Reduced Size of the Amygdala in Individuals With 47,XXY and 47,XXX Karyotypes

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The excess of 47,XXX and 47,XXY karyotypes found in cytogenetic screening studies of individuals with schizophrenia has given support for an increased risk of psychiatric illness among men and women with sex chromosomal aneuploidy (SCA). Mesial temporal lobe structures, including the amygdala and hippocampus, are thought to be associated with abnormalities of mood and behavior in humans and in the neurobiology of schizophrenia. This study focuses on variations in volumes of mesial temporal lobe structures in men and women with SCA. Utilizing an unselected birth cohort of subjects with SCA and high-resolution magnetic resonance imaging (MRI), we investigated the neuroanatomical consequences of a supernumerary X chromosome on the morphology of the amygdala and hippocampus. Regional and total brain volumes were measured in 10 subjects with 47,XXY, 10 subjects with 47,XXX, and 20 euploid controls. Amygdala volumes were significantly reduced in men with 47,XXY, compared to control men, while the decrease in women with 47,XXX was not as pronounced. Hippocampus volumes were preserved in both groups, compared to same-gender controls. Longitudinal studies of SCA individuals have shown an increased incidence of mild psychopathology and behavioral dysfunction in men with 47,XXY and more overt psychiatric illness in women with 47,XXX, compared to control populations. The alteration in amygdala volumes in individuals with a supernumerary X chromosome may provide a neuroanatomic basis for these findings. © 2001 Wiley-Liss, Inc.

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INTRODUCTION

The presence of a supernumerary X chromosome in humans is often associated with atypical physical, cognitive, and behavioral features in both genders. In men with a 47,XXY karyotype, a constellation of physical features often occurs and is referred to as Klinefelter syndrome (KS). Hypogonadism and infertility due to reduced or absent spermatogenesis are hallmark features of KS. Men with KS also have taller statures and lower serum testosterone levels than euploid men and occasionally exhibit gynecomastia. Women with the 47,XXX karyotype often have a normal appearance with relatively tall stature and exhibit typical pubertal development, hormone levels, and reproductive competency [Harmon et al., 1998].

Neurodevelopmental abnormalities associated with sex chromosomal aneuploidy (SCA) are directly or indirectly related to the presence of additional X chromosome material. Both 47,XXY and 47,XXX forms of aneuploidy are at risk for developmental delay, including language and learning impairment [Netley and Rovet, 1982; Bender et al., 1986; Harmon et al., 1998]. Most men with KS perform normally on tests of nonverbal abilities and general intelligence but are often specifically impaired on measures of language skills. These deficits are most apparent in areas of verbal fluency and expression [Netley and Rovet, 1982; Ratcliffe et al., 1994] and are consistent with the high incidence of reading disabilities in boys and men with KS [Bender et al., 1986]. Women with 47,XXX are generally noted to have mild global intellectual deficits with full-scale IQs (FSIQs) falling below that of sibling
control groups. Although their deficits are often non-specific [Linden et al., 1988], impairments in tasks of spatial processing and linguistic skills have been documented [Netley and Rovet, 1982; Bender et al., 1989].

Several studies have investigated the behavioral phenotype of males with KS and females with 47,XXX. There is an increased incidence of psychiatric disorders in KS, including anxiety, depression, and conduct disorder [Mandoki et al., 1991; Bender et al., 1995], although in most cases these disorders are relatively mild in severity. Prospective cohort studies [Walzer et al., 1978; Stewart et al., 1986] and studies of adults [Theilgaard, 1984] also have characterized men with KS as having passive personalities and being less assertive, social, and active than euploid men. Some boys with KS also are noted to have attention and impulse control difficulties [Walzer et al., 1978, 1991]. Women with 47,XXX have an increased incidence and severity of psychiatric disturbances than other forms of SCA, although phenotypic variability also is relatively large. As a group, they are less well adapted than sibling controls, while experiencing more work, leisure, and relationship problems [Harmon et al., 1998].

Epidemiological studies [Heaton-Ward, 1977; Doody et al., 1998] have shown that populations with mild learning disabilities, a feature common in individuals with SCA [Geschwind et al., 2000], also have higher rates of mental illness than that observed in the general population. Additionally, one study reported on the excess of the 47,XXX and 47,XXY karyotypes in schizophrenia [DeLisi et al., 1994b], suggesting a possible role of a sex chromosome gene or genes in the neurodevelopmental disturbances influencing the risk for psychotic disorder. Although the individual risk for psychosis in a person with SCA might be small, the elucidation of biological mechanisms leading to supernumerary X chromosome material as a risk factor for psychosis would be of great potential value.

Bilateral reduction of mesial temporal lobe structures is perhaps the most consistent finding in neuroimaging studies of schizophrenia [Lawrie and Abukmeil, 1998; McCrory et al., 1999; Wright et al., 2000], with preferential loss of gray matter tissue in these regions [Lawrie and Abukmeil, 1998]. Previous studies of individuals with SCA have shown decreased cerebral gray volumes in areas of the temporal lobe in KS [Patwardhan et al., 2000] and normal bilateral volume of a conjoined amygdalo-hippocampal complex (AHC) [Warwick et al., 1999] in men and women with SCA. However, no study has yet measured separate amygdala and hippocampus volumes in patients with 47,XXX or 47,XXY. An increased risk for psychiatric illness in SCA [DeLisi et al., 1994b; Everman and Stoudemire, 1994], combined with the increased incidence of mild psychopathology in 47,XXX and more severe illness in women with 47,XXX [Linden et al., 1988], suggests that the morphology of the amygdala and hippocampus also may be altered in persons with SCA. In this study we test this hypothesis with a highly unique cohort of individuals with 47,XXY and 47,XXX ascertained at birth.

MATERIALS AND METHODS

Subjects

Subjects with 47,XXY and 47,XXX were recruited from an unselected cohort of 40,000 consecutive newborns screened for SCA by X chromatin examination from 1964–1974 [Robinson et al., 1982]. Among the 61 subjects with SCA recruited by the Denver group and followed longitudinally, 13 had the 47,XXY karyotype and 12 had the 47,XXX karyotype. Ten of the 47,XXY subjects and 10 of the 47,XXX subjects received research magnetic resonance imaging (MRI) scans at the University of Colorado Health Sciences Center in Denver, Colorado. Subjects with 47,XXY (mean age at MR scan, 27.3 years; SD, 3.00) were individually age matched to control men within 2 years of age (mean age at MR scan, 27.3 years; SD, 3.49). Subjects with 47,XXX (mean age at MR scan, 29.1 years; SD, 2.31) also were individually age matched to control women within 2 years of age (mean age at MR scan, 28.5 years; SD, 3.21). Informed consent was obtained from all subjects prior to scanning.

Handedness information was also obtained from all subjects involved in the study. Of the 20 subjects with aneuploidy, only 1 subject with 47,XXY and 2 subjects with 47,XXX were left-handed. Wechsler Intelligence Scale (WISC-R) scores of FSIQ also were previously obtained for both men with 47,XXY (mean, 91.30; SD, 13.86) and women with 47,XXX (mean, 81.73; SD, 15.64) during childhood.

Neuroimaging

MR data were acquired using 1.5-Tesla GE Signa scanners. Coronal 3D volumetric spoiled gradient echo (SPGR) series were acquired (TR = 35, TE = 6, flip angle = 45°, number of excitations = 1, field of view (FOV) = 24, matrix = 256 × 192, 124–1.5-mm contiguous slices) and used for all measurements and analysis.

The volumetric assessment of image data in Brain-Image® [Reiss, 2000] required a stepwise process of data importation, removal of nonbrain voxels, correction of image nonuniformity, positional normalization, and manual delineation of regions of interest (ROIs). Brain tissue was isolated [Subramaniam et al., 1997] and coronal images were oriented perpendicular to the anterior commissure–posterior commissure plane. The intraclass correlation coefficient was used to evaluate inter-rater reliability between two raters trained to circumscribe areas of the hippocampus (.91) and amygdala (.97) to ensure accuracy in measurements. A single rater, blinded to group membership, then circumscribed regions of the amygdala and hippocampus for subjects with 47,XXY or 47,XXX and the 20 control group subjects on coronal images oriented perpendicular to the anterior commissure–posterior commissure plane and according to a protocol previously developed in our lab [Kates et al., 1997]. To increase the resolution at which the ROIs could be drawn, the matrix sizes of the coronal data sets were expanded from 256² pixels to 512² pixels using a bicubic...
interpolation algorithm. Volume measures recorded total tissue volumes (white and gray matter) for all ROIs.

The amygdala was circumscribed coronally and proceeded posteriorly, beginning on the slice where the anterior commissure first crosses the midline of the brain. The surrounding white matter tract defined the inferior border of the amygdala, while the medial border of the amygdala was defined as the cerebral spinal fluid (CSF)/gray border. The delineation continued superiorly and laterally around the amygdala following the gray/white border. In the posterior regions of the amygdala, the superior border was partially defined by the presence of the entorhinal sulcus. The amygdala was drawn until it disappeared posteriorly.

The anterior-most slice of the hippocampus was determined by the presence of the alveus and by the development of a laminar structure that distinguishes the hippocampus from the amygdala. The borders were defined by the surrounding white matter and CSF and superiorly by the amygdala. The hippocampus excluded the tail of the caudate nucleus anteriorly and excluded the thalamus posteriorly. Circumscription of the hippocampus continued until it disappeared posteriorly, approximately at the point where the corpus callosum fuses with the fornix.

**Statistics**

Analysis of variance (ANOVA) was used to assess differences in age between groups and comparisons of whole-brain tissue volumes among 47,XXY, 47,XXX, and control subjects. Analysis of covariance (ANCOVA) was used to assess comparisons of hippocampus and amygdala tissue volumes and used whole-brain tissue as a covariate to adjust for differences in total brain volumes between groups. Group differences took diagnoses as a between-subject factor. A two-sided $P$ value of 0.05 was the significance threshold for all analyses.

**RESULTS**

Total brain volumes were distributed normally among the 47,XXY, 47,XXX, and control groups. ANOVA revealed no significant differences between subjects with X chromosome aneuploidy and gender-matched controls for MR scan age (men, $P < .9892$, $F = 1.885 \times 10^{-4}$; women, $P < .6650$, $F = .194$).

### 47,XXY

Whole-brain tissue volumes were reduced in men with 47,XXY, compared to control men (Fig. 1), although this difference did not reach significance (Table I). ANCOVA analysis revealed significantly reduced amygdala tissue volumes in men with KS ($P < .0010$, $F = 15.755$). Hippocampus volumes in the 47,XXY group were not significantly different from volumes measured in control men ($P < .3089$, $F = 1.100$).

### 47,XXX

Whole-brain tissue volumes were significantly reduced in women with 47,XXX, compared to control women (Fig. 1) ($P < .0040$, $F = 10.897$) (Table I). Hippocampus volumes were not significantly reduced in women with 47,XXX, compared to control women ($P < .0538$, $F = .064$). Although amygdala volumes in the 47,XXX group showed a trend for reduced volumes, compared to controls, this difference was also not significant ($P < .0614$, $F = 4.012$).
DISCUSSION

An imbalance of gene products in SCA may leave affected individuals susceptible to psychosis [DeLisi et al., 1994a] or other forms of milder psychiatric illness. Previous cytogenetic screening studies have found an increased representation of the 47,XXX and 47,XXY karyotypes among children and adults with schizophrenia and unspecified psychotic disorder [Nielsen and Wohlert, 1991; DeLisi et al., 1994b; Kumra et al., 1998]. Despite these findings, longitudinal studies following small samples of individuals with SCA karyotyped at birth have found that most subjects perform relatively well into adulthood, with only a few instances of serious psychiatric illness. Psychotic disorders in the current population of SCA men and women have been rare [Linden et al., 1988]. Nevertheless, the elucidation of neuroanatomical variation that represents a risk factor for psychopathology in this population is of potential importance.

Variations in mesial temporal lobe volumes in populations at risk for or diagnosed with schizophrenia have been consistently reported. For example, both Lawrie et al. [1999] and Seidman et al. [1997] have reported reduced combined AHC volumes or subcortical volumes, respectively, in subjects with two or more first- or second-degree relatives with schizophrenia, compared to general population controls. Several morphometric MR studies also have reported reduced amygdala or AHC volumes in schizophrenic patient populations [Bogerts et al., 1990; Breier et al., 1992; Shenton et al., 1992; Pearlson et al., 1997; Bryant et al., 1999] and in patient populations co-morbid for schizophrenia and learning disability [Sanderson et al., 1999].

The sex chromosomes have numerous genes implicated in the development of higher intelligence [Crow, 1993; Turner, 1996; Skuse et al., 1997]. However, the few reports [Warwick et al., 1999; Patwardhan et al., 2000] of the effects of sex chromosomal polysomy on brain development have produced conflicting results. In the present study, men with 47,XXX demonstrated significant reductions in amygdala tissue volumes, compared to control men, yet showed relative preservation of hippocampus and whole-brain tissue volumes. These results are in apparent contrast to recent reports of preserved AHC volumes in another group of unselected young adults with 47,XXX [Warwick et al., 1999]. However, measures of composite structures like the AHC may be less sensitive to individual variations in amygdala volumes than separate and discrete measures of the amygdala and hippocampus. Warwick et al. [1999] also described enlarged ventricular volumes in men with KS, a finding often reported in studies of schizophrenia yet not found in the current cohort of aneuploid men [Patwardhan et al., 2000]. Differences in these results may be due to differences in the methods used for isolating ventricular ROIs or in sampling [Warwick et al., 1999; Patwardhan et al., 2000].

The presence of atypical mood or temperament [Bancroft et al., 1982; Stewart et al., 1982] in individuals with KS also suggests that temporal lobe development and function may be abnormal, particularly in mesial temporal regions. Amygdaloidal nuclei project to a variety of regions of the brain thought to influence neuroendocrine, cognitive, and emotional aspects of information processing. The amygdala is known to play an integral role in the mediation of affective behaviors in primates [Aggleton, 1993] and complex emotional states, such as depression and aggression, in humans [Drevets et al., 1992; LeDoux, 1995; Amen et al., 1996]. The destruction of the amygdala, whether by bilateral amygdalec-tomy in monkeys [Kluver and Bucy, 1997] or by selective surgery in humans [Kiloh and Smith, 1978], results in a loss of aggressive behavior and reduced sexual drive [Kluver and Bucy, 1997]. Adolescents and young men with KS have reduced activity levels, self-esteem, and sexual interest, compared to euploid peer groups [Bancroft et al., 1982; Ratcliffe, 1982], and often describe themselves as being more sensitive, introspective, and insecure [Ratcliffe et al., 1982; Mandoki et al., 1991]. Accordingly, altered amygdala volumes in KS may provide a possible neuroanatomic basis for the atypical temperament and passivity associated with this condition. Decreased amygdala volume also may be associated with the decreased sexual drive often described in 47,XXX men prior to testosterone therapy given that the medial posterior dorsal nucleus (MePD)
of the amygdala is implicated in reproductive behavior [Kondo et al., 1997] and is richly endowed with androgen and estrogen receptors.

Although women with 47,XXX show a reduction in amygdala volumes, compared to controls (effect size of 0.9), this result was not significant after covarying for overall reductions in whole-brain volume. Like men with 47,XXX, the hippocampus was relatively preserved in women with 47,XXX, compared to controls, despite the presence of whole-brain reduction. The behavioral phenotype seen in women with 47,XXX does not appear to be associated with as dramatic a reduction in mesial temporal lobe volumes and may instead also be attributable to regions of the brain not assessed in this study or to a more global loss of tissue. In contrast to men with 47,XXX, women with the 47,XXX karyotype undergo brain development within the context of a normal hormonal environment. This may be a moderating factor associated with the more modest reductions in amygdala volumes (relative to whole brain) observed in this group.

During fetal life, the brain is organized by its hormonal environment to form a sexually dimorphic organ. In addition to the sexual differentiation prescribed by sex-determining genes, the neurodevelopmental roles of sex steroids also are thought to be influential during neonatal, peripubertal, and adult life [McClelland, 1999]. Cooke et al. [1999] recently demonstrated the sensitivity of the MePD to circulating androgens in adult rats. The MePD is larger in volume in male rats than in female rats. Castration of adult males, however, causes a reduction in amygdala volume to control female levels within 4 weeks. Conversely, exogenous androgen supplementation of female rats increases amygdala volumes to levels found in control male rats.

In the study presented here, gender differences in regional brain volumes between control men and control women were observed for amygdala tissue. Reduced volumes in men with 47,XXX may be due to interactions between genetic influences and hormone action in early developmental life and possibly into adulthood. Atypical hormonal levels in men with KS may affect development and maintenance of mesial temporal lobe regions given the strong evidence of androgen and estrogen receptor localization in mammalian hippocampus [Gould et al., 1990] and amygdala [Simerly et al., 1990; Yokosuka et al., 1997] tissue and human temporal cortex [Sarrieau et al., 1990]. It also is possible that reduced amygdala volumes are independent of the sex steroid environment. Other explanations for reduced size of the amygdala in 47,XXX include elevations in glucocorticoid and cortisol levels during repeated depressive or high-anxiety states, a model that has previously been proposed to explain hippocampus volume loss during major depression [Axelson et al., 1993; Drevets, 1999].

While the causative factor in reduced amygdala volumes in 47,XXX is not yet established, the role of sex steroid hormones in forming what is normally a sexually dimorphic region of the brain merits further investigation.

An increased risk for schizophrenia and psychosis in SCA has been reported through previous screening studies of psychiatric patient populations. Longitudinal studies of individuals with SCA followed from birth provide a unique unselected sample population for investigating genetic and hormonal risk factors for cognitive, behavioral, and psychiatric disability. Most individuals in the current SCA cohort have adapted relatively well into adulthood [Bender et al., 1995; Harmon et al., 1998], although there is an increased incidence of severe psychopathology, particularly among women with 47,XXX. Future structural and functional MRI studies should focus on these individuals in mid-adulthood and on the functional consequences of reduced volumes in the temporal lobe regions.

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