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SEX-TYPICAL & SEX-ATYPICAL WHITE MATTER DIFFUSION CHARACTERISTICS IN GENDER DYSPHORIC BOYS & GIRLS

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Abstract

White matter microstructure, assessed by means of diffusion tensor imaging, varies as a function of gender. Men show higher overall, and region-specific fractional anisotropy values compared with women, suggesting differences in axonal organization and myelination. Diffusion measures are highly sensitive to neurodevelopmental changes of white matter cellular architecture during adolescence. In the present study we investigated whether 21 adolescent girls and 17 adolescent boys, diagnosed with Gender Dysphoria (GD; DSM-5) exhibit sex-atypical (in accordance with their experienced gender), rather than sex-typical (in accordance with their natal sex) white matter microstructural characteristics. We first identified sexually dimorphic white matter brain areas in two age-matched control groups (21 girls and 20 boys) and then compared the mean diffusion values for each of these regions between groups. Boys with GD did not differ significantly from either the control boys or the control girls in the majority of the sexually dimorphic areas, indicating they had intermediate values between the sexes. In contrast, girls with GD predominantly had sex-typical white matter diffusion characteristics showing only slight masculinization in fiber organization. Our findings provide new evidence for the hypothesis that individuals with GD may have undergone atypical neuronal sexual differentiation and possess certain neurobiological characteristics of their experienced gender.

Diffusion tensor imaging (DTI) has been applied as a powerful and sensitive magnetic resonance technique to characterize and map the three-dimensional diffusion of water in brain tissue. Diffusion measures are highly sensitive to changes of white matter cellular architecture and have therefore been used to characterize neuropathology, as well as normative neurodevelopmental microstructural changes (Alexander et al. 2007). *Fractional anisotropy* (FA) is the most widely used measure for anisotropic diffusion. FA values have been shown to increase from infancy to early adulthood (Lebel et al. 2010; Lebel and Beaulieu 2011; Geng et al. 2012), reflecting white matter maturation, axonal fiber organization and myelination, peaking

around the age of 30, followed by a gradual decline of FA and increase in *mean diffusivity* (MD) during late adulthood (Salat, Tuch, Greve, et al. 2005; Salat, Tuch, Hevelone, et al. 2005; Hsu et al. 2008).

Diffusion is considered isotropic when motion of water is uniform and non-restricted. In contrast, water diffusion is highly restricted and hindered, thus less uniform and anisotropic in fibrous tissue such as white matter. FA is derived from three eigenvalues ($\lambda 1$, $\lambda 2$, $\lambda 3$,) and describes the degree of anisotropy of a diffusion process within a voxel (values can range between .1–1.0), and is thus modulated by the microstructural cell and fiber characteristics within that particular voxel (Basser and Pierpaoli 1996). Other common diffusion measures are MD, which is the average of the three eigenvalues, *axial diffusivity* (AD) characterizes diffusion parallel to the fiber bundle ($\lambda 1$), and *radial diffusivity* (RD) the diffusion direction perpendicular to the axonal fiber bundle ($(\lambda 2 + \lambda 3)/2$).

Of note, white matter diffusion characteristics were found to vary as a function of gender. Men consistently show higher overall, as well as region-specific FA values, often concomitant with lower MD and RD values, compared with women (Hsu et al. 2008; Inano et al. 2011; Menzler et al. 2011; Westerhausen et al. 2011). It is thought that higher FA in men reflects more organized fiber bundles, whereas women may possess a greater degree of white matter fiber crossing (Schmithorst, Holland, & Dardzinski, 2008), resulting in higher intra- and inter-hemispheric connectivity in women. Accordingly, regional diffusion parameters differing between men and women, suggesting differences in axonal organization and myelination, were predictive for gender-specific personality characteristics (Chou et al. 2011; Peper et al. 2013) and cognitive functions (Clayden et al., 2012; Fryer et al., 2008; Schmithorst, Wilke, Dardzinski, & Holland, 2005; Silveri et al., 2006). Moreover, pubertal development (Bava et al., 2011; Schmithorst, Holland, & Dardzinski, 2008), as well as sex hormones (Herting et al. 2012; Peper et al. 2013) were differentially associated with white matter diffusion characteristics in males and females.

Individuals with Gender Dysphoria (GD; DSM-5 (American Psychiatric Association 2013)); also referred to as transsexualism (ICD-10 (World Health Organization 1992)) are characterized by a profound feeling of incongruence between their natal sex and expressed/experienced gender. It has been hypothesized that atypical levels of perinatal sex steroids during a critical period of sexual differentiation of the brain may be involved in the development of GD (van Goozen et al. 2002; Swaab 2007).

So far, two previous DTI studies investigated FA values in treatment-naïve transgender populations. Rametti et al. (2011-a) found that adult women with GD had significantly higher, thus male-typical FA values compared with control women in most pre-defined sexually dimorphic areas. Similarly, adult men with GD differed from both male and female controls, and thus revealed an intermediate pattern of white matter diffusion characteristics (Rametti, Carrillo, Gomez-Gil, Junque, Zubiaurre-Elorza, et al., 2011b). Of note, these effects were found prior to any hormonal treatment, suggesting a priori sex-atypical differentiation of brain structures.

Likewise, a previous study reported sex-atypical measures in the corpus callosum shape, the major inter-hemispheric fiber bundle, in a group of adult men and women diagnosed with GD. Whereas men with GD had a more female-typical shape, that of women with GD was more similar to that of control men (Yokota et al. 2005). However, another study focusing on volumes of the corpus callosum, found no differences between adult individuals with GD and controls (Emory et al. 1991).

At the *Center of Expertise on Gender Dysphoria* in Amsterdam, the current treatment protocol allows adolescents diagnosed with GD that persisted from childhood into adolescence to start treatment with *gonadotropin-releasing hormone analogues* (GnRHa) from the age of 12 years on, to suppress endogenous gonadal stimulation and thus the development of irreversible sex-characteristics of the natal sex (Kreukels and Cohen-Kettenis 2011). From the age of 16 years on, as a first step in sex reassignment they receive cross-sex hormone treatment, i.e. biological boys receive estrogens and biological girls receive androgens (Delemarre-van de Waal and Cohen-Kettenis 2006; Hembree et al. 2009).

The present study aimed to investigate whether adolescent boys and girls with GD, prior to the start of the cross-sex hormonal treatment, would show sex-atypical white matter diffusion characteristics in predefined areas showing significant sex differences between control girls and boys. Similar to the studies by Rametti et al. (2011-a, 2011-b), our adolescent participants were unanimously diagnosed with early onset GD (Nieder et al. 2011). Participants in the current study, however, were not exposed to endogenous sex hormones at the time of measurement, but were receiving GnRHa to suppress pubertal development. Examining white matter microstructure in adolescents with GD on GnRHa enabled us to control for possible activational effects of endogenous sex

hormones on white matter brain development. Based on the previous studies in adult individuals with GD, we hypothesized that both, boys and girls with GD would show sex-atypical white matter diffusion characteristics, similar to their experienced gender control groups.

Materials and Methods

SUBJECTS

SEVENTEEN ADOLESCENT BOYS (mean age 15.3 years, standard deviation (SD) = 1.2) and 21 adolescent girls (mean age 16.1 years, SD = .8) were recruited via the *Center of Expertise on Gender Dysphoria* at the VU University Medical Center in Amsterdam. The control subjects, 20 boys (mean age 15.9, SD = .6) and 21 girls (mean age 16.3 years, SD = 1.0), were recruited via several secondary schools in the Netherlands, and by inviting friends of the participants with GD. All participants with GD had been treated with monthly 3.75 mg Triptorelin (Decapeptyl-CR®, Ferring, Hoofddorp, the Netherlands) injections for, on average, 23.5 months (range 2–48 months) resulting in complete suppression of gonadal hormone production.

ASSESSMENTS AND SUBJECT CHARACTERISTICS

IN CONTROLS, PUBERTAL staging (pubic hair growth, breast (in girls) and genital (in boys) development) was assessed by means of the five-point (1 = prepubertal, 5 = postpubertal) Tanner Maturation Scale self-report questionnaire (Marshall and Tanner 1969, 1970), which correlates highly with physician assessments (Duke et al. 1980; Morris and Udry 1980). In all participants with GD, as part of their regular medical check-ups at the clinic, pubertal stages were determined by a pediatric endocrinologist (DTK). All participants completed four subtests (arithmetic, vocabulary, picture arrangement, and block design) of the Wechsler Intelligence Scale for Children (Wechsler 2005–a) or, if older than 16 years of age, the Wechsler Intelligence Scale for Adults (Wechsler 2005–b). Each four-subtest sum score was converted to an individual's estimated *Intelligence Quotient* (IQ) score. All participants and their legal guardians gave their informed consent according to the Declaration of Helsinki, and the study was

approved by the Ethics Committee of the VU University Medical Center Amsterdam (application number NL31283.029.10).

IMAGE ACQUISITION

THE SCANS WERE ACQUIRED on a 3.0 Tesla GE Signa HDxt scanner (General Electric, Milwaukee, WI, USA). An echo-planar imaging sequence was used for diffusion-weighted imaging. Thirty diffusion-weighted and five images without diffusion weighting were obtained (b value = 1000 mm^2/s, TR = 13000 ms, TR = 85 ms, field of view = 256 × 256 mm, 45 slices, slice thickness = 2.4 mm).

IMAGE PROCESSING

DIFFUSION IMAGES WERE pre-processed using DTIfit, part of the FMRIB's Diffusion Toolbox as implemented in the FMRIB Software Library (FSL) version 4.1.9 (Smith et al., 2004). Images were corrected for susceptibility distortions, eddy current distortions, realigned to one of the non-diffusion images (b = 0) using affine registration (Jenkinson et al. 2002), and non-brain tissue removal using BET (Smith, 2002). By fitting a tensor model to the diffusion data, the eigenvalues (λ1, λ2, λ3,) of the tensor for each voxel were identified, and FA, AD, RD, and MD maps were calculated. All images were corrected for motion artifacts.

Voxel-wise statistical analyses were performed on FA images using *Tract-Based Spatial Statistics* (TBSS) (Smith et al. 2006). All subjects' FA maps were normalized via non-linear registration to MNI152 standard space using FNIRT (Rueckert et al. 1999). These normalized images were then averaged to create a group-wise mean FA map which was thinned so that the centers of all tracts common to the whole group were represented in a white matter skeleton. An FA threshold of .2 was applied to reduce partial volume effects. Finally, each subject's aligned FA map was projected onto the white matter skeleton for subsequent voxel-wise group-level statistics. AD, RD, and MD maps were also normalized to standard space and projected onto the FA-derived white matter skeleton for subsequent group-level statistics. Anatomical locations were identified with the *Johns Hopkins University* (JHU) White-Matter Tractography Atlas and JHU ICBM-DTI-81 White-Matter Labels (Mori et al. 2005; Wakana et al. 2007; Hua et al. 2008).

STATISTICAL ANALYSES

FIRST, WE DEFINED SEXUALLY dimorphic *regions of interest* (ROI) by means of voxel-wise statistical analyses for each of the FA, AD, RD, and MD maps by contrasting control boys and control girls using two-sample *t* tests. Permutation-based non-parametric testing (10,000 permutations) was performed, as implemented in the Randomise Tool of FSL (Nichols and Holmes 2002). The resultant statistical maps were thresholded for statistical significance at $p < .05$ family-wise error (FWE) corrected, applying the *Threshold-Free Cluster Enhancement* (TFCE) option (Smith & Nichols, 2009) and ROIs with a minimum cluster size of 10 voxels were defined.

Second, we extracted each subject's mean FA value as well as AD, RD, MD values for each of the previously defined sexually dimorphic ROIs. Using the Statistical Package for the Social Sciences, version 20 (SPSS Inc., Chicago, IL, USA), we conducted multivariate analyses of variance (MANOVA), treating group as between subject factor with four levels (control boys, control girls, girls with GD, and boys with GD). Age, IQ, and pubertal stages were added to the model as covariates of no interest. Post-hoc *t* test comparisons tested whether diffusion parameters of adolescent boys and girls with GD would be more in line with those of their natal sex control group (sex-typical), or more in accordance with their experienced gender (sex-atypical). Bonferroni correction for multiple comparisons was applied, considering a threshold of $p < .05$ as statistically significant.

Results

DEMOGRAPHICS & SUBJECT CHARACTERISTICS

DEMOGRAPHIC, SELF-REPORT and subject characteristics are presented in Table 1. The boys with GD were significantly younger and less physically mature (lower Tanner stages) compared with the three other groups. The IQ scores of both the boys and the girls with GD were significantly lower than those of the control groups. Age, pubertal stages, and IQ thus differed between the groups and, in addition, have previously been shown to affect diffusion parameters (e.g. Hasan et al., 2010; Herting et al., 2012; Schmithorst, Wilke, Dardzinski, & Holland, 2005); we therefore included these variables as covariates in further analyses.

Table 1 Demographics and subject characteristics

		BOYS W. GD	GIRLS W. GD	CTRL GIRLS	CTRL BOYS	*F* (df)	*P*-VALUE
N		17	21	21	20		
Age (mean (SD))		15.3 (1.2)	16.1 (0.8)	16.3 (1.0)	15.9 (0.6)	4.6 (3, 75)	**.005**
IQ (mean (SD))		99.3 (17.8)	10.5 (12.7)	11.3 (14.7)	113.4 (14.5)	4.3 (3, 75)	**.007**
Pubertal stage (mean (SD))	P	3.1 (1.1)	4.7 (0.6)	4.2 (0.7)	4.7 (0.7)	17.4 (3, 74)	**< .001**
	G/B*	3.1 (0.8)	4.1 (1.1)	4.1 (0.8)	4.1 (0.8)	5.1 (3, 74)	**.003**

GD = Gender Dysphoria; Ctrl = control; P = pubic hair growth; G = genital development; B = breast development; *G applies for natal boys and B for natal girls; pubertal stages were assessed by means of the five-point (1 = prepubertal, 5 = postpubertal) Tanner Maturation Scale.

SEX DIFFERENCES IN CONTROLS

THE TWO-SAMPLE *t* tests comparing control boys and girls on each of the four diffusion parameters (FA, AD, RD, MD) revealed eight areas in which control boys had significantly higher FA values than control girls (see Figure 6.1 and Table 2). We found no areas where girls had higher FA values than boys. No significant differences between boys and girls were found with respect to AD, RD, and MD at our a priori threshold of $p < .05$ FWE-corrected. Eleven clusters were identified, in which we found a trend ($p < .1$ FWE-corrected) for higher RD values in control girls compared with control boys. The brain areas showing significant sex differences in FA overlapped for the most part with those in RD (see Figure 1 and Table 2). All sexually dimorphic regions were located in the left hemisphere, specifically in white matter projection tracts such as the *cortico-spinal tract* (CST) and the *anterior thalamic radiation* (ATR), and in association fiber tracts such as the *superior longitudinal fasciculus* (SLF), the *uncinate fasciculus* (UF), and the anterior part of the *inferior fronto-occipital fasciculus* (IFOF).

< FIGURE 6.1
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GROUP COMPARISONS REVEALED that girls with GD had predominantly sex-typical FA values; they differed significantly from the control boys in five out of eight clusters (located in the ATR, CST, UF, and IFOF). In the three remaining ROIs, FA values of girls with GD were not significantly different from either of both control groups (see Figure 6.2A). Boys with GD had significantly lower, thus female-typical FA values compared with control boys in two out of eight clusters (both in the IFOF and UF), and had significantly higher FA values than control girls,

Table 2 Locations of areas showing significant differences between control boys and control girls

	FA Ctrl Boys > Ctrl Girls						
JHU WHITE-MATTER TRACTOGRAPHY ATLAS	**JHU ICBM-DTI-81 WHITE-MATTER LABELS**	**X**	**Y**	**Z**	**N**	***Z*MAX**	***P* VALUE**
CST	posterior limb of the internal capsule	117	106	87	941	4.9	**.023**
IFOF/UF	anterior corona radiata	111	153	67	266	4.3	**.037**
UF/IFOF	unclassified /frontal lobe	121	160	69	12	3.1	**.049**
UF	unclassified /frontal lobe	115	155	65	10	3.5	**.049**
ATR/IFOF	anterior limb of the internal capsule	109	144	74	11	3.2	**.050**
ATR	anterior limb of the internal capsule	102	135	75	39	4.3	**.047**
ATR	anterior limb of the internal capsule	105	139	75	15	3.2	**.049**
SLF temporal part/SLF	unclassified /superior corona radiata	115	129	107	44	3.1	**.049**
	RD Ctrl Girls > Ctrl Boys						
JHU WHITE-MATTER TRACTOGRAPHY ATLAS	**JHU ICBM-DTI-81 WHITE-MATTER LABELS**	**X**	**Y**	**Z**	**N**	***Z*MAX**	***P* VALUE**
SLF /SLF temporal part	superior corona radiata	116	119	91	345	4.8	.055
SLF /SLF temporal part	superior longitudinal fasciculus	125	129	94	22	3.7	.083
UF /IFOF	anterior corona radiata	110	152	63	188	5.1	.061
IFOF /UF	external capsule	124	126	68	13	4.2	.070
IFOF /UF	unclassified /frontal lobe	121	161	70	12	3.1	.078
UF	external capsule	118	138	82	12	3.5	.077
UF	unclassified /frontal lobe	115	153	65	16	3.3	.074
ATR /IFOF	anterior limb of the internal capsule	109	144	75	12	3.5	.075
ATR /SLF	superior corona radiata	114	129	91	13	3.4	.069
ATR /SLF	unclassified /superior thalamic radiation	115	128	109	13	3.1	.091
CST	superior corona radiata	117	104	92	12	2.9	.072

FA = fractional anisotropy; RD = radial diffusivity; Ctrl = control; CST = cortico-spinal tract; IFOF = inferior fronto-occipital fasciculus; UF = uncinate fasciculus; ATR = anterior thalamic radiation; SLF = superior longitudinal fasciculus; x y z = coordinates in Montreal Neurological Institute space; N = number of voxels; Zmax = peak voxel z statistic; all cluster were located in the left hemisphere

thus male-typical, in one ROI (ATR). In the remaining five sexually dimorphic areas, boys with GD differed significantly from neither the control boys nor the control girls (see Figure 6.2A). Sex difference comparisons between the girls and boys with GD revealed only one cluster (in the ATR) in which the boys with GD

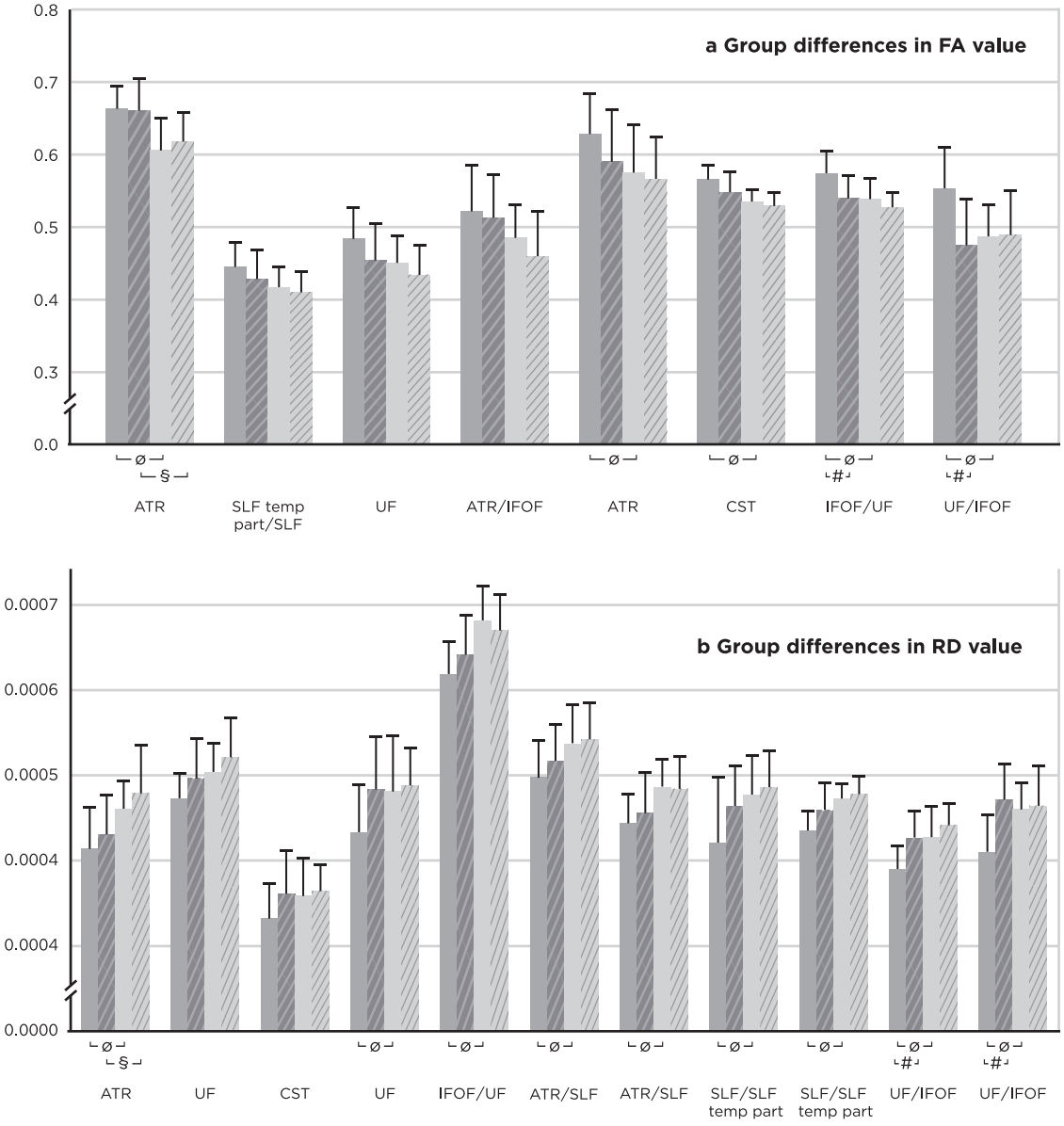

Figure 6.2 Histograms depicting group differences in **a** mean fractional anisotropy (FA) and **b** mean radial diffusivity (RD) values in those brain areas where significant sex differences between the control groups were found. CST = corticospinal tract; SLF = superior longitudinal fasciculus; SLF temp = temporal part of the SLF; ATR = anterior thalamic radiation; UF = uncinate fasciculus; IFOF = inferior fronto-occipital fasciculus; ø = significant differences between girls with Gender Dysphoria (GD) and control boys; # = significant differences between boys with GD and control boys; § = significant differences between boys with GD and control girls; all cluster were located in the left hemisphere.

showed significantly higher, thus male-typical FA values compared with the girls with GD.

Similarly to the FA results, girls with GD showed female-typical RD values, thus significantly higher RD compared with control boys, in eight out of eleven sexually dimorphic clusters (located in the SLF, IFOF, UF, and ATR). In the three remaining areas (in the UF and CST), RD values of the girls with GD were not significantly different from either control group (see Figure 6.2b), and thus intermediate to those of the controls. The boys with GD showed sex-atypical RD values, thus similar to control girls, in two out of eleven clusters (both in the UF/IFOF), and had male-typical RD values in one cluster (in the ATR). Boys with GD differed significantly from neither control group in the remaining eight ROIs (see Figure 6.2b) and had values in between those of the control boys and girls. Significant sex differences in RD between the boys and girls with GD in any of the predefined ROIs were absent.

Discussion

THE PRESENT STUDY SET out to determine whether gender dysphoric adolescent boys and girls exhibit sex-atypical (in accordance with their experienced gender), rather than sex-typical (in accordance with their natal sex) white matter microstructural characteristics. We first identified sexually dimorphic white matter brain areas in two age-matched control groups and then compared the mean FA and RD values for each of these regions between groups. In contrast to the sex differences found in the control groups, the typical sexual dimorphism in diffusion parameters was virtually absent in boys and girls with GD. Boys with GD did not differ significantly from either the control boys or the control girls in the majority of the sexually dimorphic ROIs, indicating they had intermediate values between the sexes. In contrast, girls with GD showed a predominantly female-typical pattern of diffusion characteristics; they thus had values similar to their natal sex control group, i.e. control girls.

Our results corroborate a number of previous studies (e.g. Inano et al., 2011; Menzler et al., 2011; Schmithorst et al., 2008), reporting sex differences in white matter microstructure, with control boys showing significantly higher FA than control girls in several projection and association fiber tracts. Higher FA is likely to result from lower diffusion in the direction perpendicular to the axonal

fiber bundle, i.e. RD (see Figure 1 for overlapping effects of FA and RD), reflecting a higher degree of myelination of these fiber tracts in boys as compared with girls (Eluvathingal et al. 2007; Peper et al. 2013). We found no sex differences for AD or MD. AD is thought to be unaffected by changes in the myelin sheet, but is specifically associated with axonal injury (Song et al. 2002, 2005). Therefore, sex differences or changes in AD may not be expected to be found in a group of healthy adolescents as in the current study. Changes in MD have been suggested to reflect changes in fiber density, associated with maturation of fiber tracts. Accordingly, sex differences in MD have been reported previously (Herting et al., 2012; Schmithorst et al., 2008), but were mostly related to aging and found in groups of participants with a broader age range compared with our study.

Our results in boys with GD are in line with one earlier DTI study in treatment-naïve adult men with GD (Rametti, Carrillo, Gomez-Gil, Junque, Zubiaurre-Elorza, et al., 2011b), who had FA values that were intermediate to those of control men and women in sexually dimorphic brain areas such as the SLF bilaterally, the right CST, forceps minor, and the anterior cingulum. Thus, both adolescent boys with GD receiving puberty suppressing medication and adult men with (early onset) GD exposed to endogenous sex hormones, showed a pattern of white matter fiber organization that was neither typically male nor typically female. In both age groups, only a small number of brain areas showed diffusion values compatible with full feminization or masculinization, whereas most areas had intermediate values relative to control males and females. This suggests that males with early onset GD may have had insufficient masculinization of their white matter fiber tissue during brain development, supporting the hypothesis of an atypical early sexual differentiation of the brain in individuals with GD (van Goozen et al. 2002; Swaab 2007). Moreover, this process seems to be unaffected by endogenous, circulating sex hormones.

Our results in girls with GD are at odds with those of Rametti et al. (2011-a) who found that treatment-naïve adult women with GD had a white matter microstructural pattern similar to control men, in two areas of the right SLF and the right forceps minor. In one cluster, located in the CST, women with GD had intermediate FA values to control men and women, similar to our findings. Visual inspection of the data (see Figure 2a and 2b) reveals that in most of the sexually dimorphic regions, adolescent girls with GD had numerical FA and RD values that are intermediate to those of control girls and control boys. However,

since they differed significantly from the control boys in some of the clusters, we conclude that girls with GD showed a more sex-typical pattern of white matter microstructure.

Our participants with GD differed from those tested by Rametti et al. (2011-a, 2011-b) with respect to age and with respect to their hormonal status. Age, as well as pubertal stage and sex hormones have been shown to differentially affect white matter architecture in males and females (Herting et al., 2012; Lebel & Beaulieu, 2011; Schmithorst et al., 2008). In interaction with their GD diagnosis, natal girls, in contrast to natal boys, may therefore be more susceptible to activational effects of sex hormones or other neurodevelopmental changes occurring during adolescence. As suggested by Berenbaum and Beltz (2011), puberty may be regarded as a second sensitive period during which sex hormones exert gender-specific influences on brain development. Thus, factors such as sex hormones, natal sex, and GD diagnosis may interact differently during adolescent brain maturation as compared with the adult situation (Paus, Keshavan, & Giedd, 2008; Perrin et al., 2009). This may explain why we found the expected *intermediate* pattern of fiber organization in boys with GD, but a more sex-typical pattern in our group of adolescent girls with GD receiving puberty suppressing medication. In line with this suggestion, Rametti et al. (2012) showed that 10 months of androgen treatment resulted in further masculinization of white matter fiber tracts such as the CST and the SLF in adult women with GD. Accordingly, androgen signaling and androgen receptor functioning have been shown to significantly modulate white matter (Perrin et al., 2008) as well as cortical brain maturation (Raznahan, Lee, Stidd, Long, Greenstein, Clasen, and Addington 2010) during adolescence.

In addition to the difference in subject characteristics, our predefined sexually dimorphic regions were not identical to those of Rametti et al. (2011-a, 2011-b). Whereas nearly all their predefined sexually dimorphic areas were located in the right hemisphere (only the SLF showed bilateral sex differences), such areas in our study were exclusively left-lateralized. When lowering the threshold to $p < .03$ (uncorrected), bilateral effects were observed, although the differences remained stronger within the left hemisphere. Previous studies investigating laterality effects of FA and other diffusion characteristics have reported inconsistent findings. Schmithorst et al. (2008), in a large sample of children and adolescents found predominantly right-lateralized, but also bilateral sex differences in FA. In contrast, Huster et al. (2009) found bilateral sex

differences in FA and MD within the cingulum bundle, with stronger effects in the left compared with the right hemisphere. Furthermore, numerous previous studies in adult as well as adolescent populations found widespread bilateral sex differences in FA and RD (Inano et al. 2011; Menzler et al. 2011; Herting et al. 2012). A study, combining magnetic transfer imaging and DTI, reported highly consistent leftward hemispheric asymmetry effects of FA (Kang, Herron, et al. 2011), similar to our results. The authors interpreted the systematic leftward asymmetry effects in fiber organization as being underlying to functional hemispheric specialization, such as left-sided language dominance. In addition, Kang et al. (2011) found sex differences in hemispheric lateralization of diffusion characteristics in motor areas, which may be associated with functional gender differences on complex movement and coordination tasks, as has been suggested by Muetzel et al. (2008).

Apart from the differences in location, the sex differences in diffusion values within our ROIs were less pronounced compared with those reported by Rametti et al. (2011-a, 2011-b), and in case of RD only reached a trend for statistical significance. This indicates that the gender differences in our population were more variable and may explain why most of the mean FA and RD values in GD individuals were not significantly different from either of both control groups, whereas Rametti et al. (2011-a, 2011-b) found significant effects in their groups diagnosed with GD compared with controls. Sex differences in white matter volume have been shown to gradually increase until early adulthood (Lenroot et al. 2007; Lenroot and Giedd 2010). Therefore, our participants' adolescent age may have contributed to the greater variability in sexual dimorphism in our population, resulting in smaller effect sizes.

Recently, it has been noted that Gender Dysphoria and disorders from the autistic spectrum are often comorbid (de Vries et al. 2010; Bejerot et al. 2011; Lemaire et al. 2013). Both conditions are suggested to reflect aberrant endocrine perinatal developments (Whitehouse et al. 2010; Baron-Cohen et al. 2011; Cheslack-Postava and Jordan-Young 2012). In this context, a DTI study by Beacher et al. (2012) is worth mentioning. They compared a group of adult male and female participants diagnosed with Asperger syndrome to age- and sex-matched control groups and found significant sex by diagnosis interactions in pre-defined areas of the major white matter tracts: the normative sex difference, thus higher FA values in control men compared with control women, was absent or attenuated in male and female participants diagnosed with Asperger syndrome. Thus,

in a similar fashion as our participants diagnosed with GD (who were not co-diagnosed with an autism spectrum disorder), male and female participants with Asperger showed intermediate values on their white matter diffusion parameters compared with male and female controls. The results of Beacher et al. (2012), together with the findings of the present study add evidence to the hypothesis that GD and autism spectrum conditions share certain structural brain correlates, and therefore may share some common neurodevelopmental pathway.

In conclusion, we found that boys with GD overall showed neither a typically male – nor female pattern of white matter microstructure, whereas girls with GD predominantly had sex-typical white matter diffusion characteristics with only slight masculinization in fiber organization. Our findings provide new preliminary evidence for the hypothesis that natal boys with GD may have undergone atypical neuronal sexual differentiation and possess certain neurobiological characteristics of their experienced gender.

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