Origins of gender differences in the human brain

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Chapter 4

The influence of sex hormones and sex chromosome genes on the sexual differentiation of human brain structure

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ABSTRACT

There is much interest in the relative importance of sex hormones and sex chromosome genes in the sexual differentiation of human brain structure and function. A unique opportunity to study this question is provided by women with complete androgen insensitivity syndrome (CAIS), who have a 46,XY karyotype but are resistant to androgens. Therefore, we compared regional gray matter (GM) volumes in 46,XY women with CAIS ($n = 34$) to control men ($n = 56$) and control women ($n = 53$), using both univariate and multivariate approaches. In regions that showed sex differences in the control groups, women with CAIS showed a female-typical regional GM volume in the right pre- and postcentral gyrus, arguing against a direct role for genes on the sex chromosomes in this region. However, the multivariate pattern recognition analyses showed that the overall pattern of spatially distributed GM volume throughout the brain was neither female- nor male-typical in women with CAIS. Our results suggest that sex differences in regional GM volume do not solely reflect differences in androgen exposure, but likely reflect a combination of sex hormone influences, direct effects of genes on the sex chromosomes, and socialization effects, with varying effects across brain regions.

INTRODUCTION

Structural sex differences in the human brain have been consistently described. These include sex differences in overall brain structure and in specific brain regions. The male brain is on average 11% larger than the female brain (as seen in meta-analysis; Ruigrok et al., 2014), which is only partially accounted for by differences in body size (Peters 1991; Ankney 1992). With respect to sex differences in regional brain structure, a recent meta-analysis of 16 whole-brain voxel-based magnetic resonance imaging (MRI) studies (Ruigrok et al. 2014) showed larger bilateral GM volumes in men in limbic regions, including the amygdala, hippocampus, parahippocampal, and cingulate gyrus, the temporal pole, precuneus, putamen, and cerebellum but larger GM volumes in women in the bilateral thalamus and precuneus, right planum temporale/parietal operculum, insula, Heschl's and anterior cingulate gyrus, parts of the frontal cortex, and in the left parahippocampal gyrus and lateral occipital cortex. Results from multivariate pattern recognition (MPR) methods, in which spatially distributed GM patterns are used to differentiate between the sexes, complement the findings obtained in studies using a mass-univariate statistical approach. A general advantage of MPR methods is that they can detect subtle, spatially distributed, patterns in the data (Schrouff et al. 2013), which might reflect differences between groups difficult to detect with a mass-univariate analysis. Based on neuroanatomical spatial patterns, male and female brains were seen to be discriminated with accuracies significantly above chance level (e.g., Feis et al. 2013), even when groups were matched for brain size (Wang et al. 2012).

Delineating the mechanisms underlying sexual differentiation of the human brain is important for understanding origins of brain development and the etiology of neuropsychiatric disorders with a sex difference in prevalence, such as depression, anxiety disorders, autism, and attention-deficit hyperactivity disorder (ADHD) (e.g., reviewed in Zahn-Waxler et al. 2006; Bao and Swaab 2011). The classic theory of brain sexual differentiation was first presented by Phoenix et al. (1959), who showed in rodents that exposure to high levels of testosterone during prenatal development permanently organizes reproductive behavior by masculinizing and/or defeminizing actions. These findings have now been replicated in many species with respect to brain and behavior (Wallen 2009), and in humans with respect to behavior (Hines 2010; Berenbaum and Beltz 2011). In rodents, androgens act through aromatization to estrogens for many of these organizational effects (reviewed in Baum 1979), whereas in nonhuman primates and humans, sexual differentiation of the brain is hypothesized to result from direct androgen actions (Cohen-Bendahan et al. 2005; Wallen 2005; Baum 2006; Hamann et al. 2014; van Hemmen, Veltman, et al. 2016). Although the classic theory of brain sexual differentiation further implied that steroid hormones only have activational (transient) effects on the brain during puberty and adulthood, puberty has more recently been proposed as a possible second period of brain organization (Sisk and Zehr 2005; Schulz et al. 2009).

Evidence regarding early sex hormone effects on GM volume in sexually differentiated brain regions is inconsistent. In one study of boys (Lombardo et al. 2012), testosterone levels in amniotic fluid were associated with GM volume at age 8 to 11 in some, but not all, sexually differentiated brain regions. In contrast, no masculinizing effect of fetal testosterone was observed in brain structure of girls with congenital adrenal hyperplasia (CAH) due to 21α-hydroxylase deficiency (Merke et al. 2003), who are exposed to high (sex-atypical) prenatal androgens. However, findings in girls with CAH might be confounded by other factors related to the disease or its treatment, including altered glucocorticoids. In addition to early sex hormone effects, associations between regional GM volume and circulating sex hormones have been studied in (non-CAH) adolescents (Neufang et al. 2009; Peper et al. 2009; Bramen et al. 2011; Herting et al. 2014) and adults (Lessov-Schlaggar et al. 2005; Witte et al. 2010; Lentini et al. 2012). These studies showed associations between regional GM volume and circulating concentrations of estradiol, testosterone and, to a lesser extent, progesterone; some of these associations were found in GM regions that demonstrated sex differences. Unfortunately, results differed across studies, perhaps due to methodological variations, such as regression analyses performed within versus across the sexes and whole brain versus region of interest (ROI) approaches. Hormonal factors related to the menstrual cycle and hormonal contraceptive use also have been associated with changes in GM volume (Protopopescu et al. 2008; Pletzer et al. 2010; Hagemann et al. 2011; De Bondt et al. 2013; Franke et al. 2015), which might modulate sex differences found in adults (Pletzer et al. 2010).

There is growing interest in the role of genes on the sex chromosomes in the sexual differentiation of the brain (McCarthy and Arnold 2011). However, it is difficult to separate these genetic effects from sex hormone effects, because the genes on the sex chromosomes (in addition to many autosomal genes) are involved in aspects of gonadal development and are therefore intrinsically related to sex hormone levels. Therefore, most evidence comes from mouse models specifically created to disentangle effects of genes on the sex chromosomes from hormonal effects (for review see Cox et al. 2014). Human studies of brain structure in individuals with sex chromosomal aneuploidies are difficult to interpret in terms of sex chromosome gene effects, because the sex chromosomal aneuploidies are usually accompanied by hormonal differences.

The complete androgen insensitivity syndrome (CAIS) provides a unique opportunity to investigate the role of sex hormones versus sex chromosome genes in the sexual differentiation of the human brain. Women with CAIS have a 46,XY karyotype and normally functioning abdominal, pelvic or inguinal testes that secrete normal or exaggerated amounts of testosterone while *in situ*. However, mutation(s) in the androgen receptor (AR) gene lead to absent or dysfunctional androgen receptors and complete androgen resistance, resulting in a female phenotype regardless of testosterone concentrations within or above the male range (Melo et al. 2003; Doehnert et al. 2015). Nearly all women with CAIS are androphilic

(sexually attracted to men), have a female gender identity, and show female-typical gender role behavior (Masica et al. 1971; Wisniewski et al. 2000; Hines et al. 2003). Functional MRI (fMRI) studies revealed a female-typical neural activation pattern in women with CAIS in response to sexual images (Hamann et al. 2014) and during mental rotation (van Hemmen, Veltman, et al. 2016). These findings indicate that sex differences in regional brain function related to these tasks are most likely not directly driven by genes on the X and Y chromosomes, but rather reflect differences in gonadal hormone exposure, or female-typical socialization. Specifically, they might reflect the absence of masculinizing actions through the androgen receptor, or the presence of feminizing actions by estrogens derived from androgens produced by the testes while still *in situ,* and/or estrogen replacement therapy following gonadectomy. A study of white matter microstructure showed similar overall findings; a female-typical fractional anisotropy in women with CAIS throughout extended white matter regions indicating a predominant role for sex hormones. In contrast, however, a more subtle contribution of sex chromosome genes or masculinizing androgen effects not mediated by the androgen receptor could not be ruled out for some aspects of white matter microstructure (van Hemmen, Saris, et al. 2016).

In the present study we analyzed GM volume in women with CAIS. We used structural MRI scans from 34 women with CAIS, 56 control men, and 53 control women to investigate the role of sex hormones versus sex chromosome genes in the sexual differentiation of regional GM volume, using several approaches. First, we used voxel-based morphometry (VBM) in combination with a mass-univariate approach to compare regional GM volume of women with CAIS to control men and women within regions showing sex differences in the control groups. Second, we performed an MPR analysis in which we trained a classifier to differentiate control men and women based on spatially distributed GM volume patterns, and then applied the resulting classifier model to women with CAIS.

MATERIALS AND METHODS

Subjects

A total of 143 subjects were included in this study; 34 women with CAIS (46,XY), 56 control men, and 53 control women. Subjects were scanned at two centers: 21 women with CAIS, 43 control men, and 47 control women at the VU University Medical Center Amsterdam, the Netherlands (referred to as the NL sample or study) and 13 women with CAIS, 13 control men, and 6 control women at Emory University, Atlanta, USA (referred to as the USA sample or study). Initial samples were larger, but, data from 19 subjects were excluded because of left-handedness or ambidexterity (n = 9), the use of hormonal contraceptives in control women (n = 7), anatomical abnormalities seen on MRI (n = 2), or reduced image quality due to excessive head movement (n = 1). Participants included in the study were right-handed

(according to the Edinburgh Handedness Inventory (Oldfield 1971) or the Dutch Handedness Inventory (van Strien 1992)), and control women did not use hormonal contraceptives. Previous data from these samples have been published, with respect to neural activation to sexual stimuli in the USA sample (Hamann et al. 2014) and neural activation during mental rotation and diffusion tensor imaging in the NL sample (van Hemmen, Saris, et al. 2016; van Hemmen, Veltman, et al. 2016).

In women with CAIS from the NL sample, the diagnosis of CAIS was based on clinical characteristics, and mutation analysis of the androgen receptor (AR) gene using genomic DNA revealed a confirmed mutation in 13 women, an unclassified variant in 7 women, and was inconclusive in 1 woman. In the USA sample, the diagnosis of CAIS was based on self-report. Participants were recruited through the Androgen Insensitivity Syndrome-Differences of Sex Development (AIS-DSD) support group, and members are typically well educated about the specifics of their diagnosis, based on detailed research into their personal medical histories, including karyotype, hormonal profile, and clinical features. All women with CAIS were androphilic, i.e., sexually attracted to men. All women with CAIS from the NL sample were gonadectomized, and, with one exception, used hormone replacement therapy (HRT) consisting of estrogens (n = 16) or a combination of estrogens and progestins (n = 4) to compensate for the lack of gonadal sex hormone production. Information regarding HRT and whether or not a gonadectomy had been performed was not available for women with CAIS from the USA sample.

For the NL sample, women with CAIS were recruited from the databases of the VU University Medical Center and the Erasmus University Medical Center-Sophia's Children's Hospital Rotterdam and from the support group DSDNederland. For the USA sample, women with CAIS were recruited from the AIS-DSD support group. Control subjects were recruited using flyers and advertisements. The NL study was approved by the Medical Ethics Committee of the VU University Medical Center Amsterdam (application number NL32740.029.10 + NL29233.029.09) and the Erasmus University Medical Center (application number MEC-2010-350), and the USA study by the Institutional Review Boards of the participating institutions. All participants gave their written informed consent according to the Declaration of Helsinki.

MRI data acquisition

For the NL sample, MRI data were acquired with a 3.0T GE Signa HDxt scanner (General Electric, Milwaukee, WI, USA). High-resolution T1-weighted anatomical images were acquired using a 3D fast spoiled gradient echo (FSPGR) sequence (TR 7.8 ms, TE 3.0 ms, inversion time (TI) 450 ms, 1 mm isotropic resolution). Scanning of the USA sample was conducted using a 3.0T Siemens Tim Trio MRI system with a 12-channel parallel head coil. A magnetization-prepared rapid acquisition sequence with gradient echo (MPRAGE) was used to acquire

high-resolution T1-weighted images (TR 2,300 ms, TE 3.02 ms, inversion time (TI) 1,100 ms, 1 mm isotropic resolution).

Data analysis

Sample characteristics and overall tissue volumes

Statistical analyses of the sample characteristics and overall tissue volumes derived from VBM preprocessing (see VBM preprocessing section for further details) were performed with IBM SPSS Statistics for Windows, Version 22 (IBM Corp., Armonk, NY, USA), using parametric tests (height, overall tissue volumes) and, when assumptions for parametric tests were violated, non-parametric tests (age, level of education). Between-group analyses for possible age differences were performed between the three groups for both the complete and NL sample, and in addition between the NL and USA sample to examine possible age differences between the samples from the two scan sites. Analyses of covariance (ANCOVA) were used to compare groups for overall tissue volumes, covarying age, age and height, or age and total intracranial volume (TIV).

VBM preprocessing

Individual T1-weighted structural images were manually reoriented to roughly match the AC-PC line, and non-brain tissue was removed using the brain extraction tool (BET; Smith 2002, implemented in FSL v5.04 http://www.fmrib.ox.au.uk/fsl, Smith et al. 2004) with image bias and residual neck voxel reduction and a 0.1 fractional intensity threshold. These images were further processed with a VBM approach (Ashburner and Friston 2000), using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm8/) and Statistical Parametric Mapping 8 (SPM8; Wellcome Trust Center for Neuroimaging, Institute of Neurology at UCL, UK), implemented in MATLAB (version R2011a; The MathWorks, Inc., Natick, MA, USA). The default parameters for the VBM8 pipeline were used (for a detailed description see http://dbm.neuro.uni-jena.de/vbm8/VBM8-Manual.pdf). In short, images were segmented into different tissue classes and brought into standard space using high-dimensional DARTEL normalization (Ashburner 2007) to the IXI550 template. The voxel values of the normalized GM images were then modulated using only the nonlinear component of the Jacobian determinant derived from spatial normalization. The resulting modulated normalized GM images, representing the absolute amount of tissue corrected for individual brain size, were smoothed with an 8 mm full width at half maximum (FWHM) Gaussian kernel. In addition, the VBM8 pipeline estimated the raw overall volumes of GM, white matter (WM), and cerebrospinal fluid (CSF) segments, as well as total intracranial volume (TIV = GM + WM + CSF).

Univariate analysis

For all group comparisons of regional GM volume an absolute threshold mask of 0.2 was applied, and age and scanner were included as covariates of no interest. First, we compared control men and women to identify sexually differentiated brain regions. A statistical voxel-level threshold of $P < 0.001$ (uncorrected) and an extent threshold of $k > 98$ adjacent voxels (based on the expected number of voxels per cluster calculated by SPM) was applied, and only clusters with a cluster-level FWE-corrected $P < 0.05$ were considered significant.

In line with our research question, we focused on regions that showed sex differences when comparing regional GM volume of women with CAIS to both control groups. Therefore, the sex difference images, thresholded at $P < 0.001$ (uncorrected) and an extent threshold of $k > 98$ adjacent voxels, were used as a mask image and search region for these comparisons. An initial whole-brain uncorrected voxel-level threshold of $P < 0.001$ was used, and results were considered significant at a cluster-level $P < 0.05$ FWE-corrected for the size of the sex difference mask image. Because we had no *a priori* hypothesis about differences between women with CAIS and control groups outside regions showing sex differences, clusters outside the sex difference mask image were considered significant using a whole-brain FWE-corrected $P < 0.05$ threshold at cluster-level.

Information regarding level of education was not available from women with CAIS in the USA sample. To examine whether any differences resulting from the comparisons involving women with CAIS were influenced by potential differences in level of education, these comparisons were repeated in the NL sample alone, in which groups were matched for level of education (Table 1). The results were masked with the same sex difference mask image as was used in the complete sample, and a more lenient uncorrected whole-brain threshold of $P < 0.001$ was applied given the reduced sample size and exploratory purpose of these comparisons.

Multivariate pattern recognition analysis

In addition to the univariate analyses, a multivariate pattern recognition (MPR) analysis of the GM data was performed using the Pattern Recognition for Neuroimaging Toolbox (PRoNTo v2.0; www.mlnl.cs.ucl.ac.uk/pronto). In the current version of PRoNTo it is not yet possible to include covariates in the model. Therefore, we calculated residual GM images using a multiple regression model in SPM12 with all subjects' smoothed modulated (non-linear only) normalized GM images, covariates age and scanner, and a 0.2 absolute threshold. These residual images were used as input for the MPR analyses, and the whole-brain mask image created with this regression model was used as a mask image. Four clusters that showed higher voxel values in control men than women in non-GM areas (likely resulting from suboptimal segmentation, see Table 2 and results section for further details) were removed from this mask image to prevent an erroneous contribution of these voxels to the classifier function. In order to also remove subthreshold voxels immediately surrounding these clusters, the clusters that were removed from the mask image were thresholded with an uncorrected $P < 0.05$.

We followed several steps in this MPR approach. First, we applied a classification model to assess the accuracy with which control men and control women could be distinguished based on their GM images. For this purpose, we selected the binary Gaussian Process Classification (GPC) machine, which is a probabilistic pattern recognition algorithm, and a leave-one-subject-out (LOSO) cross-validation strategy. LOSO cross-validation computes the accuracy of classifiers by excluding one subject for testing and using all other subjects for training. This procedure is repeated until all subjects have been tested. The performance of the classifier is expressed in balanced accuracy values, representing the percentage of correctly classified subjects while taking into account the number of subjects in each class, and the area under the ROC curve (AUC). Statistical significance of the classification result was determined by permutation testing, using 1000 permutations. A weight map averaged across all cross-validation folds was computed to display the contribution of each voxel to the model.

Second, if the classification performance in the control groups was significantly above chance level, we used this classification function to predict group membership in women with CAIS. For this purpose, the binary GPC was used with a custom cross-validation scheme with 34 iterations. In each iteration one woman with CAIS was tested using the classifier that was trained using all control men and women. Individual function values, which are used for the binary class prediction, represent the class membership probability for each subject (we used control women as the reference class). A < 0.5 individual probability resulted in a classification prediction for class 1 (control men), while a > 0.5 probability resulted in a classification prediction for class 2 (control women). These individual function values were extracted for all participants to perform further statistical analyses by means of non-parametric tests, because assumptions for the use of parametric tests were violated. Class membership probabilities were compared between the three groups (Kruskal-Wallis and post hoc Mann-Whitney U tests), and a one-sample Wilcoxon singed rank test was used for each group independently to test if the median class membership probability differed significantly from 0.5.

Third, to further investigate the contribution of the sex difference regions from the univariate analysis to the classification model, we repeated the same procedure using only information from voxels within the sexually differentiated brain regions. Therefore, the sex difference mask, which was used for women with CAIS versus control group comparisons in the univariate analyses, was applied to the MPR analysis. The classification of control men and women was performed only to establish whether this model produces a significant classification result, since this is a prerequisite to perform classification of women with CAIS. In addition to the statistical analyses performed on the class membership probabilities derived from this classification model (between-group comparisons and within-group comparisons against a probability of 0.5), we performed non-parametric between-model comparisons (Wilcoxon signed rank tests) for each group separately to examine differences

in class membership probabilities when using GM volume information from voxels within the whole-brain versus within sex difference regions.

RESULTS

Sample characteristics

Sample characteristics for both the complete and the NL sample are summarized in Table 1. In both samples, groups did not differ significantly in age and level of education. Level of education was, however, unknown for the women with CAIS in the USA sample, so the comparison is based on the control groups from the complete sample and women with CAIS from the NL sample (n = 21). There was no age difference between the NL (mean age 30.3; SD = 9.3) and USA (mean age 32.4; SD = 9.3) sample (U = 1487.00, P = 0.160). Groups differed significantly in height: control men were taller than control women and women with CAIS, and women with CAIS were taller than control women (all P's < 0.001).

Neuroimaging data

Overall tissue volumes

Absolute and corrected overall tissue volume statistics are summarized in Table 1. Absolute TIV and tissue segment volumes showed no significant differences between control men and women with CAIS, but both groups had significantly larger volumes than control women. Similar results were found in TIV after correcting for differences in body height. To further characterize this finding in TIV, between-group comparisons of GM, WM, and CSF corrected for body height were performed. These showed significantly larger WM volume in control men and women with CAIS than in control women, significantly larger CSF volume in control men than in control women with no significant differences between women with CAIS and either control group for CSF volume, and no between-group differences in GM volume. When using TIV as a covariate, there were no between-group differences in GM, WM, and CSF volume.

Univariate analysis

Results from the univariate analyses are summarized in Table 2 and 3 and displayed in Figure 1. First, differences in regional GM volume between control men and women were examined to define sex difference regions (Table 2 and Fig. 1A). Three clusters with larger GM volume in control women survived the cluster-level FWE-corrected threshold of P < 0.05 and showed large effect sizes at peak-voxel level. These clusters were located bilaterally in the pre- and postcentral gyrus, also extending into the left inferior parietal gyrus, and in the bilateral cerebellum. In addition, a trend towards a significantly larger GM volume, with a medium effect size at peak-voxel level, was found in the left caudate nucleus.

Table 1 Sample characteristics for the complete sample and NL sample

	M	W	CAIS	F/χ^2	P-value	Post hoc
	Mean (SD)					
COMPLETE SAMPLE						
Demographics						
N (USA/NL)	56 (13/43)	53 (6/47)	34 (13/21)			
Age	29.4 (7.8)	29.9 (8.7)	34.2 (11.6)	3.747 [c]	0.156	—
Level of education	6.0 (1.7)	6.3 (1.4)	5.9 (1.6) [b]	1.082 [c]	0.589	—
Body height (cm)	181.4 (7.6) [a]	167.7 (6.1)	174.3 (7.8)	50.296	**< 0.001**	M>C>W
Absolute volumes						
TIV (ml)	1525.7 (14.5)	1388.1 (14.8)	1496.6 (18.8)	23.737	**< 0.001**	M=C>W
GM (ml)	733.5 (8.0)	678.3 (8.2)	718.4 (10.3)	12.318	**< 0.001**	M=C>W
WM (ml)	565.5 (7.1)	505.0 (7.3)	561.0 (9.2)	20.628	**< 0.001**	M=C>W
CSF (ml)	226.7 (2.9)	204.9 (3.0)	217.2 (3.8)	14.024	**< 0.001**	M=C>W
Height-corrected volumes						
TIV (ml)	1491.5 (16.4) [a]	1419.8 (16.5)	1499.4 (18.0)	6.117	**0.003**	M=C>W
GM (ml)	710.1 (8.7) [a]	700.0 (8.8)	720.3 (9.6)	1.230	0.295	—
WM (ml)	557.0 (8.4) [a]	512.8 (8.4)	561.7 (9.2)	8.882	**< 0.001**	M=C>W
CSF (ml)	224.4 (3.4) [a]	207.0 (3.5)	217.4 (3.8)	5.171	**0.007**	M>W, C=M/W
TIV-corrected volumes						
GM (ml)	706.4 (4.4)	715.6 (4.7)	704.9 (5.5)	1.265	0.285	—
WM (ml)	541.4 (4.0)	538.2 (4.3)	549.0 (4.9)	1.382	0.254	—
CSF (ml)	220.0 (2.5)	214.1 (2.7)	213.9 (3.1)	1.678	0.190	—
NL SAMPLE						
Demographics						
N	43	47	21			
Age	30.1 (8.4)	29.6 (8.9)	32.1 (11.8)	0.354 [c]	0.844	—
Level of education	5.8 (1.8)	6.2 (1.4)	5.9 (1.6)	1.077 [c]	0.586	—

Note: All volume means are estimated marginal means corrected for age (absolute volumes), age and body height (height-corrected volumes) or age and TIV (TIV-corrected volumes). Level of education ranged from 1 (primary school) to 8 (university degree). Bold P-values represent a significant ($P < 0.05$) main effect of group. The post hoc column represents the results of post hoc pair-wise group comparisons; = indicates P's > 0.05, > or < indicates direction of difference with $P < 0.05$, using a Bonferroni correction for multiple comparisons. M: control men; W: control women; C/CAIS: women with CAIS; TIV: Total intracranial volume (GM + WM + CSF); GM: Gray matter; WM: White matter; CSF: Cerebrospinal fluid

[a] for body height n = 55 (1 missing value)

[b] n = 21 (data only available for the NL sample)

[c] = χ^2-test statistic

Table 2 Whole-brain sex differences in GM volume

Comparison	Cluster size (mm^3)	Brain region	Cluster-level P (FWE-corrected)	MNI-coordinates x	y	z	Peak-level T	Peak-level Effect size
M > W	2379	—	*0.061*	2	-94	-9	6.56	0.76
	1836	—	0.121	44	-79	-18	6.50	—
	1272	—	0.254	-47	-72	-20	5.95	—
	591	—	0.618	2	-45	9	5.96	—
W > M	8947	R Postcentral gyrus	**< 0.001**	47	-10	39	6.60	1.00
		R Precentral gyrus						
	6473	L Postcentral gyrus	**0.001**	-36	-25	39	5.78	0.86
		L Inferior parietal gyrus						
		L Precentral gyrus						
	5704	L+R Superior posterior cerebellum	**0.002**	-5	-81	-45	5.44	0.81
	2012	*L Caudate nucleus*	*0.097*	*-11*	*14*	*12*	*3.89*	0.59
	1580	R Caudate nucleus	0.169	21	11	13	3.96	—
	1374	L Anterior cerebellum	0.222	-12	-48	-12	3.96	—
		Vermis						
		L Lingual gyrus						
	803	L+R Precuneus	0.474	8	-45	60	3.61	—
	658	L+R Midcingulate cortex	0.569	2	-25	45	3.98	—
		L+R Supplementary motor area						
	486	R Superior posterior cerebellum	0.697	29	-87	-33	3.94	—
	466	L+R Precuneus	0.713	-2	-57	51	4.03	—
	395	R Superior frontal gyrus	0.768	29	47	36	3.70	—
		R Middle frontal gyrus						
	375	L Superior medial frontal gyrus	0.784	-11	63	24	3.91	—
		L Superior frontal gyrus						

Note: Results based on whole brain between-group comparisons using an uncorrected threshold of $P < 0.001$ and an extent threshold of 98 voxels. Bold P-values represent significant clusters at a $P < 0.05$ FWE-corrected cluster-level threshold, P-values printed in italics show a trend towards significance. Peak-level effect sizes, expressed in Cohen's *d*, are presented for all clusters with a cluster-level $P < 0.1$ FWE-corrected. M: control men; W: control women; L: left hemisphere; R: right hemisphere.

None of the clusters from the comparison examining larger GM volumes in control men than women reached significance at the FWE-corrected cluster-level, although in one cluster a trend was observed. More importantly, all four clusters resulting from this comparison using the uncorrected threshold (Table 2) appear to represent non-GM voxels. Based on the location of these clusters (Table 2 and Fig. 1A), they are most likely the result of imperfect segmentation of dural venous sinuses and veins (e.g., Good et al. 2001b). Therefore, no sex difference mask image based on this comparison was used for the subsequent comparisons of regional GM volume between control groups and women with CAIS, or for the multivariate analysis.

Within the sex difference regions that showed a larger regional GM volume in control women, women with CAIS were compared with both control groups (Table 3 and Fig. 1B). Women with CAIS had more GM volume than control men in a cluster within the right pre- and postcentral gyrus, with a medium to large effect size at peak-voxel level. Although the other comparisons did not reveal any significant differences, a trend with a medium effect size at peak-voxel level was observed for a smaller GM volume in the left caudate nucleus compared with control women. Whole-brain comparisons between women with CAIS and both control groups did not reveal clusters with significant between-group differences outside these sex difference regions.

Table 3 GM volume in women with CAIS versus control groups within sex difference regions

Comparison	Cluster size (mm^3)	Brain region	Cluster-level P (FWE-corrected)	MNI-coordinates x	y	z	Peak-level T	Peak-level Effect size
M > CAIS	—	—	—	—	—	—	—	—
CAIS > M	1745	R Precentral gyrus	**0.010**	47	-15	39	4.73	0.72
		R Postcentral gyrus		44	-6	36	3.71	
W > CAIS	358	L Caudate nucleus	*0.099*	-12	15	13	3.59	0.54
CAIS > W	—	—	—	—	—	—	—	—

Note: Results based on between-group comparisons using an initial whole-brain uncorrected threshold of $P < 0.001$ and subsequent small volume correction for the size of the W > M mask image. All clusters with a minimum cluster size of 98 voxels are presented. Bold P-values represent a significant difference using a cluster-level $P < 0.05$ FWE-corrected for the size of the W > M mask image. *P*-values printed in italics show a trend towards significance ($P < 0.1$ FWE-corrected). Peak-level effect sizes are expressed in Cohen's *d*. M: control men; W: control women; CAIS: women with CAIS; L: left hemisphere; R: right hemisphere.

The comparisons within the sex difference regions were subsequently performed in subjects from the NL sample only, in which groups were matched for level of education and, in addition, all women with CAIS were known to be gonadectomized (a factor unknown in the USA sample). These explorative comparisons revealed GM differences in similar brain regions as found in the complete sample, albeit in smaller clusters. A larger GM volume in women with CAIS than in control men was found within the right pre- and postcentral gyrus

(cluster size at P < 0.001 uncorrected = 172 mm^3; peak-level T = 3.54; MNI-coordinates 48, -14, 41), and a smaller GM volume in women with CAIS than in control women was found in the left caudate nucleus (cluster size at P < 0.001 uncorrected = 294 mm^3; peak-level T = 3.47; MNI-coordinates -14, 15, 14). Results were similar when the only woman with CAIS from the NL sample who was not using estrogen HRT was additionally excluded. These results substantiate that the differences in regional GM volume between women with CAIS and control groups in the complete sample are not due to factors related to potential differences in level of education and potential heterogeneity in gonadectomy and HRT.

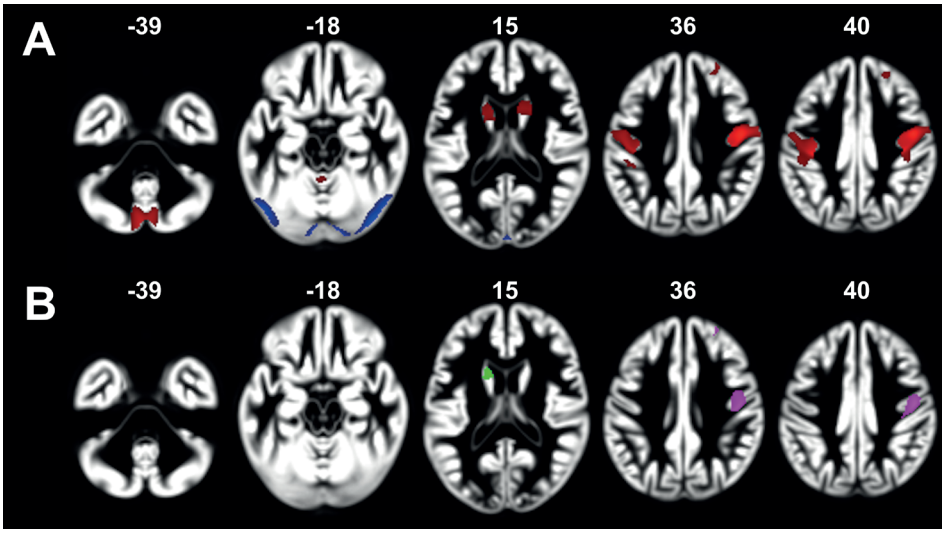

Figure 1 Differences in regional GM volume between control men, control women and women with CAIS. A: Whole-brain sex differences in regional GM volume, displayed with a whole-brain uncorrected P < 0.001 (k = 98). Red = control women (W) > control men (M); Blue = M > W. B: Differences in regional GM volume between control groups and women with CAIS within W > M sex difference regions (red in A), displayed with a whole-brain uncorrected P < 0.001. Violet = CAIS > M; Green = W > CAIS.

Multivariate pattern recognition analysis

Based on the whole-brain GM information in the control groups, the binary GPC accurately discriminated between the sexes with a significant balanced accuracy of 73% and an AUC of 0.80 (Table 4). Therefore, a classifier trained with the GM information from the control groups was subsequently used to compute class predictions in women with CAIS. A total of 20 (59%) women with CAIS received a class prediction for the class representing control men and 14 (41%) for the class representing control women (Fig. 2A).

The class membership probability scores, using the class representing control women as the reference class, showed significant differences for all between-group comparisons (Table 4). The probabilities were highest in control women, followed by women with CAIS,

and lowest in control men (all P's < 0.05 Bonferroni-corrected). Class membership probabilities were significantly higher than 0.5 in control women and lower than 0.5 in control men (P's < 0.001 Bonferroni-corrected), and did not differ significantly from 0.5 in women with CAIS (P = 0.544 Bonferroni-corrected).

When using the sex difference mask (control women > control men), instead of the whole-brain mask, the binary GPC also resulted in accurate sex discrimination for subjects in the control groups (Table 4). Classification of women with CAIS according to this classifier resulted in a total of 15 (44%) women with CAIS receiving a class prediction for the class representing control men and 19 (56%) for the class representing control women (Fig. 2B).

The class membership probabilities, using the class representing control women as the reference class, were again largest in control women, followed by women with CAIS, and lowest in control men (Table 4, all P's < 0.05 Bonferroni-corrected). Class membership probabilities were significantly higher than 0.5 in control women, lower than 0.5 in control men, and did not differ significantly from 0.5 in women with CAIS (Bonferroni corrected P's < 0.001, < 0.001, and 0.567, respectively). Comparisons of the use of GM information from voxels within the sex difference mask, rather than from the whole brain, tested with Wilcoxon signed rank tests, showed that these class membership probabilities were significantly increased in control women (Z = -2.82, P = 0.015, Bonferroni-corrected), decreased in control men (Z = -3.09, P = 0.006, Bonferroni-corrected), but unchanged in women with CAIS (Z = -0.71, P = 1.000, Bonferroni-corrected).

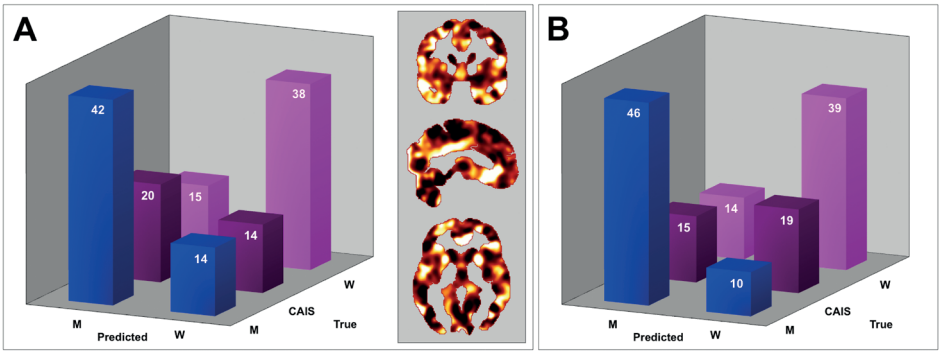

Figure 2 Classification results. Confusion matrices resulting from binary GPC models using GM information from (A) the whole-brain, or (B) voxels within the sex difference mask (control women > control men). Weight map presented for the whole-brain model (A) is based on the classification of control groups. The bright yellow colors indicate voxels that drive the classifier towards class 1 (i.e., control men) and dark black colors indicate voxels that favor class 2 (i.e., control women). M: control men; W: control women; CAIS; women with CAIS.

Table 4 Classifier performance and mean class membership probability per group

		M vs W classifier performance			Class probability			
Model	Group	AUC	Balanced accuracy	P-value	Class W	χ^2	P-value	Post hoc
Binary GPC: Whole brain	M	0.80	73%	0.001	0.36	31.362	< 0.001	W>CAIS>M
	W				0.62			
	CAIS	N/A	N/A	N/A	0.49			
Binary GPC: Sex difference regions	M	0.86	78%	0.001	0.29	45.436	< 0.001	W>CAIS>M
	W				0.69			
	CAIS	N/A	N/A	N/A	0.52			

Note: Post hoc column represents the results of post hoc pair-wise Mann-Whitney U comparisons; > or < indicates direction of difference with $P < 0.05$, using a Bonferroni correction for multiple comparisons. Class W: mean class membership probability with the class representing control women as the reference class.
AUC: area under the ROC curve; M: control men; W: control women; CAIS: women with CAIS.

DISCUSSION

The present study compared regional GM volume in 46,XY women with CAIS to control men and control women, with the goal to investigate the influence of sex hormones versus sex chromosome genes on the sexual differentiation of regional GM volume. Both a mass-univariate and an MPR approach were used. The results demonstrate that GM sex differences are not solely driven by masculinizing androgenic, feminizing estrogenic, or socialization-related effects, but likely also reflect other sex hormone influences and/or direct effects of sex chromosome genes. The relative role and importance of the underlying mechanisms is likely to vary across different brain regions.

The present findings replicated the sex difference in TIV in control subjects, also when accounting for differences in body size (Peters 1991; Ankney 1992). TIV in women with CAIS, also after correcting for height, was not significantly different from TIV in control men, and both groups had a larger TIV than did control women. This is predominantly explained by a larger WM volume in women with CAIS than in control women when correcting for body height, whereas GM volume did not differ between groups when taking height into account. This might indicate that overall WM volume, but not GM volume, is affected by direct genetic effects related to sex chromosome composition or by masculinizing effects of androgens after aromatization to estrogens, although allometric effects of body size cannot be ruled out. The sex differences in compartmental volumes did not survive a correction for

TIV, which is in agreement with previous studies that applied a similar correction (e.g., Chen et al. 2007; Leonard et al. 2008).

Regardless of similar TIV-corrected total GM volumes, region-specific sex differences in GM volume were observed. The most prominent sex differences were found in the bilateral pre- and postcentral gyrus, superior posterior lobes of the cerebellum, and a trend was observed in the left caudate nucleus. In these regions, control women had larger GM volumes than did control men. These findings are broadly in line with findings from previous studies (Filipek et al. 1994; Goldstein et al. 2001; Good et al. 2001; Luders et al. 2009; Pletzer et al. 2010; Savic and Arver 2011; Lentini et al. 2012; Den Braber et al. 2013; but see Rijpkema et al. 2012; Tang et al. 2013), although our findings regarding the cerebellum are in the opposite direction of most (Ruigrok et al. 2014), but not all studies (Welborn et al. 2009).

Within these regions that showed a sex difference, women with CAIS showed a female-typical GM volume in a cluster covering part of the right pre- and postcentral gyrus. This female-typical neural characteristic is consistent with previous findings in the same women, showing female-typical neural activation patterns, albeit in other brain regions, in response to sexual images (Hamann et al. 2014) and mental rotation (van Hemmen, Veltman, et al. 2016) and a predominantly, but not completely, female-typical white matter microstructure throughout extended white matter regions (van Hemmen, Saris, et al. 2016). The present finding suggests that the sexual differentiation of GM in the right pre- and postcentral gyrus is not directly influenced by genetic effects related to sex chromosomal composition, but is likely to be determined by sex differences in gonadal hormone exposure. This result is consistent with findings in other species showing a high density of sex steroid receptors in homologous brain regions during early development (see Goldstein et al. 2001). Due to the complete androgen resistance in women with CAIS, androgenic factors might play a key role in the sexually differentiated GM development of this brain region. A potential role for androgens was also found in boys with familial male precocious puberty (FMPP), who have early androgen excess and were found to have lower GM volume in the right precentral gyrus relative to control boys (Mueller et al. 2011). However, feminizing effects of estrogens cannot be ruled out based on the current results, because: a) women with CAIS produce androgens when the gonads are still *in situ*, which can be aromatized to estrogens, and b) after gonadectomy women with CAIS in general use estrogen HRT from adolescence onwards. Furthermore, all women with CAIS were reared as girls , so we cannot rule out a role for female-typical socialization in these findings.

Data from individuals with sex chromosome aneuploidies have been interpreted to suggest a role for X-chromosomal gene dosage in the sexual differentiation of the precentral gyrus, because a female-typical GM volume was observed in 47,XXY men with KS (Lentini et al. 2012). However, these findings might also reflect hypogonadism in men with KS before they start testosterone supplementation in adolescence or adulthood (Davis et al. 2015). In general, individuals with sex chromosome aneuploidies have atypical sex chromosomes

and early hormone exposure, making it difficult to disentangle sex chromosome dosage effects from early sex hormone deficiency.

With the exception of the findings in the right pre- and postcentral gyrus, regional GM volume in women with CAIS did not significantly differ from either control group in the other brain regions that demonstrated a sex difference in the control groups. Apart from the interpretation that women with CAIS indeed do not differ from the control groups in these brain regions, this could also be the result of lower statistical power for the comparisons involving women with CAIS, because this group was relatively small compared to the control groups. Although the absence of significant differences between women with CAIS and control groups prevents us from drawing any definite conclusions regarding the exact mechanisms underlying the sexual differentiation of these specific brain regions, it does not necessarily reflect a sub-threshold female-typical regional GM volume in women with CAIS that fails to reach significance due to a lack of power. This is indicated by the trend-level finding in the left caudate nucleus, in which regional GM volume in women with CAIS showed a non-significant deviation from control women in the direction of the male control group with a medium effect size. Thus, our findings from the univariate analysis support the hypothesis that sex differences in regional GM volume in the right pre- and postcentral gyrus predominantly reflect sex hormone effects, more specifically androgenic masculinizing and/or estrogenic feminizing effects, and potential effects related to female-typical socialization. In other sexually differentiated brain regions we cannot rule out a direct role for sex chromosome genes or masculinizing effects of androgens that are not mediated by the androgen receptor.

Results from the MPR analyses provide further insight into the factors involved in the sexual differentiation of GM volume. Although control men and women could be successfully discriminated from each other based on the whole-brain GM volume distribution, the classification of women with CAIS into either of the control groups was not above chance level. The absolute classification resulted in a small majority of women with CAIS receiving a class prediction for the class representing control men. However, the classification was based on a binary decision process, driven by individual class membership probability scores. These individual probability scores for women with CAIS did not differ significantly from 0.5, which indicates that women with CAIS were equally likely to belong to the class representing control men as to the class representing control women based on spatially distributed patterns of GM volume. Further, both control groups showed class membership probabilities in the direction of their own class, i.e., a high probability for control women and a low probability for control men when using the class representing control women as the reference class, but this probability score for women with CAIS was significantly in between that of the control groups.

When the MPR analysis was repeated while only using GM volume information from sexually differentiated brain regions, there was an increased classification performance for

the control groups. The individual class membership probabilities underlying the binary classification significantly increased in control women and decreased in control men when using the class representing control women as the reference class. These improved classification results in the control groups were expected based on the selected regions, which only include voxels showing a sex difference in GM volume. The classification of women with CAIS based on spatially distributed GM volume information within these regions was, however, unchanged. Although the absolute number of women with CAIS receiving a class prediction for the class representing control men was reduced, the probability score underlying this binary classification process was still close to 0.5 and was unchanged relative to the whole-brain classification procedure. If effective androgen exposure is the prominent factor underlying the spatially distributed GM volume patterns that resulted in the accurate discrimination of control men and women, the majority of women with CAIS would be in the class representing control women. In addition, this classification would be based on probability scores resembling those in control women. However, the classification results in women with CAIS, both whole-brain and within sex difference regions, neither resembled the female nor the male control subjects. These findings argue against a dominant role for one factor in the overall sexual differentiation of GM volume, and rather show that this is more likely the result of a combination of influences.

A limitation of the present study is the absence of brain regions in which the GM volume was found to be larger in control men than in control women, while this has been reported previously (Ruigrok et al. 2014). This prevented us from analyzing GM volume in women with CAIS relative to both control groups in these specific regions. Nevertheless, this limitation was balanced by the strength of the whole-brain MPR analysis, allowing us to detect differences between groups with a higher sensitivity than the conventional univariate approach. Therefore, subtle sex differences that might not have been detected using the mass-univariate analysis may have contributed to the classifier function that was able to successfully discriminate control men from control women.

Taken together, the present results from univariate and multivariate analyses of GM volume in women with CAIS demonstrate that complete androgen resistance in the presence of a 46,XY karyotype results in a female-typical regional GM volume in the right pre- and postcentral gyrus, while the overall pattern of regional GM volume throughout the brain is neither predominantly female- nor male-typical. This indicates that the sexual differentiation of regional GM volume is not only under the influence of masculinizing androgenic effects, feminizing estrogenic effects and/or effects related to female-typical socialization, as observed in the right pre- and postcentral gyrus. Instead, these findings suggest that sex differences in GM volume develop under the influence of a more complex combination of factors. These factors might include the sex hormone and/or socialization-related effects as described with regard to the right pre- and postcentral gyrus, as well as direct effects of genes carried on the sex chromosomes and/or masculinizing androgen effects that are not

mediated by the androgen receptor. It is important to note that the pattern we observed could reflect multiple influences acting on all regions, or different influences acting on different regions (e.g., androgen effects on some regions, sex chromosome gene effects on others). Thus, the relative influence of these factors might vary throughout different brain regions.

REFERENCES

Ankney CD. 1992. Sex differences in relative brain size: The mismeasure of woman, too? Intelligence. 16:329–336.

Ashburner J. 2007. A fast diffeomorphic image registration algorithm. Neuroimage. 38:95–113.

Ashburner J, Friston KJ. 2000. Voxel-based morphometry--the methods. Neuroimage. 11:805–821.

Bao A-M, Swaab DF. 2011. Sexual differentiation of the human brain: relation to gender identity, sexual orientation and neuropsychiatric disorders. Front Neuroendocrinol. 32:214–226.

Baum M. 1979. Differentiation of coital behavior in mammals: a comparative analysis. Neurosci Biobehav Rev. 3:265–284.

Baum MJ. 2006. Mammalian animal models of psychosexual differentiation: When is 'translation' to the human situation possible? Horm Behav. 50:579–588.

Berenbaum SA, Beltz AM. 2011. Sexual differentiation of human behavior: effects of prenatal and pubertal organizational hormones. Front Neuroendocrinol. 32:183–200.

Bramen JE, Hranilovich JA, Dahl RE, Forbes EE, Chen J, Toga AW, Dinov ID, Worthman CM, Sowell ER. 2011. Puberty influences medial temporal lobe and cortical gray matter maturation differently in boys than girls matched for sexual maturity. Cereb Cortex. 21:636–646.

Chen X, Sachdev PS, Wen W, Anstey KJ. 2007. Sex differences in regional gray matter in healthy individuals aged 44-48 years: a voxel-based morphometric study. Neuroimage. 36:691–699.

Cohen-Bendahan CCC, Van De Beek C, Berenbaum SA. 2005. Prenatal sex hormone effects on child and adult sex-typed behavior: Methods and findings. Neurosci Biobehav Rev. 29:353–384.

Cox KH, Bonthuis PJ, Rissman EF. 2014. Mouse model systems to study sex chromosome genes and behavior: relevance to humans. Front Neuroendocrinol. 35:405–419.

Davis SM, Rogol AD, Ross JL. 2015. Testis Development and Fertility Potential in Boys with Klinefelter Syndrome. Endocrinol Metab Clin North Am. 44:843–865.

De Bondt T, Jacquemyn Y, Van Hecke W, Sijbers J, Sunaert S, Parizel PM. 2013. Regional gray matter volume differences and sex-hormone correlations as a function of menstrual cycle phase and hormonal contraceptives use. Brain Res. 1530:22–31.

Den Braber A, Van't Ent D, Stoffers D, Linkenkaer-Hansen K, Boomsma DI, De Geus EJC. 2013. Sex differences in gray and white matter structure in age-matched unrelated males and females and opposite-sex siblings. Int J Psychol Res. 6:7–21.

Doehnert U, Bertelloni S, Werner R, Dati E, Hiort O. 2015. Characteristic features of reproductive hormone profiles in late adolescent and adult females with complete androgen insensitivity syndrome. Sex Dev. 9:69–74.

Feis D-L, Brodersen KH, von Cramon DY, Luders E, Tittgemeyer M. 2013. Decoding gender dimorphism of the human brain using multimodal anatomical and diffusion MRI data. Neuroimage. 70:250–257.

Filipek PA, Richelme C, Kennedy DN, Caviness VS. 1994. The Young Adult Human Brain: An MRI-based Morphometric Analysis. Cereb Cortex. 4:344–360.

Franke K, Hagemann G, Schleussner E, Gaser C. 2015. Changes of individual BrainAGE during the course of the menstrual cycle. Neuroimage. 115:1–6.

Goldstein JM, Seidman LJ, Horton NJ, Makris N, Kennedy DN, Caviness Jr. VS, Faraone S V, Tsuang MT. 2001. Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. Cereb Cortex. 11:490–497.

Good CD, Johnsrude I, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. 2001a. Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. Neuroimage. 14:685–700.

Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. 2001b. A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage. 14:21–36.

Hagemann G, Ugur T, Schleussner E, Mentzel H-J, Fitzek C, Witte OW, Gaser C. 2011. Changes in brain size during the menstrual cycle. PLoS One. 6:e14655.

Hamann S, Stevens J, Vick JH, Bryk K, Quigley CA, Berenbaum SA, Wallen K. 2014. Brain responses to sexual images in 46,XY women with complete androgen insensitivity syndrome are female-typical. Horm Behav. 66:724–730.

Herting MM, Gautam P, Spielberg JM, Kan E, Dahl RE, Sowell ER. 2014. The role of testosterone and estradiol in brain volume changes across adolescence: a longitudinal structural MRI study. Hum Brain Mapp. 35:5633–5645.

Hines M. 2010. Sex-related variation in human behavior and the brain. Trends Cogn Sci. 14:448–456.

Hines M, Ahmed SF, Hughes IA. 2003. Psychological outcomes and gender-related development in complete androgen insensitivity syndrome. Arch Sex Behav. 32:93–101.

Lentini E, Kasahara M, Arver S, Savic I. 2012. Sex Differences in the Human Brain and the Impact of Sex Chromosomes and Sex Hormones. Cereb Cortex. 23:2322–2336.

Leonard CM, Towler S, Welcome S, Halderman LK, Otto R, Eckert MA, Chiarello C. 2008. Size matters: cerebral volume influences sex differences in neuroanatomy. Cereb Cortex. 18:2920–2931.

Lessov-Schlaggar CN, Reed T, Swan GE, Krasnow RE, DeCarli C, Marcus R, Holloway L, Wolf PA, Carmelli D. 2005. Association of sex steroid hormones with brain morphology and cognition in healthy elderly men. Neurology. 65:1591–1596.

Lombardo M V, Ashwin E, Auyeung B, Chakrabarti B, Taylor K, Hackett G, Bullmore ET, Baron-Cohen S. 2012. Fetal testosterone influences sexually dimorphic gray matter in the human brain. J Neurosci. 32:674–680.

Luders E, Gaser C, Narr KL, Toga AW. 2009. Why sex matters: brain size independent differences in gray matter distributions between men and women. J Neurosci. 29:14265–14270.

Masica DN, Money J, Ehrhardt AA. 1971. Fetal feminization and female gender identity in the testicular feminizing syndrome of androgen insensitivity. Arch Sex Behav. 1:131–142.

McCarthy MM, Arnold AP. 2011. Reframing sexual differentiation of the brain. Nat Neurosci. 14:677–683.

Melo KFS, Mendonca BB, Billerbeck AEC, Costa EMF, Inácio M, Silva FAQ, Leal AMO, Latronico AC, Arnhold IJP. 2003. Clinical, hormonal, behavioral, and genetic characteristics of androgen insensitivity syndrome in a Brazilian cohort: five novel mutations in the androgen receptor gene. J Clin Endocrinol Metab. 88:3241–3250.

Merke DP, Fields JD, Keil MF, Vaituzis AC, Chrousos GP, Giedd JN. 2003. Children with classic congenital adrenal hyperplasia have decreased amygdala volume: potential prenatal and postnatal hormonal effects. J Clin Endocrinol Metab. 88:1760–1765.

Mueller SC, Merke DP, Leschek EW, Fromm S, VanRyzin C, Ernst M. 2011. Increased medial temporal lobe and striatal grey-matter volume in a rare disorder of androgen excess: a voxel-based morphometry (VBM) study. Int J Neuropsychopharmacol. 14:445–457.

Neufang S, Specht K, Hausmann M, Güntürkün O, Herpertz-Dahlmann B, Fink GR, Konrad K. 2009. Sex differences and the impact of steroid hormones on the developing human brain. Cereb Cortex. 19:464–473.

Oldfield RC. 1971. The assessment and analysis of handedness: The Edinburgh inventory. Neuropsychologia. 9:97–113.

Peper JS, Brouwer RM, Schnack HG, van Baal GC, van Leeuwen M, van den Berg SM, Delemarre-Van de Waal HA, Boomsma DI, Kahn RS, Hulshoff Pol HE. 2009. Sex steroids and brain structure in pubertal boys and girls. Psychoneuroendocrinology. 34:332–342.

Peters M. 1991. Sex differences in human brain size and the general meaning of differences in brain size. Can J Psychol. 45:507–522.

Phoenix CH, Goy RW, Gerall AA, Young WC. 1959. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology. 65:369–382.

Pletzer B, Kronbichler M, Aichhorn M, Bergmann J, Ladurner G, Kerschbaum HH. 2010. Menstrual cycle and hormonal contraceptive use modulate human brain structure. Brain Res. 1348:55–62.

Protopopescu X, Butler T, Pan H, Root J, Altemus M, Polanecsky M, McEwen B, Silbersweig D, Stern E. 2008. Hippocampal structural changes across the menstrual cycle. Hippocampus. 18:985–988.

Rijpkema M, Everaerd D, van der Pol C, Franke B, Tendolkar I, Fernández G. 2012. Normal sexual dimorphism in the human basal ganglia. Hum Brain Mapp. 33:1246–1252.

Ruigrok AN V, Salimi-Khorshidi G, Lai M-C, Baron-Cohen S, Lombardo M V, Tait RJ, Suckling J. 2014. A meta-analysis of sex differences in human brain structure. Neurosci Biobehav Rev. 39:34–50.

Savic I, Arver S. 2011. Sex dimorphism of the brain in male-to-female transsexuals. Cereb Cortex. 21:2525–2533.

Schrouff J, Rosa MJ, Rondina JM, Marquand AF, Chu C, Ashburner J, Phillips C, Richiardi J, Mourão-Miranda J. 2013. PRoNTo: pattern recognition for neuroimaging toolbox. Neuroinformatics. 11:319–337.

Schulz KM, Molenda-Figueira H a, Sisk CL. 2009. Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. Horm Behav. 55:597–604.

Sisk CL, Zehr JL. 2005. Pubertal hormones organize the adolescent brain and behavior. Front Neuroendocrinol. 26:163–174.

Smith SM. 2002. Fast robust automated brain extraction. Hum Brain Mapp. 17:143–155.

Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazy RK, Saunders J, Vickers J, Zhang Y, De Stefano N, Brady JM, Matthews PM. 2004. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 23 Suppl 1:S208-19.

Tang T, Jiao Y, Wang X, Lu Z. 2013. Gender versus brain size effects on subcortical gray matter volumes in the human brain. Neurosci Lett. 556:79–83.

van Hemmen J, Saris IMJ, Cohen-Kettenis PT, Veltman DJ, Pouwels PJW, Bakker J. 2016. Sex Differences in White Matter Microstructure in the Human Brain Predominantly Reflect Differences in Sex Hormone Exposure. Cereb Cortex.

van Hemmen J, Veltman DJ, Hoekzema E, Cohen-Kettenis PT, Dessens AB, Bakker J. 2016. Neural Activation During Mental Rotation in Complete Androgen Insensitivity Syndrome: The Influence of Sex Hormones and Sex Chromosomes. Cereb Cortex. 26:1036–1045.

van Strien JW. 1992. Classificatie van links- en rechtshandige proefpersonen. / Classification of left-handed and right-handed test subjects. Ned Tijdschr Psychol. 47:88–92.

Wallen K. 2005. Hormonal influences on sexually differentiated behavior in nonhuman primates. Front Neuroendocrinol. 26:7–26.

Wallen K. 2009. The Organizational Hypothesis: Reflections on the 50th anniversary of the publication of Phoenix, Goy, Gerall, and Young (1959). Horm Behav. 55:561–565.

Wang L, Shen H, Tang F, Zang Y, Hu D. 2012. Combined structural and resting-state functional MRI analysis of sexual dimorphism in the young adult human brain: an MVPA approach. Neuroimage. 61:931–940.

Welborn BL, Papademetris X, Reis DL, Rajeevan N, Bloise SM, Gray JR. 2009. Variation in orbitofrontal cortex volume: relation to sex, emotion regulation and affect. Soc Cogn Affect Neurosci. 4:328–339.

Wisniewski AB, Migeon CJ, Meyer-Bahlburg HF, Gearhart JP, Berkovitz GD, Brown TR, Money J. 2000. Complete androgen insensitivity syndrome: long-term medical, surgical, and psychosexual outcome. J Clin Endocrinol Metab. 85:2664–2669.

Witte AV, Savli M, Holik A, Kasper S, Lanzenberger R. 2010. Regional sex differences in grey matter volume are associated with sex hormones in the young adult human brain. Neuroimage. 49:1205–1212.

Zahn-Waxler C, Crick NR, Shirtcliff E a, Woods KE. 2006. The origins and development of psychopathology in females and males. In: Cicchetti D,, Cohen DJ, editors. Developmental psychopathology, Vol 1: Theory and method. 2nd ed. Hoboken, NJ, USA: John Wiley & Sons, Inc. p. 76–138.