# Morphological Differences in Adolescent Female to Male Transsexuals before Cross-Hormone Treatment

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## Abstract

Early-onset gender identity disorder (EO-GID) describes a strong and persistent development of cross-gender identification. Using structural magnetic resonance imaging (sMRI) and blood samples, we studied 13 female to male patients with EO-GID and compared them to 11 biological female controls. We found that the EO-GID group in comparison to its control group showed several significant differences in regional brain volumes. These include an increase in cerebral gray matter and a decrease in volume of cerebellar white matter in the mid anterior and posterior part of the corpus callosum. Furthermore, we showed statistically significant relationships between hormone levels and regional brain volume. These include relationships between the free thyroid hormone thyroxine (T4) and volumes of the frontal lobe, the temporal lobe and cerebral white matter; between sex-hormone binding globulin (SHGB) and the frontal lobe; as well as between thyroid-stimulating hormone (TSH) and cerebral gray matter. The results of regression analyses indicate that brain volume (outcome variables) decreases with the lower thyroid hormone levels (predictor variables). We propose that abnormal hormonal development of thyroid hormones influences white matter volume in our EO-GID group. Such an abnormal development further might affect both structural and functional properties of the brain.

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# Introduction

Puberty is a developmental stage that includes vast hormonal, behavioral and morphological changes (Paus, 2005; Durston and Casey, 2006; Blakemore et al., 2010). Here, adolescents develop a preference for gender-typical activities (Serbin et al., 1993) and exclude or reject others who deviate significantly from the norm (Zucker et al., 1995). Nevertheless, not all adolescents experience their gender identity and biological sex to develop in the same direction, but some do the opposite way. This study aims to explore brain morphology of individuals with such an incongruence between biological sex and gender identity.

A strong and persistent development of cross-gender identification is called gender identity disorder (GID; Diagnostic and Statistical Manual of Mental Disorders (DSM IV-R), American Psychiatric Association, 2000) or transsexualism (International Classification of Diseases (ICD-10), World Health Organization, 1993). Many individuals diagnosed with GID report significant symptoms of psychological distress (Sánchez & Vilain, 2009) and as a result will take steps to alter features of their bodies (e.g., through the use of sex hormones and/ or plastic surgery) in order to make them more congruent with their sense of gender. GID furthermore is a rare disorder with variable prevalence rates ranging from 1:100,000 to 1:2900 in European and Asian countries (DeCuypere et al., 2007). Even though GID persists for many individuals during their lives (Zucker, 2003, 2004), its diagnosis is made only empirically and therefore lacks reliability (Asscheman, 2009). However, there is relatively little research on the etiology of this disorder.

Regarding social and psychological risk factors, it has been reported that male individuals with GID who wish to convert to a female body (called male to female transsexuals (MtFs)) are sometimes characterized by distant relationships with their fathers. Female to male transsexuals (FtMs) further have reported a higher incidence of childhood abuse (Bradley & Zucker, 1997). However, such childhood experiences are not universal across individuals diagnosed with GID. Children dressing up in clothes of the opposite sex may be rewarded by the attention they receive, but they will rarely be diagnosed with gender identity disorder (Zucker et al., 1984). Similarly, prenatal hopes and expectations of parents do not appear to influence a development of GID (Zucker et al., 1995). As a result, evidence of social risk factors possibly playing a role in the development of GID has been found to be equivocal and the

focus in recent research has been shifting towards biological variables as the underlying mechanisms causing transsexualism (Cohen-Kettenis and Gooren, 1999; Swaab, 2004; Savic et al., 2010).

The dominant theory regarding biological risk factors suggests that sexual differentiation of the brain during embryonic development correlates with the sexual differentiation of the body (Zhou et al., 1995). Their hypothesis therewith presumes that the perception of the own sex or gender identity is linked to sexual differentiation of the brain, which occurs later in embryonic development than genital sex differentiation. A discrepancy between biological sex and gender identity is therefore believed to be possible, if prenatal sex differentiation of the brain is disturbed (Swaab, 2007). It has been proposed that disturbed genetic differentiation inducing abnormal neuroendocrinological effects could be the cause for such an alteration of sex differentiation of the prenatal brain (Giedd et al., 1997; van Goozen et al., 2002; Bentz et al., 2007).

Postmortem studies comparing MtFs to controls show that MtFs display a smaller cell volume and number of neurons in the bed nucleus of the stria terminalis (BSTc), a structure that is important for male sexual behavior (Claro et al., 1995). It has been proposed that the development of this structure is very similar in MtFs to that of biological females. Furthermore, these structural differences seem not to be influenced by adult sex hormone levels or sexual orientation (Zhou et al., 1995; Kruijver et al., 2000). In addition, a recent study by Garcia-Falgueras and Swaab (2008) found a comparable ‘‘feminized’’ size of the sexually dimorphic third interstitial nucleus of the anterior hypothalamus (INAH3) in male to female transsexuals (Berglund et al., 2008). The hypothalamus is one of the most investigated brain structures with regard to sexual dimorphism and reproduction, and plays an important role in controlling endocrine, autonomic and behavioral functions (Toni et al., 2004; Card, 2009). Being a part of the hypothalamic-pituitary-gonadal axis (HPG), the hypothalamus also produces thyroid stimulating hormone (TSH), which is believed to play an important role in pubertal brain development (Tran et al., 2004). Thyroid hormones, such as free thyroxine (T4 free) are essential for normal development, growth, neural differentiation, and metabolic regulation in mammals (Williams, 2008; Cheng et al., 2010; Tata, 2012) and can lead in case of thyroid hormone deficiency during brain development to profound neurological deficits and

growth retardation (Zimmermann, 2009). Nevertheless, the structural findings of the here reviewed postmortem studies are limited by selection bias (for a review see Roulson et al., 2005), such as the inclusion of only male to female transsexuals.

With regard to functional brain activation studies, transsexuals seem to share more features with those of the experienced gender than those of their biological sex, even before treatment. These functional brain patterns were observed during functional magnetic resonance imaging (fMRI) procedures for example during pheromone exposure (Berglund et al., 2008), while participants viewed erotic film excerpts (Gizewski et al., 2009) or when participants were asked to exercise a cognitive mental rotation task (Schöning et al., 2010).

Another approach regards the possible cerebral networks involved in the own body perception of the self (for gray matter: Blanke et al., 2002; Adamovich et al., 2009; Hodzic et al., 2009; for white matter: Lewis & Carmody, 2008; Takeuchi et al., 2012). One study (Luders et al., 2009) recently addressed these issues by applying an explorative voxel-based morphological (VBM) analysis to magnetic resonance imaging (MRI) data of gray matter (GM) fractions. They found that GM volume in the putamen was more pronounced in male to female transsexuals than in male and female controls, particularly on the right side (Luders et al. 2009). While the GM in the putamen did not differ significantly between male and female controls, these results emphasize the possibility that GID could be associated with an altered anatomy in brain regions located even located outside the sex different hypothalamus structures. Whether this particular role of the putamen is reduced only to MtFs or also expands to FtMs requires further investigations.

Recently, Rametti et al. (2011) used diffusion tensor imaging (DTI) with MRI scanning to investigate whether white matter patterns of untreated FtMs were more similar to those of their biological sex or to those of their desired gender identity, compared to control. They found that biological females diagnosed as FtMs showed higher fractional anisotropy (FA) values in several white matter microstructures, indicating an increase of axon caliber myelination and/ or fiber organization in the white matter pathways involved comparable to biological males (Beaulieu, 2002; Alexander et al., 2007). These structures are located in the anterior and posterior parts of the right superior longitudinal fasciculus and the forceps minor, structures usually involved in higher cognitive functions. The forceps minor is a part of the anterior

region of the corpus callosum (CC) and connects orbitofrontal regions via the genu of the CC (Park et al., 2008). The authors further suggested that the white matter microstructure pattern in FtMs was closer to the pattern of subjects who share their gender identity (males) rather than to those who share their biological sex (females).

Structural MRI studies looking at morphological differences further investigated the corpus callosum – the largest white matter structure of the human brain. It connects cortical regions of both hemispheres due to numerous intra- and interhemispheric myelinated axonal projections. This way, the CC enables hemispheric transfer and communication between the different cortical areas, integrating sensory, motor, cognitive and emotional functions from both hemispheres (Witelson et al., 2008). Altered morphology and function of the CC has been related to the development of many psychopathologies, such as schizophrenia, autism, attention deficit hyperactivity disorder (ADHD), alien hand syndrome, bipolar affective disorder and personality disorders (for a review: van der Knaap & van der Ham, 2011). Early MRI results found no differences for the whole corpus callosum or splenum region between MtFs, FtMs and controls (Emory et al., 1991). However, over a decade later by using more sophisticated MRI measure of the CC shape, Yokota et al. (2005) were able to show that the pattern of the shape of the corpus callosum in transsexuals is closer to that of individuals with the same gender identity than to individuals of the same biological sex.

To conclude, cumulative evidence from studies using a variety of methods ranging from postmortem studies to diffusion tensor imaging suggests that specific neuroanatomical features may be associated with transsexual identity. These areas include the BSTc (for MtFs), certain gray matter fractions (for MtFs), white matter patterns of the forceps minor (FtMs) and the corpus callosum (both FtMs and MtFs). A weakness of these former studies has been that most studies have mainly focused on adult treated male to female transsexuals (Zhou et al., 1995; Berglund et al., 2008; Garcia-Falgueras & Swaab, 2008; Gizewski et al., 2009; Luders et al., 2009; Schöning et al., 2010). Also, in order to identify the underlying neural correlates of GID, it is important to investigate untreated and operationally diagnosed patients, therewith excluding subjective clinical impression classifications (Zhou et al., 1995; Garcia-Falgueras & Swaab, 2008; no information for Emory et al., 1991; Yokota et al., 2005; Kruijver et al., 2010). This is important, as selection bias, such as age and

treatment differences will significantly impact research results, especially when investigating changes in brain morphology possibly resulting from abnormal brain development. Sex specific brain development includes a rapid increase of myelinated white matter during early childhood (Sampaio & Truwit, 2001), with the process of myelination believed to be accelerated by thyroid hormones (Brent & Davies, 2012). Therefore, our neuroendocrinological study provides needed insights into the brain morphology of untreated adolescent FtM transsexuals with the help of structural magnetic resonance imaging (sMRI) and hormone levels with the help of blood sample analyses.

Our hypotheses were that 1) there will be significant morphological differences in size between the FtMs and the control group with regard to gray and white matter distribution. 2) In addition, a significant difference in size is expected also for structures located outside of the hypothalamus, namely the corpus callosum and possibly the putamen. 3) Furthermore, we hypothesize that the differences in the size of the corpus callosum cannot be explained by hormones involved in pubertal development, namely estradiol, testosterone, sex-hormone binding globulin (SHBG), TSH, and T4 free.

# Methods

## Sample

A total of twenty-four female subjects were included in this study. The sample consisted of a patient group ($n$ = 13) that was operationalized diagnosed by two clinicians with early onset gender identity disorder (EO-GID) and compared to age-matched healthy control subjects ($n$ = 11) between the ages of 12 and 20.

*EO-GID group*

The selected children and adolescents had been patients between 2006 and 2012 at the National Clinic for Transsexualism at Oslo University Hospital (OUS-Rikshospitalet), Norway, were untreated and received a diagnosis for early onset gender identity disorder (F 64.0) in conjunction with the Diagnostic and Statistical Manual of Mental Disorders (4th ed., text revision [DSM–IV–TR]; American Psychiatric Association [APA], 2000). All participants were MRI scanned after being assessed by at least two different senior psychologists or psychiatrists over a minimum period of 6 months. In line with the Harry Benjamin International Gender Dysphoria Association's Standards of Care [1990 and 1998 (Levine et al., 1998)], two independent structured interviews were used, with all patients fulfilling criteria A to D in the DSM-IV-TR from childhood on. Participation in the study was voluntary, with no incentives offered. All subjects involved already had real-life experience in the desired role (females to male) by the time of the assessment.

*Control group*

The control subjects were either high school graduates, military recruits from the armed forces, college students or employees of the University of Oslo. They were recruited by advertisement and were age matched to the patient group. As can be seen in table 1, the control group did not differ significantly in age ($F$ = .003, $p$ = .987) and showed a similar standard distribution. Furthermore, they had no lifetime diagnosis of gender identity disorder, self- or parent-reported history of neurological or psychiatric disorders, chronic illness, premature birth, learning disabilities, use of medicines known to affect nervous system functioning, or any MRI contraindications.

In addition, all participants were chromosomally and endocrinologically screened, and medication-free. No GID patient had ever received previous cross-sex

hormone treatment. Participants with any endocrinological, genetic, neurological or major psychiatric comorbidity or left-handedness were excluded from the study. All participants were Caucasians. All subjects gave written informed consent to participate in the study prior assessment and their permission to use the data for research purposes. The design was approved by the Regional Ethical Committee of South Norway. Written informed consent was obtained from all participants from 12 years of age and from the parent/guardian for participants under 18 years of age. Oral informed consent was given by participants over 12 years of age.

All participants' scans were examined by a neuroradiologist, which led to the exclusion of one additional participant, reducing the total number of participants to 24.

**Table 1.** Age distribution

| | N | Mean | Std. Deviation | Std. Error mean |
|---|---|---|---|---|
| GID | 13 | 18.43 | 1.83 | .50 |
| Control | 11 | 18.41 | 1.91 | .57 |

## Design

Using a between-subject design, all participants of the EO-GID group were matched by age and biological sex with the control group. In order to look at differences between both groups, group (EO-GID, control) was the between-subjects factor with gray and white matter volume and respectively regions of interest as the dependent measures. 21 total brain volume (TBV) corrected brain volumes for white matter (cerebral WM, cerebellum WM), gray matter (cerebral GM, cerebellum GM) and regions of interest (cerebral cortex, cerebellum cortex, thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens area, choroid plexus, brainstem, corpus callosum posterior, corpus callosum mid posterior, corpus callosum central, corpus callosum mid anterior, corpus callosum anterior) were used as the within-subjects factors. Total brain volume (TBV) was calculated based on all brain regions (cerebral cortex, cerebellum cortex, thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens area, choroid plexus, brainstem, corpus callosum posterior, corpus callosum mid posterior, corpus callosum central, corpus callosum

mid anterior, corpus callosum anterior) and ventricular (lateral ventricle, inferior lateral ventricle, CSF, ventral DC, vessel, 3rd, 4th and 5th ventricle) volumes.

**Procedure**

MRI scans of the EO-GID group were acquired between 12:30 and 19:00 within one week prior to blood sampling at the Department of Radiology at Rikshospitalet, Oslo. All participants were informed by a trained radiograph about the risks involving MRI scanning and the possibility to withdraw from the study at any given time. Furthermore, written and oral consent was given. No participant refused from participation. Blood samples were drawn by a trained nurse between 9:00 and 12:00 at the Department of Endocrinology at OUS without regard to the phase of the menstrual cycle.

**MRI acquisition and analysis**

Imaging data were collected using a 12-channel head coil on a 1.5 tesla Siemens Avanto scanner (Siemens Medical Solutions). The pulse sequences used for the morphometric analyses were two three-dimensional, T1-weighted [magnetization prepared rapid gradient echo (MP-RAGE)] scans, with the following parameters: repetition time, 2400 ms; echo time, 3.61 ms; inversion time, 1000 ms; flip angle, 8°; matrix, 192 x 192; field of view, 192. Each scan took 7 min, 42 s. Each volume consisted of 160 sagittal slices with voxel sizes of 1.25 x 1.25 x 1.20 mm. Each MP-RAGE was visually inspected, and only scans deemed to have no or minimal movement artifacts were included in analyses. The two MP-RAGEs were averaged to increase the signal-to-noise-ratio. Where there were problems achieving two high-quality scans attributable to motion artifacts, etc., only one scan was used in the analysis. This was the case for 2 EO-GID participants. All datasets were processed and analyzed into the following segmentations: volumes of the caudate, putamen, pallidum, accumbens, thalamus, hippocampus, amygdala, cerebellum cortex (also for white and grey matter), ventricles, and total cerebral cortex (also for white and grey matter). Unfortunately, different versions were used for the analyses of the groups. The EO-GID group used the recon-all command by FreeSurfer 5.1.0 (http://surfer.nmr.mgh.harvard.edu/fswiki). The datasets from the control group were processed and analyzed at the Neuroimaging Analysis Lab, Center for the Study of

Human Cognition, University of Oslo, with additional use of computing resources from the Titan High Performance Computing facilities (http://hpc.uio.no/index.php/Titan) at the University of Oslo, using an older version, FreeSurfer 4.0.5 (http://surfer.nmr.mgh.harvard.edu/fswiki).

FreeSurfer is a program using powerful tools that provide extensive and automated analysis of key features in the human brain. This includes volumetric segmentation of most macroscopically visible brain structures (Fischl et al., 2002). In order to resolve issues, such as variability within boundaries, FreeSurfer uses a Bayesian approach and decomposes the problem into formulating a more realistic image likelihood term. For the image likelihood, it uses a separate model for each structure for each point in space. This allows it to account for within-structure heterogeneity, while keeping the distributions of smaller brain regions separately. Instead of modeling all "gray matter" together, it is able to use significantly sharper and hence more informative distributions, making the segmentation problem less ambiguous.

Total brain volume (TBV) was calculated based on all brain region and ventricular volumes. Automated segmentations have been found to be statistically indistinguishable from manual labeling (Fischl et al., 2002), and correlations between FreeSurfer segmentation and manual labeling of hippocampal volume reached 0.85 in a study by Tae et al. (2008). Reproducibility errors between scan sessions have been shown to be up to 2.3% in young adults, with higher error estimates for the smallest structures (Jovicich et al., 2009). Furthermore, the segmentations were visually inspected for accuracy, and none were discarded.

**Statistical analysis**

Brain size substantially differs individually, which is bound to affect individual brain volumes both within and across age groups. This variance is, among other things, related to physical parameters such as height (Peters et al., 1998) and sex (Giedd et al., 1997; Sowell et al., 2007). To account for these differences in brain size, two types of analyses have been made. An independent t-test revealed no significant difference in TBV between both groups ($F$ = .466; $p$ = .700) and table 2 shows that the accompanying standard deviation was very similar indicating a very similar distribution of TBV. Furthermore, Q-Q-Plots were run and inspected in order to

ensure normal distribution of the different brain regions. All statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, Illinois). The level of significance for all analyses was set at $p < .05$.

**Table 2.** TBV distribution

| Group | N | Mean | Std. Deviation | Std. Error mean |
|---|---|---|---|---|
| GID | 13 | 745306.23 | 68035.096 | 18869.541 |
| Control | 11 | 755511.27 | 17619.224 | 17619.224 |

## Blood sampling

Differences in brain size can also be explained by hormonally influenced developmental processes. To reach a better insight into these underlying processes, we took blood samples of all of the patients between a week to the day prior to MRI scanning. Unfortunately, only references to the normal population, but not the control group could be used as comparisons, as no blood samples were taken for the control group. All serum samples were stored at -20 °C until measurement at the Hormone Laboratory, Centre of Endocrinology, Aker University Hospital, Oslo. Further, the serum concentrations were analyzed for estradiol (E2), free thyroxine (FT4), thyroid-stimulating hormone (TSH), sex hormone binding globulin (SHBG) and testosterone. All results were further inspected by the head of the Endocrinology Department at OUS and set into comparison with normal hormonal distributions. None of the serum concentrations were found to be outside the normal range.

# Results

## Repeated measures ANOVA

A repeated measures analysis of variance (ANOVA) with 14 TBV corrected bilateral brain volumes (cerebral WM, cerebral GM, cerebral cortex, cerebellum WM, cerebellum GM, cerebellum cortex, thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens area, choroid plexus) and two hemispheres as within-subjects factors, group (GID, control) as the between-subjects factor and both age and TBV as covariates was done in order to look at difference between both groups with regard to the different brain volumes.

No significant interaction effect was found for hemisphere x age ($F$ = .711, $p$ = .409), or hemisphere x group ($F$ = .261, $p$ = .615), but an interaction was found for hemisphere x TBV ($F$ = 957.084, $p$ = .000). Besides the significant interaction effects between TBV and the hemispheres as well as between volumes and TBV, there was no significant interaction hemisphere x volume effect ($F$ = 1.325, $p$ = .198). Thus, hemispheres as an intervening factor has been excluded in the following analyses.
There was a significant interaction effect between volumes x TBV ($F$ = 16.408, $p$ = .000) and for volume x group ($F$ = 3.650, $p$ = .000) if sphericity assumed, but not for volume x age ($F$ = 2.544, $p$ = .116). Significant interaction effects have been found for hemisphere x volumes x age ($F$ = 2.406, $p$ = .004), hemisphere x volumes x group ($F$ = 3.543, $p$ = .000), and as expected hemisphere x volumes x TBV ($F$ = 16.221, $p$ = .000).

The same analysis was run for all ventricle volumes (lateral ventricle, inferior lateral ventricle, CSF, ventral DC, vessel, 3rd, 4th and 5th ventricle), revealing no significant interaction effects of ventricles x age ($F$ = .386, $p$ = .910), ventricles x group ($F$ = .557, $p$ = .790), but for ventricle x TBV ($F$ = 3.299, $p$ = .003).

As there was no significant effect of hemisphere or of ventricle, but one of TBV, a third repeated measures ANOVA was run with 21 TBV corrected brain volumes (cerebral WM, cerebral GM, cerebral cortex, cerebellum WM, cerebellum GM, cerebellum cortex, thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens area, choroid plexus, brainstem, corpus callosum posterior, corpus callosum mid posterior, corpus callosum central, corpus callosum mid anterior, corpus callosum anterior), including brain areas that were not divided into hemispheres, but excluding ventricle volumes, with group (GID, control) as the

between-subjects factor. The correction was done by calculation of the proportional volumes of TBV, as it illustrated in table 3. As age has been shown to be evenly distributed in both groups (see table 1), and possible variations in volume can also be controlled by TBV correction, it has been excluded as a covariate in this analysis. With corrected TBV volumes, a highly significant interaction effect of volumes x group ($F = 4.268$, $p = .000$) could be observed.

**MANOVA**

To take a closer look at the areas involved in this interaction, a multivariate ANOVA (MANOVA) was run with 21 TBV corrected volumes (cerebral WM, cerebral GM, cerebral cortex, cerebellum WM, cerebellum GM, cerebellum cortex, thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens area, choroid plexus, brainstem, corpus callosum posterior, corpus callosum mid posterior, corpus callosum central, corpus callosum mid anterior, corpus callosum anterior) as dependent variables and group (EO-GID, control) as the fixed factor. This one-way MANOVA (table 3) revealed a significant multivariate main effects for group, Wilks' Lambda = .026, $F$ (19, 4) = 8.024, $p$ = .028. Thus, the variable group (i.e. being diagnosed with EO-GID or not) has a direct influence on brain volume. Taking a closer look at the volumes involved, cerebral GM ($F = 4.703$, $p = .041$), cerebellum WM ($F = 6.291$, $p = .020$), and both the posterior ($F = 5.851$, $p = .024$) and the mid anterior part of the corpus callosum ($F = 4.511$, $p = .045$) showed significant results after an adjustment for multiple comparisons with Bonferroni correction.

**Table 3.** Raw and TBV corrected brain volumes and MANOVA results

| | Raw volumes | | | | TBV corrected volumes | | | | MANOVA | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | | Std. Dev. | | Mean | | Std. Dev. | | *p* | *F* |
| | Controls (*n* = 11) | GID (*n* = 13) | Controls (*n* = 11) | GID (*n* = 13) | Controls (*n* = 11) | GID (*n* = 13) | Controls (*n* = 11) | GID (*n* = 13) | | |
| Cerebral WM | 448351 | 415735 | 48387 | 54972 | .59295 | .55794 | .03666 | .05240 | .076 | 3.463 |
| Cerebral GM | 92403 | 122946 | 29776 | 44971 | .12192 | .16489 | .03466 | .05733 | **.041*** | **4.703** |
| Cerebral Cortex | 540755 | 538682 | 53044 | 48874 | .71488 | .72284 | .01653 | .01163 | .181 | 1.905 |
| Cerebellum WM | 29537 | 26819 | 2822 | 2905 | .03920 | .03601 | .00378 | .00241 | **.020*** | **6.291** |
| Cerebellum GM | 85644 | 83013 | 5382 | 9319 | .11377 | .11139 | .00903 | .00748 | .486 | .501 |
| Cerebellum Cortex | 115181 | 109832 | 7101 | 11297 | .15298 | .14739 | .01144 | .00762 | .168 | 2.301 |
| Thalamus | 13663 | 13489 | 1059 | 1546 | .01809 | .01810 | .00062 | .00134 | .981 | .001 |
| Caudate | 7480 | 7439 | 587 | 823 | .00994 | .01001 | .00084 | .00107 | .845 | .039 |
| Putamen | 11459 | 11591 | 956 | 1100 | .01519 | .01560 | .00107 | .00142 | .438 | .624 |
| Pallidum | 3517 | 3431 | 356 | 380 | .00466 | .00461 | .00029 | .00041 | .779 | .080 |
| Brainstem | 19983 | 19826 | 1270 | 1878 | .02652 | .02663 | .00168 | .00138 | .862 | .031 |
| Hippocampus | 8662 | 8395 | 477 | 505 | .01151 | .01131 | .00091 | .00073 | .550 | .369 |
| Amygdala | 3146 | 3208 | 256 | 303 | .00417 | .00342 | .00034 | .00039 | .351 | .907 |
| Accumbens area | 1411 | 1372 | 162 | 195 | .00187 | .00186 | .00019 | .00031 | .892 | .019 |
| Choroidplexus | 3211 | 2941 | 720 | 857 | .00426 | .00329 | .00099 | .00093 | .394 | .754 |
| Optic chiasm | 258 | 275 | 46 | 52 | .00034 | .00037 | .00007 | .00005 | .351 | .909 |
| CC posterior | 1002 | 826 | 161 | 142 | .00133 | .00111 | .00024 | .00019 | **.024*** | **5.851** |
| CC mid posterior | 487 | 421 | 135 | 98 | .00065 | .00057 | .00019. | .00013 | .235 | 1.490 |
| CC central | 535 | 435 | 153 | 69 | .00071 | .00059 | .00021 | .00011 | .082 | 3.324 |
| CC mid anterior | 519 | 413 | 150 | 72 | .00069 | .00055 | .00021 | .00009 | **.045*** | **4.511** |
| CC anterior | 920 | 838 | 111 | 150 | .00122 | .00113 | .00013 | .00019 | .192 | 1.816 |

*WM = white matter, GM = gray matter, CC = corpus callosum*

## Correlation analyses

*Correlation analysis within blood sample results*

A bivariate two-tailed correlation was run with SHBG, estradiol, testosterone, progesterone, TSH and T4 free for the patient group only. No significant correlation between TBV and the different hormonal levels were found (see table 4). However, an expected significant positive correlation between estradiol and testosterone ($r$ = .629, $R^2$ = .396, $p$ < .05), indicating that estradiol shares 39.6 % of variability with testosterone. Progesterone and SHBG were significantly positively correlated and shared 49.7 % ($r$ = .705, $R^2$ = .497, $p$ < .05), whereas progesterone and testosterone showed a significant negative correlation and share 55.6 % ($r$ = -.746, $R^2$ = .556, $p$ < .05) of variability. Testosterone was negatively correlated and shared 34.8 % ($r$ = -.590, $R^2$ = .348, $p$ < .05) of variability with TSH. All of the significant correlations therefore were either connected to testosterone or to progesterone.

**Table 4.** Results from the correlation analysis within blood samples

| | SHBG | | Estradiol | | Testosterone | | TSH | | T4 free | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *r* | $R^2$ | *r* | $R^2$ | *r* | $R^2$ | *r* | $R^2$ | *r* | $R^2$ |
| SHBG | 1 | 1 | -.022 | .000 | -.179 | .032 | -.141 | .019 | .275 | .076 |
| Estradiol | -.022 | .000 | 1 | 1 | **.629*** | **.396** | -.331 | .109 | .180 | .032 |
| Testosterone | -.179 | .032 | **.629*** | **.396** | 1 | 1 | **-.590*** | **.348** | .016 | .000 |
| TSH | -.141 | .019 | -.331 | .109 | **-.590*** | **.348** | 1 | 1 | -.392 | .154 |
| T4 free | .275 | .076 | .180 | .032 | .016 | .000 | -.392 | .154 | 1 | 1 |

*SHBG = sex-hormone binding globulin, TSH = thyroid-stimulating hormone, T4 free = free thyroxine*

*Correlation analyses between blood sample results and brain volumes*

Three bivariate correlation analyses between the different lobes of the brain (frontal lobe, parietal lobe, temporal lobe, occipital lobe), total brain volume (TBV), the cerebral cortex (WM, GM, all), the cerebellum (WM, GM, all) or respectively CC posterior, mid posterior, central, mid anterior and anterior with the blood sample results (SHBG, estradiol, testosterone, progesterone, TSH and T4 free) were run. T4

free was significantly correlated to both the frontal lobe ($r$ = .604, $p$ = .029), explaining 36.5 % of variance, and the temporal lobe ($r$ = .569, $p$ = .043), explaining 32.4 % of variance. Furthermore, the frontal lobe showed a significant correlation with sex hormone binding globulin (SHBG) ($r$ = .658, $p$ = .015), explaining 43.3 % of variance (see table 5). In addition, both a significant positive correlation between TSH and the cerebral gray matter ($r$ = .642, $R^2$ = .556, $p$ = .018), explaining 41.2 % of their shared variance and a significant positive correlation between T4 free and the cerebral white matter ($r$ = .598, $R^2$ = .556, $p$ = .031), explaining 35.8 % of their shared variance, could be observed. These correlations though are reduced to the white matter-gray matter structure only and do not show an effect on the main volume of the structure itself ($r$ = .225, $R^2$ = .051; respectively $r$ = .254, $R^2$ = .064; $p$ > .05; see table 6). Furthermore, no significant correlation was found between the blood sample results and the different volumes of the corpus callosum (see table 8).

**Regression analyses**

*Regression analyses between blood sample results and brain volumes*

Several linear regression analyses were done in order to further investigate the nature of the significant correlations shown in tables 5 and 6, with the different brain regions (frontal lobe, temporal lobe, cerebral white and gray matter) as dependent or outcome variables and hormone levels (T4 free, TSH and SHBG) as independent or predictor variables. All brain volumes showed positive statistically significant hormone relationships (all $p$ < .05; see table 7). The $F$-ratios of our data for the frontal lobe and T4 free was $F$ = 6.304 with $R^2$ = .364, indicating that T4 free can explain 36.4 % of variance in volume of the frontal lobe. For the frontal lobe and SHBG the analysis showed $F$ = 8.378 with $R^2$ = .432, indicating that SHBG can explain 43.2 % of variance in volume of the frontal lobe. The temporal lobe and T4 free showed $F$ = 5.258 with $R^2$ = .323, indicating that T4 free can explain 32.3 % of variance in volume of the temporal lobe. Furthermore, cerebral white matter and T4 free showed $F$ = 6.136 with $R^2$ = .358, indicating that T4 free can explain 35.8 % of variance in volume of the cerebral white matter. TSH and cerebral gray matter showed $F$ = 7.714 with $R^2$ = .412, indicating that TSH free can explain 41.2 % of variance in volume of the cerebral gray matter. Furthermore, as all $\beta$-values showed significant results (all $p$ < .05; see table 7), the different hormone levels (predictor variables) significantly

predict the different volumes of the brain regions (outcome variables), which means that brain volume increases with the different hormone levels.

**Table 5.** Results from the correlation analyses between blood samples and volumes of the different lobes (frontal, parietal, temporal and occipital lobe)

| | TBV | | | Frontal Lobe | | | Parietal Lobe | | | Temporal Lobe | | | Occipital Lobe | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* |
| SHBG | .411 | .169 | .163 | **.658*** | **.433** | **.015** | .513 | .263 | .073 | .208 | .043 | .494 | .087 | .007 | .778 |
| Estradiol | -.072 | .005 | .814 | .210 | .044 | .491 | .122 | .015 | .691 | -.131 | .017 | .670 | -.252 | .063 | .406 |
| Testosterone | -.408 | .166 | .166 | -.043 | .002 | .889 | .013 | .000 | .966 | -.123 | .015 | .688 | -.058 | .003 | .850 |
| TSH | .164 | .027 | .592 | -.382 | .146 | .198 | -.396 | .157 | .181 | -.362 | .131 | .224 | .106 | .011 | .730 |
| T4 free | .336 | .113 | .262 | **.604*** | **.365** | **.029** | .449 | .202 | .123 | **.569*** | **.324** | **.043** | .139 | .019 | .650 |

*TBV = total brain volume, SHBG = sex-hormone binding globulin, TSH = thyroid-stimulating hormone, T4 free = free thyroxine*

**Table 6a.** Results from the correlation analyses between blood samples and brain volumes of the cerebral white and gray matter, as well as the cerebellar white and gray matter

| | Cerebral White Matter | | | Cerebral Gray Matter | | | Cerebral Cortex | | |
|---|---|---|---|---|---|---|---|---|---|
| | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* |
| SHBG | .144 | .021 | .640 | .275 | .076 | .363 | .414 | .013 | .159 |
| Estradiol | -.026 | .001 | .934 | -.019 | .000 | .949 | -.047 | .002 | .879 |
| Testosterone | -.152 | .023 | .619 | -.252 | .063 | .406 | -.403 | .162 | .172 |
| TSH | -.325 | .106 | .278 | **.642*** | **.412** | **.018** | .225 | .051 | .460 |
| T4 free | **.598*** | **.358** | **.031** | -.456 | .208 | .117 | .254 | .064 | .403 |

*SHBG = sex-hormone binding globulin, TSH = thyroid-stimulating hormone, T4 free = free thyroxine*

**Table 6b.** Results from the correlation analyses between blood samples and brain volumes of the cerebral white and gray matter, as well as the cerebellar white and gray matter

| | Cerebellum White Matter | | | Cerebellum Gray Matter | | | Cerebellum Cortex | | |
|---|---|---|---|---|---|---|---|---|---|
| | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* |
| SHBG | .225 | .051 | .460 | .081 | .006 | .793 | .124 | .015 | .685 |
| Estradiol | -.261 | .068 | .390 | -.106 | .011 | .730 | -.155 | .024 | .614 |
| Testosterone | -.148 | .022 | .629 | -.398 | .158 | .178 | -.366 | .134 | .218 |
| TSH | -.255 | .065 | .401 | .177 | .031 | .562 | .081 | .006 | .793 |
| T4 free | .401 | .161 | .174 | .252 | .016 | .407 | .311 | .097 | .302 |

*SHBG = sex-hormone binding globulin, TSH = thyroid-stimulating hormone, T4 free = free thyroxine*

**Table 7.** Results from the regression analyses between the frontal lobe and T4, respective SHBG; the temporal lobe and TSH; the cerebral white matter and T4; and the cerebral gray matter and TSH

| Variables<br>outcome variables | predictors | *β* | *F* | *p* | $R^2$ |
|---|---|---|---|---|---|
| T4 free | Frontal Lobe | .604 | 6.304 | **.029*** | .364 |
| SHBG | Frontal Lobe | .658 | 8.378 | **.015*** | .432 |
| T4 free | Temporal Lobe | .569 | 5.258 | **.043*** | .323 |
| T4 free | Cerebral White Matter | .598 | 6.136 | **.031*** | .358 |
| TSH | Cerebral Gray Matter | .642 | 7.714 | **.018*** | .412 |

*SHBG = sex-hormone binding globulin, TSH = thyroid-stimulating hormone, T4 free = free thyroxine*

**Table 8.** Results from the correlation analyses between blood samples and the segmented corpus callosum volumes

| | CC posterior | | | CC mid posterior | | | CC central | | | CC mid anterior | | | CC anterior | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* | *r* | $R^2$ | *p* |
| SHBG | .123 | .015 | .689 | -.096 | .009 | .754 | -.215 | .046 | .481 | .024 | .000 | .938 | .040 | .002 | .896 |
| Estradiol | -.118 | .014 | .701 | -.129 | .017 | .675 | -.288 | .083 | .340 | -.386 | .149 | .192 | -.281 | .079 | .352 |
| Testosterone | -.211 | .044 | .488 | -.180 | .032 | .556 | -.227 | .051 | .456 | -.452 | .204 | .121 | -.127 | .016 | .678 |
| TSH | -.083 | .007 | .789 | -.203 | .041 | .507 | -.125 | .016 | .683 | -.091 | .008 | .767 | -.346 | .119 | .246 |
| T4 free | .510 | .260 | .075 | .414 | .171 | .159 | .082 | .007 | .790 | .306 | .094 | .309 | .467 | .218 | .108 |

*CC = corpus callosum, SHBG = sex-hormone binding globulin, TSH = thyroid-stimulating hormone, T4 free = free thyroxine*

## Discussion

Our study aimed to investigate morphological brain differences in a sample of young female to male early onset transsexuals before cross-sex treatment, using both structural MRI and blood samples. The results from this study show significant differences with regard to gray and white matter distribution within certain brain regions. Specifically, compared to the control group, the gray matter of the cerebral cortex was significantly bigger in the EO-GID group whereas the cerebellum white matter was significantly smaller in size. Therewith, our first hypothesis was met that there will be significant morphological differences in size between the FtMs and the control group with regard to gray and white matter distribution. This is especially interesting as gray and white matter distribution has been described to be different for transsexuals, reflecting more the distribution of the gender identity than the biological sex (Yokota et al., 2005; Luders et al., 2009; Rametti et al., 2011). These differences though have mainly focused on distribution patterns, but not on the percentage of white and gray matter within specific brain regions.

**White and gray matter**

Structural neuroimaging studies were able to show that white matter volume (Paus et al., 2001) and white matter integrity (Asato et al., 2010) increase with development (Paus, 2010; Schmithorst & Yuan, 2010), with active myelination persisting well into early adulthood (Yakovlev et al., 1967; Huttenlocher, 1990). In line with known sex differences in puberty-onset (Delemarre-van de Waal, 2002), white matter growth increases with age slightly in girls and steeply in boys during adolescence (De Bellis et al., 2001; Lenroot et al., 2007; Perrin et al., 2009), with total cerebral volume peaking at 10.5 years in females (Lenroot et al., 2007). Therefore, it can be assumed that our EO-GID group with the youngest patient being 12 years old already reached their maximum cerebral volume. This could suggest that the found significant differences in volume in our group do not underlie variations of normal brain development simply not having reached its peak of volume yet, but rather the disorder itself. Furthermore, normal male white matter development has been shown to not only increase more rapidly across all lobes during adolescence, but also to continue longer (Lenroot et al., 2007). Nevertheless, our FtM group did not show increased white matter volume, but the opposite with a significant decrease of cerebral white matter volume. It is therefore suggested, that the white matter volume found in our FtM patient group does not resemble the one of typical male development. Nevertheless, as our study did not contain a male control group, only a suggestion and not a definite conclusion can be drawn here. The cause of these known sex-specific differences in white matter development has been suggested to be increasing pubertal hormones influencing the organization of white matter pathways between (or within) the frontal and temporal cortices (Herting et al., 2011). Furthermore, this rapid brain development happening during puberty coincides with maturation of cognitive processes and behavior. Nevertheless, with regard to the implication of the found white matter differences, one has to take into account that changes in white matter bundles do not occur independently of the connecting gray matter changes.

With regard to the development of its distribution, a global increase of cortical and subcortical gray matter volume can be noted during childhood. This development reaches its peak around the onset of puberty, though with girls reaching maximal gray matter volume in frontal and parietal brain areas at the age of 8.5 and therewith 1–2

years before boys (Lenroot et al., 2007). This difference in time of onset parallels known sex differences in puberty-onset (Delemarre-van de Waal, 2002) and therewith underlines the influence of pubertal hormones on sex-specific brain development. As a reaction to this increase, gray matter volume gradually decreases again during adolescence and early adulthood (Giedd & Rapoport, 2010; Gogtay & Thompson, 2010). With the youngest patient of our study being 12 years old, it can be expected that our EO-GID group has already reached its maximum gray matter volume and is now in the process of decreasing it. Interestingly, our results show rather the opposite, with cerebral gray matter volume being significantly bigger than in the control group. This might indicate an abnormal cerebral gray matter development in our EO-GID group. As this decrease in gray matter (called synaptic pruning) is thought to reduce the overall number of synapses and neurons in order to create more efficient synaptic configurations (Paolicelli et al., 2011), a disruption of pruning is connected to several psychopathologies such as ADHD (for a review see Duerden et al., 2012) and schizophrenia (for a review Matheson et al., 2011). Interestingly, only cerebral gray matter showed significantly higher volumes in the EO-GID group, but no other gray matter structures did, such as for example the brainstem, the putamen or cerebellar gray matter. These changes in gray matter volume therefore might just be a by-product of the abnormal white matter development, as high gray matter volumes are directly connected with reduced white matter volumes. Supporting this theory, further white matter structures such as the corpus callosum and cerebellar white matter has been showing significantly lower volumes in the EO-GID group.

Furthermore, during puberty and adolescence, pruning typically takes place in the prefrontal, parietal, and temporal cortices (Giedd et al., 1999; Sowell et al., 2002; Bramen et al., 2011) and was found to be related to increased levels of estradiol in girls and to increased levels of testosterone in boys (Peper et al., 2011). Even though neither the estradiol nor the testosterone levels were outside the normal range for the EO-GID group, both white and gray matter of the cerebral cortex were positively correlated with hormone levels (specifically free thyroxine (T4 free) for white matter and thyroid stimulating hormone (TSH) for gray matter; as shown in table 5). This correlation analysis was further followed up by simple regression analyses, explaining how the cerebral white and gray matter are related to the levels of T4 free respective TSH. In this case, cerebral white matter and T4 free showed $F = 6.136$ with $R^2 = .358$,

indicating that T4 free can explain 35.8 % of variance in volume of the cerebral white matter and TSH and cerebral gray matter showed $F = 7.714$ with $R^2 = .412$, indicating that TSH free can explain 41.2 % of variance in volume of the cerebral gray matter. Its results show a positive statistically significant relationship between cerebral white matter and T4 and between cerebral gray matter and TSH respectively. These results imply that higher thyroid hormone levels predict an increase in cerebral volume, or in this case lower thyroid hormone levels predict reduced cerebral volume. Furthermore, no correlation of the cerebral cortex to either testosterone or estradiol was found, despite of what could have been expected by cumulative evidence (as for example by the results of Peper et al., 2011).

Therefore, this regressive relationship may be a first documented indication of an abnormal hormonal development in EO-GID influencing the development of certain brain areas. In general, abnormal brain development has been thought to play a role in the development of several neuropsychiatric disorders with their onset during childhood and puberty such as (but not limited to) depression, anxiety disorders, schizophrenia, and eating disorders (Kessler et al., 2005; Paus et al., 2008). Furthermore, these disorders often display a sex-specific prevalence or course of the illness (Westberg & Eriksson, 2008; Martel et al., 2009), leaving room for speculation whether the dominant sex-aberrant development of gender identity in EO-GID is based on early abnormal brain development.

When taking a closer look at the within hormones correlation (table 4), it can be noted that TSH was also negatively correlated to testosterone, explaining 34.8 % of variance. This correlation indicates that the lower the testosterone levels are, the higher are the TSH levels, and the higher the TSH levels are, the bigger the cerebral cortex will be. Furthermore, it can be noted that the two neurohormones that were positively correlated with the white and gray matter cortex are thyroid hormones. Released by the anterior pituitary gland in the brain, the thyroid stimulating hormone (TSH) regulates the production of the hormone free thyroxine (T4). As cells of the developing brain are targeted heavily by T4, thyroid hormones play a particularly crucial role in brain maturation during fetal development (Kester et al., 2004). This has been underlined by the identification of a transport protein (OATP1C1), which is necessary for transporting T4 across the blood-brain barrier, allowing it to act directly on brain development. Therefore, thyroid function plays an important role in the

development and retention of cognitive function (Begin et al., 2008). Due to their important role in promoting neural differentiation, thyroid hormones, such as T4 free and TSH, have been shown to not only influence early but also adult brain development (Flamant & Samarut, 1998; Liu et al., 2002). Thyroid dysfunction especially is associated with disruptions in cognitive function in older age, such as in dementia and more specifically Alzheimer disease (Smith et al., 2002; Liesbeth et al., 2004). This significant relationship of both TSH and T4 to cortical gray respectively white matter (see tables 6 and 7) may lead to speculation about the role thyroid hormones might play in the development of EO-GID.

Furthermore, a significant positive relationship between the frontal respective temporal lobe and T4 free was found (see table 7). As indicated by the reduced volume of cerebral white matter and its also positive relationship to T4 free, it can be speculated that these results suggest that lower volumes of the blood brain barrier passing thyroid hormone T4 predict lower volumes of the temporal and frontal lobe. Such a possible effect on brain volume leaves room for speculation with regard to the functional implications involved. The frontal lobe plays a pivotal role in executive cognitive functioning and behavior. It as well mediates basic neurologic functions, speech and language abilities, motivational behaviors and social competency (Cummings & Miller, 2007). In addition, the temporal lobe has been associated with a number of functions. These include auditory and visual processing/discrimination (Pinto Hamuy et al., 1957; Humphries et al., 2001), learning and memory (Schiltz et al., 1973), social cognition (Olson et al., 2007), social and sexual behavior (Miller et al., 1995), language processing (Hermann & Wyler, 1988), face recognition (Kazui et al., 1995), and olfactory and gustatory perception (Schellinger et al., 1983). Consequently, possible abnormal hormonal development influencing the development of certain brain areas showing a positive statistically significant relationship with both the frontal and temporal lobe may interfere with a variety of their functions, therefore leading to possible impairments of cognitive functioning and behavioral disturbances. The maturation of the prefrontal cortex and (medial) temporal lobes as well as their connecting fibers have been implicated in the development of typical adolescent behaviors (Blakemore, 2008; Berns et al., 2009; Olson et al., 2009; Van Leijenhorst et al., 2010), which indicates that abnormal development might lead to aberrant behavior developed during adolescence. Such abnormal behavior may be reflected in a reduced

preference for gender-typical activities (as it can be seen in normal development, Serbin et al., 1993), but rather identification with gender-typical activities of the opposite sex, as it can be found in GID.

In addition, the frontal lobe was also significantly correlated to the sex-hormone binding globulin (SHBG), a glycoprotein that binds to the sex hormones androgen and estrogen, inhibiting the function of these hormones (Brand et al., 2011). As pubertal hormones are thought to influence the organization of white matter pathways between or within the frontal and temporal cortices (Herting et al., 2011), an inhibition of these hormones and therefore also the processes they induce, could produce abnormal white matter development in this structure. The frontal cortex is further connected with several brain regions, such as the cerebellum (Narender, 2012) that also showed statistically significant reduced white matter volume in the EO-GID group. Furthermore, interhemispheric communication of the frontal lobe is enabled by the corpus callosum (see figure 3; for a review: van der Knaap & van der Ham, 2011), another structure with statistically significant reduced volume (mid anterior and posterior corpus callosum) in the EO-GID group. The anterior part of this structure is further located directly in the middle of the frontal lobe (see figure 1, respective 2 for the topographical segmentation). In addition, as it can be seen in figure 1 (respectively 2 for the topographical segmentation), the corpus callosum's posterior part is closely situated to the cerebellum, whose white matter was significantly reduced as well. This topographic proximity might indicate a pattern of an abnormal hormonal development in patients with EO-GID, which in turn could result in abnormal brain development of white matter structures.

Even though caution is advisable, regression analysis can infer causal relationships between dependent and independent variables (Armstrong, 2011). All $\beta$-values of the regression analyses showed significant results ($p > .05$; see table 7), the different hormones (TSH, T4 free and SHBG respectively) significantly predicted the size of the different brain regions (frontal lobe, temporal lobe, cerebral white and respectively gray matter). Furthermore, all $p$ values were highly significant ranging from $p = .043$ (for T4 free and the temporal lobe) to $p = .015$ (for SHBG and the frontal lobe). The strength of these findings are underlined by the small group size ($n = 13$) despite its moderate statistical power (all $\beta < .80$), producing large effect sizes with all $r > .50$ (see table 7).

Nevertheless, other hormonal or developmental processes might also be in play. Therefore, these correlations might only indicate some of the underlying hormonal processes involved. More studies need to take a closer look at different brain development in dependency to thyroid hormones in patients diagnosed with early onset GID contrasted with healthy age-matched controls. Unfortunately, in this study, white and gray matter percentage was limited to two regions (the cerebral cortex and the cerebellum) and a follow-up study is strongly suggested covering different brain regions as well. It might also be interesting to compare these results to age matched biological males to further confirm whether this different distribution is similar to the ones of their preferred gender.

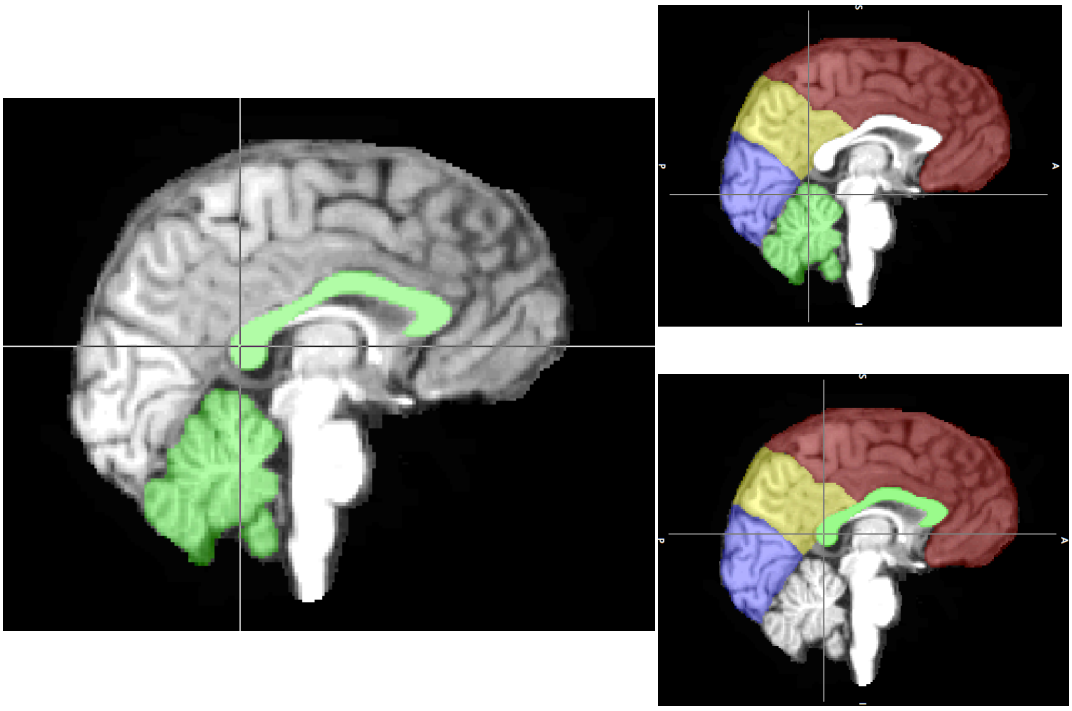

***Figure 1***. *Corpus callosum, cerebellum and the frontal, occipital and parietal lobe*
*T1 weighted MRI picture mid-sagittal view of a EO-GID patient (age 14) after visually inspected brain segmentation with FreeSurfer. The regions corpus callosum (picture on the left, lower picture on the right) and the cerebellum (picture on the left, upper picture on the right) are highlighted in green. Furthermore, the frontal lobe is highlighted in red, the parietal lobe in yellow and the occipital lobe in blue. Crosshair in the picture on the left and the lower right located on the posterior corpus callosum (CC), Talairach coordinates (127/125/75). Crosshair coordinates on the upper right picture located on the superior cerebellum, Talairach coordinates (127/104/81). Coordinates were taken from FSLView and are shown in (X/Y/Z).*

**Brain regions**

With regard to structures located outside the hypothalamus, both the posterior and the mid anterior part of the corpus callosum as well as the cerebellar white matter were significantly reduced in volume in the EO-GID group compared to the control group. Therewith also our second hypothesis was met, even though the putamen showed no difference in size. In addition, none of these volumar differences could be explained by age, but only by group and were further not correlated with any hormone levels, supporting also our third hypothesis (see tables 5 and 6). This may indicate that these group differences are rather related to the disorder itself than to other normal developmental processes. This hypothesis is supported by the results' independence of age and survival of significance despite of the small sample size and the very conservative and restricting Bonferroni correction.

*The cerebellum*

One of the structures showing significant group differences for the EO-GID group is the cerebellum, the often forgotten "little brain" at the back of the cerebrum. Even though known for its role in motor function, accumulating evidence from both human lesion (for example the research on cerebellar cognitive affective syndrome (CCAS) by Schmahmann et al., 2007) and animal studies (for example by Ramnani et al., 2006) suggest it playing a significant role in cognitive functions as well. This broad spectrum of functions is enabled by its wide connections to different brain structures, receiving and/or sending projections to all major divisions of the central nervous system (Anand et al., 1959; Harper & Heath, 1973; Heath & Harper, 1974; Dietrichs et al., 1994; Schmahmann, 1996; Clower et al., 2001; Middleton & Strick, 2001; Allen et al., 2005). While most of the cerebellum's output neurons act to excite their downstream targets, its Purkinje neurons inhibit them. In order to explain these seemingly contrasting actions, Eccles and his colleagues proposed that activity within the cerebellar nuclei is relatively unshaped and theoretically multi-dimensional until it is given form by the inhibitory 'sculpting' of the cortical input (Eccles et al., 1967). This mechanism also seems to influence behavior, as lesions of the lateral cerebellum showed impairments of cognitive functions (Schmahmann, 2007). As our patient group showed reduced white matter volume, it in turn indicates an increase of gray matter volume. In line with our previous suggestions, we propose that such a difference in gray and white matter distribution as displayed in the EO-GID group

may indicate insufficient pruning in the areas showing significantly reduced white matter volume. Such possible inssufficient pruning processes could influence brain development and therewith possibly impacting functions of the brain regions involved. Interestingly, the cerebellum's inhibiting Purkinje neurons are situated in its cortex' intermediate layer, representing one of two layers of the three-layered cerebellar cortex that consists of gray matter (Kandel et al., 2000). Whether an increase of gray matter includes that more Purkinje neurons can be found in the cerebellar gray matter of EO-GID patients and if these therefore inhibit and sculpt cerebellar functions more than the participants in the control group, can only be established with further analyses that require postmortem studies. Nevertheless, it can be speculated that these differences in white matter volume may have an impact on other structures as well, due to the cerebellum's many trajectories. Such a possible impact may further be influenced by the inhibiting Purkinje cells, which due to their role in sculpting cortical input, could further impact signal communication. With regard to possible functional implications, it is proposed that a reduction of Purkinje neurons will release the deep nuclei from inhibition, leading to aberrant activity along the cerebellum–thalamus–cerebral cortex circuit. Furthermore, such activity has been proposed to lead to an abnormal strengthening of anatomic connections between the cerebellum and other brain regions, which in turn would create deviant functional connectivity patterns with the cerebral cortex (Cao et al., 2010). In addition, such abnormal connectivity is thought to contribute to a variety of functional and anatomic abnormalities, including variable functional topography in the cerebral cortex (Evangeloo et al., 2000; Hutchinson et al., 2008), excessive growth of cerebro-cortical areas (Manson et al., 2006; Bashir et al., 2011) and overexcitation of thalamocortical projections (Walterfang et al., 2009; Cao et al., 2010), altering information processing. Consequently, an increase in Purkinje neurons might overly inhibit the deep nuclei, therewith altering the cerebellum–thalamus–cerebral cortex circuit as well. In contrast to excessive growth of cerebro-cortical areas or overexcitation of thalamocortical projections, it might rather hinder cerebro-cortical growth and possibly underexcite thalamocortical projections. Furthermore, as the thalamus is believed to act as a relay between several subcortical areas and the cerebral cortex (e.g. Llinás et al., 1998; Edelman & Tononi, 2000), a disturbance of this circuit could have altering functions on the cerebral cortex as well. Interestingly, an alteration of

cerebral white matter in our patient group could also be found (see table 3). The thalamus is further believed to play an important role in consciousness of the self (Zelazo, 2004), which has been directly connected to its connection with the cerebral cortex (e.g. Romijn, 2002; Crick & Koch, 2003). Northoff et al. (2006) further assume self-referential processing to be at the core of what is called the self. Self-referential processing accounts for distinguishing stimuli related to one's own self from those that are not relevant to one's own concerns. A disturbance of the cerebellum–thalamus–cerebral cortex circuit therefore might come with alterations of self-referential, possibly including a disturbed perception of the own sex, as it can be found in our patient group.

Nevertheless, alterations of function due to possible abnormal cerebellar development do not have to be limited to the cerebellum–thalamus–cerebral cortex circuit, but could possibly also apply to other projections, therewith sculpting behavior in an even broader way. One of these projections might be connected to interhemispheric transfer of information with the cerebellum transferring visual information on one side of the cerebral hemisphere and motor performance on the contralateral side (as demonstrated in animals: Glickstein & Sperry, 1960; Glickstein et al., 1998; Glickstein, 2009; and in humans: Gazzaniga, 2000, 2005). This role of the cerebellum in interhemispheric communication is very similar to the one of the corpus callosum, the brain's largest white matter structure, which was also significantly decreased in volume (see table 3). A reduction in size of both the cerebellar white matter and corpus callosum possibly developed by inefficient pruning may reduce signal communication between several brain regions enabled through the cerebellum's many functional projections.

*The corpus callosum*

Our study is not the first one to find differences in size of the corpus callosum in transsexuals. This area has been researched with the help of different methods, ranging from diffusion tensor imaging (Rametti et al., 2011), detecting differences in white matter microstructures of the anterior region of the corpus callosum (CC), to sophisticated structural MRI measures of the CC shape (Yokota et al., 2005), demonstrating that the pattern of the shape of the corpus callosum in transsexuals is closer to that of individuals with the same gender identity than to individuals of the

same biological sex. Nevertheless, there are some differences between the former studies and ours. One important difference includes the inclusion criteria patients had to fulfill in order to participate in the study. Even though all patients were diagnosed with EO-GID, they were also older in Rametti's study, with a mean age of 28.24 years with a range of 10.61 years. Unfortunately, there was no information available on the average age or diagnosis of the patients participating in Yokota et al.'s study.

As our patients' ages range from 12 to 20 years and were all diagnosed with early-onset gender identity disorder, it enabled us to also take a look at possible differences in brain morphology in EO-GID not only in comparison to controls, but also at possible differences in younger EO-GID patients in comparison to that former studies have shown in older patients. Since the brain undergoes reorganization during brain development by means of neural plasticity, this also allows for compensatory mechanisms to develop (Glickstein, 2009). In normal aging, these mechanisms come along with microstructural changes, which can affect interhemispheric processing and therefore also impact behavior (Schulte et al., 2005). Especially the relatively long developmental trajectory of the callosal fibers influences the connectivity between hemispheres, as they are not completely myelinated until the age of 10–13 years and just fully develop during puberty (Mayston et al., 1997; Qiu et al., 2010). Therefore, it can be assumed that our patient group (12-20 years) covers the whole spectrum of development of callosal fibers. Furthermore, the trajectories involved can be studied in figure 3, for both the anterior part (genu) and the posterior part (splenium) of the corpus callosum, the two subparts of the corpus callosum that showed significantly reduced volume in our EO-GID group. Taking a closer look at the areas affected (see figure 2), the genu contains the highest density of thin, myelinated axons, connecting the prefrontal cortex and higher order sensory areas. Fiber density increases again in the splenium, which connects visual areas in the occipital lobe (Aboitiz & Montiel, 2003; Aralasmak et al., 2006; Raybaud, 2010). Therewith, according to the topographical organization of the cortex, changes in fiber size and composition of the corpus callosum can also lead to altered function of the cortex (as can be seen by the trajectories in figure 3). Furthermore, altered development resulting in size changes of callosal volume have been connected to several psychopathologies, such as ADHD and some types of schizophrenia (van der Knaap, van der Ham, 2011). As it can be seen in figure 2, the splenium is very closely situated to the cerebellum (the

cerebellum in relation to the CC can be seen in figure 1). As no differences could be found in the occipital lobe, but in the cerebellum, possible functional impact of altered size of the splenium in our group might not have affected its trajectories to the occipital lobe, but rather the development of white matter structures in the cerebellum.

Further, morphological alterations in the anterior callosal regions have been shown to affect frontal lobe function, creating difficulties with for example face recognition, as it has been noted in patients with autism (van der Knaap, van der Ham, 2011). Interestingly, Rametti et al. (2011) also found differences in a female to male EO-GID group in the genu of the corpus callosum. As the genu is topographically placed directly in the middle of the frontal cortex and we were able to demonstrate a regressive relationship between the frontal lobe and the blood-brain barrier crossing thyroid hormone T4, it can be speculated that such an abnormal hormonal development involving T4 might be connected to abnormal brain development, namely the anterior part of the corpus callosum.

Taking a closer look at the possible functional implications of our study's results, one has to consider that the main function of the corpus callosum is to regulate transfer and communication between the different cortical areas, integrating sensory, motor, cognitive and emotional functions from both hemispheres (Witelson et al., 2008). As demonstrated in figure 3, the CC does so by connecting cortical regions of both hemispheres by the help of numerous intra- and interhemispheric myelinated axonal projections. Nevertheless, how this regulation process works remains unclear, as studies investigating the role of the corpus callosum issue conflicting statements. Some studies suggest that the corpus callosum plays an inhibitory role, whereas others claim that it rather serves an excitatory function (Clarke & Zaidel, 1994; Bloom & Hynd, 2005). These statements have to be distinguished from the neurochemical properties of the callosal fibers themselves. The axons of the corpus callosum mostly depend on glutamate as a neurotransmitter and are therefore thought to be excitatory per nature (Conti & Manzoni, 1994; Bloom & Hynd, 2005; Westerhausen & Hugdahl, 2008). However, due to the presence of inhibitory interneurons, the CC also has an inhibitory function (Kawaguchi, 1992; Clarke & Zaidel, 1994; Westerhausen & Hugdahl, 2008). Therefore, the relationship between the degree of callosal connectivity and lateralization splits views into two

models: an inhibitory and an excitatory one. The inhibitory model proposes that the corpus callosum is maintaining independent processing between the two hemispheres to increase laterality effects by hindering activity in the opposing hemisphere and causing greater connectivity. The excitatory model on the other hand poses that the corpus callosum shares and integrates information between both hemispheres in order to decrease laterality effects and enhance interhemispheric exchange by greater connectivity (Clarke & Zaidel, 1994; Bloom & Hynd, 2005). The contrast of both theories suggests that these two models may not need to be exclusive, but rather compliment each other, i.e. excite when needed but also taming the excitation when necessary, depending on the nature of communication (interhemispheric or intrahemispheric). Consequently, abnormal development of the CC could lead to a disturbance of such a regulation system. Furthermore, such a regulation system that is believe to both excite and inhibit certain projections seems very similar to the proposed sculpting mechanism of the cerebellum by Eccles et al. (1967). Therefore, with both structures showing decreased brain volume and therewith possibly disturbing sculpting procedures of the brain, the possible impact of reduced callosal volume seems to parallel the possible functional impact of reduced cerebellar volume.

In addition, both structures also play a role in hemispheric communication. It is believed that the pathologies connected to altered CC morphology result from disturbed communication between the hemispheres (van der Knaap & van der Ham, 2011), indicating that significant morphological alterations as found in our study might be responsible for dysregulation of interhemispheric transfer. Furthermore, since the degree of connectivity between hemispheres can be reflected by the size of the corpus callosum (Aboitiz, 1992; Clarke & Zaidel, 1994), it can be speculated that our group showing reduced CC volume, also might show reduced hemispheric connectivity. In addition, caused by a lack of communication between both hemispheres, absence of callosal structure has been shown to result in disturbed behavior (van der Knaap, van der Ham, 2011). Therefore, altered size of callosal structures can be believed to be connected to altered behavior as well. As a result, due to their various trajectories, the functional impact of such an abnormal development of size in both structures is very likely to not only excerpt local influences but may also affect a more global level. As early onset gender identity disorder is defined by a

strong and persistent development of cross-gender identification, this could present such a possible global functional impact.

Finally, differences in the CC can further cause or be caused by hemispheric size differences. Nevertheless, the possibility of hemisphere playing a substantial role can be neglected here, as hemisphere as an intervening factor could be excluded with the help of a repeated measure ANOVA (see results section).

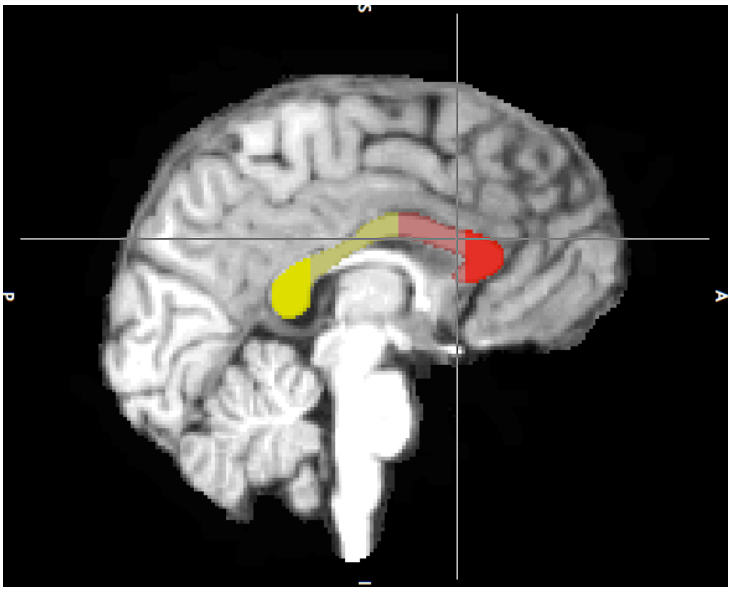

***Figure 2.*** *Corpus callosum, segmented*

*T1 weighted MRI picture mid-sagittal view of a EO-GID patient (age 14) after visually inspected brain segmentation with FreeSurfer. The different subparts of the corpus callosum are highlighted in different colors. Dark red represents the anterior part of the CC, with the lighter red being the genu and the dark yellow representing the posterior part of the CC, with the lighter yellow representing the splenium. Furthermore, the letter S indicates superior, A for anterior, I for inferior and P for posterior.*

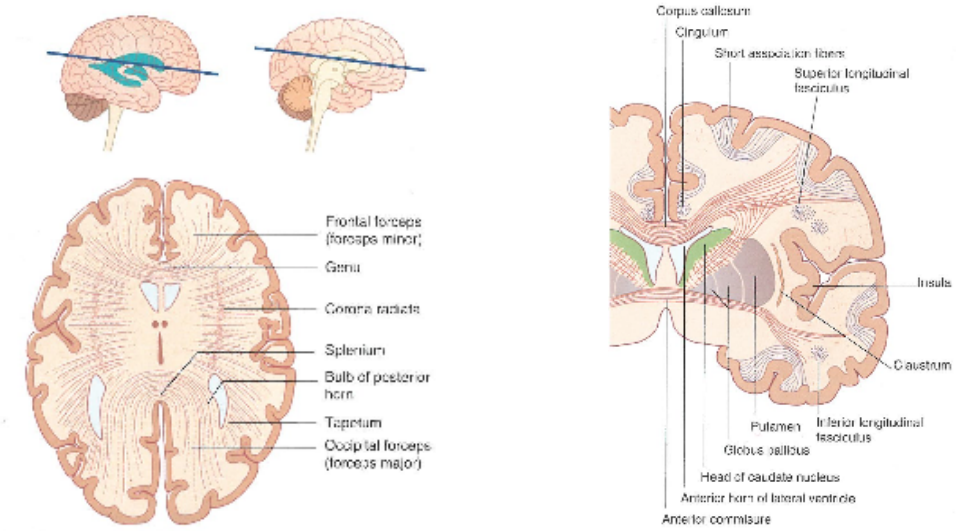


***Figure 3***. *Trajectories of the corpus callosum*

*On the left side: horizontal section of the brain through the genu and splenium of the corpus callosum showing the corpus callosum's trajectories. On the right side: coronal section of the brain showing position of short and long association bundles (images taken from FitzGerald et al., 2012).*

## Implications for GID

The results of our study have several implications. First, finding the possible indicators of the underlying neural correlates of early onset gender identity disorder might help with the diagnosis of this disorder, as not all not all children with GID will develop to be transsexuals after puberty. This might be related to the current broadness of the child criteria for GID in the DSM-IV-TR, since even children who only show gender variant behavior can obtain a diagnosis (Zucker, 2010). Furthermore, as its diagnosis mainly relies on subjective reports, the identification of biological indicators of GID is of current need. Besides the broadness of the definition of GID, the persistence of GID in this population after puberty has started is considerably higher than in normally developing children. In one qualitative study, older adolescents (14-18 years old), who had been children with a GID diagnosis but whose GID reversed, reported that their gender dysphoria had disappeared shortly after the first physical signs of puberty, and the first sexual feelings and sexual attractions (Steensma et al., 2011). It therefore seems unlikely that adolescents who have been through the first stages of puberty will experience a reversal of their GID in the late pubertal stages. As all of our patients did not only experience GID during

childhood, but also in adolescence, the results of our study might add to the diagnostic criteria of EO-GID, seperating it from children only showing gender variant behavior. The need for a more unified and specified diagnosis of GID can further be seen in the current discussion about the proposed changes by the American Psychiatric Association (APA) of diagnosis for the forthcoming fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Here, they based their recommendations on articles published in Archives of Sexual Behavior (Cohen-Kettenis & Pfäfflin, 2010; Drescher, 2010; Meyer-Bahlburg, 2010; Zucker, 2010), which underlines the impact studies investigating the underlying neural correlates of GID have.

Second, identification of the underlying biological processes that play a role in the development of EO-GID will further help to optimize treatment. Due to the difference of their developmental stage, there are different treatments available for children and adolescents. Treatment for children often consists of individual therapy with the child and parent counseling, whereas adolescents with GID can either start with puberty delaying hormone therapy or continue with therapy until they turn 18 years and can be referred to an adult gender identity clinic. Puberty delaying hormone therapy consists of the prescription of hormone analogues (GnRHa), which enables gender dysphoric adolescents under the age of 16 to explore their gender dysphoria and the wish for gender reassignment without the distress of physical puberty development (Cohen-Kettenis & van Goozen, 1998). Nevertheless, some researchers state that suppressing puberty results in an increased risk of misdiagnosis because the patient's gender identity might still change (Ross & Need, 1989; Verhulst et al., 1996). However, no distinction has been made between children and adolescents in these two studies and as suggested by the study of Steensma et al. (2011), possibly only the first signs of puberty are needed to detetct whether their gender dysphoria is persistent or not. Furthermore, the same study indicated that participants did not regret undergoing treatment. Nevertheless, one issue that has not been resolved is the identification of the best candidates for early hormonal treatments. Cohen-Kettenis and van Goozen (1997) suggested that the least risky subgroup of adolescents who have GID are those who show little evidence of psychiatric impairment. However, by adolescence, it is not clear to what extent the psychiatric impairment is a consequence of the chronic gender dysphoria (Newman, 1970). Furthermore, the use of hormone

delaying therapy has been very controversial (de Vries et al., 2011) with recent findings from animal studies indicating its influence on brain morphology (Nuruddin et al., 2013). Therefore, objective diagnostic criteria for EO-GID are needed in order to make an informed and safe treatment decision.

Third, optimized treatment might help to decrease psychopathology that resulted from late diagnosis and treatment. On the basis of the high levels of internalizing psychopathology observed in children and adolescents with GID, it has been speculated (Coates et al., l994) that inhibition and stress reactivity (Kagan et al., 1987) are important contributors to the vulnerability of EO-GID. Dynamic models for understanding the development of GID (Zucker & Bradley, 1995) propose general factors that increase the child's insecurity or anxiety about their idea of the self. The general factors proposed include a constitutionally determined reactivity to stress, early attachment difficulties that exacerbate the child's insecurity, and familial or situational factors that increase the child's anxiety. Therefore, if a biological correlate for EO-GID could be identified, one might also be able to differentiate between psychopathology that follows the development of GID and that that is directly connected with its development. In addition, the influence of such a neural correlate, as found in our study, could improve both diagnosis and treatment, which would help reduce the risk of further developing psychopathology and therefore improve the standard of life in patients diagnosed with EO-GID.

## Strengths and Limitations

*Strengths*

The current study has several strengths. All participants were officially diagnosed and patients at the same clinic, avoiding differences in diagnostic criteria and processes (as this has not always been the case in former studies: Zhou et al., 1995; Kruijver et al., 2000; Luders et al., 2009).

Moreover, none of the participants had a history of hormonal treatment. This way, we were able to exclude the potential effects of administered hormones as a confounding factor for our findings. It has been demonstrated that naturally circulating hormones in adult transsexuals at baseline do not differ significantly from hormonal levels of their biological sex (Meyer et al., 1986; Spijkstra et al., 1988; Haraldsen et al., 2007). Therefore, with the help of blood samples of the EO-GID

group, we were able to speculate of the possible underlying biological processes that might play a role in the development of GID.

However, it remains to be established whether pre-, peri-, or postnatal hormonal effects in early childhood have an influence on the aetiology of gender identity disorder. Therefore, further studies are needed to resolve the degree to which genetic variability and environmental factors influence the development of gender identity (Schweizer et al., 2009), possibly (but not necessarily) via affecting brain structures.

*Limitations*

Besides its strengths, our study also shows certain limitations. To further determine the exact nature of the underlying biological processes involved in the development of GID in contrast to normally developing adolescents, blood samples should also have been collected from the controls. In addition, it would be interesting to contrast our group not only to female controls, but also to male controls in order to detect whether they show the proposed parallel development with their aspired gender, rather than their biological sex (Claro et al., 1995; Berglund et al., 2008; Gizewski et al., 2009; Schöning et al., 2010; Rametti et al., 2011). Furthermore, adding a fourth group consisting of early onset male to female transsexuals would help determine whether the findings of our study only regard female to male transsexuals or whether these apply to early onset gender identity in general. Moreover, a group diagnosed with GID with a later time of onset contrasting this early onset GID group would help to further determine whether the biological processes believed to influence the development of GID found, can be generalized or whether there are differences between an early and a late onset of the disorder. As developmental processes have been shown to influence brain development (Paus, 2005; Durston & Casey, 2006; Blakemore et al., 2010), a bigger sample size allowing the groups to be split into different age groups, would be advised. It may also to be of interest to include another group with abnormal white matter development in order to differentiate further how this abnormal white matter development can be further differentiated and therewith clarified. Such a bigger group should further be followed up with both sMRI scans and blood samples, to allow not only between-subject, but also within-subject comparisons. Moreover, we cannot exclude the possibility that

future hormonal treatment and surgical treatments could affect brain structure. To solve this question, pre- and post- treatment studies or, at least, comparisons with treated groups are needed.

In addition, as differences in methods used determining abnormal morphology (MRI, DTI and post-mortem) as well as differences in classifications can also provide variation between patient groups, a combination of several methods might further differentiate findings and therewith set them better into context. With regard to the brain structures we looked at, more white and gray matter areas are needed. Even though all segmentation results have been manually inspected and corrected, using the same FreeSurfer version would be needed to further insure consistency of results (as suggested by Gronenschild et al., 2012). Furthermore, the population of controls and GID should be of the same size to avoid influencing statistical probability.

Alternatively, other variables may be independently affecting both the expression of a transsexual identity and the neuroanatomy in transsexuals, leading to an observed association between both. The reported high lifetime psychiatric comorbidity in GID (Hepp et al., 2005) could be such an interfering variable. The influences of these comorbidities can further produce distinct symptoms in different individuals varying with the stage of illness, which again can be attributed to morphological abnormalities. Furthermore, interfering variables can also be genetic predisposition, psychosocial and environmental influences, hormonal exposures, or most likely an interplay between these variables. Both genes and environmental demands have been demonstrated to determine brain anatomy (Draganski et al., 2004; Thompson et al., 2001) and have been shown to be applicable to transsexualism (Green, 2000; Coolidge et al., 2002; Henningsson et al., 2005; Hare et al., 2009).

## Conclusion

Our study is to our knowledge the first study to show morphological brain differences in a sample of young female to male early onset transsexuals before cross-sex treatment, using both structural MRI and blood samples.

Cumulative evidence from former studies using a variety of methods ranging from postmortem studies to diffusion tensor imaging suggested that specific neuroanatomical features are associated with transsexual identity. These areas include the BSTc (for MtFs), certain gray matter fractions (for MtFs), white matter patterns of the forceps minor (FtMs) and the corpus callosum (both FtMs and MtFs). Of these, only one study has explicitly focused on untreated early-onset female to male transsexuals (Rametti et al., 2011; although there was no information about hormone levels or treatment for Yokota at al., 2005; and Kruijver et al., 2000). Unfortunately, with all their patients showing a relatively high mean age at scanning (28.24 years versus 18.43 years in our study) and synaptic pruning in the cerebral cortex occurs at puberty and is completed during early adolescence (Huttenlocher, 1979), possible abnormal pruning of the brain might not be detected.

We were further able to demonstrate several statistically significant results; an increase of cerebral gray matter volume, reduced cerebellar white matter and reduced volume of both the posterior and the mid anterior part of the corpus callosum (see table 5). Furthermore, we were able to show a statistically significant regressive relationship between T4 and the frontal lobe, the temporal lobe and cerebral white matter, between SHBG and the frontal lobe as well as between TSH and cerebral gray matter (see table 7), indicating that brain volume (outcome variables) increases with the different hormone levels (predictor variables), or in this case that lower hormone levels predict reduced regional brain volume. Several results of our study (reduced white matter volume, as it can be seen in the cerebellum, the cerebral cortex and the corpus callosum) support the literature's notion of an abnormal white matter development in GID (Yokota et al., 2005; Rametti et al., 2011). Furthermore, the results of our study showed no increase of volume in this structure (as shown by the studies of Yokota et al., 2005; Rametti et al., 2011) but rather a significant reduction in size. Nevertheless, as our study did not have a male control group, no conclusion

can be given whether the results from the EO-GID group also rather reflects the one of the aspired gender than the biological sex, as concluded by Rametti et al. (2011).

To set the found results into context, a theory is proposed on how they are connected to the development of EO-GID. The dominant theory of GID suggests a late sex differentiation in the brain as the underlying biological correlate (Giedd et al., 1997; van Goozen et al., 2002; Bentz et al., 2007; Swaab, 2007). Our theory on the other hand, proposes that this discrepancy is not caused by sex differentiation per se, but rather by abnormal white matter development. Such an abnormal development can be caused by neuroactive steroids, which regulate physiological functions of the central nervous system (Melcangi & Panzica, 2006; Melcangi et al., 2008). Indeed, it has been put forward that puberty, a period characterized by neural development, is a sensitive period for steroids to organize the brain (Romeo, 2003; Sisk & Zehr, 2005; Ahmed et al., 2008; Schulz et al., 2009), acting on brain regions such as the cerebral cortex (Muñoz-Cueto et al., 1990, 1991) and the cerebellum (Sakamoto et al., 2003; Tsutsui et al., 2004). Furthermore, neuroimaging techniques have demonstrated that neuroactive steroids have a profound influence on structural reorganization of gray and white matter during human puberty and adolescence (Peper et al., 2011). Nevertheless, it appears that in our EO-GID group, not a direct influence of neurosteroids (such as testosterone or estradiol), but rather thyroid hormones (TSH and T4) showed significant relationships with abnormal brain development. As T4 is able to cross the blood-brain barrier, it allows it to act directly on brain development, enabling it to act on the development and retention of cognitive function (Begin et al., 2008). Thyroid function is further believed to accelerate the myelination process of white matter (Brent & Davies, 2012) and its dysfunction has been associated with disruptions in cognitive function (Smith et al., 2002; Liesbeth et al., 2004). Such a possible abnormal hormone development would therefore impact the different brain regions involved and therewith their projections to other brain regions and resulting functions. This could in turn influence the development of cerebral gray and white matter networks involved in the own body perception of the self (for gray matter: Blanke et al., 2002; Adamovich et al., 2009; Hodzic et al., 2009; for white matter: Lewis & Carmody, 2008; Takeuchi et al., 2012), as this is the dominant feature of gender identity disorder. Regression analyses of the different lobes have detected that T4 showed a positive statistically significant relationship with both the frontal and the

temporal lobe, indicating that lower T4 values predict lower volume in these regions. These two areas are further both topographically closely situated to both the cerebellum and the corpus callosum and share several trajectories with both regions, including some involved in consciousness of the self. Furthermore, both the cerebellum and the corpus callosum include regulation systems, which are believed to both excite and inhibit certain projections to the rest of the brain. With both structures showing reduced white matter volume, a possible disturbance of these sculpting procedures can be suggested. Such a disturbance could further result in altered communication between the hemispheres, as it has been shown that significant morphological alterations as found in our study might be responsible for dysregulation of interhemispheric transfer (van der Knaap & van der Ham, 2011). Furthermore, altered size of callosal structures can be believed to be connected to altered behavior as well. As a result, due to their various trajectories, the functional impact of abnormal brain development possibly resulting from abnormal hormonal development is very likely to not only excerpt local effects, but rather affect a more global level. As early onset gender identity disorder is defined by a strong and persistent development of cross-gender identification, this could present such a possible global functional impact. Supporting this theory, animal studies have revealed an important role of neuroactive hormones (Panzica et al., 2011), suggesting that they may represent good candidates for the development of neuroprotective strategies for neurodegenerative and psychiatric disorders. A disturbance of neuroactive hormone development might contribute to psychiatric disorders, as it could be suggested in this case.

Even though unlikely, this does not necessarily exclude the possibility that the brain of early onset transsexuals is developing into a direction of their aspired gender, or that these changes have been caused by it, or that this abnormal white matter development acts as a compensatory neural mechanism. Future research needs to resolve whether the observed distinct features in patients diagnosed with GID influence their gender identity or possibly are a consequence of being transsexual.

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**Figures**

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