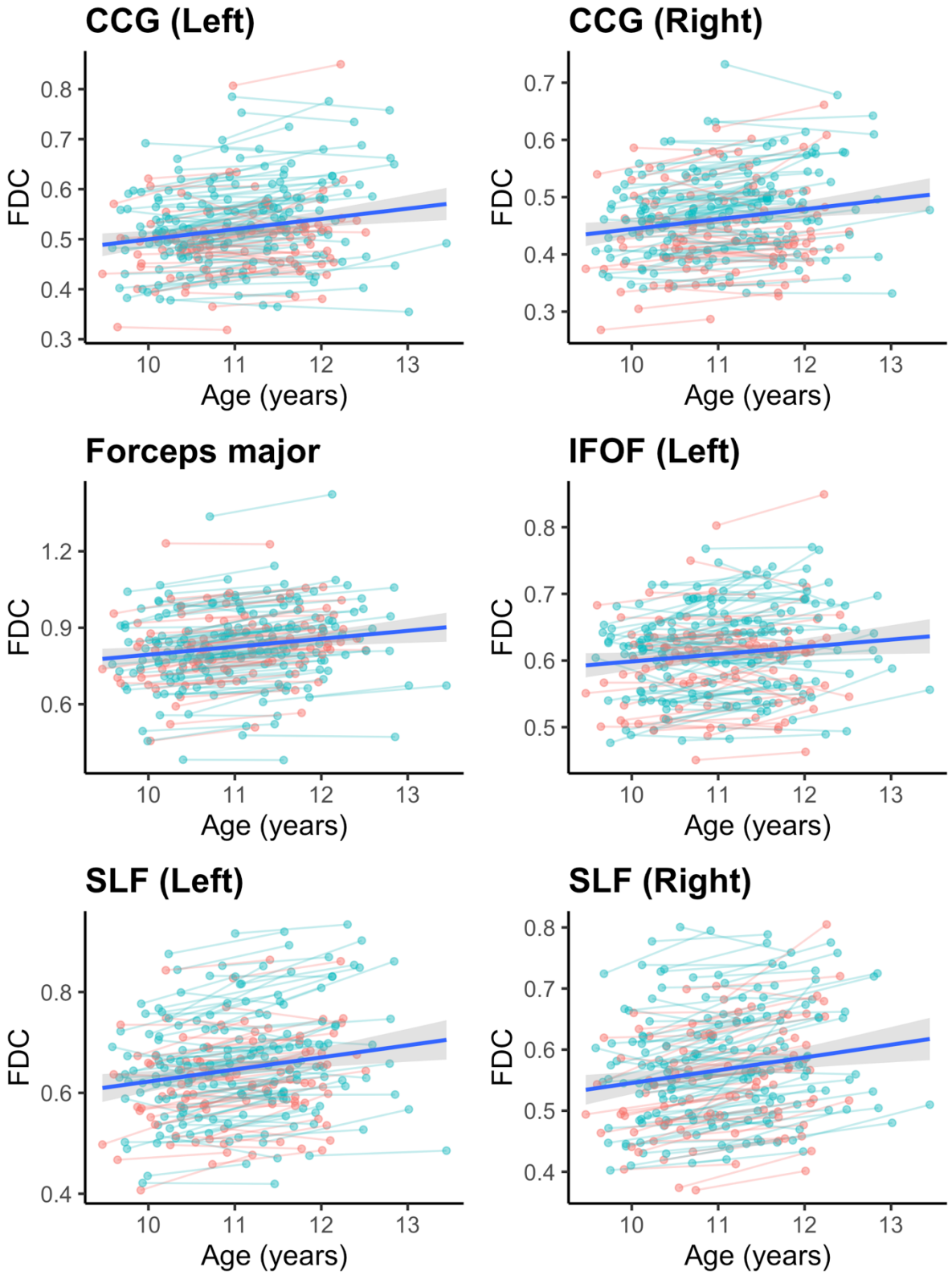


**Figure S2**: Longitudinal change in FDC for regions with significant changes in fibre properties over time

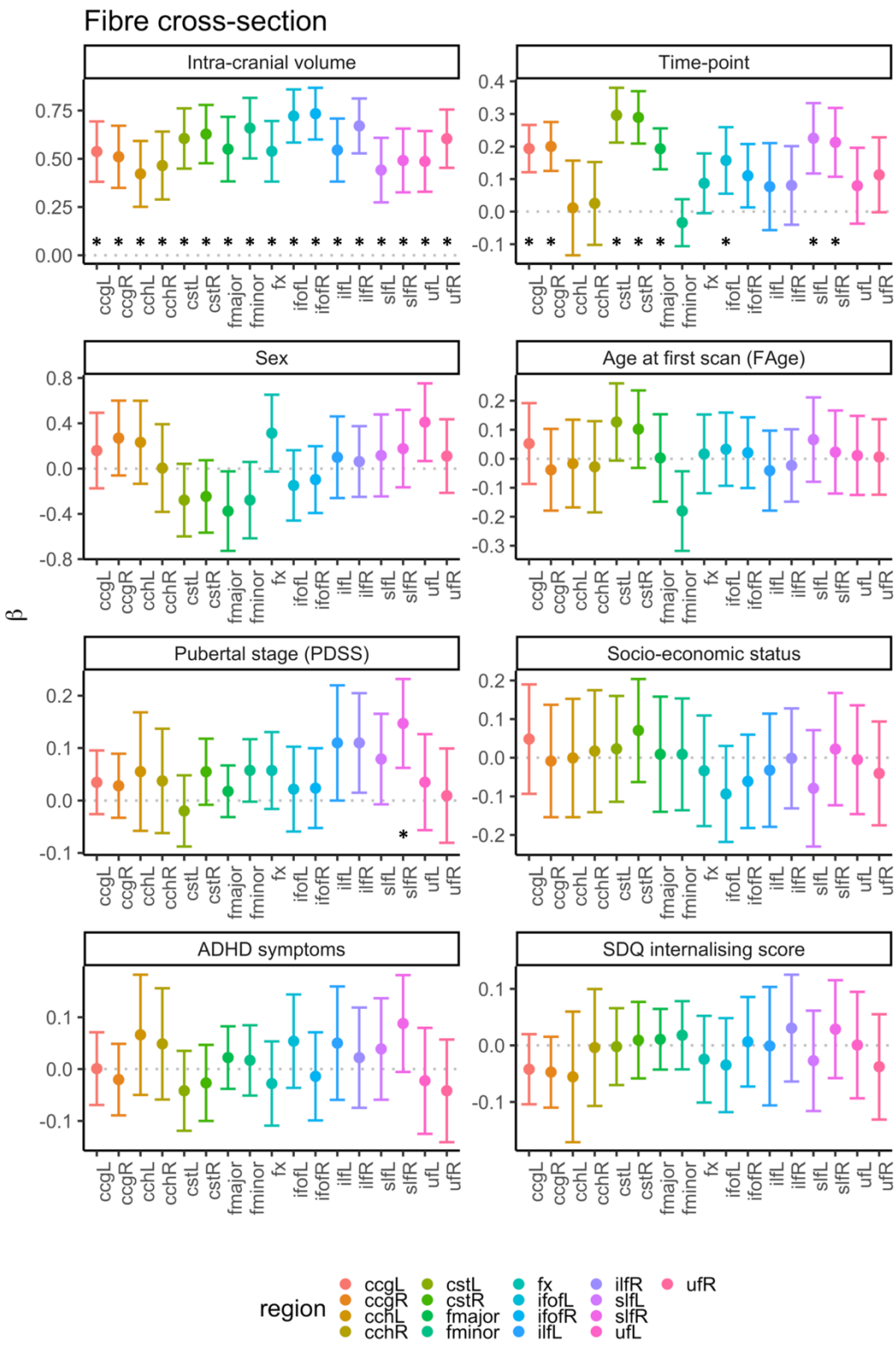


**Figure S3**: Relationships between participant characteristics and fibre cross-section across all white matter tracts. 95% confidence intervals which do not cross zero suggest a relationship between that variable and the metric of interest. Regions that reach statistical significance at $p_{FDR} < .05$ are annotated with an asterisk (*)

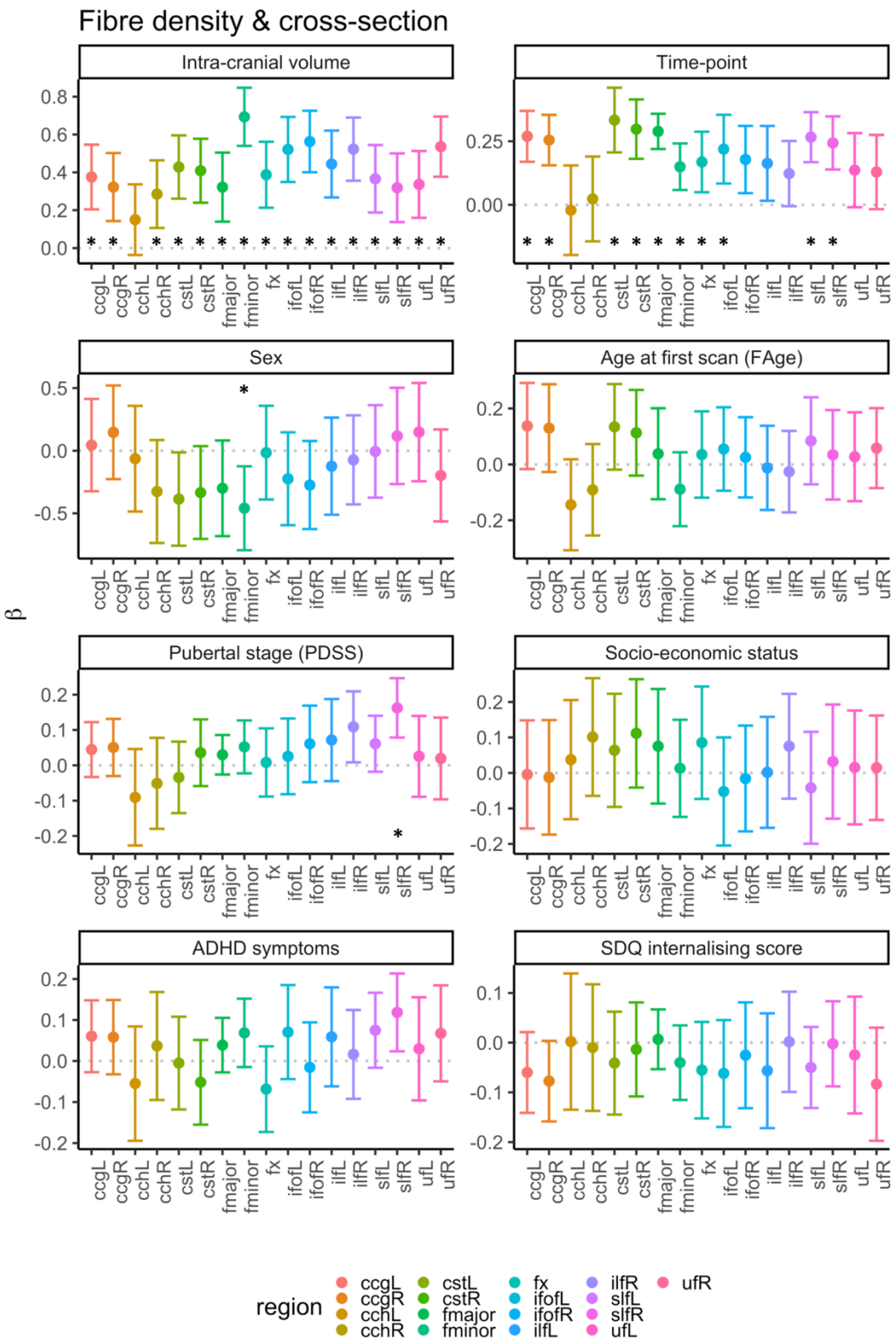


**Figure S4**: Relationships between participant characteristics and fibre density and cross- section across all white matter tracts. 95% confidence intervals which do not cross zero suggest a relationship between that variable and the metric of interest. Regions that reach statistical significance at $p_{FDR} < .05$ are annotated with an asterisk (*).