Brain morphology in Klinefelter syndrome

Extra X chromosome and testosterone supplementation

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Article abstract—Objective: This study focuses on variation in brain morphology associated with supernumerary X chromosome and Klinefelter syndrome (KS). Using an unselected birth cohort of KS subjects and high-resolution MRI, the authors investigated the neuroanatomic consequences of the 47,XXY karyotype in the presence and absence of exogenous testosterone supplementation. Methods: Regional brain volumes were measured in 10 subjects with KS and 10 age-matched control men. Five of the KS subjects had received testosterone supplementation since puberty (KS+T) and five had not (KS−T). Results: KS subjects showed significant (p < 0.01) reduction in left temporal lobe gray matter volumes compared with normal control subjects. Differences in left temporal gray volumes were also significant between the KS+T and KS−T groups (p < 0.01). Verbal fluency scores were significantly different between the KS+T and KS−T groups as well. Conclusion: Supernumerary X chromosome material in men is associated with a reduction in left temporal lobe gray matter, a finding that is consistent with the verbal and language deficits associated with KS. Also, relative preservation of gray matter in the left temporal region is associated with exposure to exogenous androgen during development. A history of testosterone supplementation also appears to be associated with increased verbal fluency scores in KS patients. Key words: Brain morphology—Klinefelter syndrome—Extra X chromosome—Testosterone supplementation.
increased cortical gray matter volumes, particularly in the areas of the temporal lobe and superior temporal gyrus (STG).

Materials and methods. Patients. Patients in the KS sample were recruited from an unselected cohort of 40,000 consecutive newborns screened for sex chromosome aneuploidy by X-chromatin examination between 1964 and 1974. Of the 68 patients with sex chromosome aneuploidy followed longitudinally, 13 had the 47,XXY karyotype. Ten of these nonmosaic patients were involved in our present study, including five who chose to receive testosterone supplementation (KS+T; mean age at supplementation, 17.4 years; SD, 2.70) and five who received no exogenous testosterone (KS−T). Cumulatively, the discontinuous intervals of testosterone supplementation ranged from 2 to 10 years for each KS+T patient.

The 10 propositi recruited by the National Jewish Medical and Research Center underwent research MRI scans at University Hospital in Denver, CO; they ranged in age from 24 to 32 years (mean, 27.32; SD, 2.99) at the time of image acquisition. Ten individually age-matched control men were recruited and scanned at both University Hospital in Denver (n = 7) and at the Stanford University School of Medicine, Stanford, CA (n = 3). Control subjects were matched within 2 years of age (mean, 26.81; SD, 3.28). Informed consent was obtained from all patients prior to scanning and testing.

Neuroimaging. All MR data were acquired using 1.5-tesla GE Signa scanners (Milwaukee, WI) located at the two imaging centers. Coronal three-dimensional volumetric spoiled gradient echo (SPGR) series were acquired with identical protocols in both centers (repetition time [TR] = 35, echo time [TE] = 6, flip angle = 45°; number of excitations = 1, field of view = 24, matrix = 256 X 192; 124 1.5-mm-thick partitions with 0-mm gap) and were used for all measurements and analysis. All raw formatted image data acquired in Colorado were transferred via digital tape to the Stanford Psychiatry Neuroimaging Laboratory for morphometric analysis.

The SPGR image data were imported into the program BrainImagel (Reiss, 1999) for semi-automated image processing, analysis, and quantification. Volumetric assessment of segmented image data in BrainImage® requires a stepwise process of data importation, removal of nonbrain voxels, correction of image nonuniformity, positional normalization, and fuzzy tissue segmentation. Brain tissue was isolated and subdivided into cerebral lobe, subcortical, cerebellum, and brainstem regions using a modified Talairach stereotactic grid.10,11 Segmentation of whole tissue into white, gray, and CSF compartments relies on a constrained fuzzy algorithm based on voxel intensity and tissue boundaries.12 Additionally, a single rater manually circumscribed regions of the STG for the 10 KS patients and 10 control subjects in coronal images oriented perpendicularly to the anterior commissure–posterior commissure plane according to a protocol previously developed in our laboratory. The boundaries of the STG were defined by the cortical surface and medially by a line connecting the deepest extent of the superior temporal sulcus to the furthest extent of the inferior ramus of the sylvian fissure. Anteriorly, the STG coincided with the half-way point between the head of the putamen and the anterior commissure. The most posterior slice of the STG was the first slice where the crus of the fornix was clearly distinct from the pulvinar. Interrater reliability for this procedure was previously established as 0.96 as measured by the intraclass correlation coefficient.

Neuropsychological testing. The relation between testosterone supplementation and IQ for patients with KS was also examined to ensure that the five KS+T patients had not been self-selected by receiving testosterone supplementation. Wechsler Intelligence Scale for Children–Revised (WISC-R) scores of performance IQ, verbal IQ, and full-scale IQ were gathered for all KS patients prior to initial testosterone treatments (mean age, 8.5 years; SD, 2.38) and at comparative ages in the KS−T group (mean, 8.2 years; SD, 1.10).

Verbal abilities of adult KS men were assessed within 3 years of the each patient’s MRI scan. Standard scores from the Memory for Sentences subtest13 of the Woodcock-Johnson Language Battery were recorded to assess deficits in verbal memory. Verbal fluency total scores were also gathered from the Controlled Oral Word Association Test (COWAT).14 All testing was done at the National Jewish Medical and Research Center in Denver.

Statistics. Total brain volumes were distributed normally among the KS and control groups. Group differences in regional volumes were assessed with analysis of variance (ANOVA) that took diagnosis as a between-subject factor. Analysis of covariance (ANCOVA) was also used to assess subregion comparisons after adjusting for the effects of total (cerebral) or regional (temporal lobe) compartmental volumes. Fisher’s protected least significant difference (PLSD) test for post hoc comparisons was used to further investigate multiple comparisons between the control subjects and the KS+T and KS−T groups for significantly different regions of interest. A two-sided p-value of 0.01 was the significance threshold for all analyses.

Unpaired Student’s t-tests were used to compare preadolescent IQ data between the KS+T and KS−T groups. Student’s t-test was also used to compare the standard Memory for Sentences subtest scores and COWAT scores between the KS+T and KS−T groups. The association between left temporal gray volumes and COWAT scores of KS patients was measured using the Spearman rank correlation coefficient (p).

Results. Control subjects versus KS patients. Whole-brain tissue (gray plus white) volumes were roughly equivalent between the control and KS groups. However, ANOVA revealed reductions (p ≤ 0.01) in total temporal gray and left temporal gray volumes in KS patients compared with control subjects (differences in right temporal gray volumes also approached significance). These differences did not persist during ANCOVA after adjusting for differences in total cerebral gray volumes between the KS and control groups.

Total STG gray, right STG gray, left STG gray, and right STG total tissue (gray + white) volumes were also reduced in the KS patients compared with control subjects (p ≤ 0.01). To assess the influence of the STG on reduced temporal lobe volumes, ANCOVA was used to analyze total, right, and left STG gray measures using total right or left temporal lobe gray volumes as covariates. None of these STG volumes remained significantly reduced in the
KS group after adjusting for reduced total temporal lobe volumes (table).

Other tissue compartments of the cerebrum and posterior fossa did not differ between groups. Differences were absent for ventricular CSF volumes, a region previously reported to be significantly increased in KS patients when compared with age-matched control subjects.15

Testosterone effects in KS morphology. Post hoc analyses revealed that total and left temporal gray, total STG gray, right STG gray, left STG gray, and right STG total tissue volumes were significantly reduced in the KS−T group compared with the control group. KS+T volumes were not significantly different from control subjects for any regions of interest. Left temporal gray volume was significantly reduced in the KS−T compared with the KS+T group (figure 1).

In order to investigate whether temporal gray volume reductions in KS reflects disproportionate regional volume loss in the brain, total temporal gray and left temporal gray volumes were further analyzed using ANCOVA with total cerebral gray volumes as the covariate. Post hoc analysis showed that KS−T patients had significantly reduced left temporal gray volumes when compared with both the control and KS+T groups. However, after covarying, total temporal gray volumes (right plus left) were no longer significantly reduced in the KS−T compared with KS+T patients.

Testosterone effects in KS cognition. IQ scores taken prior to initial testosterone treatment in KS+T patients and for comparative ages in KS−T patients indicated that the groups had comparable cognitive abilities at the time when five of the 10 KS patients received testosterone therapy. Unpaired Student’s t-test revealed no significant differences between the KS+T and KS−T groups for performance IQ, verbal IQ, and full-scale IQ scores on the WISC-R.

Verbal abilities in adult KS men were also examined in areas of verbal memory and verbal fluency (figure 2). The groups were not significantly different in scores of the Memory for Sentences subtest for assessing verbal memory. For verbal fluency, however, the unpaired Student’s t-tests revealed reduced COWAT scores (p < 0.01) for KS−T patients compared with KS+T adults.

The association between left temporal gray volumes and COWAT scores was investigated in the KS group (figure 3) using the Spearman rank correlation coefficient. A p of 0.542 was not significant indicating only a trend for increased COWAT scores to be associated with increased left temporal gray volumes. However, testosterone’s distinguishing effect on the morphology and verbal abilities of

<table>
<thead>
<tr>
<th>Tissue volume</th>
<th>Control subjects, n = 10</th>
<th>KS total, n = 10</th>
<th>KS+T, n = 5</th>
<th>KS−T, n = 5</th>
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</thead>
<tbody>
<tr>
<td>Whole brain tissue (gray plus white) volume, cm³ (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole brain</td>
<td>1267.1 (111.33)</td>
<td>1220.5 (128.93)</td>
<td>1214.1 (103.00)</td>
<td>1226.9 (163.37)</td>
</tr>
<tr>
<td>Right</td>
<td>636.0 (50.66)</td>
<td>608.3 (63.85)</td>
<td>607.3 (48.31)</td>
<td>609.2 (82.70)</td>
</tr>
<tr>
<td>Left</td>
<td>631.1 (61.14)</td>
<td>612.2 (65.62)</td>
<td>606.8 (55.40)</td>
<td>617.7 (80.89)</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>212.0 (23.02)</td>
<td>191.8 (22.19)</td>
<td>202.0 (23.57)</td>
<td>181.6 (17.10)</td>
</tr>
<tr>
<td>Right</td>
<td>105.6 (12.35)</td>
<td>95.9 (12.06)</td>
<td>100.1 (11.26)</td>
<td>91.8 (12.55)</td>
</tr>
<tr>
<td>Left</td>
<td>106.4 (11.46)</td>
<td>95.9 (10.95)</td>
<td>101.9 (12.41)</td>
<td>89.8 (4.90)</td>
</tr>
<tr>
<td>STG</td>
<td>36.8 (3.74)</td>
<td>32.3 (3.53)</td>
<td>33.8 (4.29)</td>
<td>30.8 (2.03)</td>
</tr>
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<td>Right</td>
<td>19.2 (2.23)</td>
<td>16.3 (1.67)*</td>
<td>17.1 (1.78)</td>
<td>15.5 (1.31)*</td>
</tr>
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<td>Left</td>
<td>17.6 (1.60)</td>
<td>16.0 (2.04)</td>
<td>16.7 (2.65)</td>
<td>15.2 (0.97)</td>
</tr>
<tr>
<td>Gray tissue volume, cm³ (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole brain</td>
<td>717.2 (69.62)</td>
<td>665.8 (67.62)</td>
<td>682.4 (44.21)</td>
<td>649.1 (87.40)</td>
</tr>
<tr>
<td>Right</td>
<td>362.4 (30.71)</td>
<td>332.2 (32.98)</td>
<td>343.0 (19.06)</td>
<td>323.2 (42.90)</td>
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<td>Left</td>
<td>354.8 (39.48)</td>
<td>332.6 (35.20)</td>
<td>339.4 (25.69)</td>
<td>325.8 (44.87)</td>
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<td>Temporal lobe</td>
<td>143.2 (14.60)</td>
<td>123.7 (18.73)*</td>
<td>135.6 (11.19)</td>
<td>111.9 (17.71)*</td>
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<td>Right</td>
<td>70.1 (7.40)</td>
<td>61.1 (9.66)</td>
<td>66.3 (6.39)</td>
<td>55.9 (10.11)</td>
</tr>
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<td>Left</td>
<td>73.1 (7.55)</td>
<td>62.6 (9.55)*</td>
<td>69.3 (5.05)</td>
<td>56.0 (8.27)*‡</td>
</tr>
<tr>
<td>STG</td>
<td>25.3 (2.93)</td>
<td>21.5 (2.41)*</td>
<td>23.1 (1.71)</td>
<td>20.0 (2.11)*</td>
</tr>
<tr>
<td>Right</td>
<td>12.6 (1.61)</td>
<td>10.5 (1.38)*</td>
<td>11.3 (0.80)</td>
<td>9.7 (1.42)*</td>
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<tr>
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<td>11.1 (1.28)*</td>
<td>11.8 (1.17)</td>
<td>10.4 (1.03)*</td>
</tr>
</tbody>
</table>

* Analysis of variance (ANOVA) and Fisher’s protected least significant difference (PLSD) show significance compared to controls.
† ANOVA and Fisher’s PLSD show significance compared to KS+T group.
‡ Analysis of covariance (ANCOVA) with total brain gray volume as a covariate. Fisher’s PLSD shows significance compared to control and KS+T groups.

KS = Klinefelter syndrome; KS+T = KS subjects with testosterone supplementation; KS−T = KS subjects without testosterone supplementation; STG = superior temporal gyrus.
adults with KS is reflected in figure 3, which indicates both increased left temporal gray volumes and COWAT scores in KS\(^1\) patients.

**Discussion.** The results of this study are tempered by the potential selection bias that is introduced when only five of the 10 patients with KS chose to receive testosterone therapy. The cognitive, environmental (e.g., socioeconomic status), or physiologic variables occurring in KS may predispose some individuals to seek testosterone supplementation more than others and may contribute to the segregated KS\(^1\) versus KS\(^2\) results presented in this paper. Scores of general intelligence were not significantly different between KS\(^1\) and KS\(^2\) groups as assessed by the WISC-R administered prior to puberty and testosterone supplementation. It appears, therefore, that general intelligence was not a predictor for whether a patient received testosterone treatment or not. As this is an indirect measure of intergroup compatibility, however, other unassessed variables may be relevant in selecting for testosterone-treated KS patients.

All of the KS\(^1\) patients began supplementation 5 to 10 years prior to the MR scan. However, the retrospective nature of this study makes it difficult to precisely document the timing of testosterone usage. Although the effect of testosterone supplementation is associated with preserved verbal fluency scores and left temporal lobe volumes, the supplementation was discontinuous and highly variable among these patients. Of the five KS\(^1\)+T patients, one received testosterone supplementation continuously to date, one received treatment sporadically, two received treatment continuously except for a single 6 to 12 month interval, and one had had no supplementation 2 years prior to the MRI. This variability may be a confounding factor in comparing KS\(^1\)+T and KS\(^2\)-T patients and may blur the distinction between the two groups.

Although volumetric measurements using the Talairach coordinate system are sensitive to total lobar differences between groups, they do not reflect differences that may occur in smaller subregions of the brain. Specific areas of the frontal and parietal lobes may be anomalous in KS and may correlate to deficits in phonologic processing, verbal fluency, and reading that occur in men with KS. Our manual delineation was limited to the STG—a structure thought to be integral in language processing. Future investigations, however, should explore other neuroanatomic areas that are of functional interest in KS but may not be assessed by the Talairach system.

**Implications.** Our results show evidence of significant variation in cortical gray volumes in the temporal lobe of persons with KS, particularly on the left. For KS, reduced performances on tests of retrieval, reading skill, verbal IQ,\(^16,17\) and auditory short-term memory\(^6,18\) stand in contrast to relatively preserved abilities on tests of visual-spatial and math skills. It has been proposed that left-hemisphere–associated tasks such as verbal fluency and auditory short-term memory are diminished in KS individuals due to a reduction of the normal cerebral lateralization that occurs in euploidic men.\(^19\) In normally developing men these skills are thought to be strongest in the left hemisphere with lateralized verbal abilities occurring primarily in the temporal lobe. Our finding of reduced left temporal lobe gray volumes in patients with KS provides a potential structural basis for the observed neuropsychological deficits. This result remains even after adjusting for the slight differences in whole brain volumes between KS patients and control subjects.

The high incidence of dyslexia in men with KS also implicates functional abnormalities in areas of the brain thought to be essential for reading. Reduced STG gray volumes in both hemispheres of the brain are consistent with the bilateral reduction of gray matter within the temporal lobe. However, ANCOVAs reveal that the STG does not specifically or principally account for this temporal gray matter reduction in KS patients, and that there may be more extensive loss of tissue within the entire lobe. While the STG is thought to play an essential role in reading, it is possible that the neuropathology associated with developmental reading disorders is also

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**Figure 1.** Left temporal lobe gray volumes (cm\(^3\)) for control subjects, Klinefelter syndrome (KS) subjects with testosterone supplementation (KS\(^1\)+T), and KS subjects without testosterone supplementation (KS\(^2\)-T).
distributed to areas of the temporal lobe not assessed in this study.

Subgroup KS+T and KS–T comparisons seem to indicate that androgen supplementation may affect KS morphology and cognition. Temporal lobe gray and STG measures for the KS+T group were not significantly reduced compared with control subjects, indicating a preservation of temporal lobe gray matter bilaterally in this group. Conversely, the untreated KS–T group illustrates the morphologic consequences of KS in the absence of exogenous androgen supplementation particularly in the left temporal lobe (see figure 1).

Left temporal gray matter preservation in KS+T patients may be related to a preservation of cognitive abilities associated with this area. Memory for Sentences subtest scores revealed no significant differences between KS+T and KS–T groups, indicating relatively equivalent abilities in verbal memory. However, the COWAT scores revealed higher abilities in verbal fluency among the KS+T group (see figure 2) compared with the KS–T group. An association between left temporal gray volumes and COWAT scores is difficult to assess with such a small sample size. However, the relatively segregated distribution between the KS+T and KS–T groups (see figure 3) indicates that testosterone supplementation was a potential influence on both COWAT scores and left temporal lobe gray volumes. Given the phenotypic variability that normally occurs among individuals with KS, the almost complete nonoverlap between KS+T and KS–T groups for left temporal gray volumes and COWAT scores may reflect a strong effect of exogenous testosterone.

The cerebral cortex may retain plasticity well into adulthood, allowing it to be continually modified by externally supplemented and endogenous hormones. The ability to reverse right and left patterns of cerebral asymmetry was previously demonstrated in go-

Figure 2. Verbal abilities in Klinefelter syndrome (KS). Controlled Oral Word Association Test (COWAT) total scores (verbal fluency) and Memory for Sentences Test standard scores (verbal memory) for KS subjects with testosterone supplementation (KS+T) and without testosterone supplementation (KS–T) in adulthood.

Figure 3. Left temporal lobe gray volumes and verbal fluency in Klinefelter syndrome (KS). Controlled Oral Word Association Test (COWAT) total scores (verbal fluency) versus left temporal gray volumes in KS subjects with testosterone supplementation (●) and without testosterone supplementation (■).
ment therapy in surgically menopausal and healthy postmenopausal women.24,25 Verbal memory scores were shown to be preserved or increased in women receiving estrogen replacement therapy after abdominal hysterectomy and bilateral salpingo-oophorectomy when compared with preoperative scores and control subjects who did not receive supplementation. With exogenous estrogen administration, Sherwin and Tulandi26 demonstrated a reversal of verbal memory deficits caused by decreased estrogen levels in women treated with gonadotropin-releasing hormone agonists for uterine myomas.

Although the language abilities of testosterone-treated KS men have not previously been compared with those of untreated KS men, other studies demonstrated the increased behavioral benefits after supplementation, even in adulthood.27,28 The mechanism of testosterone's effectiveness, however, is not well established, given the hormonal competition occurring in pituitary-gonadal function in KS. Early pubertal boys with KS show increased estradiol levels even when serum testosterone levels are relatively normal. These elevated levels persist when testosterone levels drop off by midpuberty.29 Exogenous testosterone may exert its influence in the brain through its competition with serum free estrogen and other hormonal factors (follicle stimulating hormone, luteinizing hormone), or may be mediated through the aromatization of testosterone into estradiol30 in specific areas of brain.

Men with KS show cognitive deficits prior to puberty, even when basal testosterone levels are comparatively normal.31 The putative role of testosterone as a primary predictor of cognitive functioning in men, therefore, is doubtful. However, our investigation of this unselected KS cohort suggests that testosterone supplementation is associated with preserved left temporal gray tissue volume, even in postpubertal men with KS. The small sample size makes it difficult to draw any conclusions about the structural basis of the cognitive phenotype of KS; however, our results support the left temporal lobe's role in diminished verbal fluency. With a larger sample size and a randomized testosterone treatment condition, future investigations using functional MRI should focus on the neurocognitive aspects of KS and the neurodevelopmental effects of exogenous testosterone supplementation.

References