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Longitudinal changes in pubertal maturation and white matter microstructure

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Abstract

Emerging evidence in the field of adolescent neurodevelopment suggests that pubertal processes may contribute to known trajectories of brain maturation, and may contribute, in part, to sex differences in related cognitive, behavioral and mental health outcomes. The current longitudinal study examined how changes in physical pubertal maturation (measured by the Peterson Developmental Scale) predict changes in white matter microstructure in 18 boys and 15 girls over an approximate 2-year follow-up period, while accounting for age. Using Tract-Based Spatial Statistics and multi-level modeling, the results showed that physical pubertal changes predict patterns of changes in fractional anisotropy (FA) in white matter regions in the thalamus, precentral gyrus, superior corona radiata, corpus callosum (genu), superior corona radiata, and superior frontal gyrus. Sex specific changes were also seen, as changes in gonadal and adrenal development related to increases in FA in the superior frontal gyrus and precentral gyrus in boys, but gonadal development related to decreases in FA in the anterior corona radiata in girls. These findings are the first to show how changes over time in pubertal development influence white matter development. In addition, they support a larger body of emerging research suggesting that pubertal processes contribute to distinct changes in boys and girls across brain development.

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Keywords

puberty; adolescence; diffusion tensor imaging; white matter; development; intra-class correlation coefficient

1.1 Introduction

Adolescence is a unique period of brain development, where changes in brain structure and function are coupled with improvements in cognitive domains important for adult-like functioning. White matter volumes continue to increase with age across adolescence. Child and adolescent neuroimaging studies using longitudinal diffusion tensor imaging (DTI) consistently show decreases in mean diffusivity (MD) with age, as well as increases in fractional anisotropy (FA) and decreases in radial diffusivity (RD) (Giorgio, Watkins et al. 2010, Bava, Boucquey et al. 2011, Lebel and Beaulieu 2011, Wang, Adamson et al. 2012, Simmonds, Hallquist et al. 2014). A number of sex differences have also been reported in white matter microstructure (Schmithorst, Holland et al. 2008, Herting, Maxwell et al. 2012, Wang, Adamson et al. 2012, Simmonds, Hallquist et al. 2014, Seunarine, Clayden et al. 2016), suggesting that patterns of white matter development differ between boys and girls as they mature through late childhood and adolescence in a region- and time- specific manner. These white matter changes are thought to reflect a combination of increased axonal diameter and continued myelination (Paus 2010). Maturation of white matter pathways is thought to play a vital role in communication between various regions throughout the brain, which may allow for improved signal transduction. Further, white matter maturation likely impacts the concurrent development of cognitive, emotional, behavioral and motor outcomes. Further understanding of major factors influencing white matter maturation is needed to improve our understanding of individual differences in risk for emotional and behavioral problems that begin to emerge during the teenage years (Ladouceur, Peper et al. 2012). While much has been learned, most existing research has examined white matter development as a function of chronological age. We aimed to address this gap in knowledge by exploring longitudinal changes in white matter as a function of puberty compared to chronological age.

Beyond chronological age, both animal and human studies have shown that pubertal maturation exerts sex-specific effects on brain structure (for review see (Juraska, Sisk et al. 2013)). A few cross-sectional studies have examined how puberty may contribute to the sex differences in white matter microstructure development seen across adolescence. Asato, Terwilliger et al. (2010) examined puberty in children (8-12 years), adolescents (13-17 years), and adults (18-28 years) using DTI. In all but one white matter region of interest, maturation seemed incomplete for individuals in mid-puberty, but were adult-like for those with completed pubertal maturation (i.e. post-pubertal stage). Using physical markers of pubertal development as a continuous variable, puberty has also been shown to relate to higher FA values in a sex-specific manner in superior frontal white matter regions (Herting, Maxwell et al. 2012). In the same study, testosterone levels were found to positively predict FA in regions where boys had higher FA compared to girls, whereas estradiol was found to show a negative relationship with FA in girls (Herting, Maxwell et al. 2012). However, a

separate study found pubertal stage and testosterone related to MD, but not FA, in various white matter regions in boys, while DHEA and estradiol did not relate to DTI outcome measures (Menzies, Goddings et al. 2015). Collectively, these cross-sectional studies show that pubertal maturation relates to white matter microstructure in a sex-specific manner. However, it remains unclear how within-subject *changes* in pubertal development may influence *changes* in white matter microstructure across adolescence in boys and girls.

The current longitudinal DTI study aimed to replicate known age-related changes in white matter across adolescence, as well as expand the existing literature by examining how changes in pubertal development influence changes in white matter development across adolescence. We examined how changes in adrenal- and gonadal-related pubertal markers influence changes in white matter diffusivity in boys and girls (FA, as well as MD, RD and axial diffusivity (AD)). Given previously reported sex differences (Asato, Terwilliger et al. 2010, Herting, Maxwell et al. 2012, Simmonds, Hallquist et al. 2014), we expected to replicate widespread age-related changes in white matter microstructure across adolescence, and for boys to show larger increases in FA as compared to girls. Furthermore, we expected that *changes* in pubertal development would predict *changes* in white matter development, after accounting for age, and in a sex-specific manner.

2. Material and Methods

2.1 Participants

The current longitudinal study design included adolescents between the ages of 10 and 18 years of age at the time of their first MRI visit (Time 1) and were asked to participate in a follow-up visit (Time 2) approximately 2 years later (Figure 1A). Participants were recruited via fliers, advertisements, and online postings. Written assent and consent were obtained from all participants and their parent or legal guardian, according to procedures outlined by the Institutional Review Board of Children's Hospital of Los Angeles (CHLA) and in accordance with The Code of Ethics of the World Medical Association. All child/adolescent participants were free of any significant medical or neurological condition (e.g. epilepsy, head injury with loss of consciousness), psychiatric or developmental disorder (e.g., attention deficit hyperactivity disorder, schizophrenia, autism, fetal alcohol syndrome disorder), and did not meet criteria for intellectual disability (i.e. obtained Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV) intelligence scaled score > 70). No siblings participated in the study to avoid genetic and environmental co-variance among participants. Only participants who had successfully completed both visits (including good quality DTI data and valid Pubertal Developmental Scale (PDS) self-reports; see sections 2.4 and 3.2 for details) were included in the subsequent set of analyses reported here. General intelligence was assessed using the WISC-IV (Wechsler 2003) and handedness for each participant was determined by the Edinburgh Handedness Inventory (Oldfield 1971). The WISC-IV only has age norms for adolescents under 17 years of age, thus, scores for individuals >17 years were scaled based on the 16 years and 11 months norms. Parents or participating individuals were asked about household income on an ordinal scale (i.e. < $5000; $5,000 - 9,999; $10,000 - 19,999; $20,000 - 29,999; $30,000 - 39,999; $40,000 - 49,999; $50,000 - 74,999; $75,000 - 100,000; $100,000+).

2.2 Petersen Pubertal Developmental Scale (PDS)

Pubertal maturation was assessed using the self-rating PDS (Petersen, Crockett et al. 1988). The PDS consists of a total of 5 distinct questions for boys and girls. For boys, the five questions are in regards to height growth, body hair, skin changes, vocal changes, and facial hair. For girls, the five questions consist of assessing changes in height growth, body hair, skin, breast development, and menarche. For each of the 5 questions, youth were asked to rate their development on a 4-point scale (1 = has not begun yet, 2 = barely begun, 3 = definitely begun, 4 = seems complete), except for the menarche question for girls, which consisted of a yes/no answer choice. Secondary sex characteristics captured by the PDS result from a milieu of hormonal processes (Shirtcliff, Dahl et al. 2009). Thus, dependent variables of interests included adrenal- versus gonadal- related PDS scores. As previously described (Shirtcliff, Dahl et al. 2009), gonadal scores were created for girls by averaging growth spurt, breast development, and menarche PDS items, and for boys by averaging growth spurt, deepening of voice, and facial hair growth PDS items. Adrenal scores were created by averaging pubic/body hair and skin changes from PDS items in both boys and girls.

2.3 Diffusion Weighted Imaging (DWI) Acquisition

All images were collected on the same 3.0 Tesla Philips Achieva system with an eight channel head coil at CHLA. Whole-brain structural anatomical images were acquired in the sagittal plane using a T_1 weighted MPRAGE scanning sequence (TE = 3.185 ms, TR = 6.8 ms, TI = 845.3 ms, Flip Angle = 8 degrees, acquisition matrix = 256×256, slice thickness = 1.2mm). DWI was acquired in the axial plane using a high-angular resolution EPI sequence (TR = 9000ms, TE = 86 ms, FOV= 240×240 mm, 60 slices, slice thickness = 2.5mm). Gradient encoding pulses were applied in 30 directions with a b-value of 1000s/mm^2, 2 diffusion-weighted runs were collected with a b0 (non-diffusion weighted) image per run. One reverse encoded and five forward encoded scans were collected for calibration purposes. This diffusion-weighted protocol was used because previous research has shown that 20 or more diffusion gradient directions allow for calculating robust and reliable FA measurements (Li, Mathews et al. 2005, Ni, Kavcic et al. 2006).

2.4 Motion Assessments and Image Exclusion

DWI runs with excess motion, as defined by both qualitative and quantitative methods, were excluded from subsequent analyses. First, all raw DWI images were visually examined for artifacts due to motion, including axial signal loss in each gradient volume. Next, motion was first assessed using frame-wise displacement (FD) via FSL to quantify the amount of motion between each of the 30 gradient volumes collected during each of the 2 runs. DTI runs showing one or more >5mm frame displacement values or 2 consecutive >2.5 mm FD values for any given volume (out of the 31 volumes (30 gradients and a b0)) were excluded from further analyses. For all remaining subjects mean FD values (average FD for the 31 volumes) were low (see Table 1) and did not significantly differ between DTI runs, Time 1 or Time 2, or between boys and girls (all p-values > .20). Moreover, FD means did not relate to age or pubertal variables of interest (p-values > .27). Using both quantitative and qualitative motion results, the single best DTI run was then selected for each subject to be

included in the age and puberty analyses, while 2 runs per time point per individual were included in intra-class correlation coefficient analyses (see section 2.6 below).

2.5 Diffusion Tensor Imaging Preprocessing

Standard DTI preprocessing was performed using FMRIB's Diffusion Toolbox. Briefly, eddy current effects, magnetic field inhomogeneities, and head motion were corrected using FMRIB's Diffusion Toolbox and Utility for Geometrically Unwarping EPIs (Jenkinson 2003). Next, images were brain-extracted using BET (Smith 2002) and FSL's *dtifit* was used to calculate the diffusion tensor and identify the eigenvalues and vectors of the tensor (λ_1, λ_2, λ_3), FA, MD, RD [(λ_2+λ_3)/2]) and AD [λ_1]) for each voxel.

In order to perform voxelwise statistical analyses, FA maps were registered and a common white matter skeleton was estimated for the sample using Tract Based Spatial Statistics (TBSS) (See Smith, Jenkinson et al. 2006, Smith, Johansen-Berg et al. 2007). Specifically, using all images from both Time 1 and Time 2 data, a common registration target image was identified out of all individual subjects' FA maps and affine aligned to standard MNI152 space. Second, each subject's FA map was nonlinearly registered to this common target using FMRIB's Non-linear Image Registration Tool (Andersson, Jenkinson et al. 2007), and aligned FA images were averaged to create a group-wise mean FA map. Third, a white matter skeleton, representing only the major tracts common across all subjects, was created (For more detail see Smith, Jenkinson et al. 2006, Smith, Johansen-Berg et al. 2007). A mean FA threshold of 0.3 was applied to the white matter skeleton to reduce partial volume effects (Smith, Jenkinson et al. 2006). Fourth, each subject's aligned FA image was projected onto the white matter skeleton for subsequent voxelwise statistics. To examine MD, MD images were affine aligned to standardized space, the nonlinear registration parameters determined by FNIRT were applied to the MD maps, and MD images were merged and projected onto the FA-derived white matter skeleton to perform statistical comparisons.

For a more comprehensive analysis of white matter microstructure, axial and radial diffusion were assessed in those clusters of FA and MD that were significantly related to sex, puberty, and sex-by-puberty interactions. In particular, FA and MD clusters were projected onto each subject's axial and radial maps, and a mean AD and RD value was calculated for each cluster.

2.6 FA Variability Between and Within Subjects

Using an ANOVA method, voxelwise intra-class correlations can be calculated to determine the proportion of FA variance across subjects relative to the total FA variance within a given voxel. This approach can be useful to examine the relative between-subject variance (Subj), as well as the within-subject variation (DTI run (DTI) and time (Time)), for FA in each voxel included in the TBSS white matter skeleton. The output of such a model gives the relative variability in FA that can be accounted for across subjects (Subj) as compared to within-subjects (DTI run or Time). Thus, a high proportion of variance explained by Subj, such as a value of .99 would reflect 99% of the FA variance in a voxel accounted for by a between-subject effect. The remaining 1% of the FA variance would then reflect a relatively small within-subject effect (DTI Run and Time), suggesting high consistency of FA values

within a subject across DTI Runs and over time. To accomplish this, AFNI's 3dLME program was used to apply this three-crossed-random-effect model to assess the relative between-subject and within-subject variance for each voxel of the FA skeleton (http://afni.nimh.nih.gov/sscc/gangc/ICC_REML.html). The between- and within-subject variances calculated using this method are similar to examining intra-class correlations as detailed by Shrout and Fleiss (1979). Using this method, the between-subject FA variance is defined as:

$$\mathrm{Subj}=\frac{\sigma\mathrm{Subj}^2}{\sigma\mathrm{Subj}^2+\sigma\mathrm{Run}^2+\sigma\mathrm{Time}^2\sigma\mathrm{Residual}^2},$$

while the within-subject variance for DTI scans within a given day would be:

$$\mathrm{Run}=\frac{\sigma\mathrm{Run}^2}{\sigma\mathrm{Subj}^2+\sigma\mathrm{Run}^2+\sigma\mathrm{Time}^2\sigma\mathrm{Residual}^2},$$

and the within-subject variance for a subject over time would be:

$$\mathrm{Time}=\frac{\sigma\mathrm{Time}^2}{\sigma\mathrm{Subj}^2+\sigma\mathrm{Run}^2+\sigma\mathrm{Time}^2\sigma\mathrm{Residual}^2}.$$

2.7 Analysis Procedure

Using the nlme R package (Pinheiro, Bates et al. 2015), repeated measures HLM were performed to examine linear longitudinal changes over time. The model was $y_i=X_i\beta+Z_ib_i+\varepsilon_i$, with y being the dependent variable of interest, X is the matrix of fixed-effect regressors (e.g. age, sex, etc.) and β is the associated parameter estimates, Z is the matrix of the random-effects repressors (individual subjects) and b is the random-effects coefficients (variances and co-variances), and ε is the within-subject measurement error (Singer and Willet 2003). To examine within- and between-subject changes in puberty, this type of linear model was performed for dependent variables (y) of total PDS scores, as well as adrenal- and gonadal- related PDS scores. In these models, the fixed effects (X) included time, sex, and a time-by-sex interaction term. Using AFNI's 3dLME (Chen, Saad et al. 2013), the same linear models were performed to include the fixed effects (X) of age, sex, and pubertal-markers (adrenal- or gonadal-related PDS scores) on the dependent variable (y) FA from the TBSS white matter skeleton at the voxelwise level. Using 3dLME, the conditional F-statistic for each explanatory fixed effect variable was computed using the marginal sum of squares approach (each fixed effect enters the model as the last one) (Chen, Saad et al. 2013). Using this approach, we aimed to replicate previous known age and sex differences in FA, where our linear model included testing the conditional F-statistic for the fixed terms of age, sex, and an age-by-sex interaction. To examine puberty effects, separate linear models were run for adrenal-related and gonadal-related PDS scores. These models tested the conditional F-statistic for the fixed terms of PDS score (adrenal or gonadal), sex, PDS-by-sex interaction,

while also controlling for age. In other words, the use of the conditional F-statistic allows for testing the significant contribution of PDS variables, beyond the predictive contribution of age. For example, a statistically significant conditional F-statistic for adrenal PDS score, would represent the significant effect of adding the adrenal PDS score to a model that included all other predictor variables (age, sex, and PDS-by-sex interaction). In order to estimate the effect sizes of the PDS, the level 1 pseudo-R^2 was calculated using the following equation: $R^2 = \frac{\sigma^2(\text{model}1) - \sigma^2(\text{full model})}{\sigma^2(\text{model}1)}$ (Singer and Willet 2003); where the model 1 fixed effects were age+sex and the full model fixed effects were age+sex+PDS. Similarly, the effect size of the PDS-by-sex interaction term was estimated using the same equation but with the model 1 fixed effects including age+sex+PDS and the full model fixed effects including age+sex+PDS+PDS*sex.

To correct for multiple comparisons, a Monte Carlo simulation was implemented using AFNI, while controlling for spatial autocorrelation (-acf option) to accurately reduce Type 1 error in determining the cluster-size threshold. The multiple comparison correction included a voxelwise threshold of $p \leq 0.01$, with a minimum cluster extent of 21 contiguous voxels (mm^3), to yield a corrected alpha ≤ 0.001 (an equivalent of a p-value per voxel < 0.00000023). White matter regions were identified, labeled, and categorized using the MRI Atlas of Human White Matter (Oishi, Faria et al. 2011). The corrected statistical results, including the spatial extent of each cluster, is available through Neurovault: Full details of all spatial maps are available through Neurovault: http://neurovault.org/collections/2245/.

For each significant white matter cluster from FA analyses, planned post-hoc analyses were performed to examine if age, sex, and puberty were related to AD, RD, and MD in these regions. In particular, mean values were extracted and identical linear models were performed while replacing FA with AD, RD, or MD as the dependent variable of interest. Only AD, RD, and MD models showing $p \leq .01$ are reported.

3. Results

3.1 Sample Characteristics

Four subjects were removed due to unusable data at one or more timepoints, whereas 10 individuals had only one (out of 2 possible) DTI runs at one of the two timepoints. The final sample included 19 boys and 25 girls for age analyses. Demographics for the final samples included 36% Caucasian, 30% African American/Black, 21% more than 1 race, 2% Native Hawaiian or other, and 11% chose not to disclose, with 50% Hispanic. The sample came from households with various income levels ($5,000 - 9,999: 6.8%; $10,000 - 19,999: 9.1%; $20,000 - 29,999: 11.4%; $30,000 - 39,999: 11.4%; $40,000 -49,999: 15.9%; $50,000 - 74,999: 13.6%; $75,000 - 100,000: 15.9%; $100,000+: 11.4%; chose not to disclose: 4.5%). Additional characteristics of the sample are reported in Table 1.

3.2 Pubertal Development

Pubertal data was missing for 3 girls and 1 boy, and another 6 girls and 1 boy were excluded from pubertal analyses, as their self-reports of pubertal development were deemed unreliable

due to reporting lower pubertal values in adrenal or gonadal scores at follow-up. Thus, 18 boys and 15 girls were included in the pubertal analyses. PDS data for the final sample are presented in Supplementary Table 1. At Time 1, boys and girls showed similar adrenal-related PDS scores (sex effect at Time 1: Beta 0.06, SD=.17, t=0.23, p=.82). Adrenal-related PDS scores also increased over time (time effect: Beta 0.69, SD=.17, t=4.18, p=.0002), and this change was similar in both boys and girls (time*sex interaction: Beta 0.14, SD=.25, t=0.56, p=.58) (Figure 1B). For gonadal-related PDS scores, girls had significantly higher values at Time 1 as compared to boys (sex effect at Time 1: Beta 0.87, SD=.25, t=3.41, p=.0018) (Figure 1B). Gonadal-related PDS scores also increased over time (time effect: Beta 0.74, SD=.17, t=4.38, p=.0001), but again this change was not different in boys and girls (time*sex interaction: Beta -0.16, SD=.25, t=0.65, p=.52). For girls, 7 out of 15 were pre-menstrual at Time 1, whereas all 15 girls had complete menarche by Time 2. Within these average changes, there was also quite a bit of individual variability with some adolescents reporting no changes in adrenal- or gonadal- related physical changes and others reporting more rapid changes over the approximate 2 year period (Figure 1B).

3.3 FA Variability Between and Within Subjects

Maps of voxelwise between- and within-subject variance for the white matter sketelon overlaid on an MNI152 atlas brain are presented in Figure 2. The variance explained by the between-subject (Subj) as compared to the within-subject factors (Run and Time) varied across the white matter skeleton, with higher between-subject effects seen in deep white matter regions as compared to the distal portions of the white matter skeleton. However, overall, the between-subject effect accounted for a much larger proportion of the variance as compared to the within-subject factors, with ~58% of the variance in FA explained by individual differences (Subj: mean=.58, SD=0.14, with 68% of values >.50), and only 1% and 2% of the variance in FA values accounted for by within-subject factors (Run: mean=.01, SD=0.01, with 99% of values <.05; Time: mean=.02, SD=0.02, with 99% of values <.25). These data indicated that the between-subject effect was largely responsible for the variance observed in the FA values included in the white matter skeleton, whereas the small amount of variance explained by within-subject predictors suggest high consistency, or reliability, in FA values measured within a given day (DTI Run) and time (Time 1 and Time 2).

3.4 Age, sex, and white matter diffusivity

Regions showing a significant relationship with age, sex, and their interaction are shown in Supplementary Tables 2-4. Age predicted widespread increases in FA within white matter (Figure 3A), including regions known to carry association fibers (cingulate, inferior fronto-occipital fasciculus, superior longitudinal fasciculus (SLF)), brainstem and cerebellar fibers (cerebellar, cerebral peduncle, pons, midbrain), commissural fibers (corpus callosum), projection fibers (anterior and posterior corona radiata, internal and external capsule, thalamic radiations), and cortical white matter regions of the frontal, precentral and postcentral gyri, parietal, temporal, and occipital lobes (Supplementary Table 2). Follow-up analyses showed that 1) most regions also showed a significant decrease in RD with age and 2) the majority of regions also showed a decrease in MD with age, whereas 3) only a few

regions showed either an increase in AD (such as the SLF, superior frontal white matter) or a decrease in AD (cerebral peduncle) with age.

Boys showed larger FA values compared to girls in a number of white matter regions including association fibers (cingulum/hippocampus), brainstem and cerebellum, projection fibers (corona radiata, external capsule, inferior fronto-occipital fasciculus), and cortical white matter regions of the superior frontal, postcentral, and inferior temporal gyri) (Supplementary Table 3). Alternatively, girls were found to have larger FA values in only one region of the precentral gyrus white matter as compared to boys (Figure 3B, Supplementary Table 3). Follow-up analyses showed that only a few of these regions showed a sex difference in AD, RD, or MD values, with boys showing an increase in these values as compared to girls.

Twelve white matter regions were detected that showed an age by sex interaction, including commissural fibers (the genu of the corpus callosum), projection fibers (anterior and posterior corona radiata, superior longitudinal fasciculus) and white matter regions in the inferior frontal, superior frontal, precentral, and middle temporal gyri (Figure 3B and Supplementary Table 4). In each of these regions, boys showed significant increases in FA with age, whereas girls showed decreases or no change with age. Follow-up analyses in these regions showed RD and MD values to decrease with age in boys (with less decrease, no change, or even increases in girls), and AD values tended to increase with age in boys and decrease in age with girls.

3.5 Puberty, sex, and white matter diffusivity

Adrenal- and gonadal- related changes in white matter microstructure are reported in Table 2. Two regions were detected where larger increases in adrenal-related pubertal changes predicted increases in FA, including white matter in the precentral gyrus and anterior to the thalamus in both sexes (Figure 4A). Follow-up analyses in these regions showed that adrenal-related changes predicted decreases in RD for the thalamic region. In addition, a significant interaction was also detected in white matter in the superior corona radiata (Figure 4B), where adrenal-related pubertal changes lead to increases in FA in boys, but a non-significant decrease in FA in girls. Follow-up analyses revealed that in this region adrenal-related increases related to a decrease in RD in boys, but increase in RD in girls. Alternatively, larger gonadal-related pubertal changes led to decreases in FA in the genu of corpus callosum and the anterior corona radiata in both boys and girls (Figure 4C). Follow-up analyses revealed no significant effect of gonadal change on AD, RD, or MD in these regions. A number of sex-specific effects were also seen, with gonadal-related increases in pubertal development led to increases in FA in superior frontal and precentral gyrus white matter in boys, but decreases in girls (Figure 4D). In addition, gonadal-related pubertal changes led to larger decreases in FA in the anterior corona radiata in girls as compared to boys (Figure 4D). Follow-up analyses revealed that gonadal changes led to greater increases in RD and MD in the anterior corona radiata in girls as compared to boys. In the superior frontal gyrus, gonadal changes predicted increases in AD but decreases in RD in boys, whereas the pattern for girls was exactly opposite (decreases in AD, but increases in RD). Lastly, in the precentral gyrus white matter region boys showed a decrease in RD whereas

girls showed an increase in RD as a function of overall change in gonadal physical characteristics.

After completing the *a priori* analyses, follow-up post-hoc analyses were performed to further clarify the interpretation of the puberty variables. First, given quadratic and cubic relationships have been previously seen in some patterns of white matter development (Lebel and Beaulieu 2011, Ladouceur, Peper et al. 2012), post-hoc analyses examined if adrenal- and gonadal- related changes in white matter microstructure remained significant if more complex age variables (i.e. quadratic and cube) were included in the model. These analyses showed that for all clusters but one (adrenal PDS thalamus finding), model fits were not significantly different from linear model estimates. More importantly, the significance of the adrenal- and gonadal-variables in every model remained significant after controlling for possible quadratic and cubic age terms. In addition, models were performed to further examine the unique variance of adrenal- vs. gonadal- for each significant cluster, especially given concerns that adrenal- and gonadal- PDS scores were highly related ($\beta=0.70\pm0.09$, $p<.0001$). Again, adrenal- and gonadal- predictors remained significant after accounting for this shared variance.

4. Discussion

The present study was the first to examine how longitudinal changes in different aspects of puberty-related markers of physical development predicted changes in white matter microstructure in adolescents. Neurodevelopment has primarily been studied in the context of age. Beyond replicating age and sex effects in white matter microstructure, we show that pubertal-markers contribute to distinct trajectories of change across adolescence after accounting for age-related change. The results suggest that changes in adrenal- and gonadal-related pubertal markers are associated with unique (i.e. non-overlapping) changes in FA within white matter of projection, commissural, and cortical regions. Further, sex-specific effects were seen in terms of how adrenal- versus gonadal- based changes influence white matter development (in terms of FA change) across adolescence.

Adrenarche and gonadarche are distinct developmental processes. As the adrenal glands mature, they produce an increase in adrenal androgens, including dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione (Grumbach and Styne 2003) during gonadarche, as well as into young adulthood (Saenger and Dimartino-Nardi 2001). Gonadarche, however, is driven by gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)), acting on the ovaries and testes to produce gonadal sex steroids of estradiol and progesterone, as well as testosterone, respectively (Grumbach and Styne 2003). By using an established method to separate a common physical maturation scale into adrenal-versus gonadal-based markers of pubertal development (Shirtcliff, Dahl et al. 2009), we found that changes in these two underlying pubertal components had distinct influences on how white matter microstructure develops across adolescence. Adrenal development was related to changes in FA in the thalamus, precentral and superior cortical white matter in boys and girls. Alternatively, gonadal development was found to show a number of changes in FA in regions known to carry fibers between the frontal lobes and subcortical structures (e.g. superior frontal gyrus anterior corona radiata), most of which

showed decreases in FA in these regions for girls. While previous cross-sectional studies have shown pubertal markers to relate to FA or MD values (Herting, Maxwell et al. 2012, Menzies, Goddings et al. 2015), the current results expand upon these earlier studies to show that greater *changes* in adrenal- and gonadal- based physical pubertal changes related to *changes* in white matter microstructure.

Sex differences in white matter development, including higher FA values in boys compared to girls, have been previously reported (Wang, Adamson et al. 2012, Simmonds, Hallquist et al. 2014). Both previous longitudinal studies found that boys continued to demonstrate white matter microstructural changes across later adolescence and young adulthood, whereas girls appeared to undergo growth in mid-adolescence and possibly reach mature levels earlier. In the current study, larger increases in gonadal-related pubertal development predicted increases in FA in precentral gyrus and superior frontal gyrus white matter in boys, but decreases in FA in girls across adolescence, suggesting that gonadal-related developmental processes may contribute to these known white matter sex differences. Our results are also in line with both human and animal data suggesting that gonadal hormones may facilitate white matter development in males, but inhibit such development in females. For example, in a cross-sectional study, adolescent study boys were found to have higher FA values in a number of white matter tracts, with testosterone levels associated with these higher FA values in boys and estradiol associated with lower FA in girls (Herting, Maxwell et al. 2012). In rats, males have a greater number of myelinated axons as compared to females, with typical pubertal increases in estradiol driving this effect by inhibiting myelination in females (Juraska and Markham, 2004). In addition, the adult male rat brain has a larger number of oligodendrocytes - essential for myelination - as compared to the female brain (Cerghet, Skoff et al. 2009), which may contribute to the large increases in FA (and lower RD and MD values) that have been linked to increases in testosterone (Herting, Maxwell et al. 2012, Menzies, Goddings et al. 2015). Beyond potential mechanisms underlying gonadal-changes, little is known in terms of adrenal-related processes on white matter development. To our knowledge, only one study has examined DHEA levels and white matter using DTI and found no significant relationship in adolescent boys (Menzies, Goddings et al. 2015).

Follow-up studies that measure longitudinal change for an array of gonadal and adrenal hormones are needed to further clarify how and which changes in sex steroid levels may contribute to pubertal and brain development. This is especially important as the hormones related to gonadal- versus adrenal- changes in physical and brain maturation may differ between males and females. Indeed, Shirtcliff and colleagues' (Shirtcliff, Dahl et al. 2009) cross-sectional study found that estradiol levels related to breast development, whereas testosterone and DHEA related to pubic hair development in girls. In boys, testosterone was found to relate to both genital development and pubic hair, whereas DHEA was found only to relate to pubic hair. Beyond changes in hormonal contexts with pubertal maturation, co-occurring changes in behavior, or how others treat the individual, may contribute to subsequent sex-specific patterns of change in brain and behavior. Thus, a profile of numerous physical, behavioral, and hormone measures is needed to advance our understanding of puberty's role in white matter development in boys and girls.

Limitations to the current study should be noted. First, the current study used a self-report measurement of pubertal development (PDS). Although this measure has been shown to be reliable and valid in assessing physical stages of pubertal maturation (Petersen, Crockett et al. 1988), there are some known self-report biases between the sexes, and as a function of pubertal stage and gonads/breast and pubic hair measurements (Dorn, Susman et al. 1990). This may help to explain why a few subjects in our study reported lower values (reflecting 'decreasing physical pubertal change') over time. However, it should be noted that these subjects were excluded from analyses in the current study. Moreover, two different reliability studies have concluded that despite its limitations, PDS indicators of maturation remain useful as predictors in assessing longitudinal changes within subjects (Brooks-Gunn, Warren et al. 1987); (Petersen, Crockett et al. 1988), as shown in the current report. Shirtcliff and colleagues found that PDS self-report scores for gonadal versus adrenal development were similar, or in some cases were more closely related, to hormone levels compared to physician reports (Shirtcliff, Granger et al. 2000). Second, the current study size is relatively small, suggesting that the current findings may be biased to large effects (while possibly missing more widespread brain changes carrying medium and small effect sizes). However, the longitudinal design and use of multi-level modeling allows for the ability to better isolate pubertal and sex-dependent influences on developmental change from other sources of variability. In fact, covariance estimates (e.g. ICC) further highlight that individual differences (i.e. due to sex, age, puberty, other) account for a large proportion of FA variability seen in the white matter skeleton, suggesting the importance of including both within- and between-subject effects to more fully understand pubertal influences on white matter trajectories. Third, the current study had only two measurements per subject, allowing for only a linear model to be examined as an estimate of change within a single individual. This is a disadvantage, as it is plausible that important non-linear changes in white matter occur within each subject as a function of pubertal development, and that specific within-subject developmental trajectories exist for the various DTI measures. Moving forward, studies implementing larger sample sizes with three time points will allow for greater power to detect medium and smaller effects and the testing of non-linear slopes at the individual-level.

5. Conclusions

The current study examined how within-subject changes in physical markers of pubertal development influence changes in white matter microstructure. The results show that both adrenal- and gonadal-based pubertal changes predict unique changes in FA. Furthermore, the results suggest that adrenal and gonadal-based changes lead to increases in FA in boys, but decreases in FA in girls, in frontal (precentral and superior frontal gyrus) as well as anterior and posterior corona radiata white matter regions. These findings support a larger body of emerging research suggesting that, beyond age, pubertal processes may also contribute to neurodevelopmental trajectories in boys and girls.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Result showed increases in FA with age and higher FA in boys versus girls
- Physical pubertal development contributes to changes in FA
- Sex-specific patterns seen between changes in physical puberty and FA

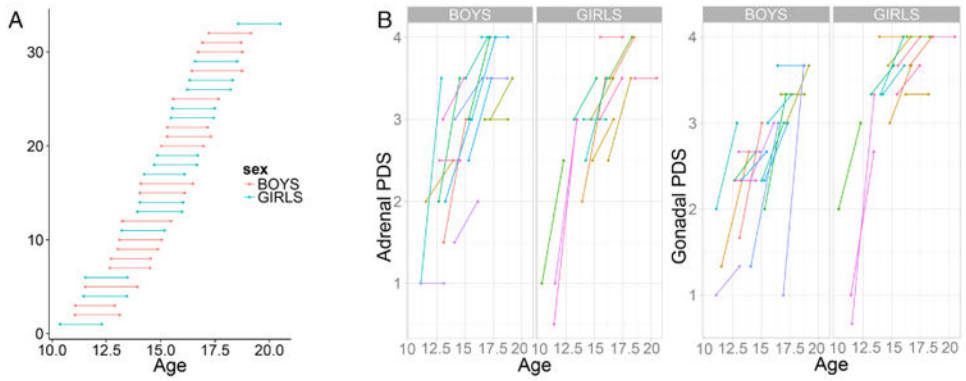

Figure 1.

A) Age distribution of the final data represented in statistical analyses. Each circle denotes a scan acquisition, and inter-scan interval for a single participant is represented by a line interconnecting the circles. B) Changes in Pubertal Development Scale (PDS) scores for adrenal- and gonadal- related values for boys and girls. Each participant is represented by a different color.

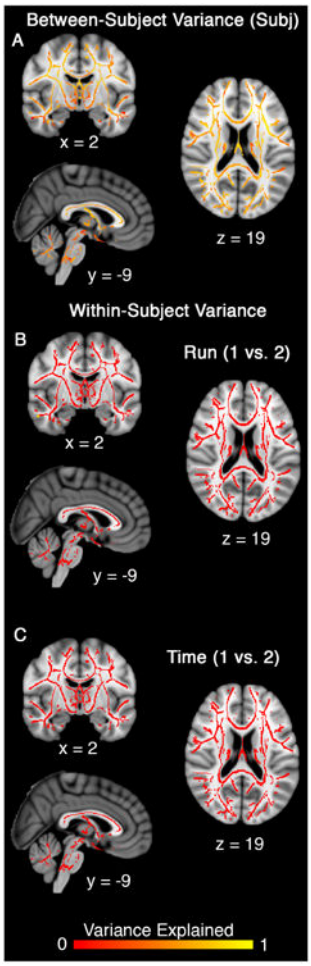

Figure 2.

Voxelwise maps for variance explained in white matter skeleton FA for A) between-subject effects (i.e. subject), and B and C) within-subject effects (i.e.DTI run and Time). Coordinates reflect MNI standard space. R=right; Left=left.

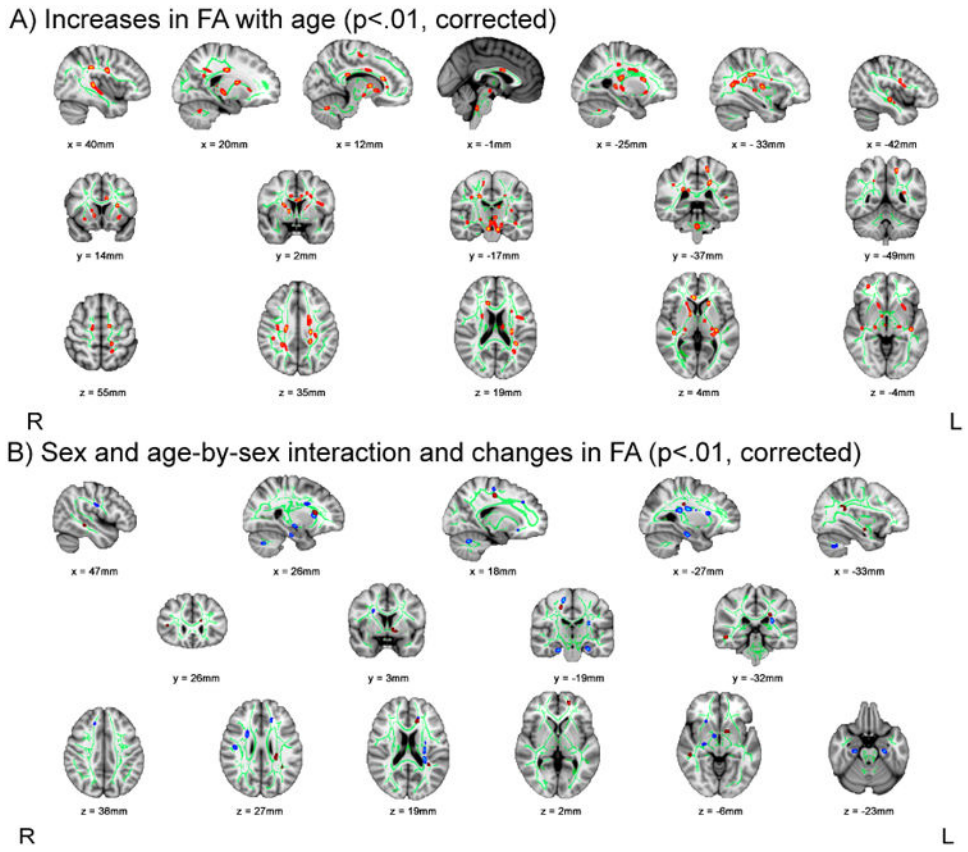

Figure 3.

White matter regions where changes in FA are significantly predicted by age, sex, and age-by-sex. A) Regions showing increases in FA values with age (red-yellow). B) Regions where FA values vary by sex (blue) as well as a function of age-by-sex (red clusters). Unconditional F-statistics maps (dilated for viewing purposes) overlaid on the mean FA skeleton (green) and a standardized brain. Coordinates reflect MNI standard space. R=right; Left=left.

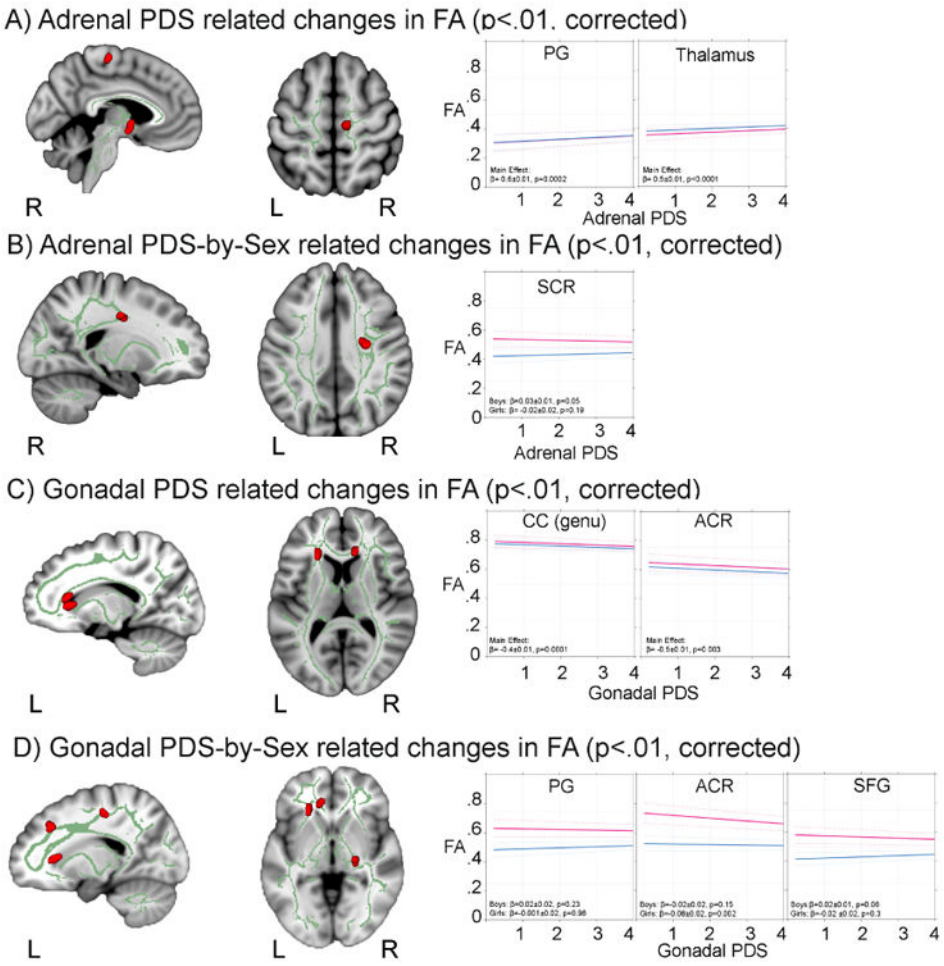

Figure 4.

Changes in FA as a function of changes in A) adrenal-PDS scores, B) adrenal-by-sex interaction, C) gonadal-PDS scores, D) gonadal-by-sex interactions, while controlling for age (p<.01, corrected). Images show regions where changes in FA are predicted by pubertal development; unconditional F-statistics maps (dilated for viewing purposes) overlaid on the mean FA skeleton (green) and a standardized brain. Plots show estimated model fits from the linear mixed model in predicting FA values for each significant region. R=right; Left=left; ACR=anterior corona radiate; CC (genu)=genu of the corpus callosum; PG=precentral gyrus; SCR=superior corona radiata; SFG=superior frontal gyrus.

Table 1

Sample demographics

Values reflect Mean ± Standard Deviation unless noted.

	All (N=44)	Boys (N=19)	Girls (N=25)
Initial Age (years)	13.93±2.19	14.12±2.07	13.79±2.31
Inter-scan interval (years)	1.98±0.19	2.03±0.19	1.95±0.13
IQ[a]	102.39±16.59	101.61±14.28	103.00±18.50
Handedness(N)[b]	R=35, L=5, A=1	R=16, L=1, A=0	R=16, L=4, A=1
Motion (Mean FD)[c]	0.49±0.14	0.50±0.15	0.49±0.14

[a] Intelligence Quotient -- Wechsler Intelligence Scale for Children, Fourth Edition

[b] Edinburgh Handedness Inventory; R=Right; L=Left; A=Ambidextrous

[c] Frame displacement (mm) between DTI gradient directions

Table 2

Regions showing a significant effect of A) adrenal-related pubertal development and adrenal-by-sex and B) gonadal-related pubertal development and gonadal-by-sex on changes in FA as well as follow-up for axial (AD), radial (RD), and mean (MD) diffusivity within these regions. Colors reflect pubertal development-by-sex interactions: Red reflects an increase in FA and AD for boys (but a decrease for girls); blue reflects decrease in RD in boys (but an increase in girls); purple reflects a greater increase in RD and MD, but decrease in FA, in girls as compared to boys.

MNI			Voxel #	White Matter Region	Effect Size (R^2)	Change in FA with puberty	Follow-up analyses		
x	y	z					AD *p-value*	RD *p-value*	MD *p-value*
A) Adrenal-related changes									
Projection Fibers									
7	-1	-2	24	thalamus	.80	Main Effect: β= 0.5±0.01, ***p<0.0001***		***0 .0002***	
Cortical White Matter Regions									
11	-20	64	30	precentral gyrus (PG)	.73	Main Effect: β= 0.6±0.01, ***p=0.0002***			
Adrenal-by-sex changes									
Cortical White Matter Regions									
25	-12	38	22	superior corona radiata (SCR)	.81	Interaction: β=-0.05±0.01, ***p=0.0006*** Boys: β=0.03±0.01, *p=0.05* Girls: β= -0.02±0.02, p=0.19		***0.004***	
B) Gonadal-related changes									
Commissural Fibers									
12	30	10	32	genu of the corpus callosum	.77	β= -0.4±0.01, ***p=0.0001***			
Projection Fibers									
-23	25	13	26	anterior corona radiata (ACR)	.81	β= -0.5±0.01, ***p=0.003***			
Gonadal-by-sex changes									
Projection Fibers									
-23	25	14	27	anterior corona radiata (ACR)	.66	Interaction: β= -0.07±0.01, ***p<.0001*** Boys: β=-0.02±0.02, p=0.15 Girls: β=-0.08±0.02, *p=0.002*		***0.0009***	***0.005***
Cortical White Matter Regions									
-11	33	41	25	superior frontal gyrus (SFG)	.70	Interaction: β= -0.07±0.01, **p=.0001** Boys: β =0.02±0.01, p=0.06 Girls: β=-0.02 ±0.02, p=0.3	***0.001***	***0.005***	
19	-21	50	30	precentral gyrus (PG)	.79	Interaction: β= -0.05±0.02, **p=.0009** Boys: β=0.02±0.02, p=0.23 Girls: β=-0.001 ±0.02, p=0.96		***0.006***	