Cerebral Cortex December 2014;24:3246–3257
doi:10.1093/cercor/bht180
Advance Access publication August 7, 2013

Sex Differences in Cortical Thickness and Their Possible Genetic and Sex Hormonal Underpinnings

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Although it has been shown that cortical thickness (Cth) differs between sexes, the underlying mechanisms are unknown. Seeing as XXY males have 1 extra X chromosome, we investigated the possible effects of X- and sex-chromosome dosage on Cth by comparing data from 31 XXY males with 39 XY and 47 XX controls. Plasma testosterone and estrogen were also measured in an effort to differentiate between possible sex-hormone and sex-chromosome gene effects. Cth was calculated with FreeSurfer software. Parietal and occipital Cth was greater in XX females than XY males. In these regions Cth was inversely correlated with *z*-normalized testosterone. In the motor strip, the cortex was thinner in XY males compared with both XX females and XXY males, indicating the possibility of an X-chromosome gene-dosage effect. XXY males had thinner right superior temporal and left middle temporal cortex, and a thicker right orbitofrontal cortex and lingual cortex than both control groups. Based on these data and previous reports from women with XO monosomy, it is hypothesized that programming of the motor cortex is influenced by processes linked to X-escapee genes, which do not have Y-chromosome homologs, and that programming of the superior temporal cortex is mediated by X-chromosome escapee genes with Y-homologs.

Keywords: brain, cortical thickness, Klinefelter's syndrome, MRI, sex chromosome, sex dimorphism, sex hormone

Introduction

Advances in imaging methods have provided new insights into the sex differences of the human brain. Sex differences have been shown in subcortical volumes (Filipek et al. 1994; Raz et al. 1995; Murphy et al. 1996; Paus et al. 1996; Giedd et al. 1997, 2006; Neufang et al. 2009), as well as in the regional volumes of gray matter (GM) and white matter (WM). With some exceptions (Goldstein et al. 2001; Carne et al. 2006), studies show that men have larger GM volumes in the mesial temporal lobe, the cerebellum, and the lingual gyrus (Good et al. 2001; Carne et al. 2006; Savic and Arver 2011; Lentini et al. 2012), whereas women have larger GM volumes in the precentral gyrus, the orbitofrontal and anterior cingulate cortices, and the right inferior parietal cortex (Nopoulos et al. 2000; Good et al. 2001; Luders et al. 2005; Luders, Gaser et al. 2009; Luders, Sanchez et al. 2009; Savic and Arver 2011; Lentini et al. 2012). These sex differences are believed to derive from specific processes that shape brain morphology during development. To unravel what underlies these processes is extremely important, especially considering that the vast majority of neuropsychiatric disorders have a skewed sex distribution with regard to age at onset, symptom presentation, and prevalence (Swaab 2007; Fernandez-Guasti et al. 2012); identifying the possible sex-specific protective agents could contribute to the development of potential therapies.

In a recent study using voxel-based morphometry, we found that some of the abovementioned sex differences in regard to GM volumes involved sex-chromosome genes whereas others seemed to be influenced by sex hormones (Lentini et al. 2012). That sex-chromosome genes could be involved in sexual dimorphism of the brain is both compelling and interesting, given that several cerebral disorders are linked to X-chromosome genes (polymicrogyria, Retts syndrome, fragile X, X-linked lissencephaly). GM volume is, however, a composite and rather unspecific metric, and several recent studies show that the 2 lower order measures of GM volume, cortical thickness, and surface area (SA), are better related to genetic processes and, therefore, more appropriate to study when trying to investigate factors shaping sex differences in cortical morphology (Kruggel et al. 2003; Sowell et al. 2003). Cth and SA have differing evolutionary histories (Rakic 1988, 1995), developmental trajectories (Armstrong et al. 1995; Sowell et al. 2007) and genetic determinants, and are differentially influenced by neuronal migration (Panizzon et al. 2009). In regard to the possible sex dimorphism of the brain and its underpinnings, it seems particularly interesting to study Cth, because Cth shows sex differences in certain regions (Luders et al. 2005; Im et al. 2006; Luders, Narr, Thompson, Rex, Jancke et al. 2006; Sowell et al. 2007; Lv et al. 2010), seems to correlate with behavioral measures (Schilling et al. 2012), and is reported to better identify genetic influences than GM volume or SA (Winkler et al. 2010).

In the present study, we set out to investigate whether we could reproduce the previously described sex differences regarding Cth, whether such differences are related to sex hormone levels and/or sex-chromosome gene dosage, and if the tentative hormonal and genetic effects are regionally different. These questions were addressed by comparing the Cth of males with Klinefelter's syndrome (47,XXY), the most common form of sex-chromosome aneuploidy, (Jacobs et al. 1988), with the Cth of 46,XY male and 46,XX female controls. The study included measurements of plasma estradiol and testosterone as well as measures of the ratio between the length of the second and fourth digits (2D:4D ratio), which, according to several studies, may serve as a proxy for fetal testosterone (Manning et al. 1998, 2004; Williams et al. 2000; Lutchmaya et al. 2004; Honekopp et al. 2007; Coates et al. 2009; Honekopp and Watson 2010). We recently reported that this type of experimental design lends itself well to investigations of the possible effects of sex hormones and sex chromosomes on cerebral tissue (Lentini et al. 2012).

Individuals with Klinefelter's syndrome are born with one or more extra X chromosomes. Their phenotype is characterized by hypogonadism, and their testosterone levels are usually normal or subnormal during the prenatal period and up to puberty (Carson et al. 1982; Ratcliffe et al. 1994) before becoming drastically reduced (Aksglaede et al. 2006) and calling for testosterone supplementation. The gender roles of 47,XXY males do not differ from other males; their identity is male, and the majority of them are heterosexual. 47,XXY males generally have impaired executive and language functions, and their IQ is in the normal to subnormal range (van Rijn et al. 2008). Structural changes in the brain have been consistently found in studies. They are characterized by reductions in the volume of the hippocampus, amygdala, and caudate, reductions in the GM volume of the superior temporal gyrus, insular cortex, and cerebellum, and increases in the GM volume of the parietal cortex and the precentral gyrus (Giedd et al. 2006; Savic 2012) (Patwardhan et al. 2002; Giedd et al. 2007; van Rijn et al. 2008; Lenroot et al. 2009; Bryant et al. 2011; Lentini et al. 2012). When it comes to Cth, there are, however, to the best of our knowledge, only 2 publications concerning 47,XXY males (Giedd et al. 2007; Lenroot et al. 2009), both involving adolescents. Neither of them included comparisons with both male and female subjects, or specifically addressed possible sex-chromosome gene dosage effects on Cth. Yet, such comparisons would not only be of great value when trying to understand the possible impact of sex chromosomes on the brain in general, and could also shed more light on the underpinnings of the reported sex differences in cortical morphology in particular. In addition, they could provide chromosome-gene dosage-structure correlates to the behavioral and cognitive impairments in men with Klinefelter's syndrome.

In the somatic cells of 46,XX females, 1 of the 2 X chromosomes is randomly inactivated. However, about 15% of X-linked genes escape this process, and are denoted as escapee genes. In 46,XX females these genes will be expressed from both X chromosomes, whereas they are only expressed from 1 X chromosome in 46,XY males. Because only a few of the escapee genes have homologs on the Y-chromosome (Xu et al. 2002; Xu and Disteche 2006), a major portion of X-linked escapee genes will be expressed in excess in 46,XX females compared with 46,XY males, and potentially play an important role in sexual differentiation.

Based on this information, we expected to find differences in Cth between 47,XXY males and controls, and hypothesized that these differences would be primarily related to 2 types of genetic mechanisms.

1. First, in 47,XXY males an excessive expression of genes that lie in the pseudoautosomal regions of the X chromosome could occur. Some of these X-escapee genes have active Y-chromosome homologues and may, consequently, be expressed in a higher dose in XXY males than in both XX and XY controls, potentially leading to differences in Cth in comparison to both control groups.
2. Second, like 46,XX females, 47,XXY males also have X-escapee genes that do not have Y-chromosome analogues, although the exact percentage of these genes has not been determined in 47,XXY males (Vawter et al. 2007). These genes should be expressed in excess in 47,XXY males only in relation to 46,XY males but not in relation to 46,XX females.

Thus, provided that chromosome-linked gene dosage processes are associated with cortical thickness, certain differences were expected only in relation to 46,XY males, while other differences were expected in relation to both control populations (see also Supplementary Table S1). We hypothesized that:

1. Differences in overall and/or regional Cth between 47,XXY and 46,XY males, which are also found between 46,XX females and 46,XY males, would be related to the genes located on the extra X chromosome.
2. Differences in Cth between 47,XXY males and both control groups would, on the other hand, be associated with trisomy (3 sex chromosomes), or represent downstream effects of this type of sex-chromosome aneuploidy.
3. Finally, we hypothesized that values in 47,XXY males which are found to be between those of 46,XX females and 46,XY males, and are detected in regions which differ between 46,XX women and 46,XY men, could be attributed to the fact that 47,XXY males prior to testosterone treatment have testosterone levels that are slightly lower than in 46,XY boys, and, thus, between those of 46,XX females and 46,XY males; furthermore, the testosterone supplement, which in the majority of our subjects was prescribed postpuberty (see further), might not provide a full compensatory effect with regard to Cth.

The population consisted of 31 XXY males (age 39 ± 11 years, range 21–50 years, education 13 ± 3 years), 39 XY males (age 35 ± 7 years, range 25–50 years, education 16 ± 2 years), and 47 XX females (age 35 ± 7 years, range 22–50 years, education 16 ± 2 years). The XXY males were recruited from the Center of Andrology, Department of Medicine, Karolinska University Hospital, Sweden; controls were recruited from the general public. Exclusion criteria included being outside the age range of 20–50 years; having karyotypic mosaicism; heredity for, history of, or current psychosis; a personality disorder; major or bipolar depression; alcohol or substance abuse problems, and a neurological disease. Because our primary purpose was to evaluate the possible effects of X- and sex-chromosome dosage on cortical sex differences, 47,XXY males with co-morbid autism and ADHD were also excluded. Mild dyslexia was present in about 60% of the patients.

All subjects underwent a medical examination at the screening visit, including an evaluation of their medical history and routine laboratory tests. Co-morbid psychiatric disorders or personality disturbances were assessed according to the Diagnostic and Statistical Manual of the American Psychiatric Association, 4th Edition (DSM–IV; 13) by a specially trained psychiatrist. This assessment used the Structured Questionnaire for DSM-IV® Axis I and II (Structured Clinical Interview for DSM-IV® (SCID-I and II) (American Psychiatric Publishing, Inc., Arlington 1997) as well as scores for depression (Beck Depression Inventory scores (Beck 1961).

Twenty-nine of the 47,XXY males were diagnosed in adolescence, while 2 were diagnosed as adults during the course of infertility investigations. All but 2 (who were deemed to not need testosterone) had been receiving testosterone supplementation subsequent to the diagnosis and were in treatment at the time of the study. The duration of substitution therapy varied from 0 to 23 years, and the age at institution of testosterone therapy ranged from 15 to 40 years. The

karyotyping to confirm Klinefelter's syndrome was performed by assessing the metaphase chromosomes in cells derived from whole blood according to standard procedure. All of the participants were right-handed [Edinburgh Handedness Inventory, (Oldfield 1971). All were heterosexual (scored Kinsey 0) according to Kinsey's Heterosexual/Homosexual Scale, (Kinsey et al. 2003; Oldfield 1971).

MR Acquisition Protocol

The magnetic resonance imaging data were acquired on a whole-body 1.5-Tesla General Electric Signa Echospeed (Milwaukee, WI, USA) MRI medical scanner equipped with an 8-channel phased array receiver coil. Three-dimensional spoiled gradient recalled acquisition was used (effective TE = 6 ms, TR = 21 ms, bandwidth 31.25 kh, FOV = 24 cm, voxel size 0.94 × 0.94 × 1.2 mm3, 156 slices. 2D T_2-weighted fast spin echo images were also acquired, (the axial plane; effective echo time = 56 ms, repetition time = 2500 ms, flip angle = 45°, field of view = 24 cm, 23 slices of 3 mm thickness, number of excitations 1), but were not used for analyses of Cth. A clinical neuroradiologist evaluated all scans for gross abnormalities.

Finger Ratios

The 2D:4D ratios of both hands were measured using a steel vernier caliper. Measurements were carried out directly on the fingers, on the ventral side of the hand between the basal crease and the fingertip (Manning et al. 1998, 2004; Lutchmaya et al. 2004; Manno 2008; Ciumas et al. 2009). The 2D:4D ratios of 15 subjects were independently measured by 2 raters, and the inter-rater correlation was calculated with linear regression (Pearson's coefficient, $P < 0.05$).

Venous Blood Samples

Venous blood samples were collected from the controls as well as the patients between 8 and 10 a.m. in the morning. Plasma testosterone levels (nmol/L), 17β-estradiol (pmol/L) (radioimmunoassay, Testosterone RIA DSL-4000, Diagnostic Systems Laboratory, Inc., TX, USA), and the sex hormone binding globulin (SHBG) were analyzed in the Chemical Diagnostics Laboratory at the Karolinska University Hospital. The levels of bioavailable testosterone (nmol/L) were calculated using an equation developed by Sodergard et al. (1982). To avoid bias due to being in the menstrual cycle phase, women were measured in the mid-follicular phase, during which levels of 17β-estradiol remain relatively stable and low. Before conducting the statistical analyses of possible correlations between hormone levels and Cth, the individual levels of 17β-estradiol and active testosterone were z-transformed within each sex group, because natural sex differences in plasma testosterone levels and 17β-estradiol (pmol/L) could lead to false correlations—considering that the respective hormone values would be located at the 2 extremes of the correlation slope (see below).

Surface-Based Image Processing

The MR volumes were processed using the default parameters of FreeSurfer software version 5.0 (www.surfer.nmr.mgh.harvard.edu). To calculate the surface-based anatomical measures, models of WM and GM surfaces were reconstructed from MR volumes. The thickness was then measured as the distance between the WM and the GM surfaces at each vertex. The vertices were arranged in ~1 mm spacing, which allows measuring cortical thickness at up to 160 000 vertices in each hemisphere with submillimeter precision. Before the surfaces were reconstructed, several preprocessing steps were done (Fischl and Dale 2000). First, the volume was registered to the Talairach atlas using affine transformation. Second, to bring about intensity normalization, the bias field was computed and used to correct intensity variability across the image caused by radiofrequency field inhomogenieties and susceptibility artifacts. Third, the skull (nonbrain) tissue was removed by deforming a tessellated ellipsoidal template into the shape of the inner surface of the skull. Fourth, the WM hemispheres were constructed; during this procedure, the overlap in intensities between WM and GM was taken into account as well as the fact that the WM/GM borders should be planar due to the laminar structure of the cortex. Fifth, the cutting planes were chosen in such a way as to separate the hemispheres from each other as well as to remove the cerebellum and brain stem. Finally, the WM surface was generated by covering the filled WM hemisphere with triangles (tessellation) and smoothing it to follow the intensity gradients between WM and GM; then the GM (pial) surface was generated by expanding the WM surface to follow the intensity gradients between the GM and CSF.

Manual Corrections

The reconstruction of the MRI images was visually inspected at several stages: after the Talairach transformation, after the skull striping, and finally, after the surfaces had been built and the volumes labeled. At each of these stages, the necessary manual corrections were made, including correcting erroneous skull striping by adjusting watershed parameters or by manually editing out the skull tissue, and adding control points to normalize intensity for erroneous WM surface reconstruction.

Statistical Analysis

The statistical analyses were carried out to address several specific issues.

1. The first was to determine whether there were any overall or regional differences between the Cth of the male and female controls.
2. The next step was to check whether any of these differences were correlated to plasma testosterone, plasma estradiol, 2D:4D ratio (proxy of fetal testosterone) or a combination of these factors.
3. The data from the 47,XXY males was then included in the analysis to determine if there were any differences between this group and controls (the 46,XY males and the 46,XX females). The clusters that the 47,XXY males had in common with the 46,XX females but not with the 46,XY males were assumed to primarily reflect an effect of the X-chromosome genes, provided that Cth in these clusters was not correlated with plasma testosterone. Those clusters, which overlapped with clusters showing a significant correlation with testosterone and also showed difference between male and female controls (see b), were assumed to primarily reflect effects of the low testosterone, as occurs in 46,XX females, and 47,XXY males during important periods of brain development (Aksglaede et al. 2006). Prior to treatment, testosterone levels in 47,XXY males are between those of 46,XX females and 46,XY males.

4. Finally, we tested whether there were any regions in which the Cth of 47,XXY males differed from the corresponding values for both 46,XY males and 46,XX females. It was assumed that any significantly different Cth value in a cluster that was found in relation to both control groups would primarily be attributable to 47,XXY males having 3 rather than 2 sex chromosomes. The individual Cth values for these specific regions were extracted to also investigate whether these values in 47,XXY males were in between those of the control groups, which might suggest an alternative (or additional) testosterone effect (Tables 2–4).

Group Comparisons in Mean Hemispheric Cth and Possible Effects of Sex Hormones and Sex Chromosomes

Possible differences in the mean cortical thicknesses of the left (L) and right (R) hemispheres among the 3 groups were tested with 2 separate 1-way ANOVA analyses, using IBM Statistical Package for Social Sciences software (SPSS Statistics) Version 20 ($P < 0.025$ with Bonferroni correction). R/L asymmetry among each group was tested with paired *t*-tests ($P < 0.05$). Possible group differences in R/L asymmetry of the mean hemispheric Cth were then calculated using 1-way ANOVA with group as the between factor and the R/L ratio as the input measure (within factor) ($P < 0.05$).

The possible effects of circulating hormones and digit ratio on "mean hemispheric Cth" and on R/L hemisphere asymmetry in Cth were calculated in SPSS using partial correlations with age as covariate of no interest ($P < 0.016$, due to the 3 separate comparisons: R hemisphere Cth, L hemisphere Cth, and R/L hemisphere asymmetry). Correlation analyses including circulating hormones were carried out only among the control groups, since the hormone levels of 47,XXY males were biased by their ongoing testosterone treatments, and were therefore unlikely to reflect possible prepubertal or pubertal effects on Cth (which could be assumed for controls). The individual values for 46,XY males and 46,XX females were *z*-normalized for each group. Linear regression with z-testosterone and z-estradiol was utilized since, according to some previous publications, it best describes the effects of sex hormones on Cth (Lange, Brain Development Cooperative group, (Nguyen et al. 2013). Considering that testosterone, according to some reports, may affect the brain hemispheres differently depending on gender (Bramen et al. 2012; Nguyen et al. 2013), linear regression using sex hormone values and hemispheric Cth values was also carried out separately among 46,XY males and 46,XX females, ($P < 0.013$; Bonferroni correction for 4 separate comparisons: regressions with testosterone and estradiol in both men and women).

Group Comparisons of Regional Cth and of the Possible Effects of Sex Hormones and Sex Chromosomes

Group comparisons of regional Cth were performed through explorative (vertex-by-vertex) analysis using the General linear model (GLM). In preprocessing, each subject's data was resampled into a common space (provided by FreeSurfer). Spatial smoothing of 10 mm FWHM was applied, considering the size of investigated population and the expected group differences (Lerch and Evans 2005, Lenroot et al. 2009).

The differences among the 3 groups (47,XXY males, 46,XY males, 46,XX females) were evaluated for each vertex using age as the nuisance variable. A different-offset-same-slope model was used (the slope of the line that represents thickness as a function of age was set to be the same for groups compared, but the offset of these 2 lines was allowed to vary during fitting to the data). Within-group analyses were carried out using the same-offset-same-slope model. Correction for multiple comparisons was performed using Monte Carlo simulation, which generates random noise fields and detects the clusters that appear at specific size and probability thresholds. In our study, after 5000 of such iterations, a frequency of how often a simulated cluster's value exceeded the value from the true data analysis was computed, and this frequency was used to indicate the results which were significant at the level corrected for multiple comparisons (here set to $P < 0.05$).

To investigate whether there were common differences between 2 groups in relation to the third, conjunctional analyses were carried out. For these analyses, the method called Minimum Statistic compared with the Global Null (MS/GN) was used. This method, while testing the conjunction of 2 comparisons, A and B, allows for the conclusion that there is an effect in both A and B (Nichols et al. 2005). As we assumed that 47,XXY males would show a "female" pattern, the *p* threshold was set at <0.05 uncorrected for the XX–XY and XXY–XY conjunction, and $P < 0.05$ corrected (Monte Carlo simulation) was used for the remaining conjunction analyses.

Next, we investigated whether there was "a regional" effect of circulating hormones and right-hand 2D:4D ratios by means of multivariate analyses. The individual values for 46,XY males and 46,XX females were *z*-normalized for each group. Z-transformed testosterone and estradiol and the right-hand 2D:4D ratio were each used as a covariate of interest to examine how the respective correlations with Cth in each vertex differed between groups (one calculation for each factor), age was nuisance variable. We employed linear regression. Based on previous reports about the effects of sex hormones on Cth (Bramen et al. 2011; Nguyen et al. 2013), it was hypothesized that a correlation between Cth and sex hormone levels, as well as the D2:D4 ratio would be found primarily in regions in which Cth differed between 46,XY males and 46,XX females. The significance level for clusters, which overlapped with those showing differences between 46,XY and 46,XX controls, was, therefore, $P < 0.05$ uncorrected, whereas a threshold of $P < 0.05$ corrected was employed for the remaining brain regions. Correlation analyses with z-estradiol and z-testosterone were carried out only among controls, as the corresponding hormone levels in 47,XXY males were biased by testosterone treatment.

Results

Population Demographics and Finger Ratios

The demographical data are presented in Table 1. None of the subjects was deemed to suffer from a co-morbid psychiatric disorder or personality disturbance. Beck Depression Inventory score was 8 ± 8 for the 47,XXY males, 2 ± 2 for the 46,XY males, and 5 ± 3 for the 46,XX females. Thus, all the 3 subject groups had values within the normal range. No significant group differences were found in respect to age ($P = 0.057$, $F = 2.93$). The groups differed with regard to years of education ($P < 0.0001$, $F = 14.1$, df = 2), as the education level was lower among the 47,XXY males compared with both control groups; there was no such difference between 46,XX females and 46,

XY males (XX vs. XY $P = 0.910$; XX vs. XXY and XY vs. XXY, $P < 0.0001$). No group differences were found in regard to handedness or sexual orientation, and no gross anatomical abnormalities were found according to an experienced neuroradiologist.

Measures of digit ratios, carried out by 2 raters, were highly correlated ($r = 0.9$; $P < 0.001$). The results presented here were based on measurements from rater 1, as rater 2 performed ratings for only 15 subjects. A significant group difference was found for the 2D:4D ratio of the right hand ($P = 0.015$, $F = 4.5$, df = 2) but not the left hand ($P = 0.460$, $F = 0.8$, df = 2). 46,XX females and 47,XXY males showed higher ratios than 46,XY males without showing any significant difference between each other (Table 1).

Table 1
Demographic data

	46, XX females (N47)	46, XY males (N39)	46, XXY males (N31)	*F*-value
Age (years)	35 ± 7	35 ± 7	39 ± 11	2.9
Education (years)	16 ± 2	16 ± 2	13 ± 3	14.1***
Right D2:D4[a] (ratio)	1.00 ± 0.03	0.97 ± 0.02	0.99 ± 0.04	4.5*
Left D2:D4 (ratio)	0.99 ± 0.03	0.98 ± 0.03	0.99 ± 0.04	0.78
Plasma oestradiol (pmol/L)	200 ± 100	70 ± 30	100 ± 50	7.9***
Bioactive testosterone (nmol/L)	0.5 ± 0.3	6 ± 2	12 ± 7	38.3***
L Hemisphere cortex; mean (cm)	2.70 ± 0.09	2.65 ± 0.07	2.7 ± 0.1	2.0
R Hemisphere cortex; mean (cm)	2.72 ± 0.09	2.68 ± 0.08	2.8 ± 0.2	3.1
Total intracranial volume[b] (cm^3)	1300 ± 100	1400 ± 100	1300 ± 100	29.7***

Note: Data are given as mean and SD: *F*-values from group comparisons (1-way ANOVA). Possible differences in digit ratios between the separate groups were calculated with Scheffe's post hoc test ($P < 0.05$).
[a]Difference between XY and XX, $P = 0.042$; difference between XXY and XX, $P = 0.998$; difference between XY and XXY, $P = 0.041$.
[b]Difference between XY and XX, $P < 0010$; difference between XXY and XX, $P = 0.01$; difference between XY and XXY, $P < 0.001$.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Group Comparison of Mean Hemispheric Cth

The 1-way ANOVA did not show any significant group differences in mean Cth for either hemisphere (left hemisphere: df = 2, $F = 3.051$, $P = 0.051$; right hemisphere: df = 2, $F = 1.950$, $P = 0.147$). The right hemisphere was thicker than the left in all 3 groups of subjects ($P = 0.001$ for XX females, $P = 0.00003$ for XY males, and $P = 0.000001$ for XXY males). There were no significant group differences in hemispheric asymmetry (1-way ANOVA, $P = 0.094$; df = 2; $F = 2.41$).

Table 1 presents the mean cortical thickness values for each group for the left and right hemispheres. The total intracranial volume, calculated with FreeSurface software, differed between the groups ($P < 0.001$, $F = 29.7$) and was significantly smaller in 46,XX women compared with 47,XXY men and 46, XY men (Table 1).

Group Comparison of Regional Cth

The Cth values for the 46,XX females were greater than for the 46,XY males in the right precentral gyrus (there was a homologous cluster, which did not pass the $P < 0.05$ corrected significance level), as well as the right and left parietal and occipital lobes; see Table 2, Figure 1*A*. There were no regions in which the cerebral cortex was thicker among 46,XY males.

Cth in the aforementioned regions was also greater among 47,XXY males than 46,XY males. In addition, the 47,XXY males had greater Cth than the 46,XY males in the right orbitofrontal and superior frontal gyri (in the XX–XY contrast showed significance at the $P < 0.05$ uncorrected level in the latter region), and in the left inferior temporal gyrus.

The 47,XXY males also showed singular features and differed from both control groups. Compared with both 46,XY males and 46,XX females, they had significantly thinner cortex in the right superior temporal gyrus and the left middle

Table 2
Clusters showing significant group difference in cortical thickness

Cluster	XX-XY (positive −log10 (*P*) values) XY-XX (negative −log10 (*P*) values)			XY-XXY (positive −log10 (*P*) values) XXY-XY (negative −log10 (*P*) values)			XX-XXY (positive −log10 (*P*) values) XXY-XX (negative −log10 (*P*) values)		
	Maximum vertexwise −log10 (*P*)	Cluster size (cm^2)	Talairach coordinates	Maximum vertexwise −log10 (*P*)	Cluster size (cm^2)	Talairach coordinates	Maximum vertexwise −log10 (*P*)	Cluster size (cm^2)	Talairach coordinates
L occipital cortex (+portion of parietal cortex)	4.6	91.2	−33 −69 −9	−3−6	23.4	−16 −79 −6			
L parietal cortex	4.3	12.5	−5 −63 37	−5.4	99.1	−5 −91 17			
				−3.4	19.7	−15 −30 69			
				−5.0	17.5	−12 −50 62			
L inferior temporal gyrus				−2.4	15.6	−49 −15 −27			
L middle temporal cortex + insular cortex				3.9	10.0	−56 −54 7	4.1	40.2	−53 −56 8
						−34 −18 3			
R cuneus and lingual cortex				−5.4	99.1	6 −90 16			
R lingual cortex							−4.7	27.2	6 −89 −2
R parietal cortex (+cuneus)	4.5	85.3	17 −78 37	−5.3	54.3	6 −90 16	4.1	22.8	47 −54 44
R precentral gyrus	3.2	15.0	42 −8 32	−2.9	14.9	51 −3 39			
R postcentral gyrus							5.8	13.7	51 −13 −28
R lateral orbitofrontal cortex + superior frontal cortex				−4.1	18.4	37 7 −8	−4.6	22.9	42 25 −12
R superior temporal cortex				5.9	16.8	47 6 −18	2.9	12.9	63 −19 1

Note: Statistical threshold is $P < 0.05$, corrected for multiple comparisons (according to Monte Carlo permutations). Age was used as a nuisance covariate. The Talairach's coordinates indicate location of maximum difference, the "Region" column describes the coverage of the respective cluster.

temporal and insular cortices, as well as significantly thicker right orbitofrontal cortex and right lingual cortex (Table 2).

Several clusters in the separate group comparisons emerged also in conjunctional analysis. Thus, the [XXY–XY and XX–XY] conjunction showed clusters in the left parietal cortex, in the cuneus, and in the precentral gyri, with significantly thinner cortex among 46,XY males. The [XY–XXY and XX–XXY] conjunction, on the other hand, showed clusters characterized by significantly thinner cortex among 47,XXY males in the left middle temporal and right superior temporal gyri, covering a portion of the right anterior insular cortex (Table 3, Fig. 2). Furthermore, clusters with thicker cortex among 47,XXY males when compared with both control groups appeared in the right lateral orbitofrontal and right lingual cortices. Finally, the [XX–XY and XX–XXY] conjunction revealed clusters in the right superior and left inferior parietal cortices (Table 3). Interestingly, among the 47,XXY males, the mean Cth values for clusters in the right and left parietal cortices were in between those for 46,XX females and 46,XY males (Table 4), not found in other ROIs.

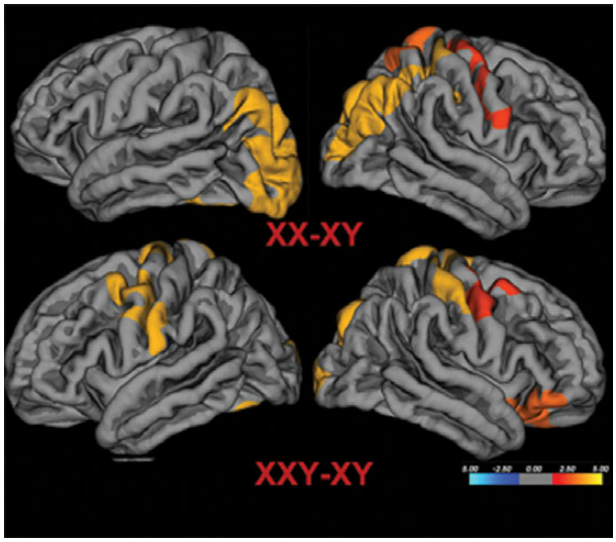

Figure 1. Clusters showing significant group differences, calculated at $P < 0.05$ corrected for multiple comparisons (Monte Carlo permutation). The projection of cerebral hemispheres (MR images of the FreeSurfer atlas) is standardized, and not all the significant clusters are reveled. Scale is logarithmic and shows −log10(*P*). Warm colors indicate positive contrasts (higher values in XX women and XXY men), cool colors negative contrasts (higher values in XY men).

Correlations Between Cortical Thickness and Sex Hormone Levels

Correlation analysis with *z*-transformed sex hormone levels showed an inverse linear correlation with Cth in the left parietal lobe, and to a lesser degree in the right occipital lobe. The Cth in the left parietal lobe was also positively correlated with z-transformed estradiol (Table 5), further indicating that sex hormones could have had an impact on the observed sex difference in this region. Both testosterone- and estradiol-related clusters remained when rerunning the regression analyses using both age and the D2:D4 ratio as nuisance variable. No other significant correlations with sex hormones were detected. No significant correlations were found between Cth and right-hand 2D:4D ratio.

Post Hoc Analyses

Several more recent studies have shown that cerebral functional networks have an intrinsically cohesive modular structure in that the connections between regions are much denser within each module than between them. The modules are mainly composed of functionally, as well as anatomically, related brain regions and can be identified by maps of covariance. This is of particular interest for investigations of clinical populations since maps of covariance, such as for Cth, may vary as a function of disease processes, and, thus, differ between patients and controls (He et al. 2008).

After observing that Cth in several regions significantly differed between 47,XYY males and both control groups, the question emerged whether also the pattern of covariance for each of these regions differed between 47,XXY males and the control groups. If regions were to be found where the cortico-cortical covariance pattern differed between 47,XXY males and both the 46,XX and 46,XY controls, we postulated, it would suggest that a wider set of networks could be involved in the 47,XXY phenotype. These regions would, together with those showing a significantly different Cth, represent strong candidate networks for further investigations of structural and functional underpinnings of the cognitive dysfunctions reported among 47,XXY males (Savic 2012).

Table 3
Conjunctional clusters

	[XX-XY and XXY-XY]			[XX-XXY and XY-XXY]			[XX-XXY and XX-XY]		
Region	Max, −log10(*P*)	Size (cm^2)	Co-ordinate	Max,-log10(*P*)	Size (cm^2)	Co-ordinate	Max,−log10(*P*)	Size (cm^2)	Co-ordinate
R parietal cortex							3.2	12.6	48 −53 43
L parietal cortex	4.3	21.0	−16 −78 35				3.6	13.8	−37 −80 16
R + L cuneus	3.2	8.9	−4 −89 16						
R precentral gyrus	*2.7*	*5.7*	*7 −10 56* *46 −6 37*						
L precentral gyrus[a]	*2.5*	*2.9*	*−50 −10 23*						
R superior temporal gyrus				3.4	7.2	52 7 −13			
L middle temporal gyrus				3.6	5.9	−55 −57 9			
R lingual gyrus				−4.4	18.6	6 −89 −3			
R lateral orbital cortex				−3.8	8.0	42 25 −12			

Note: Italics denote values significant at $P < 0.05$ uncorrected for multiple comparisons; these clusters were regarded as significant because they were covered by the a priori hypothesis. The other clusters were significant at $P < 0.05$ corrected (Monte Carlo permutation). The Talairach's coordinates indicate location of highest difference, the "Region" column describes the coverage of the respective cluster. R: right; L: left.

[a]This cluster was not formally significant as Cth in this region differed between XX and XY controls only at $P < 0.05$ uncorrected (seel also Table 2).

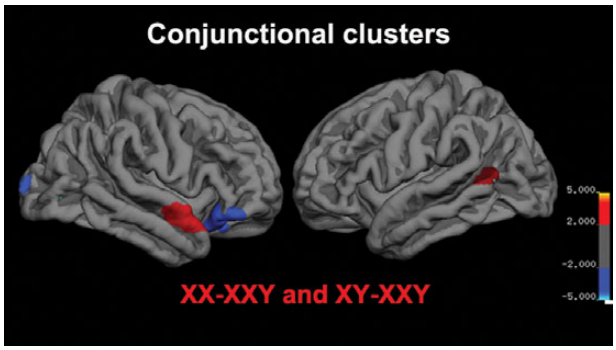

Figure 2. Conjunctional clusters illustrating regions in which 47,XXY males had different Cth in relation to both 46,XX females and 46,XY males ($P < 0.05$ corrected for multiple comparisons). Scale is logarithmic and shows −log10(P). Warm colors indicate regions with lower values in 47,XXY males (positive XX-XXY and XY-XXY contrast), and cool colors indicate regions with higher values in 47,XXY males (the reversed contrast).

Table 4
Cortical thickness in clusters showing significant group differences

Region	XX	XY	XXY
L occipital cortex	2.02 ± 0.14	1.98 ± 0.14[a]	2.05 ± 0.15
R occipital cortex	2.10 ± 0.13	2.03 ± 0.17[a]	2.15 ± 0.17
L parietal cortex	2.38 ± 0.13	2.31 ± 0.10[a]	2.37 ± 0.12
R parietal cortex	2.42 ± 0.12	2.33 ± 0.10[a]	2.40 ± 0.14
L precentral cortex	2.75 ± 0.11	2.68 ± 0.11[a]	2.76 ± 0.14
R precentral cortex	2.75 ± 0.09	2.69 ± 0.12[a]	2.77 ± 0.16
R orbitofrontal cortex	3.16 ± 0.20	3.18 ± 0.20	3.34 ± 0.23[b]
R superior temporal cortex	3.15 ± 0.21	3.20 ± 0.22	2.97 ± 0.15[b]
L middle temporal cortex	2.86 ± 0.20	2.84 ± 0.24	2.61 ± 0.21[b]
R lingual gyrus	2.25 ± 0.13	2.24 ± 0.15	2.33 ± 0.12[b]

Note: Mean cortical thickness calculated from the individual mean values in selected ROIs, which initially showed significant differences between the groups investigated. Data are presented as mean and SD. The presented values are strictly descriptive and not used in any statistical operations.
[a]Regions in which cortical thickness was lower in XY men in comparison to both XX women and XYY men, $P < 0.05$, (the 2 latter groups did not differ).
[b]Regions in which cortical thickness differed in XXY men in relation to both control groups ($P < 0.05$).

Table 5
Co-variation between cortical thickness and sex hormone levels

Cluster	z-testosterone			z-oestradiol		
	Maximum vertex-wise log10(P)	Cluster size (cm^2)	Talairach Coordinates	Maximum vertex-wise −log10(P)	Cluster size (cm^2)	Talairach Coordinates
L parietal cortex	−3.5	6.1	−33 −52 53 −10 −65 27	3.9	5.3	−35 −30 22
R occipital cortex	−2.5	2.4	4 −78 13			

Note: Clusters calculated at $P < 0.05$, uncorrected for multiple comparisons. The left parietal cluster survived also the multiple comparison correction at $P < 0.05$. Only 46,XX female and 46,XY male controls were used in the analysis. Age and right hands D2:D4 ratio was employed as nuisance variable to control for their effect. The Talairach's coordinates indicate location of the vertex with highest difference, the "Region: column describes coverage of the respective cluster.

Measures of Cth at different vertices were inter-related, thereby indicating patterns of structural covariance across the cortex. Significant clusters for within- and between-group covariance analyses were calculated at $P < 0.01$ corrected after Monte Carlo permutations (the higher threshold of significance was used to avoid large confluent clusters).

47,XXY males generally showed fewer clusters with significant correlations from the 4 seed regions than the controls (Supplementary Table S2 and Supplementary Fig. S1). They had, on the other hand, generally more pronounced covariations between these seed regions than the controls, see Supplementary Table S3.

Discussion

The present study investigates sex differences in respect to cortical thickness and how these differences may be related to sex chromosomes and sex hormones. Three principal observations were made:

1. The thickness of the parietal cortex, and to a lesser extent in occipital cortex area was significantly greater in 46,XX females than 46,XY males. In these regions, the values of Cth were inversely correlated with z-testosterone, and in the parietal cortex there was also positive correlation with z-estradiol.
2. In the motor strip, the cortex was thinner in 46,XY males compared with both 46,XX females and 47,XXY males, indicating the possibility of an X-chromosome gene-dosage effect.
3. In the right superior temporal gyrus, right orbitofrontal cortex and lingual gyrus, and in the left middle temporal gyrus, the Cth in 47,XXY males differed from that of both control groups, raising the question about possible sex-chromosome gene-dosage-related effects in these regions,

These results concerning 46,XX females and 46,XY males accord with previous reports showing that Cth varies between different regions and is slightly greater in the temporal compared with occipital lobe (Luders, Narr, Zaidel et al. 2006). They also accord with earlier findings of a thicker parietal, occipital, and precentral cortex in 46,XX females compared with 46,XY males (Sowell et al. 2004; Im et al. 2006; Luders, Narr, Thompson, Rex, Woods et al. 2006; Lv et al. 2010), irrespective of whether correction for total brain volume was carried out (Luders, Narr, Zaidel et al. 2006). Also, the presently observed cortico-cortical covariations, with maximal covariations between neighboring areas in the same hemisphere, and the homologous areas in the opposite hemispheres, concur with the correlation patterns found by several other researchers (Mechelli et al. 2005; He et al. 2008; Lv et al. 2010; Raznahan et al. 2010; Tijms et al. 2012).

The present data on men with Klinefelter's syndrome are more difficult to relate to previous investigations. To the best of our knowledge, the only other available data were based on investigations of adolescents who had, in the main, not yet begun to receive testosterone supplementation (Giedd et al. 2007), making it difficult to compare the findings with our adults who were already on testosterone treatment. Compared with age-matched controls, these adolescents showed cortical thinning not only in the temporal lobe (as with our 47,XXY males), but also in the frontal and the superior parietal lobes (Giedd et al. 2007). These seemingly incongruent results may be more easily understood in light of findings that parietal and frontal Cth increases with testosterone levels in pubertal boys (Bramen et al. 2012) and that testosterone levels are lower in

pubescent 47,XXY males than in age-matched controls (as further discussed below).

Sex Hormone Interaction with Cortical Thickness

In accordance with previous MR studies of GM volume (Nopoulos et al. 2000; Neufang et al. 2009; Lentini et al. 2012), we found that the levels of z-transformed bioactive testosterone were inversely, and significantly correlated with the parietal Cth. A significant inverse correlation with testosterone levels was detected also in the occipital cortex. This correlation was less pronounced (maximum vertexwise $\log10(P) = -2.5$), and while supported by some previous reports (see further), it should be taken with some precaution; we will, therefore, primarily discuss the correlation in the parietal cortex.

That testosterone may have an effect on neuronal tissue in occipitoparietal lobe is congruent with the notion that steroid receptors are expressed in these parts of the brain (Simerly et al. 1990; Abdelgadir et al. 1999; Goldstein et al. 2001). With regard to Cth, there seems to exist an interesting sex by age interaction with testosterone. Bramen et al. (2012) recently reported that correlations between testosterone levels and Cth vary between different regions of the brain, pointing in partly opposing directions in prepubertal boys and girls. Whereas a testosterone-related thinning was detected in parts of the parieto-occipital cortex and in the frontal pole in prepubertal girls, a testosterone-related increase was found in the Cth of the corresponding areas in boys. Notably, this relationship becomes inverse for both sexes after puberty, (Nguyen et al. 2013). Testosterone may, thus, have some impact on Cth into adulthood, as has been previously reported in respect to cerebral volume (Patwardhan et al. 2000; Pol et al. 2006). Since testosterone treatment in our XXY population in general started at late puberty or even later no prepubertal effects should occur from the supplement. We tested whether this treatment had impact on Cth in the specific regions where Cth differed between XXY men and XY men. This was done in a post hoc correlation analysis between age at institution of testosterone supplement, duration of treatment, and Cth (using the extracted ROI data and Pearsson's correlation analysis, $P < 0.05$). No significant correlations were found in any of the regions.

The inverse correlation regarding parietal Cth found in the present study may be attributed to the well-known pruning effects of testosterone. Such a mechanism may also explain why the parietal cortex was thicker in our 47,XXY males than in 46,XY males, as 47,XXY males have low testosterone levels prior to receiving testosterone substitution (starting in late puberty or even later). Because testosterone level in 47,XXY during puberty is higher than that of age-matched female controls, it also concurs with the present observation that parietal and occipital cortex was thinner in 47,XXY men than in 46, XX females (Table 4).

Our adult 47,XXY men had significantly higher, and more variable testosterone levels than male controls, while the variability in Cth was similar to that of controls (Table 1). One possible reason to these seemingly contradictory findings is that among 47,XXY men, there is preference for inactivation of androgen receptors that have shorter CAG repeat chain on exon 1 (Zitzmann et al. 2004). The androgen receptor is located on the X chromosome, and 47,XXY men, thus, have 2 androgen receptor alleles; if they are heterozygous with respect to number of CAG, the shorter receptor is methylated and inactivated leaving the less active receptor to be expressed. Therefore, some 47XXY males are less sensitive to androgens, and require higher testosterone levels to achieve an adequate response. Titration of testosterone dose is based on clinical response and luteinic hormone (LH) suppression. This scenario provides an explanation to the higher variability in blood testosterone among our XXY population.

Parallel to the inverse correlation with z-testosterone, there was a positive correlation between parietal Cth and z-estradiol. Although unprecedented, this observation is comprehensible, considering that estrogen is reported to have a protective effect on GM tissue (Wise et al. 2001), and that an elevation of estrogen levels during ovulation, (Hagemann et al. 2011), or through postmenopausal substitution (Erickson et al. 2005), leads to an increase in GM volumes. GM volume is also been reported to correlate to plasma estradiol, albeit not selectively in the parietal lobe (Witte et al. 2010).

Cortical Thickness and Digit Ratio

Although the 2D:4D ratio was lower in 47,XXY males, possibly pointing to fetal testosterone having an impact on this anthropomorphic measure, no significant link was found between digit ratio and cerebral Cth. The use of the 2D:4D ratio as a proxy for fetal testosterone is, despite the over 300 related works on it in the literature (Voracek and Loibl 2009), not fully accepted, mainly because the exact mechanisms by which the 2D:4D ratio and prenatal androgen exposure related are not entirely clear. Knickmeyer et al. (2011) recently suggested that it may be more appropriate to interpret the 2D:4D ratio in adulthood as an index of early testosterone exposure rather than prenatal exposure per se. The failure to find a correlation with digit ratio in the present study should therefore not be taken as an argument against the influence of fetal testosterone on Cth.

Taken together, the present data from the control groups confirm that there are sex differences in respect to cortical thickness and add to the previous findings by suggesting that the sex differences regarding the parietal Cth are related to sex hormone governed processes.

Sex Chromosome Interaction with Cth

The findings related to the possible effects of sex chromosomes will be discussed in terms of X-chromosome and sex-chromosome dosage, notwithstanding that this is a simplification.

Although the long chain of downstream mediators between the number of X chromosomes, number of sex chromosomes, and cortical thickness evidently precludes drawing any detailed conclusions about the mechanisms involved, the present observations nonetheless show a regional genetic influence on some of the observed sex differences. This notion is in accordance with several recent reports on the heritability of regional Cth (Rogers et al. 2007; Schmitt et al. 2008), and provides additional substance to these reports by specifically addressing the issue of sex differences, which has, to the best of our knowledge, not been done earlier.

Cth in the precentral gyrus was significantly higher in 47, XXY males and 46,XX females compared with 46,XY males. Interestingly, in women with Turner syndrome, (45,X0 females), precentral Cth is found to be reduced (Lepage et al. 2013),

suggesting that Cth in the motor cortex may be influenced by X-chromosome genes that have escaped inactivation and do not have an Y-chromosome homolog. This postulation concurs with the present and previous reports of sex differences in Cth as well as in GM volumes in this region (Raz et al. 2001; Savic and Arver 2011; Luders et al. 2005) and also with the recently reported relation between X-chromosome number and the GM volume of the precentral gyrus (Lentini et al. 2012). The link found between the anatomy of the precentral (motor) cortex and X-chromosome genes is of particular interest, considering that several hereditary disorders of the motor system are linked to the X chromosome (Fragile X, familiar X-linked dystonias, X-linked cerebellar hypoplasia, adrenoleukodystrophy), (Palazzolo et al. 2008), and emphasizes the importance of investigating how gene-mediated processes shape motor circuitry.

The findings of cortical thinning in the right superior temporal gyrus and left middle temporal gyrus and of cortical thickening in the right lingual and right orbitofrontal cortices in 47,XXY males, suggests possible regional effects of sex-chromosome gene dosage. Such effects have been implied in previous findings regarding subjects with sex-chromosome aneuploidy. Several independent studies carried out in different populations of subjects, and using different methodologies, suggest that the thickness of the temporal cortex may be related to sex-chromosome gene dosage. In 1 study, a comparative analysis of the Cth of the superior temporal gyrus showed that during childhood and during adolescence (the latter with some accentuation) 45,X0 females have a significantly thicker cortex than 46,XX females (Lepage et al. 2012). This finding is reciprocal to the comparatively thinner cortex presently found in 47,XXY males. A similar reciprocity between women with Turner's syndrome and men with Klinefelter's syndrome has been found regarding temporal GM volume, which was increased in 45,X0 females versus 46,XX females (Good et al. 2003; Molko et al. 2003; Kesler et al. 2004; Marzelli et al. 2011), but decreased in 47,XXY males versus 46, XY males (Lentini et al. 2012; Lepage et al. 2012). These observations make it plausible that PAR genes of the X-chromosome that have Y-chromosome homologs (Vawter et al. 2007) could be expressed in triplets in 47,XXY males and lead to aberrant regional brain maturation in the temporal cortex, which, in turn, may be enhanced by low testosterone levels during adolescence (Patwardhan et al. 2000). An isolated effect of testosterone seems less probable, considering that the reported changes in temporal lobe Cth seem to have occurred already in prepuberty (Giedd et al. 2007; Lepage et al. 2012), and that estrogen as well as testosterone is low in both 47,XXY males and 45,X0 females, which is incompatible with the finding of opposing patterns of temporal lobe Cth in these 2 populations (Gravholt et al. 1999; Kates and Singer 2000; Patwardhan et al. 2000).

The greater Cth observed in the right orbitofrontal and lingual gyri of 47,XXY males is more difficult to attribute to sex-chromosome gene dosage (mainly because the information is sparse), although the recent report about thinning of the right lingual cortex in prepubertal 45,X0 females provides some support for such an explanation (Lepage et al. 2012).

One notable observation is that the most pronounced changes presently detected in the 47,XXY males, including their pattern of cortico-cortical correlations were those that are closely associated with the cognitive–behavioral domains reported to be particularly impaired in this population. Orbitofrontal changes relate to impulse control (Feinberg et al. 1994; Coccaro et al. 2007; Zeeb et al. 2010), which can be impaired in 47,XXY males, and changes in the temporal cortex and lingual cortex are relevant for dyslectic and sociocognitive problems described in this population (Feinberg et al. 1994; Savic 2012). The present findings seem, thus, pertinent for functional deficits in Klienefelter's men, and deserve further attention when trying to understand their exact underpinnings.

Another issue worth a comment is that although, in general, there is a phenotype variation among XXY men, the hitherto reported cerebral differences in relation to male controls seem rather consistent (Giedd et al. 2007; Lenroot et al. 2009). We investigated the distribution of Cth values in various ROIs, but found it to be similar to that of controls, (see also Table 4). Whether, and how "the cerebral phenotype" relates to other features (body mass, height, skeleton) is of interest, and needs to be addressed in further investigations.

Methodological Limitations

One of the strengths of this study is the use of a new methodology, which incorporates measurements of sex hormone levels and comparisons of data from 47,XXY males with that of both male and female controls. The fact that hormone levels were, as in several previous studies (Peper et al. 2008; Neufang et al. 2009; Witte et al. 2010), measured on only 1 occasion is a limitation. Hormone levels vary with activity, stress, and sleeping patterns. Although we tried to standardize these factors, and can claim that the measures of hormone levels and cerebral Cth were temporally related (blood samples were taken on the same day as the MRI scans), it should be acknowledged that multiple measurements of serum hormone levels over time might have been more precise for determining the link between circulating hormones and brain morphology.

One may wonder if the significant differences in total brain volume (with smaller brains in the XX group, Table 1) as well as the finer gyrification pattern of the female brain might result in increased partial volume effects, which could appear as a thicker cortex in MRI. There are several arguments against this concern. First of all, the thickness mapping approach is rather immune to partial volume effects (Fischl and Dale 2000), because it allows manual corrections. Second, the super sampling of the data and fitting of the intensities at the GM and WM interface is at a slightly finer resolution than, for example, voxel-based morphometry. Third, it has been shown that Cth is greater in females than males regardless of the scaling of brain volume (Luders, Narr, Thompson, Rex, Woods et al. 2006). Fourth, common space was used in all of the statistical comparisons. Finally, the total brain volume did not significantly differ between 46,XY males and 47,XXY males, and yet there were several clusters with thicker cortex in the latter group.

The duration of education was equal for the XX and XY groups, but significantly lower in the XXY group, which raises the question as to whether lower education could have contributed to the observed regional differences in Cth in 47,XXY men. To the best of our knowledge, lower education is reported to be associated with a significantly thicker cortex (Querbes et al. 2009), particularly in the right lateral occipital and right middle temporal lobe (not in the frontal areas). Our XXY men showed both thicker and thinner cortex compared with male controls, and the finding that their temporal lobe cortex was thinner argues against education as an explanatory

factor. Lower education could, theoretically, have contributed only to the higher orbitofrontal Cth in XXY men. However, a post hoc correlation analysis showed no significant correlation (Pearson's correlation, $P < 0.05$) between years of education and Cth in this region, nor in any of the other ROIs analyzed (Table 4). Together, these observations suggest that education was of minor or no importance for the observed group differences.

The study offered no option to directly examine the cellular correlates of the neuroimaging differences identified, and the observed hormone correlations could not be translated to exact biological mechanisms. Testosterone could act via several mechanisms, such as via androgen receptors, which are coded by genes located on the X chromosome.

Cortical thickness is related to cell packing density, cell size, and number of cortical neurons (Kruggel et al. 2003). Although an increasing number of studies show a relationship between cortical thickness and function, theoretically, it is also possible that there is no clear functional correlate to the increased regional thickness of the cortex. Because we have not measured behavioral correlates, it is not evident whether increased or decreased thickness in a certain region by itself is disadvantageous for functioning. Increased numbers of cortical neurons in functional units might be advantageous by contributing to an efficient processing of ingoing and outgoing information. On the other hand, women have a thicker parietal lobe cortex but consistently lower visuospatial performance, and thinning of the frontal cortex during brain development with a loss of redundant synapses through pruning seems to improve computational efficiency in related cerebral networks (Sowell et al. 2001).

Summary

In summary, the present study expands the previous literature on neuroimaging by proposing that processes linked to X-chromosome gene dosage affect Cth in the precentral gyrus, and that processes related to sex-chromosome gene dosage may have an impact on Cth in the superior temporal gyrus (see also the summarizing Supplementary Table S1). By identifying brain areas that seem to exhibit the effects of sex chromosomes, the present results add to the animal data about the genes located on X and Y chromosomes that could contribute to sex differences in the Cth.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Funding

We thank the Insurance Council for Working Life and Social Science (FAS), the Swedish Research Council, and VINNOVA for their financial support.

Notes

Special acknowledgment is given to Tetiana Dadakova who did all the preprocessing of FreeSurfer data. Emilia Lentini is acknowledged for the statistical analyses. We are also very grateful to Professor Hans Forsberg for all the fruitful discussions. I.S. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Conflict of Interest*: None declared.

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