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Review

The microstructure of white matter in male to female transsexuals before cross-sex hormonal treatment. A DTI study

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ABSTRACT

Background: Diffusion tensor imaging (DTI) has been shown to be sensitive in detecting white matter differences between sexes. Before cross-sex hormone treatment female to male transsexuals (FtM) differ from females but not from males in several brain fibers. The purpose of this paper is to investigate whether white matter patterns in male to female (MtF) transsexuals before commencing cross-sex hormone treatment are also more similar to those of their biological sex or whether they are more similar to those of their gender identity.
Method: DTI was performed in 18 MtF transsexuals and 19 male and 19 female controls scanned with a 3 T Trio Tim Magneton. Fractional anisotropy (FA) was performed on white matter of the whole brain, which was spatially analyzed using Tract-Based Spatial Statistics.
Results: MtF transsexuals differed from both male and female controls bilaterally in the superior longitudinal fasciculus, the right anterior cingulum, the right forceps minor, and the right corticospinal tract.
Conclusions: Our results show that the white matter microstructure pattern in untreated MtF transsexuals falls halfway between the pattern of male and female controls. The nature of these differences suggests that some fasciculi do not complete the masculinization process in MtF transsexuals during brain development.

1. Introduction

Transsexualism is an extreme form of gender identity disorder (American Psychiatric Association, 2000). Male to female (MtF) transsexuals are characterized by persistent cross-gender identification, discomfort with their assigned gender, cross-dressing and a search for hormonal and surgical sex reassignment to the desired anatomical sex to become females. The etiology of transsexualism is unknown but biological variables could play a role in its development (Cohen-Kettenis and Gooren, 1999; Gooren, 2006; Swaab, 2004).

Postmortem anatomical studies have shown that some subcortical structures are feminized in MtF transsexuals. The volume and the number of neurons of the central part of the bed nucleus of the stria terminalis (BSTc) and the third interstitial nucleus of the anterior hypothalamus (INAH3), which present sex differences in control subjects, are feminized in MtF transsexuals (Garcia-Falgueras and Swaab, 2008; Kruijver et al., 2000; Zhou et al., 1995). These studies all suggest that the feminization of the BSTc and the INAH3 in MtF transsexuals is related to neither their sexual orientation nor their cross-hormonal treatment.

Only a few structural and functional MRI studies focus on MtF transsexuals. Luders et al. (2009) found that, before cross-sex hormone administration, the regional structure of the gray matter in MtF transsexuals was more similar to the pattern found in males than in females. Nevertheless, the transsexuals did show a significantly larger volume of gray matter in the right putamen than did control males.

MRI functional studies of transsexuals analyze the brain while performing tasks, such as mental rotation, in which males and females consistently differ (Kimura, 1999). There are only three fMRI studies of mental rotation in transsexuals. Sommer et al. (2008), using a longitudinal design, found that activation during mental

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rotation did not increase during cross-sex hormone treatments, probably because of their small sample size. However, in a sample of treated and untreated MtF transsexuals and control males, it was reported that both transsexual groups had increased activation in the temporo-occipital regions and decreased activation in the left parietal lobe, suggesting *a priori* differences between control males and MtF transsexuals (Schöning et al., 2010). In addition, comparing chronically hormone treated MtF and FtM transsexuals to male and female controls, we found a parietal hypoactivation in the MtF transsexuals, but no significant differences for FtM transsexuals (Carrillo et al., 2010).

There are two additional functional neuroimaging works that addressed the cerebral patterns of MtF transsexuals. Gizewski et al. (2009) studied the cerebral activation produced by visual erotic stimuli in MtF transsexuals before treatment and found an activation pattern in MtF transsexuals similar to that observed in females. They suggested that MtF transsexuals show a tendency toward female-like cerebral processing. Moreover, Berglund et al. (2008), using positron emission tomography (PET), studied brain activation in MtF transsexuals when smelling 4, 16-androstendien-3-one (AND) and estra-1,3,5(10),16-tetraen-3-ol (EST). These steroids activate the hypothalamus in a sex-differentiated manner (Savic et al., 2001). Smelling AND and EST, MtF transsexuals showed a pattern of activation that was different from their biological sex and was situated in an intermediate position with predominantly female-like features (Berglund et al., 2008).

There are two structural MRI studies focused on white matter in transsexuals. The earliest (Emory et al., 1991) found no differences for the whole corpus callosum (CC) or the splenium region between transsexuals and controls. A more recent work, measuring CC shape, concluded that the pattern of CC shape in transsexuals is closer to that in individuals with the same gender identity than to that in individuals with the same biological sex (Yokota et al., 2005).

Diffusion Tensor Imaging (DTI) is the most suitable technique for detecting subtle changes in the white matter of patients with psychiatric disorders (Nucifora et al., 2007). DTI has been used to investigate sex differences in adults (Huster et al., 2009; Westerhausen et al., 2003) and in developmental studies (Schmithorst et al., 2008). Recently, we used DTI to investigate whether white matter patterns of female to male (FtM) transsexuals, who had not begun cross-sex hormone treatment, were more similar to that of their biological sex or to that of their gender identity (Rametti et al., in press). We found sex differences in the white matter microstructure of some brain fasciculi. Compared to control females, FtM transsexuals showed higher fractional anisotropy (FA) values in the anterior and posterior parts of the right superior longitudinal fasciculus and the forceps minor. However, in the corticospinal tract, FA values in FtM transsexuals are significantly less than in males and significantly greater than in females. Thus, we suggested that, for some fasciculi involved in higher cognitive functions, the white matter microstructure pattern in FtM transsexuals is closer to the pattern of subjects who share their gender identity (males) than to those who share their biological sex (females).

Mammalian brain sex differences, even in humans, occur in complex networks (Garcia-Falgueras et al., 2006; Segovia and Guillamon, 1993). If FtM transsexuals show a tendency to have masculinized FA values in some brain fasciculi it could be expected that the opposite would be true for MtF transsexuals. Therefore, the purpose of the present study was to test if the pattern of the white matter microstructure in MtF transsexuals shows a trend toward feminization. We measured FA, which is related to the ordered arrangement of myelinated fibers (Beaulieu, 2002), as an indicator of white matter coherence and axonal organization (Lebel et al., 2008). To the best of our knowledge there are no previous studies in the literature describing white matter microstructure in MtF transsexuals.

2. Materials and methods

2.1. Subjects

We recruited 18 untreated MtF transsexuals, 19 female and 19 male controls for this study. Two mental health professionals from the Gender Identity Unit (GIU) at the Hospital Clínic of Barcelona performed several semi-structured clinical interviews (Bobes et al., 1997; Gómez-Gil et al., 2009a). The estimated prevalence rate of transsexualism in Catalonia (Spain) is 1:21,031 males and 1:48,096 females and the sex ratio is 2.6 (Gómez-Gil et al., 2005, 2009b).

Psychological criteria for the diagnosis and treatment followed the guidelines provided by the Harry Benjamin International Gender Dysphoria Association (Meyer et al., 2002). The diagnosis of transsexualism was confirmed following the revised fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2000) and the tenth revision of the International Classification of Diseases (World Health Organization, 1993). For study inclusion, transsexual subjects needed to self-identify as an MtF transsexual, deny any history of hormonal treatment and declare their intention of undergoing cross-sex hormonal therapy. The hormonal levels of the untreated MtF transsexual group were obtained before the study (Table 1). All MtF transsexuals selected had early-onset gender nonconformity (before puberty), were erotically attracted to males, and affirmed that they aspired to sex reassignment. Sexual orientation of transsexual subjects was determined by asking what partner (a man, a woman, both or neither) the subjects would prefer or feel sexual attraction to if their body did not interfere.

The control subjects were recruited through responses to advertisements. They were free of any neurological, systemic, or psychiatric disease, as verified by a detailed interview. An inclusion criterion for all participants was to be free of psychotropic medication and/or illegal drug use. The study only included heterosexual controls. Informed consent was obtained from all participants, and

Table 1
Characteristics of the sample and group comparisons.

	MtF Transsexuals[a] ($n = 18$)	Control Females ($n = 19$)	Control Males ($n = 19$)	F	p values
Age (years)	24.71 ± 8.15[b]	33.00 ± 8.23	31.94 ± 6.11	5.51	0.007
Hormonal levels[c]					
Testosterone (ng/dl)	559.06 ± 163.91				
Free testosterone index (%)	70.50 ± 22.87				
Sex steroid binding globulin (nmol/L)	29.99 ± 9.17				
17-b-estradiol (pg/ml)	19.06 ± 16.57				

[a] Male to Female transsexuals (MtF). Results are expressed as mean ± standard deviation.
[b] At least $p < 0.01$ with respect to male and female controls.
[c] Normal testosterone levels: adult males (275–850 ng/dL) and females (10–80 ng/dL). Normal free testosterone index: adult males (38–123%) and females (1–7%). Normal levels of sex steroid binding globulin: adult males (10–60 nmol/L) and females (35–135 nmol/L). Normal estradiol levels: males (10–41 pg/mL); females: follicular phase (22–55 pg/mL), luteal phase (68–196 pg/mL).

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Table 2
Gray matter, white matter and cerebrospinal fluid volumes.

	MtF[a]		Female controls		Male controls		F	*p*
	Mean	SD	Mean	SD	Mean	SD		
Gray matter (cm^3)	675.40[c]	135.19	580.49[b]	43.98	662.48	52.40	6.66	0.003
White matter (cm^3)	591.21[c]	92.32	520.44[b]	39.65	602.20	54.01	8.73	0.001
Cerebrospinal fluid (cm^3)	305.34[c]	66.71	275.43[b]	28.03	309.43	24.08	3.44	0.04
Intracranial volume (cm^3)	1571.96[c]	283.67	1376.37[b]	82.42	1574.12	123.31	7.29	0.002

[a] MtF: Male to Female transsexuals.
[b] Differences between female and male controls are at least $p < 0.001$.
[c] Differences between MtF and female controls are at least $p < 0.008$.

the study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethical Committee of the Hospital Clínic of Barcelona.

2.2. Imaging protocol

MRI scans were performed using a 3-T magnet (SIEMENS Trio Tim Magneton, Erlangen, Germany). Diffusion tensor images were acquired with singleshot diffusion weighted echo-planar imaging (EPI) in the axial plane with diffusion sensitization gradients applied in 64 noncolinear directions with a b-value of 1000 mm^2/s (TE = 94 ms, TR = 9300 ms, flip angle 90, slice thickness = 2 mm, providing 1.97 mm in-plane resolution). The approximate scanning time for the DTI acquisition was 10 min.

In addition, we acquired a high-resolution T1-weighted magnetization prepared rapid gradient-echo (MP-RAGE) 3D MRI sequence in sagittal plane to use as a reference image for signal attenuation measurement. The echo time was 2.98 ms, the repetition time was 2300 ms, and the inversion time was 900. A set of slices covering the whole brain, including the cerebellum, was acquired with matrix size 256 × 256, field of view (FOV) = 25 × 25 cm and voxel size $1 \times 1 \times 1$ mm^3.

2.3. Tract-based spatial statistics (TBSS)

Following image acquisition, the diffusion images were transferred to a Linux-based workstation and further processed. Image data processing was performed with FSL v4.1.2 (http://www.fmrib.ox.ac.uk/fsl/) (Smith et al., 2004). First, we used SIENAX (Smith, 2002) to estimate gray matter (GM), white matter (WM), cerebrospinal fluid (CSF) and intracranial (ICV) volumes.

For the TBSS analysis, the entire image sets were visually inspected and corrected for motion and eddy currents. The next step involved extraction of the brain matter on the B0 image, using the Brain Extraction Tool (BET) available with the FSL software; a fractional intensity threshold of 0.2 was used for this step. Using the brain extracted B0 image, we then extracted the brain for the FA images. The brain extracted FA images of all participants were used as the input images for TBSS processing (Smith et al., 2006).

The initial step of TBSS analysis consisted of voxelwise nonlinear registration of all subjects' FA data into a common space using the FNIRT registration tool (Andersson et al., 2007a,b). The transformed FA images of all participants were averaged to create a mean FA image. This mean FA image was then used to create a skeleton image, which represents the centers of all the white matter tracts the groups have in common. An FA threshold of 0.2 was used to differentiate between gray and white matter. Each subject's aligned FA data were then projected onto this skeleton.

2.4. Statistical analysis

Using TBSS, voxelwise statistical analysis of individual skeleton images of the three groups was performed using a non-parametric permutation test (randomized) and a standard GLM design. We applied a two-sample *t*-test with a *p* value of <0.05 FWE, after correcting for multiple comparisons. We used the Threshold-Free Cluster Enhancement (TFCE) method to define the clusters (Smith et al., 2006). To identify the fasciculi involved in each significant cluster we used the JHU White-Matter Tractography Atlas for the MNI 152 brain. We selected the clusters that achieved statistical significance between male and females to obtain a mask for each fasciculus involved. By means of these masks we extracted the FA values of each subject. These tract FA values were analyzed using age and intracranial volume across groups as covariates. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, Illinois). For group comparisons of FA values we used ANCOVA followed by Bonferroni post-hoc contrasts. The level of significance was set at $p < 0.05$.

3. Results

As expected, male controls have greater gray and white matter as well as CSF volumes than female controls. MtF have similar global volumes to male controls and these volumes differed significantly from those of the females (Table 2).

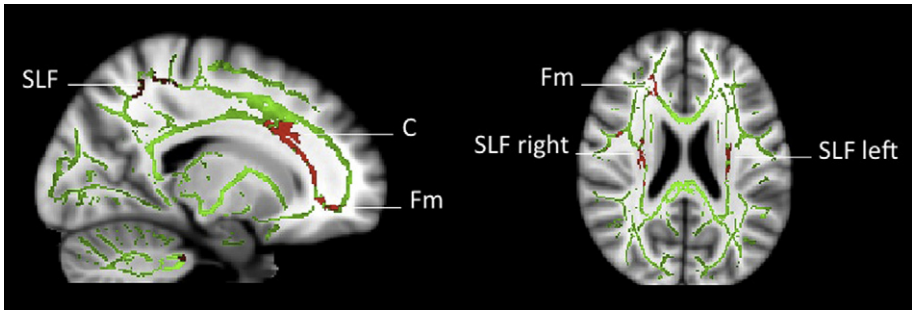

Fig. 1. Sagittal and axial maps of fractional anisotropy (FA) showing sex differences. FA is bilaterally lower in female than in male controls in the superior longitudinal fasciculus (SLF). Control females also show lower than control male FA values in the forceps minor (Fm) and the cingulum (C). The group skeleton used for the between group contrast study is green. The red color shows the clusters of significantly decreased FA in female compared to male controls. The threshold for significance was set at $p < 0.05$ corrected for multiple comparisons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

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Table 3
Coordinates of the clusters showing significant differences between male and female controls.

Locations	MNI coordinates					Cluster size
	x	y	z	t	p	
Control males > Control females						
Superior longitudinal fasciculus (right)	18	−53	50	4.6	<0.004	1227
Forceps minor (right)	18	46	−6	4.89	<0.005	1115
Inferior frontooccipital fasciculus (right)	33	9	2	5.14	<0.01	859
Superior longitudinal fasciculus (left)	−28	−8	22	4.49	<0.02	628
Corticospinal tract (right)	6	−28	−31	4.08	<0.04	466
Cingulum (right)	18	25	28	4.83	<0.04	456

Location x, y and z coordinates are based on the atlas of the Montreal Neurological Institute (MNI).

The whole TBSS analysis showed that control males have significantly higher FA values than control females in the left and the right superior longitudinal fasciculus, in the right inferior fronto-occipital fasciculus, the left cingulum, the forceps minor, and the corticospinal tract (Fig. 1 and Table 3). The contrast analysis testing for females being greater than males did not show any significant differences.

We performed ANCOVA analyses of the FA mean values for each cluster that showed sexual differences, taking as covariates age and intracranial volume. MtF transsexuals showed a constant pattern of differences for all the fasciculi. As can be seen in Fig. 2, the FA values of MtF transsexuals fall between those of male and female controls. Except for the inferior frontooccipital fasciculus (right), the FA values of MtF transsexuals for all the fasciculi were significantly different from those male and female controls (see Table 4).

4. Discussion

4.1. General discussion

From the analysis of FA, which indicates white matter coherence and axonal organization (Lebel et al., 2008), the main result of our study is that MtF transsexuals differ from both male and female controls in almost all the fascicles that showed sex differences. Interestingly the FA values of these fasciculi in MtF transsexuals fall halfway between those of the fasciculi in the male and female controls and are significantly different from either of the control FA values.

MtF transsexuals differed from male and female controls in the right and the left superior longitudinal fasciculus. The SLF connects complex cortical regions that subserve higher cognitive functions and that are sexually dimorphic. Sex differences in cognition are consistently found in spatial abilities and verbal fluency (Kimura, 1999); males outshine females in the former but the females outshine males in the latter. It has been reported that the performance of untreated MtF transsexuals in mental rotation tasks is consistent with that of their biological sex (Haraldsen et al., 2003; Slabbekoorn et al., 1999). Schöning et al. (2010) studied spatial cognition using fMRI and found that untreated and treated MtF transsexuals had increased activation in the temporo-occipital regions and decreased activation in the left parietal lobe compared to control men. We have investigated brain activation during mental rotation in chronically hormone treated MtF transsexuals. These MtF transsexuals present less activation than male controls in the superior parietal lobe (Brodman's area 7) and higher activation than females in the superior part of the gyrus frontalis (Brodman's area 9) (Carrillo et al., 2010). Interestingly, these two cerebral regions are connected by the SLF (Makris et al., 2005; Hua et al., 2009).

We found significant differences between MtF transsexuals and male and female controls in the forceps minor and the anterior region of the cingulum, both in the right hemisphere. The forceps minor connects orbitofrontal regions (Park et al., 2008) and the cingulum is an associative bundle that runs from the anterior temporal gyrus to the orbitofrontal cortex (Catani and Thiebaut de Schotten, 2008) and both form part of the emotional networks (Kober et al., 2008). There is evidence that the orbitofrontal cortex and anterior cingulate cortices are involved in reinforcement processing and the reward value of reinforcers and punishers (Cohen, 2008; Kringelbach and Rolls, 2004). Moreover, it has been suggested that the anterior cingulated cortex relates current information with an extended history of reward (Walton et al., 2007).

The FA values of the corticospinal tract in MtF transsexuals also differed from male and female controls. Studies performed in non-human primates (Lemon, 2008) have shown that this tract is

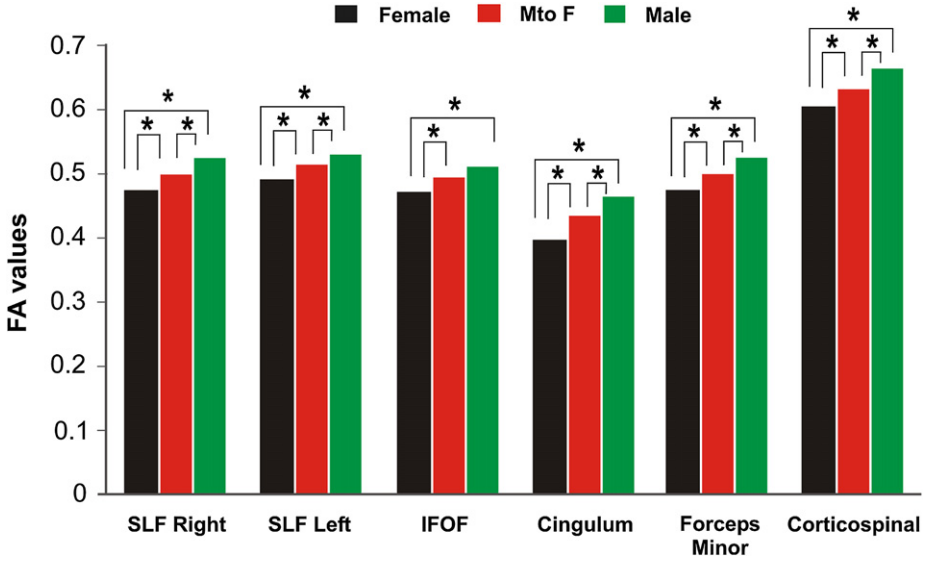

Fig. 2. Histograms showing the FA means between control females (black), male to female transsexuals (MtF) (red) and control males (green). MtF transsexuals significantly differed from female and male controls in almost of all the fascicles in which control males differed from control females. SLF: superior longitudinal fasciculus; IFOF: infero frontooccipital fasciculus. *At least $p < 0.01$. For SD see Table 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

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Table 4
Group comparisons of the fractional anisotropy in the clusters of the fasciculi presenting sex differences.

	MtF[a]		Female		Male		F	*P*
	Mean	SD	Mean	SD	Mean	SD		
Superior longitudinal fasciculus (right)	0.504[b,c]	0.022	0.478	0.019	0.526	0.023	22.21	0.001
Forceps minor (right)	0.501[b,c]	0.024	0.473	0.020	0.527	0.023	21.05	0.001
Inferior frontooccipital fasciculus (right)	0.496[b]	0.027	0.476	0.015	0.514	0.023	13.14	0.001
Superior longitudinal fasciculus (left)	0.520[b,c]	0.020	0.497	0.019	0.536	0.020	13.51	0.001
Corticospinal tract (right)	0.639[b,c]	0.031	0.611	0.029	0.668	0.035	13.43	0.001
Cingulum (right)	0.439[b,c]	0.021	0.403	0.025	0.468	0.021	33.66	0.001

At least $p < 0.01$ with respect to female (b) and males (c) controls.
[a] MtF: Male to Female transsexuals.

a descending motor pathway originated from several cortical regions (primary motor cortex, premotor cortices, supplementary motor area and cingulate motor area, primary somatosensory cortex, posterior parietal cortex and the parietal operculum). Limb movements that require a high degree of skill and flexibility are controlled by these motor fibers. Lesions of this tract affect fine sensoriomotor function of the hand (Lemon and Griffith, 2005). The maturation of the corticospinal tract depends on motor experience and genetic factors (Cheeran et al., 2009; Martin et al., 2007).

In a previous work we found that FA values of the corticospinal tract in FtM transsexuals also differed from male and female controls in the same way that we have found for the MtF transsexuals in the current work, but in the former case the FA value was higher than in control females. In contrast, the FA values of SLF and the forceps minor in the FtM differed from control females but not from control males (Rametti et al., in press).

In so far as masculinization and feminization processes, it seems that before cross-sex treatment in FtM transsexuals, the SLF and the forceps minor are masculinized, while the corticospinal tract seems to be incompletely feminized (Rametti et al., in press). However, in MtF transsexuals the SLF, the forceps minor, the cingulum and the corticospinal tract seem to present an incomplete masculinization because the FA values were halfway between those of these structures in the male and female controls and the difference with each of the latter was significant.

Considering the present work and the data available in the literature, what can we say of the brain of MtF transsexuals? Most importantly, we would suggest that MtF transsexuals do not show a simple feminization of their brain —rather, they present a complex picture in which feminization and incomplete masculinization are present depending on the brain region studied and the kind of measurements taken.

The histological measurements of the BSTc and the INH3, which are located in regions related to sexual behavior in mammals (Claro et al., 1995; De Jonge et al., 1989), are feminized in MtF transsexuals (Garcia-Falgueras and Swaab, 2008; Kruijver et al., 2000; Zhou et al., 1995) and an MRI study also showed a feminization of the putamen (Luders et al., 2009).

Focusing our attention on the white matter, MtF transsexuals show a feminization of the shape of the corpus callosum (Yokota et al., 2005). In addition, we have shown the existence of incomplete masculinization FA values in forceps minor, superior longitudinal fasciculus, cingulum and corticospinal tract.

An fMRI study of the effects of erotic stimuli on cerebral activation, shows that MtF transsexuals, prior to treatment, present a feminized activation pattern in thalamus, amygdala and orbitofrontal and insular cortices (Gizewski et al., 2009). Besides, when smelling odorous steroids, MtF transsexuals present a pattern of hypothalamic activation that differs from their biological sex (Berglund et al., 2008).

A complex picture of the brain of the MtF transsexuals emerges from the above data. Prior to their cross-sex hormonal treatment MtF transsexuals do not present a simple feminization of the brain; rather they present a mixture of feminized and incompletely masculinized structures in those regions in which male and female controls differ.

4.2. Strengths and limitations

The current study has several strengths. It is the first to study the white matter microstructure in MtF transsexuals. Second, the subjects had never received cross-sex hormone treatment. Third, the hormone assays show that the gonadal hormone levels of the MtF transsexuals reflected no endocrine pathology. Finally, for the FA analyses we used the application of automatic masks that were extracted from the significant clusters obtained in the male-female contrast. This procedure avoids the methodological problems associated with classic ROI analyses, such as the variability in the localization of the ROI in several brain structures, and the difficulty for repeatability of DTI measurements (see Brander et al., 2010).

Although we can conclude that there are *a priori* structural brain differences in untreated MtF transsexuals that seem to have occurred during brain maturation, these differences are not seen in the entire brain, but in specific regions of four fascicles. Moreover, we cannot exclude the possibility that future hormonal treatment and surgical treatments could affect brain white matter microstructure in these individuals after the treatment. To solve this question pre and post treatment studies or, at least, comparisons with cross-sex hormone treated groups are needed.

5. Conclusion

In conclusion, our results show that the white matter microstructure pattern in untreated MtF transsexuals is intermediate between male and female controls. The direction of the differences suggests that some fasciculi do not complete the masculinization process during brain development before the individual seeks treatments.

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Contributors

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Conflict of interest

None.

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