Influence of X Chromosome and Hormones on Human Brain Development: A Magnetic Resonance Imaging and Proton Magnetic Resonance Spectroscopy Study of Turner Syndrome

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Background: *Women with Turner syndrome (TS; 45,X) lack a normal second X chromosome, and many are prescribed exogenous sex and growth hormones (GH). Hence, they allow us an opportunity to investigate genetic and endocrine influences on brain development.*

Methods: *We examined brain anatomy and metabolism in 27 adult monosomic TS women and 21 control subjects with volumetric magnetic resonance imaging and magnetic resonance spectroscopy.*

Results: *In TS women, regional gray matter volume was significantly smaller in parieto-occipital cortex and caudate nucleus and larger in cerebellar hemispheres. White matter was reduced in the cerebellar hemispheres, parieto-occipital regions, and splenium of the corpus callosum but was increased in the temporal and orbitofrontal lobes and genui of corpus callosum. Women with TS had a significantly lower parietal lobe concentration of N-acetyl aspartate, and higher hippocampal choline. Also, among women with TS, there were significant differences in regional gray matter volumes and/or neuronal integrity, depending upon parental origin of X chromosome and oxandrolone and GH use.*

Conclusions: *X chromosome monosomy, imprinting and neuroendocrine milieu modulate human brain development—perhaps in a regionally specific manner.*

Key Words: Turner syndrome, brain, magnetic resonance imaging, magnetic resonance spectroscopy, x chromosome

Women with Turner syndrome (TS) (45,X) provide one of the few models that allow us to examine the relative effects of the X chromosome and hormones on the human brain, because they: 1) have a characteristic cognitive phenotype (Collaer et al 2002; Lawrence et al 2003; Murphy et al 1994; Ross et al 2002), 2) do not possess a normal second X chromosome, 3) lack endogenous sex hormones owing to gonadal dysgenesis and, 4) are prescribed exogenous hormones to encourage growth and puberty. Furthermore, TS is a common condition (1:2000 live female births) (Lippe 1991).

Nevertheless, few studies have investigated the effect of X chromosome monosomy on brain. Previous anatomic region of interest (ROI) investigations report that women with TS have a significantly smaller volume of total brain tissue (gray + white matter) in parieto-occipital lobes (Murphy et al 1993; Reiss et al 1995), hippocampus, caudate nucleus, and thalamus (Murphy et al 1993); however, ROI studies (unlike voxel-based morphometry [VBM]) are not able to investigate subtle differences in gray and white matter and are operator-dependent and non-automated. Voxel-based morphometry has only been employed in one study (Good et al 2003): women with TS had significantly larger volume of gray matter in the amygdala and orbitofrontal cortex. They did not report, however, on the rest of the brain. Thus, there is a need for whole-brain studies in TS. We hypothesized that adults with TS have differences in the anatomy of brain regions previously reported as abnormal but that, also, VBM would detect subtle differences in gray and white matter that are not measurable with ROI approaches.

Women with TS also have glucose hypometabolism in parieto-occipital and medial temporal regions (Clark et al 1990; Murphy et al 1997). The basis of this is unknown but could be secondary to differences in neuronal integrity (i.e., neuronal density and/or metabolism). One method to investigate this is proton magnetic resonance spectroscopy (^{1}H MRS), which can measure N-acetyl aspartate (NAA), creatine + phosphocreatine (Cr+PCr), and choline (Cho)-containing substances; NAA, Cho, and Cr+PCr are generally accepted as measures of neuronal density/mitochondrial metabolism, membrane turnover/degradation, and phosphate metabolism, respectively (Miller et al 1996). Therefore, we employed ^{1}H MRS to determine if brain regions reported to be metabolically abnormal in TS (i.e., parietal and medial temporal regions) have differences in neuronal integrity.

In female subjects without TS (46,XX), one X chromosome is randomly inactivated (Lyon 1961), although some genes on the second "suppressed" X chromosome escape inactivation. We and others have previously suggested that haploinsufficiency of genes escaping inactivation underpins the characteristic cognitive and neuroanatomic phenotype of TS people (Murphy et al 1993, 1994, 1997; Therman and Patau 1974); however, the phenotypic variability of genetically similar (monosomic) women with TS

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has prompted a search for additional modulatory factors, such as genetic imprinting (i.e., genes that are preferentially expressed depending on their parent of origin) and endocrine milieu.

Girls with TS with a maternally derived X chromosome (X^m) might differ from those with a paternally derived X chromosome (X^p) in social behavior and memory (Bishop et al 2000; Skuse et al 1997). Studies of brain anatomy in X^m and X^p, however, have been inconsistent; one ROI study (Brown et al 2002) and a VBM study (Good et al 2003) found no differences, whereas another ROI study reported a significantly greater volume of superior temporal gyrus in X^m than X^p (Kesler et al 2003). Thus, the effect of imprinting on brain is poorly understood. We hypothesized that imprinting would be associated with differences in cerebral hemispheres and basal ganglia—brain regions we previously reported were under substantial developmental control from the X chromosome in fragile X syndrome and TS (Moore et al 2004; Murphy et al 1993).

Endocrine factors also modulate brain development (Arnold and Gorski 1984), and girls with TS lack estrogen and androgen. Established clinical practice is to give estrogen replacement therapy (ERT) and androgen (oxandrolone) during puberty (Karim et al 1973; Nilsson et al 1996; Rudman et al 1980). Estrogens and androgens both affect brain, (e.g., androgens have neurodevelopmental [Arnold and Gorski 1984] and neuroprotective effects [Hammond et al 2001; Tirassa et al 1997]). Also, ERT and oxandrolone modulate cognitive function in girls with TS (Ross et al 1998, 2000, 2003); however, the biological basis for this is unknown, because nobody has reported the effect of oxandrolone and ERT on brain anatomy and metabolism in TS. All the women we studied were taking ERT, but only some had been prescribed oxandrolone. Therefore, we were only able to test the hypothesis that, in women with TS, oxandrolone modulates anatomy and metabolism.

Endogenous growth hormone (GH) is not low in TS; nevertheless, supra-physiological doses are given to promote growth. In humans, GH receptors (GHR) are present throughout brain (Castro et al 2000; Lai et al 1993), and in rats, GH affects brain development (for review, see Harvey et al 1993); however, there are no studies on the effect of GH on brain anatomy in humans. Therefore, we investigated whether TS women prescribed GH have significant differences in brain anatomy.

In summary, we tested the main hypothesis that, compared with healthy control subjects, women with TS have: 1) abnormalities in the gross anatomy of the parieto-occipital region, basal ganglia, and cerebellum, measured with ROI approaches, but that VBM would show more subtle changes in grey and white matter in these regions; and 2) differences in neuronal integrity of amygdala-hippocampal complex and parietal lobe. We also tested the subsidiary hypothesis that, within women with TS, brain is further modified by mode of inheritance of X chromosome and endocrine factors. Thus, we carried out a preliminary post hoc comparison between TS people who were X^m and X^p and women who were and were not treated with oxandrolone and GH.

Methods and Materials

Subjects

All subjects were participants in a behavioral genetics research program conducted by the South London and Maudsley NHS (National Health Service) Trust, the Institute of Psychiatry, and the Middlesex Hospital. We included 27 adult women with monosomic TS (mean age 27 ± 8 years), determined by blood karyotyping. At least 30 metaphase spreads were analyzed in each woman with conventional cytogenetic techniques including Giemsa / trypsin banding (Seabright 1971). Twenty healthy female control subjects were recruited from the local area (mean age 27 ± 7). Ethical approval was obtained from the Institute of Psychiatry Ethics Committee, and written informed consent was obtained from all participants.

All women underwent medical and psychiatric interview by a physician, and we excluded women with disorders grossly affecting brain function (e.g., epilepsy, neurosurgery, head injury, hypertension, schizophrenia). History of hormone use was obtained from both subjects and parents and, where possible, confirmed from the medical notes.

MRI and ^{1}H MRS Data Acquisition

All MRI and ^{1}H MRS data were obtained with a GE Signa 1.5T Neuro-optimised MR system (General Electric, Milwaukee, Wisconsin). Whole-head coronal three-dimensional (3D) SPGR images (repetition time [TR] = 14 msec, echo time [TE] = 3 msec, 256 × 192 acquisition matrix, 124-mm × 1.5-mm slices) were obtained from all subjects.

For ^{1}H MRS, a right parietal voxel of interest (VOI), measuring $2 \times 2 \times 2$ cm^3, and a hippocampal VOI, measuring $7.7 \times 15.5 \times 20$ mm^3, were placed in standard locations with previously published methods (Murphy et al 2002; Robertson et al 2001) and contained both gray and white matter. Point resolved spectroscopy (PRESS) spectra (TE 136 msec, TR 2000 msec, 256 averages) were obtained from the VOIs after chemical shift selective (CHESS) water suppression. Spectra were not gathered in all women, owing to time constraints: parietal spectra were obtained in 15 women with TS and 15 control subjects; hippocampal spectra were obtained in 14 women with TS and 14 control subjects. There were no significant differences in demographic characteristics between women who did and did not undergo ^{1}H MRS.

ROI Approach

Manual tracing of brain structures (except the amygdala) was performed on SPGR data sets, with Measure software (Barta et al 1997) (Johns Hopkins University, Baltimore, Maryland) with previously published anatomic definitions (e.g., see van Amelsvoort et al 2001). The amygdala was measured with the DISPIM software package (BIL Solutions, Risley, United Kingdom) (Plummer 1992) and the anatomic definitions of Brierley et al (2002). Raters were blind to subject status, and inter-rater correlation coefficients were r .9 for all regions traced. We measured total intracranial space and bulk tissue volume (i.e., gray + white matter) of cerebellum, right and left cerebral hemispheres, frontal, parietal, temporal and occipital lobes, amygdala, hippocampus, caudate nucleus, and putamen. Women with TS had a significantly greater total intracranial volume (see ROI results). Thus, we expressed brain volumes as a percentage of intracranial volume to account for individual variation in head size.

VBM Pre-Processing

The VBM pre-processing was performed on the SPGR data with Statistical Parametric Mapping software (SPM2, Wellcome Department of Imaging Neurosciences, University College London). The image processing steps have been described in detail elsewhere (Ashburner and Friston 2000; Good et al 2001). The segmentation algorithm implemented in SPM incorporates a priori knowledge of the likely spatial distribution of tissue types in the brain with prior probability tissue maps

derived from a large number of subjects. To ensure the most accurate segmentation possible, we created study-specific customized prior probability maps based on all 47 subjects. The pre-processing stages were as follows: 1) scans were segmented into probabilistic maps of gray and white matter and cerebrospinal fluid with a modified mixture model clustering algorithm; 2) the segmented gray matter map was mapped to a gray matter template, and the derived warping parameters were applied to the original T1-weighted image to map it into standard space (this procedure prevents skull and other non-brain voxels from contributing to the registration, while avoiding the need for explicit skull-stripping); 3) the registered image was then re-segmented, which is necessary, because the a priori knowledge incorporated into the SPM2 segmentation algorithm means that it works optimally on images in standard space. The segmented maps were then corrected for volume changes introduced during registration and smoothed with a Gaussian filter of 5 mm full width at half maximum (FWHM). Total gray and white matter volumes were calculated from the segmented maps in native space.

^{1}H MRS

The ^{1}H MRS data were analyzed with SAGE/IDL software, provided by the manufacturer (General Electric Medical Systems, Milwaukee, Wisconsin), to integrate the major resonances. In addition to peak area ratios of the metabolites NAA, Cr+PCr and Cho, metabolite concentrations were determined with their documented relaxation characteristics at 1.5 Tesla, together with the total water signal of the VOI (Simmons et al 1998). To allow us to determine if between-group differences in metabolites were confounded by differences in regional brain volume, volumes of right parietal lobe and hippocampus were measured (as previously mentioned).

Genetic Studies

Deoxyribonucleic acid (DNA) samples were obtained from women with TS and both parents (where available) with buccal swabs or blood samples; DNA was extracted with the method described by Freeman et al (2003). Standard primers (www.gdb.org) were used to amplify microsatellite markers on the X chromosome (DXS984; DXS996; DXS451; DXS999; DXS993) and polymerase chain reaction was carried out with standard denaturation and reaction steps. The DNA samples were analyzed for genotypes with Genescan and Genotyper software (Applied Biosystems, Foster City, California), version 3.7. Parent of origin was assigned by matching of offspring X chromosome marker alleles to parental X chromosome alleles to assign the transmitting parent. Where only one parent was available, they were assumed to be the transmitting parent if their alleles matched; if there were alleles not present in the available parent, the missing parent was assumed to be the transmitting parent.

Statistics

The analysis of IQ, manually traced brain volumes, and ^{1}H MRS was carried out with SPSS (SPSS 11.0 for windows; SPSS, Chicago, Illinois). Between-group differences among women with TS for hormone therapy and X chromosome origin were compared with χ^2 or Fisher's Exact tests—depending on cell size. For between-group comparisons of hand-traced regional brain volumes, total gray and white matter volume, and ^{1}H MRS data, we used independent samples t tests (two-tailed) or Mann–Whitney U tests according to the normality of data distribution. Level of statistical significance was defined as $p < .05$.

For the VBM analyses, between-group differences in gray and white matter volume were estimated by fitting an analysis of covariance (ANCOVA) model at each intracerebral voxel in standard space, covarying for total gray (or white) matter volume. Age at scan was covaried for where there was a significant between-group difference (women who previously took GH [GH+] vs. women who had never taken GH [GH−] and women who previously took oxandrolone [oxandrolone+] vs. women who never took oxandrolone [oxandrolone−]). Given that structural brain changes are likely to extend over a number of contiguous voxels, test statistics incorporating spatial information, such as 3D cluster mass (the sum of suprathreshold voxel statistics), are generally more powerful than other possible test statistics, which are informed only by data at a single voxel. Therefore, our approach is to initially set a relatively lenient p value ($p \leq .05$) to detect voxels putatively demonstrating differences between groups. We then search for spatial clusters of such voxels and test the cluster mass of each cluster for significance at a level of $p \leq .05$. Permutation testing is used to assess statistical significance at both the voxel and cluster levels (Bullmore 1999; Sigmundsson et al 2001). At the cluster level, rather than set a single a priori p value, below which we regard findings as significant, we calculate, for a range of p values, the number of clusters that would be expected by chance alone. We then set the statistical threshold for cluster significance for each analysis such that the expected number of false positive clusters is < 1, and quote the p value at which this occurs.

Our main comparison was between women with TS and healthy control subjects. Within the TS group, we also carried out a preliminary analysis in which we compared: 1) X^m versus X^p, 2) GH+ versus GH−, and (3) oxandrolone+ versus oxandrolone−.

Results

Subject Characteristics

Thirteen women with TS were GH+ and 14 were GH−. Twelve were oxandrolone+ and 15 were oxandrolone−. There was a significant difference in mean age between GH+ (mean age 22.4, SD 5.1) and GH− (mean age 31.4, SD 8.1) [$t = -3.45$, $p < .002$] and between oxandrolone+ (mean age 22.9, SD 4.7) and oxandrolone− (mean age 30.4, SD 8.9) [$t = -2.64$, $p < .02$]. Among the women with TS, one had controlled hypertension and four individuals had a history of hypothyroidism (all receiving thyroxine replacement). Two women with TS had a history of depression and were taking antidepressants (Table 1).

Table 1. Subject Characteristics

Demographic Variables	TS ($n = 27$)	Controls ($n = 20$)	p Value
Age, Years (mean ± SD)	27 ± 8	27 ± 7	.91
Current ERT users	27	1	—
Past GH Use	13	0	—
Past oxandrolone Use	12	0	—
Right Handed	26	20	—

ERT, estrogen replacement therapy; GH, growth hormone; TS, Turner's syndrome.

Genetic Studies

Cheek swabs or blood samples were collected from 25 women with TS and 1 or both of their parents; 2 families declined the test. The DNA analysis showed 18 women with TS were X^m and 7 were X^p, as determined by inheritance of alleles from the parent(s). Four families had DNA from the mother only; where the offspring had alleles not held by the mother (two cases), they were scored as having X^p. There were no significant differences between X^m and X^p in mean age and GH status; however, there was a significant difference in the frequency of oxandrolone+ (X^m = 6, X^p = 6) and oxandrolone− (X^m = 12, X^p = 1) (p = .03).

Adjusted ROI Volumes

In our main comparison, women with TS had a significantly greater % volume of cerebellum, compared with control subjects (Table 2). In contrast, women with TS had a significantly smaller % volume of bilateral parieto-occipital lobes and right hippocampus and caudate nucleus. In our exploratory analyses, among women with TS, there were no significant differences in brain matter volumes between GH+ and GH−. Women who were oxandrolone+, however, had significantly larger left caudate nuclear volume than oxandrolone− (mean ± SD = .31 ± .03 vs. .29 ± .03, p = .043). Furthermore, those who were X^m had a significantly larger volume of total and right hippocampus as compared with X^p (mean ± SD = .43 ± .04 vs. .39 ± .04, p = .03; and .22 ± .02 vs. .20 ± .02, p = .013, respectively) (Table 2).

Total Gray and White Matter Volumes

There was no significant difference in total brain gray and white matter volumes (in native space generated by SPM2) between TS women and control subjects.

Voxel-Based Morphometry: Main Comparisons

TS Compared With Control Subjects: Gray Matter. All gray matter changes were significant at $p \leq .003$, the value at which < 1 false positive cluster was expected by chance alone (Table 3 and Figure 1):

Decreased gray matter volume: women with TS had a significantly smaller gray matter volume bilaterally in the parieto-occipital cortex, inferior temporal lobes, caudate nucleus and thalamus, and in cerebellar vermis and left prefrontal cortex.

Increased gray matter volume: women with TS had a significantly increased gray matter volume bilaterally in the cerebellar hemispheres and anterior putamen.

TS Compared With Control Subjects: White Matter. All white matter changes were significant at $p \leq .006$, the value at which < 1 false

Table 2. Region of Interest Volumes of Brain Structures, % Total Intracranial Volume

Structure	TS Mean % of Intracranial Volume ± SD	Controls Mean % of Intracranial Volume ± SD	Significance (2-tailed)
Intracranial Volume Total (cm^3)	1333.43 ± 105.31	1238.91 ± 121.14	.007[a]
Cerebral Hemispheres (%) Total	78.6 ± 2.48	79.5 ± 2.86	.278
Right	39.3 ± 1.43	39.4 ± 1.51	.830
Left	39.3 ± 1.30	39.8 ± 1.61	.261
Frontal Lobes Total	38.7 ± 1.72	38.2 ± 2.34	.407
Right	19.4 ± 1.11	19.0 ± 1.19	.189
Left	19.2 ± 0.83	19.0 ± 1.29	.677
Temporal Lobes Total	10.8 ± 0.75	10.4 ± 0.91	.160
Right	5.55 ± 0.43	5.34 ± 0.56	.155
Left	5.22 ± 0.40	5.07 ± 0.40	.275
Parietal Lobes Total	18.6 ± 2.07	18.9 ± 3.38	.702
Right	9.24 ± 1.03	9.49 ± 1.61	.554
Left	9.32 ± 1.05	9.38 ± 1.83	.747
Occipital Lobes Total	10.5 ± 2.24	12.1 ± 2.80	.021[a]
Right	4.99 ± 1.11	5.82 ± 1.54	.041[a]
Left	5.48 ± 1.22	6.24 ± 1.36	.050[a]
Parietal + Occipital Lobes Total	29.0 ± 2.53	31.0 ± 3.30	.025[a]
Right	14.2 ± 1.30	15.3 ± 1.66	.017[a]
Left	14.8 ± 1.34	15.6 ± 1.72	.074
Cerebellum Total	10.6 ± 1.20	9.80 ± 1.04	.027[a]
Hippocampus Total	0.42 ± 0.04	0.45 ± 0.07	.108
Right	0.22 ± 0.02	0.23 ± 0.03	.036[a]
Left	0.21 ± 0.05	0.22 ± 0.04	.312
Amygdala Total	0.34 ± 0.05	0.33 ± 0.05	.814
Right	0.17 ± 0.02	0.17 ± 0.02	.891
Left	0.17 ± 0.02	0.17 ± 0.03	.562
Caudate Total	0.59 ± 0.06	0.63 ± 0.07	.061
Right	0.29 ± 0.03	0.31 ± 0.04	.039[a]
Left	0.30 ± 0.03	0.31 ± 0.04	.220
Putamen Total	0.53 ± 0.07	0.56 ± 0.06	.132
Right	0.26 ± 0.04	0.28 ± 0.03	.067
Left	0.27 ± 0.04	0.28 ± 0.03	.337
Lateral Ventricles Total	0.90 ± 0.60	0.92 ± 0.46	.667
Right	0.45 ± 0.30	0.42 ± 0.22	.830
Left	0.45 ± 0.30	0.49 ± 0.26	.333

[a]Significant result.

Table 3. Voxel-based Morphometry: Clusters of Significantly Decreased and Increased Gray ($p = .003$) and White ($p = .006$) Matter Volume in Women with Turner Syndrome as Compared With Control Subjects

Cluster Number (from inferior to superior)	Cluster Centroid (and other regions included in cluster)	Brodmann Area (BA) of Centroid (and other BAs included in cluster)	x	y	z	No. Voxels
Gray matter						
Decreases						
1	Cerebellar vermis	—	24	−44	−40	142
2	Right cerebellum	—	33	−42	−31	91
3	Left inferior temporal gyrus (inferior/middle/superior temporal/fusiform gyri)	20 (21, 36, 37, 38)	44	−6	−28	509
4	Right inferior temporal gyrus (inferior temporal/ fusiform gyri)	20 (18)	50	−8	−29	172
5	Left cerebellum	—	21	−39	−26	112
6	Right cuneus (R + L cunei, lingual/inferior occipital gyri, hippocampus, parahippocampal gyri, posterior cingulate, inferior/superior parietal/ paracentral lobules/precuneus, precentral gyri, L postcentral gyrus, R fusiform gyrus)	18 (1, 2, 3, 4, 5, 7, 17, 19, 23, 27, 28, 30, 31, 35, 40)	0	−80	16	8273
7	Right thalamus (R + L thalamus/caudate nuclei/ anterior cingulate gyri, L posterior cingulate gyrus)	− (25, 30)	0	−6	12	771
8	Left inferior frontal gyrus (L inferior/middle frontal/precentral gyri)	− (6, 9, 45)	49	10	24	489
9	Left postcentral gyrus (L postcentral/precentral gyri)	1 (4, 6)	48	13	46	263
Increases						
1	Left cerebellar hemisphere	—	27	−68	−38	154
2	Right cerebellar hemisphere	—	26	−71	−38	180
3	Left putamen (L putamen/caudate nuclcus)	—	16	15	2	221
4	Right putamen (R putamen/caudate nucleus)	—	19	13	6	243
White matter						
Decreases						
1	Cerebellum (cerebellum, brainstem)	—	2	−45	−34	241
2	Right cerebellum	—	25	−63	−33	125
3	Right cerebellum	—	26	−58	−28	119
4	Right occipital white matter	—	24	−78	6	260
5	Left occipitofrontal fasciculus	—	16	−12	21	164
6	Right external capsule	—	33	−15	15	97
7	Right splenium of corpus callosum	—	11	−37	30	167
8	Right superior parietal white matter	—	20	−49	58	156
Increases						
1	Brainstem	—	1	−21	−24	682
2	Right middle temporal lobe	—	43	−12	−13	435
3	Left middle temporal lobe	—	45	−36	−2	605
4	Left orbito-frontal white matter	—	12	26	−10	171
5	Right orbito-frontal white matter	—	16	23	−12	112
6	Left genu of corpus callosum	—	24	28	3	201
7	Right genu of corpus callosum	—	21	28	8	736
8	Right precentral white matter	—	47	−12	32	212
9	Right centrum semi-ovale	—	16	−19	43	105

All gray matter changes were significant at $p \leq .003$ and white matter changes at $p \leq .006$, the value at which <1 false positive cluster was expected by chance alone.

positive cluster was expected by chance alone (Table 3 and Figure 1):

Decreased white matter volume: women with TS had a significantly smaller white matter volume bilaterally in the cerebellar hemispheres, in right superior parietal lobe, occipital lobe, external capsule, and splenium of the corpus callosum, and in left occipito-frontal fasciculus.

Increased white matter volume: women with TS had significantly increased white matter volume in: brainstem; bilaterally, in the temporal lobe, orbitofrontal white matter and genui of corpus callosum, and adjacent to the right precentral gyrus and right centrum semi-ovale.

Exploratory Analyses Within TS Female Subjects

X^p compared with X^m: Gray and White Matter. All gray matter changes were significant at $p \leq .002$, and white matter changes were significant at $p \leq .01$, the values at which < 1 false positive cluster was expected by chance alone. Women with TS who were X^m had a significantly decreased gray matter volume bilaterally in the caudate nuclei, extending posteriorly into the thalamus and, in white matter volume, bilaterally in the temporal lobes (Table 4 and Figure 2).

Oxandrolone and Women With TS. Compared with oxandrolone+, oxandrolone− TS women had no significant

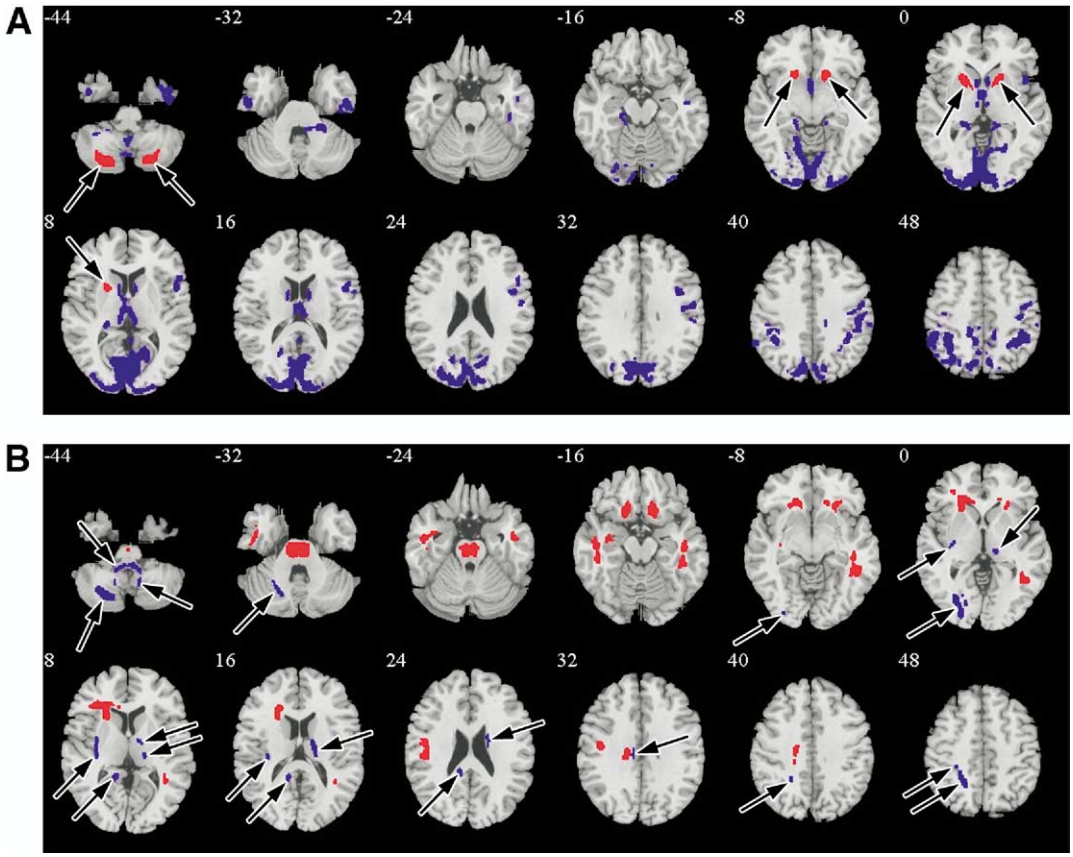

Figure 1. Voxel-based morphometry: areas of **(A)** gray matter volume significantly decreased (blue) and increased (red, with arrows) ($p \leq .003$) and **(B)** white matter volume significantly decreased (blue, with arrows) and increased (red) ($p \leq .006$) in women with Turner syndrome compared with control subjects. All changes were significant at the above values, at which <1 false positive cluster was expected by chance alone.

differences (co-varied for age) in gray or white matter volume.

GH and Women With TS. All gray matter differences were significant at $p \leq .002$, the value at which no more than one false positive cluster was expected by chance alone: Compared with GH+, TS women who were GH− had (co-varied for age) a significantly decreased gray matter volume bilaterally in the parieto-occipital and posterior temporal lobes and basal ganglia, but no significant differences in white matter volume (Table 5 and Figure 3).

^{1}H MRS

In the right parietal lobe, women with TS had a significantly lower concentration of NAA compared with control subjects (12.2 ± 2.74 vs. 13.9 ± 1.73, respectively; $p = .05$) and lower ratio of NAA:Cr+PCr and NAA:Cho. In right hippocampus,

Table 4. Voxel-based Morphology: Clusters of Significantly Decreased Gray Matter ($p = .002$) and White Matter ($p = .01$) Volume Among Women with Turner Syndrome Who Have Maternally Inherited X Chromosome Compared with Paternally Inherited X Chromosome

Cluster Number (from inferior to superior)	Cluster Centroid (and other regions included in cluster)	BA's Included in Cluster	x	y	z	No. Voxels
Gray Matter Decrease						
1	Caudate nucleus (R + L caudate nucleus/globus pallidus/thalamus/ anterior cingulate gyrus)	33	1	4	12	1555
White Matter Decrease						
1	Left temporal lobe (L temporal lobe/ lentiform nucleus)	—	35	−21	−4	180
2	Right temporal lobe (R temporal lobe/ external capsule)	—	36	−16	−2	226

All gray matter changes were significant at $p \leq .002$, and white matter changes at $p \leq .01$, the value at which < 1 false positive cluster was expected by chance alone.

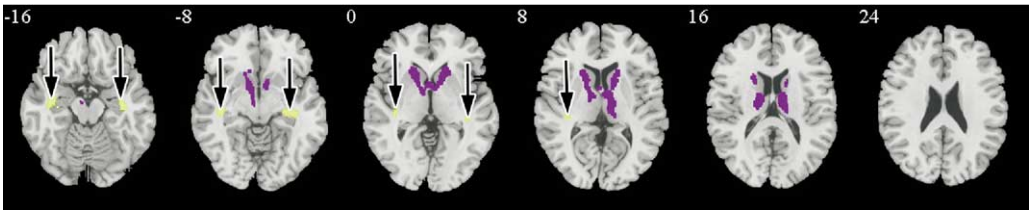

Figure 2. Voxel-based morphometry: areas of significantly decreased gray matter (purple) volume ($p \leq .002$) and white matter (yellow, with arrows) volume ($p \leq .01$) in women with Turner syndrome who have maternally inherited X chromosome compared with paternally inherited X chromosome. All changes were significant at the above values, at which < 1 false positive cluster was expected by chance alone.

women with TS had a significantly higher concentration of Cho than control subjects (3.87 ± .87 vs. 3.11 ± .87, respectively; $p = .028$) and a significantly lower NAA:Cho ratio. Among women with TS, there were no significant differences in metabolite concentrations or ratios between X^m and X^p or GH+ and GH−. Women with TS who were oxandrolone+, however, had a significantly higher concentration of parietal NAA than those who were oxandrolone− (13.75 ± 1.39 vs. 10.88 ± 2.99, respectively; $p = .038$) (Table 6).

Discussion

Our results support the hypothesis that our women with TS have differences in the anatomy of the parieto-occipital region, cerebellum, and basal ganglia and that these appear complex using VBM. Also, adults with TS have differences in hippocampal and parietal lobe neuronal membrane turnover and neuronal density/mitochondrial metabolism, which might partially explain prior reports of abnormalities in brain function and glucose metabolism. Furthermore, we report preliminary evidence that parental origin of X chromosome and postnatal hormone milieu are associated with differences in brain.

Study Limitations

Our study was observational and might be confounded by differences in physical/mental health. For example, we included euthyroid women with a past history of hypothyroidism and two taking antidepressants; however, when we excluded these individuals our results were unchanged. We did not correct for multiple comparisons, raising the possibility of type I error (e.g., we compared 34 brain regions with ROI data). By chance, making 34 comparisons, we would expect one or two false positives; however, we found nine significant differences. Furthermore, two techniques (ROI and VBM) were applied to the same data set, with good concordance between the results, making chance findings less likely. We also cannot be certain that women with TS were fully adherent to hormone treatments, although there was good agreement between self-reports of compliance and clinical records when both were available. Furthermore, in our preliminary analyses on the effect of imprinting and endocrine factors, there was "crossover" between groups. For example, in the X^m versus X^p comparison, oxandrolone status was not equally distributed and, in the ROI analysis, oxandrolone+ had significantly larger caudate nuclear volume than oxandrolone−. The comparison of oxandrolone+

Table 5. Voxel-based Morphometry: Clusters of Significantly Decreased Gray Matter Volume Among Women with Turner Syndrome Who Did Not Take Growth Hormone Compared with Those Who Did ($p = .002$)

Cluster Number (from inferior to superior)	Cluster Centroid (and other regions included in cluster)	BA of Centroid (and other BAs included in cluster)	x	y	z	No. Voxels
Decreases						
1	Brain stem	N/A	−2	−40	−39	74
2	Right putamen (R inferior frontal/anterior cingulate gyri/putamen/globus pallidus/caudate nucleus)	N/A (11, 25)	19	14	−3	604
3	Left middle temporal gyrus (L fusiform gyrus, L inferior/middle/superior temporal gyri, L inferior parietal lobule, L middle occipital gyrus)	37 (19, 21, 22, 39)	53	−65	4	653
4	Left putamen (L inferior frontal gyrus, L anterior cingulate gyrus, L fusiform gyrus, L putamen, L globus pallidus, L caudate nucleus)	N/A (19, 25, 37, 47)	17	11	−3	745
5	Right middle temporal gyrus (R inferior/middle/superior temporal gyri)	37 (19, 22, 23, 39)	55	−64	8	432
6	Left cuneus (R + L posterior cingulate gyri/inferior parietal lobule/cuneus/precuncus, L superior parietal lobule, L postcentral gyrus)	19 (7, 17, 18, 23, 30, 31)	2	−70	34	1996

All gray matter changes were significant at $p \leq .002$, the value at which < 1 false positive cluster was expected by chance alone.

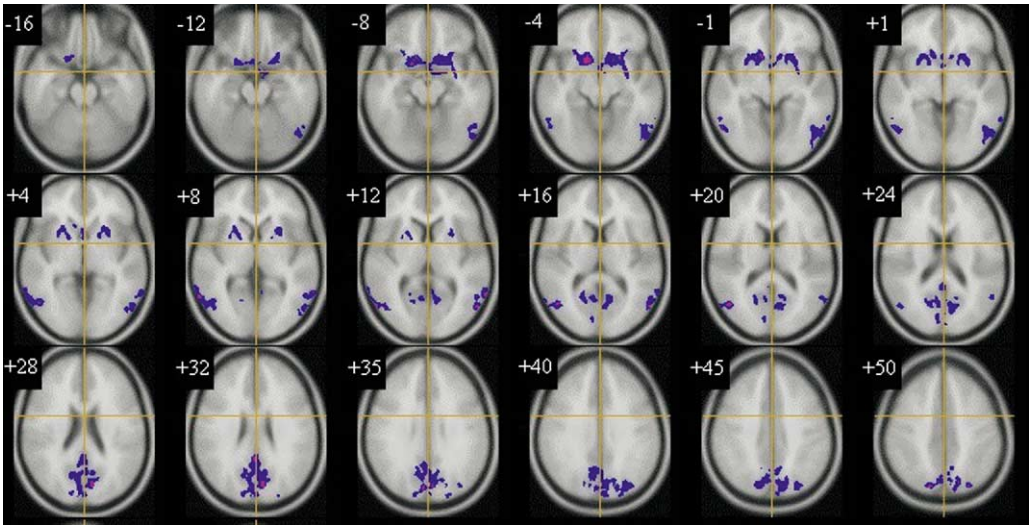

Figure 3. Voxel-based morphometry: areas of significantly decreased gray matter volume (pink represents more highly significant voxels than blue) among women with Turner syndrome who took growth hormone compared with those who did not ($p \leq .002$) All changes and correlations were significant at the above values, at which < 1 false positive cluster was expected by chance alone.

with oxandrolone− using VBM, however, revealed no significant between-group differences. Thus, differences in oxandrolone status of X^m and X^p women are unlikely to fully explain our results.

Main Comparisons

Differences in Brain Anatomy Between Women With TS and Control Subjects. In our main comparison, we found differences in the anatomy of similar brain regions with two different analytic techniques. For example, women with TS had a significantly smaller volume of parieto-occipital regions (with a right-sided predominance), hippocampus and caudate nucleus, and an increased volume of cerebellum as measured with ROI. These findings were all confirmed with VBM and are in agreement with prior work by ourselves and others (Murphy et al 1993; Reiss et al 1995). Thus, there is now increasing evidence that these brain regions are especially affected in TS.

By using both VBM and ROI approaches in the same sample, however, we have been able to elaborate on these anatomic differences. First, people with TS had an overall increase in bulk cerebellar volume (gray + white matter) as detected by ROI approach, although VBM demonstrated that women with TS had a significantly greater volume of gray matter in the cerebellar hemispheres but a significantly smaller volume in the vermis (as seen by Fryer et al 2003) and decreased cerebellar white matter volume. Second, VBM was able to demonstrate subtle differences in regional brain anatomy that were undetectable with ROI approaches, such as decreased gray matter volume of the dorsolateral prefrontal cortex and inferior temporal lobes. Last, with VBM, we found that people with TS have both decreases and increases in white matter volume of brain regions containing tracts that connect the dorsal and ventral visual processing streams (such as the short associative fibres of the right dorsal stream and the right inferior fronto-occipital fasciculus [Catani et al 2002, 2003]). Thus, people with TS have abnormalities in brain anatomy, and the regions most affected include the association neocortex, cerebellum, and basal ganglia. Also, people with TS have more subtle abnormalities than ROI approaches

Table 6. ^{1}H Spectroscopy Results (a Subgroup of Women Underwent ^{1}H Spectroscopy)

	TS	Control Subjects	*P* Values
Right Parietal ^{1}H Spectroscopy	(n = 15)	(n = 15)	
Cho (mean ± SD) mmol/L	2.36 ± 0.38	2.15 ± 0.32	.985
Cr+PCr (mean ± SD) mmol/L	10.2 ± 0.87	10.1 ± 1.20	.829
NAA (mean ± SD) mmol/L	12.2 ± 2.74	13.9 ± 1.73	.050
NAA:Cr+PCr (mean ± SD)	1.64 ± 0.34	1.89 ± 0.15	.014
NAA:Cho (mean ± SD)	1.65 ± 0.34	1.88 ± 0.21	.041
Cho:Cr+PCr (mean ± SD)	1.00 ± 0.11	1.01 ± 0.12	.696
Right Hippocampal ^{1}H Spectroscopy	(n = 14)	(n = 14)	
Cho (mean ± SD) mmol/L	3.87 ± 0.87	3.11 ± 0.87	.028
Cr+PCr (mean ± SD) mmol/L	11.1 ± 2.47	10.6 ± 3.13	.748
NAA (mean ± SD) mmol/L	11.2 ± 2.17	10.5 ± 2.73	.456
NAA:Cr+PCr (mean ± SD)	1.44 ± 0.30	1.42 ± 0.35	.898
NAA:Cho (mean ± SD)	0.93 ± 0.13	1.10 ± 0.20	.015
Cho:Cr+PCr (mean ± SD)	1.58 ± 0.44	1.31 ± 0.25	.058

Cho, choline metabolite concentration; Cr+PCr, creatine and phosphocreatine metabolite concentration; NAA, N-acetyl aspartate metabolite concentration; TS, Turner syndrome.

would suggest: they have a complex pattern of both increased and decreased gray and white matter volume in closely connected brain regions. Furthermore, abnormalities occur in regions containing important white matter tracts, and this might help explain prior reports of impaired connectivity in TS (Clark et al 1990; Murphy et al 1997).

Differences in Neuronal Integrity. The approach of combining structural MRI with ^{1}H MRS in the same individuals allows us to speculate on the biological mechanisms that underlie anatomic differences we detected. For example, women with TS had a significantly higher concentration of hippocampal Cho, and lower parietal NAA, than control subjects. In healthy adults, older age is associated with increased Cho and decreased NAA (e.g., see Robertson et al 2001). Choline-related compounds are components of neuronal cell membranes and are found in highest concentration in neuroglia and myelin sheaths (Miller et al 1996). Moreover, in rats, membrane turnover is increased by abnormal patterns of neuronal activation (Arai et al 1996), and we previously reported that women with TS have abnormal hippocampal connectivity (Murphy et al 1997). Thus, differences in Cho might indicate differences in neuroglial number, neuronal membrane turnover, neuronal connectivity, and/or be linked to hypermetabolism involving phosphatidyl choline via the action of phospholipase A2. As noted previously, increased NAA might reflect differences in neuronal density and/or mitochondrial metabolism (Clark 1998). Thus, our findings suggest that people with TS might have: 1) increased brain ageing; 2) differences in neuronal membrane turnover and signal transduction that modify cell survival; and 3) reduced neuronal density/mitochondrial function in parietal lobe—and this might partially explain prior reports of decreased glucose metabolism in this region (Clark et al 1990; Murphy et al 1997). Nevertheless, this was a cross-sectional investigation of relatively young women, and so we cannot directly address whether women with TS have increased brain ageing. Further longitudinal studies including older women are required.

Exploratory Comparisons: What Underlies the Anatomic and Metabolic Differences?

The neurobiological differences we found between people with TS and control subjects are most likely caused by a complex interaction of factors, including genetic and endocrine variables.

The Role of Parental Origin of the X Chromosome. In view of reports of cognitive and behavioral differences between X^{p} and X^{m} TS women (Bishop et al 2000; Skuse et al 1997), we hypothesized that there would be anatomic differences in brain regions that are under substantial developmental control from the X chromosome (Murphy et al 1993). In support of this, TS women with X^{m} and X^{p} had significant differences in bulk volume of right amygdala-hippocampal complex, gray matter volume in the caudate nucleus and thalamus, and white matter bilaterally in the temporal lobes. Thus, our data tentatively support the suggestion that mode of inheritance of the X chromosome affects the development of specific brain regions. Further investigations are needed to determine if differences in brain anatomy between X^{m} and X^{p} are related to their social and cognitive phenotype (Bishop et al 2000; Skuse et al 1997).

Androgens. Oxandrolone use was associated with a larger left caudate nucleus and increased NAA (a measure of neuronal density and/or mitochondrial metabolism [Clark 1998]) in the parietal lobe. In rats, androgens increase soma diameter (Breedlove and Arnold 1981) and dendrite length (DeVoogd and Nottebohm 1981). Also, in rats and in humans, androgens modulate visuospatial function (Hampson et al 1998; Hier and Crowley Jr. 1982; Resnick 1986) and brain development beyond infancy (Arnold and Gorski 1984; Chung et al 2002; Roselli 1991; Sato et al 2004). Furthermore, testosterone and non-aromatiseable androgens have neuroprotective effects (Hammond et al 2001) and increase brain NGF levels (Tirassa et al 1997). Thus, oxandrolone might be acting as both a developmental modulator and a neuroprotectant in TS; however, our results are preliminary, and longitudinal studies are required, investigating the effect of oxandrolone on brain maturation in TS.

GH. There were significant differences between women with TS who were GH+ and GH−. Women who were GH− had a significantly smaller gray matter volume in bilateral temporal, parietal, and occipital cortices, and basal ganglia. To our knowledge, we are the first to demonstrate, in vivo, an effect of GH on human brain. These regions contain GHR in humans (Castro et al 2000; Lai et al 1993). We did not find an effect of GH in the hippocampus or frontal lobe, however, where GHR are also located. There are differences in GHR molecular mass between brain regions (Lai et al 1993); thus, the lack of effect in these regions might be related to regional functional differences in receptors.

Growth hormone stimulates neuronal and glial proliferation and increases brain size in young animals (Harvey et al 1993); GH-deficient animals are microcephalic, with hypomyelination and reduced neuronal growth and synaptogenesis (Noguchi 1996). Also, in humans, GH modulates monoaminergic activity (Burman et al 1993) and insulin-like growth factor 1 (IGF-1; which has multiple neurotrophic and protective actions [Sonntag et al 2000]). The only study examining GH effects on cognition in girls with TS found no difference (Ross et al 1997), although neuropsychological tests are an indirect measure of brain, and the differences we report might be too subtle to be detected with cognitive testing. Again, our results require replication, preferably with longitudinal studies.

Conclusions

X chromosome monosomy has specific effects on brain anatomy, neuronal integrity, and metabolism. Nevertheless, we found an overlap of putative "endocrine" and "genetic" effects in some brain regions. This is to be expected, because individual brain regions are subject to multiple influences at different stages of development (Xu et al 2002). Thus, we suggest that the neuroanatomic phenotype of TS is consequent on a complex interaction between haploinsufficiency of genes escaping X inactivation, genomic imprinting, and hormonal milieu. Future studies in TS should address the longitudinal effects of hormone supplementation, the impact of genes that escape inactivation, and white matter tractography. Furthermore, clinicians prescribing hormones should be aware that they might affect brain development; and studies of human brain need to account for potential confounds introduced by hormonal milieu.

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