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Klinefelter's Syndrome (XXY) as a Genetic Model for Psychotic Disorders

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Males with an extra-X chromosome (Klinefelter's syndrome) frequently, although not always, have an increased prevalence of psychiatric disturbances that range from attention deficit disorder in childhood to schizophrenia or severe affective disorders during adulthood. In addition, they frequently have characteristic verbal deficits. Thus, examining brain magnetic resonance imaging (MRI) scans of these individuals may yield clues to the influence of X chromosome genes on brain structural variation corresponding to psychiatric and cognitive disorders. Eleven adult XXY and 11 age matched XY male controls were examined with a structured psychiatric interview, battery of cognitive tests, and an MRI scan. Ten of eleven of the XXY men had some form of psychiatric disturbance, four of whom had auditory hallucinations compared with none of the XY controls. Significantly smaller frontal lobe, temporal lobe, and superior temporal gyrus (STG) cortical volumes were observed bilaterally in the XXY men. In addition, diffusion tensor imaging (DTI) of white matter integrity resulted in four regions of reduced fractional anisotropy (FA) in XXY men compared with controls, three in the left hemisphere, and one on the right. These correspond to the left posterior limb of the internal capsule, bilateral anterior cingulate, and left arcuate bundle. Specific cognitive deficits in executive functioning attributable to frontal lobe integrity and verbal comprehension were noted. Thus, excess expression of one or more X chromosome genes influences both gray and white matter development in frontal and temporal lobes, as well as white matter tracts leading to them, and may in this way contribute to the executive and language deficits observed in these adults. Future prospective studies are needed to determine which gene or genes are involved and whether their expression could be modified with appropriate treatments early in life. Brain expressed genes that are known to escape inactivation on extra-X chromosomes would be prime candidates.

KEY WORDS: MRI; DTI; verbal IQ; cognition; schizophrenia; bipolar disorder

BACKGROUND

An increased frequency of a variety of psychiatric disturbances, often found severe enough to lead to hospitalization, are found among individuals with Klinefelter's syndrome (an XXY karyotype) and XXY men are found in about 0.8–1% of males hospitalized with schizophrenia, a four- to five-fold excess over general live birth rates [DeLisi et al., 1994]. This may indicate that genes that are overexpressed in the brains of XXY males may also be relevant to schizophrenia and other psychiatric disorders. Since the search for genes leading to susceptibility to major psychiatric disorders has produced inconsistent results thus far, the XXY karyotype may serve as a naturally occurring genetic model to provide clues to the inherited abnormalities that cause these disorders in individuals with normal chromosomal numbers.

Similarly, approximately 1% of all individuals institutionalized with mental retardation have an XXY karyotype [Rosen et al., 1970] and approximately half of all mental retardation in males originates from a defective gene on the X chromosome. While the fragile-X site has been implicated in most X-linked mental retardation disorders [e.g., Reiss et al., 1995], other regions of the X chromosome have also been linked to some mental retardation syndromes [reviewed in Chiurazzi et al., 2000]. Thus, genes involved in human cognition reside on the X chromosome.

Klinefelter et al. [1942] described a syndrome in males characterized by gynaecomastia, aspermatogenesis, and increased urinary excretion of FSH. In 1956, it was subsequently shown [Bradbury et al., 1956; Jackson et al., 1956; Plunkett and Barr, 1956; Riis et al., 1956] that patients with Klinefelter's syndrome had positive sex chromatin material in epithelial cells similar to normal females; and then in 1959, Jacobs and Strong [1959] found that a chromatin-positive patient with Klinefelter's syndrome had 47 chromosomes or an XXY karyotype.

It is now known that the XXY karyotype occurs in 1 in 500 live male births and is the most common type of human chromosome anomaly [Smyth and Bremmer, 1998]. Unlike chromosomal duplications or translocations on autosomes, the XXY karyotype leads to relatively mild clinically noticeable deviations. This mildness is probably due to inactivation of most genes on the extra X chromosome [Heard et al., 1997]. However, there is a class of X chromosome genes that have homologies on the Y chromosome [Jegalian and Page, 1998], and tend to escape the normal extra X chromosome inactivation process, as do some other X-specific genes [Carrel et al., 1999; Sudbrak et al., 2001]. It is thought that the characteristic features of Klinefelter's syndrome originate from genes that escape inactivation, and are expressed in excess. Thus the tall stature, testicular and sex hormone deficiencies, reduction of

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secondary sex characteristics, and in some cases, breast development in post-pubertal Klinefelter's men [Smyth and Bremmer, 1998] may be due to expression of X-Y homologous genes. Similarly, behavioral and cognitive symptoms of Klinefelter's syndrome are likely to result from this class of genes as well [Carrel et al., 1999]. It has been suggested [Crow, 2002] that at least one gene or genes in X-Y homologous regions of the sex chromosomes that escape normal X-inactivation are crucial for language functioning.

Several studies in the literature have reported cognitive (particularly verbal) abnormalities, in Klinefelter's males [Nielsen, 1969; Netley and Rovet, 1982a; Theilgaard, 1986; Bender et al., 2001; Boone et al., 2001; Fales et al., 2003] and some [reviewed in DeLisi et al., 1994], but not all [Mors et al., 2001], have described associated psychiatric disturbances, in particular, schizophrenia [Ratcliffe, 1999; reviewed in DeLisi et al., 1994]. In addition, some recent studies examining brain structure in XXY men reported varying findings [i.e., amygdala, temporal lobe, and whole brain volumes, as well as enlarged ventricles and reversed cerebral asymmetries: Warwick et al., 1999, 2003; Patwardhan et al., 2000, 2002; Reiss et al., 2000] and one fMRI study found abnormalities in normal asymmetric brain functioning [Itti et al., 2003]. However, most, but not all, studies of Klinefelter's men have been in children, and none have evaluated white matter microstructure using newer MRI techniques, such as Diffusion Tensor Imaging (DTI). Thus, the present study adds additional data on brain structural, functional, and behavioral deficits in adult XXY men.

MATERIALS AND METHODS

Adult males with a previously diagnosed XXY karyotype were recruited primarily from advertisements on websites for regional USA support groups designed specifically to aid people with Klinefelter's syndrome and other chromosomal anomalies, and by contacting the leadership of these organizations directly. XY controls were selected for similarity to patients in age, race, years of education, and social class as closely as possible since these variables could have an effect on cognitive ability and brain structure that is unrelated to having an extra-X chromosome. In addition, since most XXY males are administered testosterone beginning in their adolescence, an attempt was made to find XY males that were taking testosterone for other reasons, such as infertility, as testosterone may have an effect on brain structure and functioning. Thus, controls were recruited by advertisements in New York City neighborhood gymnasiums, infertility clinics (attempting to recruit XY hypogonadal males) and local newspaper ads for males who have been administrated testosterone for non-genetic deficits. They were unscreened for psychiatric disorders and the advertisements did not mention that the study took place within a psychiatry department. Eleven males with previous written documentation of an XXY karyotype agreed to participate in this study and were matched by age, race, and social class 1:1 to controls. Since only four males volunteered who were medicated with testosterone and matched in other characteristics to XXY males, the remaining seven male controls were selected from testosterone-free individuals.

This study was approved by the New York University School of Medicine and The Nathan S. Kline Institute for Psychiatric Research human subjects' review boards. All participants gave written informed consent prior to their participation.

A blood sample was drawn from all subjects for confirmation of XXY or XY karyotypes and high-resolution karyotyping was performed by J.L. at Coriell Institute in Camden, New Jersey directly on cultured T lymphocytes using standard techniques. A minimum of 15 cells were microscopically studied on XY and 50 cells for XXY individuals using an Applied Imaging Cytovision System to capture and enhance digital images of chromosome spreads, prepare karyotypes, and archive these images to an optical disk. A minimum of five karyotypes was analyzed at the 550-band stage or higher. A partial karyotype included examples from five or more prometaphase cells at the 850-band level to provide fine structural details of the sex chromosomes and any autosome of suspicious morphology. Images were interpreted and described in conformance to internationally accepted standards [ISCN, 1995]. This provided an overview of the integrity of the genome with a resolution of about 3 megabases and excluded mosaicism for chromosomal anomalies present in 19% or more of the cells with 95% confidence [Hook, 1977].

A structured psychiatric interview was performed on all subjects using the DIGS [Diagnostic Interview for Genetic Studies, Nurnberger et al., 1994] and a psychiatric diagnosis made according to DSM-IV Criteria for both Axis I and Axis II psychiatric disorders. The Scale for Assessment of Thought, Language and Communication [TLC; Andreasen, 1986] was used to rate formal thought disorder, The Scale for Assessment of Negative Symptoms (SANS) and the Scale for Assessment of Positive Symptoms (SAPS) was used to determine any evidence of psychosis [Andreasen, 1989; N. Andersen, Iowa City, Iowa].

A battery of cognitive tests was performed that included: (1) General Cognitive Ability (Full-Scale, Verbal, and Performance IQs obtained from the full WAIS-III); (2) Receptive Language [the Goldman Fristoe Woodcock Test of Auditory Comprehension (during quiet and with noise) and the Token Test (a series of oral commands about colored circles and squares given in increasing complexity)]. (3) Expressive Language and Academic Skills [The Wide Range Achievement Test, 3rd Edition (WRAT), The Boston Naming Test, and Controlled Oral Word Association (examined with both category fluency and phonological fluency)]; (4) Executive Function [Wisconsin Card Sort Test (WCST) and the Stroop Color-Word Test]. The Stroop test measures processing speed and inhibition with simple task demands; the WCST measures more problem solving and working memory. (5) Verbal Memory (Weschler Memory Scale-III Logical Memory I and II and the California Verbal Learning Test); (6) Spatial Memory (Visual Reproduction immediate and delayed from Wechsler Memory Scale-III and the Benton Visual Retention Test); (7) Concentration/Attention [Trail-making Tests Parts A and B and the Continuous Performance Task (CPT)]; (8) Sensory-Perceptual Functioning (finger agnosis and fingertip number writing); (9) Functional Asymmetry [measured by relative hand preference using the Annett Scale (Annett, 1985), relative hand-skill according to Tapley and Bryden (1985) and the Purdue Pegboard Test].

MRI Acquisition and Processing

Brain imaging was conducted on a 1.5 T Siemens Vision MRI system at the New York University School of Medicine, Tisch Hospital and stored on optical discs for future analyses. A 3D T1-weighted sagittal MP-RAGE scan was acquired (TR = 9.7 msec, TE = 4 msec, matrix = 256×256, FOV = 210 mm, 128 slices, 1.5-mm slice thickness). In addition, an oblique axial dual echo turbo spin echo scan was acquired aligned to the plane containing the anterior and posterior commissures (AC-PC plane; TR = 5,000 msec, TE = 22/90 msec, 256×256 matrix, FOV = 240 mm, 26 slices, 5-mm slice thickness, 0-mm gap). This scan provided a proton density (PD) and a T2-weighted (T2) volume. The T2 volume was used for distortion correction of the DTI images as described below. AC-PC aligned axial DTI scans were acquired with

a pulsed gradient, double spin echo, echo planar imaging (EPI) sequence (TR/TE = 4,400/100 msec, 128 × 128 matrix interpolated to 256 × 256, FOV = 240 mm, $\underline{b} = 900$ sec/mm^2, NEX = 4, 20 slices, 5-mm slice thickness, 0-mm gap, interleaved). The double spin echo method substantially reduces image distortion artifacts due to eddy currents [Reese et al., 2003]. Diffusion was measured along six non-collinear directions (Gx, Gy, Gz) = {(1, 1, 0), (1, 0, 1), (0, 1, 1), (1, −1, 0), (−1, 0, 1), (0, 1, −1)}. For each of these six gradient directions, four acquisitions were averaged. Two acquisitions without diffusion weighting (b = 0) were also averaged. Thus, seven DTI volumes were obtained in total for each imaged slice. Manufacturer-supplied automated shimming procedures were performed prior to collection of scans to improve field homogeneity. Movement artifacts were minimized by stabilizing head position with cushions.

Image Processing for Volumetric Measurements

MPRAGE scans were re-orientated along the AC-PC plane using the MEDx software package (Sensor Systems, Sterling, VA). Scans were then manually stripped of the extracranial tissue and the intensity information from the MPRAGE images was used to automatically classify voxels into gray matter, white matter, and cerebrospinal fluid using the FAST segmentation program (FMRIB Software Library, available at http://www.fmrib.ox.ac.uk/fsl/). The segmentation program first corrected for spatial intensity variations (i.e., RF in homogeneities). The segmentation output was superimposed on the co-registered grayscale images and edited slice-by-slice using the 3D Slicer software package (available at http://www.slicer.org) to isolate the region of interest (ROI). The slice editing program allows for manual drawing and automatic reformatting of sagittally acquired images into coronal and axial views, which facilitated visualization of the appropriate sulcal landmarks.

Volumetric Measurements

Atlas [Ono et al., 1990; Duvernoy, 1999] assisted volumetric measurements were performed on coronal slices from the MPRAGE sequence that was segmented into gray matter. Measurements were guided by information from other views. On completion of anatomical ROIs slice measurements, each was summed by the slice editor to provide total structure volumes.

Frontal lobes. The posterior border began three slices anterior to the separation of the frontal lobe and temporal lobe at the temporal stem [Wible et al., 1997]. All gray matter anterior to this coronal plane was included until tissue could not be seen in the coronal view. The boundaries were then verified on sagittal and axial views. The frontal lobe was separated into right and left using the inter-hemispheric fissure.

Temporal lobes. To obtain the posterior border, all three slice orientations were used as aids and measurements were performed on coronal images. A short horizontal line going through the top of the superior temporal gyrus (STG) was drawn on the last coronal slice containing the STG.

Using the axial plane, the slice with the most visible occipital-parietal notch was then chosen and a short horizontal line was drawn through the occipital-parietal notch. The lines were then connected on the sagittal view. This line formed a cut-off point, visible on each coronal slice containing the temporal lobe. The temporal lobe below the line was then measured. The temporal lobe was traced to the most anterior point on the coronal view until there was no remaining tissue visible. The accuracy of the boundaries was checked on the sagittal plane in order to verify that parietal lobe tissue was not included.

Superior temporal gyrus (STG). The anterior boundary began at the coronal slice in which the white matter of the temporal stem is first visible. The ventral border was the superior temporal sulcus throughout its extent. All gray matter was included using the post-sylvian dorsal border, as described above. If the terminus of the sylvian fissure was branched the more prominent segment was followed to its end; thus, if a dorsal extension of the STG was present, it was included in the volume measurement. The gray matter of the STG was then divided into anterior and posterior sections using the anterior border of the first transverse temporal (i.e., Heschl's) gyrus as the dividing plane. The first coronal slice of the posterior subdivision was defined as the most anterior slice in which Heschl's sulcus (separating Heschl's gyrus from the planum temporale) was clearly present.

Hippocampus/amygdala complex. Since the gray matter of the anterior hippocampus could not be reliably differentiated from the amygdale, the hippocampus, and amygdala, excluding the parahippocampal gyrus, are reported here and measured as one structure. Tracing began at the posterior most slice, when the fornix separates from the parahippocampal white matter track. Tracing continued until the amygdala was no longer clearly distinguished from the temporal pole.

Ventricles. Right and left lateral ventricles were measured on all coronal slices where visible and checked on both axial and sagittal slices.

Reliability of anatomical measurements. Only one rater measured each structure on all scans. The intraclass correlations for measurements on 10 scans performed twice were: 0.999 for intracranial contents, 0.993 for left and 0.990 for right frontal lobes, and 0.999 for left and 0.997 for right ventricles, 0.926 for left and 0.967 for right temporal lobe, 0.909 for left and 0.934 for right STG, and 0.848 for left and 0.887 for right hippocampal/amydala complex.

Image processing for voxelwise analysis of DTI. All image processing and analyses were performed on a 2.4 GHz Dell Precision Workstation 530 computer (Dell Computer Co., Austin, TX) running the SUSE Linux 7.3 operating system (SUSE, Inc., Oakland, CA) (see Fig. 1) The MPRAGE (256 × 256 × 128) volume was registered to the T2/PD volumes

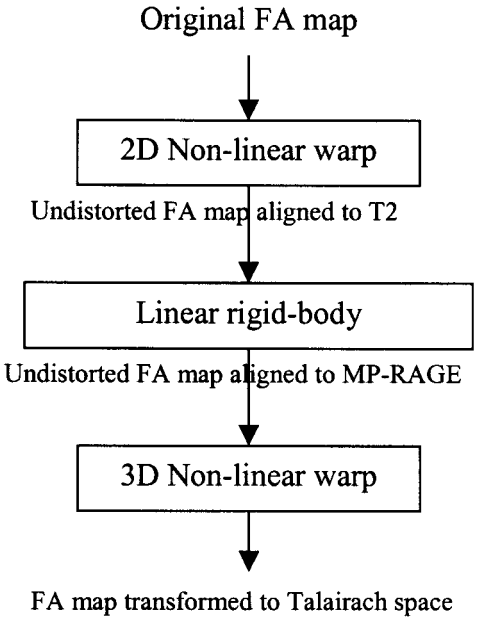

Fig. 1. Sequential method for analysis of DTI scan sequences.

($256 \times 256 \times 26$) using a linear rigid-body transformation. The 4×4 transformation matrix (M) for this registration was obtained as a product of two independently computed matrices M_1 and M_2 ($M = M_2 \times M_1$). The transformation M_1 performs a gross registration that corrects for the different image orientations between the sagittal MPRAGE and axial T2/PD volumes and slice placements. This matrix was computed directly based on the information stored in the Siemens Vision image file format. Equivalent information is available in file formats of other MR scanner manufacturers (e.g., GE) and is also part of the DICOM standard format. The M_2 transformation corrects for small registration errors due subject motion. This transformation matrix was found using the registration algorithm described by Ardekani et al. [1995]. The matrix M was stored for subsequent application in registering the fractional anisotropy (FA) maps of all subjects as described below.

The non-brain areas on the T2 volume were removed semi-automatically. The edited T2 volume was used to correct the susceptibility induced spatial distortions of the b0 ($b = 0$) DTI volume using a non-linear 2D warping algorithm as previously described [Ardekani, 2003]. In this method, an object volume, in this case b0, is iteratively deformed to match a template image, in this case the edited T2 volume, using a multi-resolution approach. The resulting 2D deformation fields (W2D) were approximated by truncated Fourier-Legendre series [Kaplan, 1973] and their coefficients were saved for use in subsequent analysis. Using the 3D version of the same warping algorithm [Ardekani, 2003], the MPRAGE volumes of all subjects were registered to a single template image. In this case, the template image ($161 \times 191 \times 151$, 1 mm^3) was the MP-RAGE volume of one (healthy volunteer) subject that had been transformed to the Talairach space using the AFNI software package (Cox, 1996). The warp field for each subject, denoted as W3D, was approximated by a truncated Fourier-Legendre series and its coefficients were saved for subsequent analysis.

An FA map was computed from the 7 DTI volumes for each subject using previously described methods [Basser et al., 1994; Basser, 1995; Basser and Pierpaoli, 1998] prior to distortion correction or any other image manipulation. The FA map of each subject was then transformed to the Talairach coordinates by combining the transformations M, W2D, and W3D into a single transformation and applying it to the original FA map by a single interpolation operation. This process is depicted graphically in Figure 1. This approach reduces interpolation errors, because only a single interpolation representing the combination of all registrations and distortion corrections is applied to the FA maps.

Thus, 22 FA maps (11 maps per group) were obtained of matrix size $161 \times 191 \times 151$ and voxel size 1 mm^3 in a common Talairach space. The average FA map of the patient group was subtracted from that of the healthy comparison subjects and divided by the estimated standard deviation at each voxel to obtain a t-map with 20 degrees of freedom. A threshold criterion was applied to the resulting t-map at ($P < 0.001$, two-tailed) followed by an extent threshold criterion of at least 100 contiguous voxels. Those voxels that met both criteria were considered to have a significantly reduced (or increased depending on the sign of the difference) mean FA value in the XXY relative to XY males.

Statistical Analyses

All statistical testing was conducted using the Statistical Package for the Social Sciences (SPSS version 11.5 for PC). Anatomical volumes for left and right sides separately for each ROI were compared between groups with an analysis of covariance (ANCOVA) controlling for age and intracranial contents. Asymmetric structural changes were examined by calculating a laterality index $(L - R)/(0.5(L + R))$ for each structure. Thus the coefficient was positive when left was greater than right, and negative when right was greater than left. Exploratory analyses were also performed correlating anatomical measurements with clinical and cognitive variables using Pearson's correlation coefficients.

RESULTS

High-Resolution Karyotyping

Of the 11 controls, all were confirmed to be XY males. However, of the 11 Klinefelter's individuals who all were previously informed by doctors based on "blood screening" that they had Klinefelter's syndrome, only 6 had all 50 cells screened showing an XXY karyotype. Of the other five individuals, in four, the majority of cells were an XXY karyotype with varying degrees of other combinations: One individual had 1 48,XXXY cell; 1 individual had 11 of 50 cells either 46XX or a translocation of the Yq12 to chromosome 18q23; one individual had 48 XXY cells; but 1 XX and 1 XY cell; 1 had 47 XXY cells, but 2XX and 1 XXXY. One individual had 17XXYs with a mixed mosaicism, mostly XYY cells. None of the data reported below was associated with either having or not having some mosaicism.

Clinical Variables

Some of the XXY and XY men satisfied criteria for Axis I DSM-IV major psychiatric disorders. However, only two of the XXY men and none of the controls had illness severe enough to require psychiatric hospitalization. Only four of the XXY men and none of the controls reported auditory hallucinations. Seven of the XXY and none of the XY males reported dyslexia in childhood Table I.

Cognitive Variables

In order to control for type II errors, an a priori significance level of $P < 0.01$ was established. Given the size of the database, data in Table II are only presented for those tests where this level of significance was obtained. The first analysis used t-tests to compare group differences in performance. Consistent with previous findings, there were statistically significant differences in a variety of intellectual and verbal skills measured, with the XXY group being more impaired on all variables. These included the WRAT-R reading test, information and vocabulary from the WAIS, and the Peabody picture vocabulary test. There were also significant differences in memory measures including sentence repetition and visual reproduction, part II from the WMS. Purdue pegboard performance on the non-dominant hand was impaired, as were all Stroop test measures, including word reading, color naming, and the color-word interference condition.

Since several indices of verbal intellectual functioning were impaired and verbal skills are central to previous conceptions of the impairments in XXY syndromes, WRAT-R reading scores were used as a covariate and all of the other variables were compared again across the two groups with analysis of covariance. In this analysis, there was only one variable that remained statistically significant for between group differences: the Stroop interference condition, $F(1,19) = 4.50$, $P < 0.04$. Thus, the array of cognitive differences between the two samples is reduced to two significant domains: verbal intelligence as indexed by reading performance and executive functioning as indexed by the Stroop test.

Brain Structure

Volumetric analyses. Subjects with Klinefelter's syndrome had significantly smaller brain volumes as measured by total intracranial contents (ICC; $F = 4.5$, $P < 0.05$). In addition

TABLE I. Demographic and Clinical Variables for XXY and XY Males (±SD)

	Klinefelter's males (XXY) (N = 11)	Control males (XY) (N = 11)
Mean age in years (range)	34.6 ± 12 (19–54)	36.5 ± 13 (20–58)
Years of education (range)	15.6 ± 3 (11–19)	16.2 ± 2 (12–20)
Employed full-time	8	9
Hand preference for writing	3 left; 8 right	0 left; 11 right
Height (inches)	71.4 ± 4.1; range, 65–77	71.3 ± 4.9; range, 60–79
Primary psychiatric diagnoses	Anxiety disorder: 1 Attention deficit disorder: 2 Bipolar disorder: 2 Major depression: 3 Schizoaffective disorder: 1 Normal: 2	 Bipolar disorder: 3 Major depression: 1 Substance abuse: 3 Normal: 4
Psychiatric hospitalizations	2	0
Auditory hallucinations	4	0
Formal thought disorder	1	0
Dyslexia	7	0
Alcohol abuse	4	2
Drug abuse	2	1
Medications: Some taken in combination	Testosterone: 9 Anxiolytic: 1 Antidepressant: 0 Neuroleptic: 1 Lithium: 0 Antiepiletic: 1 None: 1	Testosterone: 4 Anxiolytic: 1 Antidepressant: 3 Neuroleptic: Lithium:1 None: 6

when ICC and age were controlled for in the analysis, they had significantly smaller frontal lobes on the left [$F(3,18) = 6.03$, $P < 0.01$] and right [$F(3,18) = 5.93$, $P < 0.01$]; temporal lobes [left: $F(3,18) = 5.40$, $P < 0.01$ and right: $F(3,18) = 6.32$, $P < 0.005$]; the total STG [left: $F(3,18) = 7.00$, $P < 0.003$ and right: $F(3,18) = 5.80$, $P < 0.006$]; and the posterior STG [left: $F(3,18) = 9.22$, $P < 0.005$ and right: $F(3,18) = 5.92$, $P < 0.01$]. There were no significant differences in the anterior STG, amygdala hippocampus complex, or the ventricles between groups Table III.

There was a non-significant trend for the XXY males to have less rightward STG asymmetry than controls (laterality indices: 0.01 ± -0.13 vs. -0.08 ± 0.13; $F(2,19) = 2.963$, $P = 0.076$) using the asymmetry coefficients calculated. No other asymmetry differences were found.

No clinical correlations to any regional brain measurements were significant when controlled for number of tests performed. The length of time and amount of testosterone treatment were also uncorrelated with any clinical measurements.

Structure-cognition correlations. The cognitive variables that differed between the groups following covariance analysis were examined for their correlations with the brain volumetric measures. These correlations were computed within each of the two groups separately. For the XY controls, Stroop test color-word interference was significantly correlated with whole brain volume ($r = 0.77$), left ($r = 0.81$) and right ($r = 0.71$) frontal lobe volume and right hippocampal volume($r = 0.61$). In contrast, there was only one correlation between reading scores and brain volume that was significant,

TABLE II. Neuropsychological Test Results—Cognitive Performance Scores: Significant Differences Only

	XXY males	Control males		
Variable	Mean ± SD	Mean ± SD	*t*	*P*
Stroop test				
Word reading	42.73 ± 12.42	54.27 ± 5.04	2.86	0.009
Color naming	35.36 ± 14.44	49.00 ± 8.46	2.70	0.010
Interference	35.09 ± 11.06	52.18 ± 13.25	3.28	0.004
WAIS-R scaled scores				
Information	9.55 ± 3.50	13.90 ± 2.74	3.25	0.004
Vocabulary	9.18 ± 4.00	13.59 ± 3.30	2.79	0.010
WMS-R visual reprod. II	29.27 ± 6.40	35.36 ± 5.12	2.46	0.020[a]
Sentence repetition test	12.81 ± 1.94	17.45 ± 3.25	4.10	0.001
Peabody picture vocabulary test	91.75 ± 12.62	116.00 ± 19.01	3.05	0.008
Wide-range achievement test reading scaled score	97.90 ± 13.79	114.91 ± 16.75	2.60	0.010

Note: No other differences achieved statistical significance, including Full-Scale, Verbal, and Performance IQs. Auditory Comprehension, Token Test, The Boston Naming Test, Controlled Oral Word Association, the Wisconsin Card Sort Test, Verbal Memory, the Benton Visual Retention Test, and Trail-Making Tests Parts A and B.
[a]Does not meet Bonferroni criteria.

TABLE III. Brain Structural Measurements

	Klinefelter's males (XXY)	Control males (XY)	F for diagnosis, controlling for age and intracranial contents (df 3,18)	$P<$
	Volume of structure (mm^3) (mean ± SD)			
Intracranial contents** (ANCOVA)	1472.974 ± 135.11	1593.650 ± 137.511	4.5	0.05
Frontal lobe				
Left	68.0 ± 6.3	72.0 ± 15.3	6.0	0.01
Right	70.0 ± 5.5	73.8 ± 16.4	5.9	0.01
Temporal lobe				
Left	70.0 ± 10.1	72.4 ± 13.0	5.4	0.01
Right	67.3 ± 8.4	74.6 ± 15.5	6.3	0.005
Superior temporal gyrus				
Left total	11.7 ± 2.6	12.2 ± 1.5	7.0	0.003
Anterior	2.6 ± 0.88	2.0 ± .72	1.7	0.209
Posterior	9.2 ± 2.4	10.2 ± 1.8	9.2	0.001
Right total	11.5 ± 2.2	13.2 ± 2.1	5.8	0.001
Anterior	2.9 ± 1.1	2.6 ± 1.1	0.45	0.724
Posterior	8.6 ± 1.9	10.6 ± 2.2	5.9	0.001
Hippocampus/amygdala				
Left	3.9 ± 0.8	4.2 ± 0.6	1.5	0.24
Right	4.1 ± 0.6	4.5 ± 0.8	3.1	0.06
Ventricles				
Left	12.2 ± 6.3	12.1 ± 7.1	2.0	0.15
Right	13.6 ± 6.7	13.2 ± 8.3	0.4	0.77

ANCOVAs were performed controlling for age and total intracranial contents.
**Controlled for age only.

right hippocampal volume ($r = 0.62$, $P < 0.01$). For the XXY cases, there no statistically significant correlations for either cognitive variable or any brain volume measure.

Diffusion tensor imaging (DTI). There were four clusters where XXY males had significantly decreased FA values compared with XY males. They were defined as a cluster of greater than 100 voxels where all voxels had a $P < 0.001$ (two-tailed) using to a two-tailed *t*-test. The Talairach coordinates corresponding to these four clusters were: (a) x = 20 (left), y = −10, z = 8; (b) x = −9 (right), y = −26, z = 3; (c) x = 35 (left), y = 35, z = 33; (d) x = 8(left), y = 1, z = 31. These, in turn, corresponded to (a) left posterior limb of the internal capsule subcortically between the lentiform nucleus and thalamus; (b) right anterior cingulate; (c) left arcuate bundle connecting the STG and dorsolateral frontal cortices; and (d) the left anterior cingulate (see Fig. 2).

DISCUSSION

Much attention has previously been given to the association of the XXY karyotype with psychiatric disorders, particularly affective psychoses and schizophrenia [e.g., Rohde, 1963; Kvale and Fishman, 1965; Hambert, 1966; Nielsen, 1969; Nielsen et al., 1969, 1980; Forsmann, 1970; Rosen et al., 1970; Sorensen and Nielsen, 1977; Roy, 1981; reviewed in DeLisi et al., 1994] Of the 11 subjects with Klinefelter's syndrome in the present study, 1 was diagnosed with chronic schizoaffective disorder, and 3 others had a predominant affective disorder with psychotic features. None of the controls had any evidence of psychosis, although four of them had a major affective disorder. Thus, these data are consistent with earlier reports, and suggest that psychotic symptoms, particularly auditory hallucinations, may be associated with having an extra X chromosome.

Nevertheless, evidence of cognitive functional disturbances may provide a more direct window into the biological anomalies that are a consequence of having, in the case of XXY men, an excess expression of genes responsible for brain growth and differentiation. Studies of XXY children consistently show that although they generally do not have mental retardation, specific verbal skills are lacking, including reduction in those for reading and language comprehension [reviewed in Rovet et al., 1996; Bender et al., 2001]. The studies that exist of adult XXY men are less consistent, reporting a heterogeneous pattern whereby some of these men have non-verbal IQs that are worse than verbal IQ's and others the reverse [e.g., Theilgaard, 1986; Boone et al., 2001]. One study [Fales et al., 2003] reported specific deficits in verbal working memory in XXY adults. Our current results indicate that the majority of cognitive impairments in adult XXY men can be reduced to impairments in verbal skills related to word recognition in reading (as indexed by WRAT reading scores). This is consistent with the observation that as many as 7 of the 11 XXY men reported dyslexia as a child compared with none of the controls, despite both groups having achieved the same educational levels. It was also interesting that the specific color-word interference task, as measured in the Stroop test, was abnormal in these men. Although the latter may reflect the decisional capacity attributed to frontal lobe functioning (i.e., executive function), it may also be associated with language comprehension (i.e., complex verbal directions).

It is relevant that the current study also found frontal and temporal lobe (particularly STG) gray matter volume reductions with corresponding white matter tract abnormalities (e.g., arcuate bundle). Previous MRI studies of XXY men also reported loss of temporal lobe volume [Patwardhan et al., 2000] and smaller overall brain volumes [Warwick et al., 1999; Patwardhan et al., 2000], although in contrast to the Warwick et al. study, we failed to detect a difference in ventricular size. The reduction in the STG, particularly its posterior portion, is of interest because this region includes the planum temporale, a structure implicated specifically in language processing [Galaburda et al., 1978] and thought disorder in schizophrenia [Shenton et al., 1992]. Abnormalities in these structures may thus underlie the verbal, cognitive, and behavioral deficits seen.

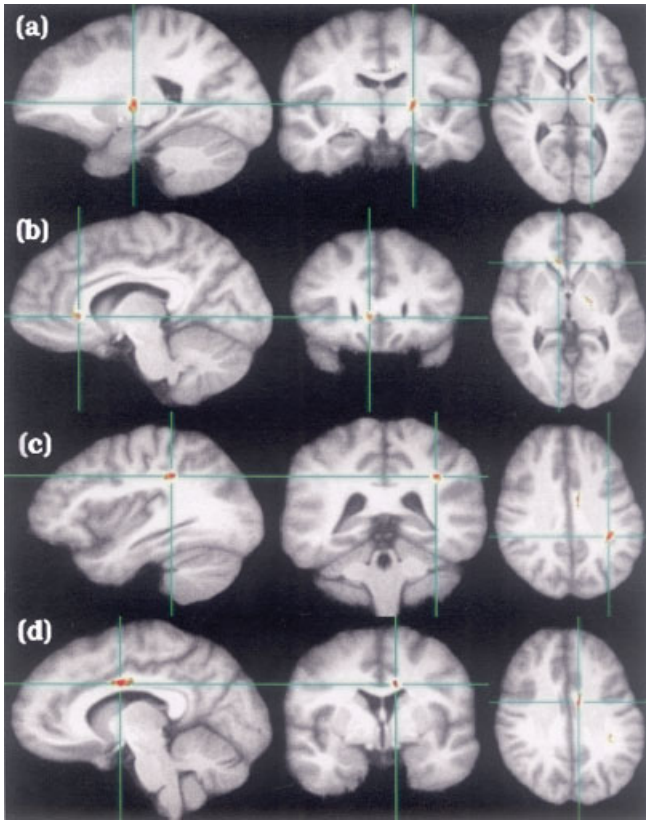

Fig. 2. Illustration of DTI results. There were four clusters where XXY males had significantly decreased FA values compared with XY males. They are defined as a cluster of voxels with a size greater that 100 voxels where all voxels have a $P < 0.001$ according to a two-sample t-test. The Talairach coordinates corresponding to these four clusters are: (a) x = 20 (**left**), y = −10, z = 8; (b) x = −9 (**right**), y = −26, z = 3; (c) x = 35 (**left**) y = 35, z = 33; (d) x = 8 (**left**), y = 1, z = 31. (a) Left posterior limb of the internal capsule subcortically between the lentiform nucleus and thalamus; (b) right anterior cingulate; (c) left arcuate bundle connecting the superior temporal gyrus and dorsolateral prefrontal cortex; and (d) the left anterior cingulate.

Some investigators have suggested that anomalous cerebral asymmetry is present in individuals with an XXY karyotype [Netley and Rovet, 1982b; Geschwind et al., 1998] and that cerebral dominance is determined by an X-Y homologous set of genes [Crow, 1994]. It is also present in schizophrenia [reviewed by Crow, 2004]. Since Warwick et al. [2003] described anomalous prefrontal and temporal cortex structural asymmetries in one case of Klinefelter's syndrome with schizophrenia, and Itti et al. [2003] found reduced functional asymmetries in upper left temporal and parietal regions, we examined whether any indication of anomalous asymmetry in brain structure was present. However, none was detected. Nevertheless, it is interesting that three of the XXY males had predominant left-hand preference, compared to none of the controls, and three of the four white matter findings by DTI were left-sided.

Although reduced testosterone production is known to be a hallmark of Klinefelter's syndrome, it is unlikely to explain the brain structural and functional differences noted above since testosterone levels are thought to be normal in XXY individuals prenatally and throughout childhood before puberty [Stewart et al., 1986; Ratcliffe et al., 1994] when maximal brain growth occurs. XXY males are not placed on testosterone therapy until adolescence. At least by this later age, as in the current study, treatment would be unlikely to reverse any of the brain deficits, despite the report by Patwardhan et al. [2000] describing gray matter volume reductions only in a small number of XXY males who were not taking testosterone. In addition, the fact that XXX females and XYY males *do not* have hormonal deficiencies, but *do* have similar verbal deficits to XXY men [Netley and Rovet, 1982a; Netley, 1986; Robinson et al., 1986] suggests that excessive production of genes on the sex chromosomes, not hormonal deficits, may be involved in producing an abnormal pattern of brain growth.

Finally, we note that although all of the Klinefelter's males in the current study had repeat karyotypes performed to confirm their XXY status, some were shown to have varying degrees of mosaicism. Most of the other publications quoted in this manuscript do not report results of such karyotyping and thus it is possible that past reports were of men who also had some degree of mosacism. It is unclear what impact this heterogeneity in individual cell karyotypes might have on the results reported here and particularly on brain development. We do not actually know whether a degree of mosaicism would also be present in brain cells. However, a big variation does exist in the range of cognitive functioning, behavioral changes, and brain structural size among these men. Our sample size is too small to be able to clarify whether the degree of mosaicism is associated with this variation.

In summary, we have confirmed that adults with an XXY karyotype frequently have significant psychopathology, verbal language deficits, and corresponding structural brain deficits, both in frontal and temporal lobes. In addition, we now add, using the newer MRI methods of DTI that abnormalities are also present in white matter. These data suggest that the presence of an extra X chromosome leads to excess expression of one or more X chromosome genes influencing both gray and white matter development in the frontal and temporal lobes, and white matter tracts leading to them. This, in turn, may contribute to the executive and language deficits observed in these adults. The similarities of these findings with those in individuals with schizophrenia, and conversely the excess of psychotic symptoms in XXY men, further suggest that Klinefelter's syndrome may be a genetic model for the study of the contribution of genes on the X chromosome to the development of psychotic disorders. Future studies in a larger cohort of males with the XXY karyotype are needed to determine which gene or genes are involved and whether their expression could be modified with appropriate treatments early in life.

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